UNIVERSITY OF SOUTHAMPTON

FACULTY OF HEALTH SCIENCES

Nursing

Examining biofilm development within fine-bore nasogastric tubes used by adults and exploring the nursing perspective of tube management

by

Michelle Baker-Moffatt

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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The consequences of malnutrition are wide ranging, making it an important patient safety issue. Enteral tube feeding can support adults unable to meet their nutritional requirements through oral intake, with fine bore nasogastric tubes preferred for patient comfort. Over a third of nasogastric tubes in use block, resulting in compromised nutrient provision and poor patient experience. Biofilm can interrupt fluid flow through indwelling devices. Evidence of biofilm on the inner surface of nasogastric tubes and its potential to contribute to blockage development had not been investigated. The purpose of this thesis was to determine the presence and distribution of biofilm on the inner surface of fine bore nasogastric tubes used by adults. In addition, to explore the nursing perspective of nasogastric tube management and the maintenance of patency.

A series of laboratory studies enabled the control of known variables, allowing detailed investigation of characteristics of biofilm development. A convenience sample of patient-used tubes were used to investigate the potential for patient variables to influence biofilm development. Statistical analysis was undertaken. A purposive sample of acute care nurses participated in a qualitative semi-structured interview study exploring the nursing perspective of nasogastric tube management through nurses' beliefs and reported practices. Thematic analysis was conducted.

The findings demonstrate the rapid development of biofilm, which can influence the pH of its environment. No advantage was demonstrated to flushing tubes with sterile water compared with tap water. No correlation between patient variables and bacterial colonisation was indicated. The semi-structured interviews illustrate current practice on busy modern hospital wards with competing priorities. Results were examined using thematic analysis, finding three main themes; looking after the patient, using the nasogastric tube, and stopping the NG tube blocking.

This research has achieved its aim of investigating the presence and distribution of biofilm within nasogastric tubes used by adults, and of exploring the nursing perspective of NG tube management and the maintenance of patency. A greater understanding of the optimal care for nasogastric tubes and the rationale behind that care could potentially lead to a reduction in biofilm development. Historical literature identified little significant change to UK practice regarding nasogastric tube management in the past 20 years. Further research is required investigating the potential role of biofilm in the development of fine bore nasogastric tube blockage.

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DECLARATION OF AUTHORSHIP

I, MICHELLE BAKER-MOFFATT

declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

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I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
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7. [Delete as appropriate] None of this work has been published before submission [or] Parts of this work have been published as: [please list references below]:

Signed: .....

Date:.....

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Chapter 1: Introduction

1.1 Introduction

Malnutrition is estimated to affect over 3 million people in the UK, of which 1.3 million are aged over 65 years (BAPEN, 2016). Up to 34 % of patients admitted to hospital are at risk of malnutrition, particularly those with chronic progressive conditions such as dementia or cancer (BAPEN, 2016). Malnutrition, and in particular undernutrition, is a state in which there is a deficiency of nutrients such as protein, vitamins and minerals resulting in measureable adverse effects on body composition, function or clinical outcome (NICE, 2012; BAPEN, 2016). In 2011 to 2012 the total cost of malnutrition in the UK was estimated to be £19.6 billion, a dramatic rise on previous estimates, with an expectation of further rises in future in response to an ageing population (NICE, 2012; NIHR, 2015; NHS England, 2015). The consequences of malnutrition are wide-ranging, as indicated in Table 1.1, making it an important patient safety issue (Saunders & Smith, 2010; NICE, 2012; BAPEN, 2016).

Table 1.1: The consequences of malnutrition

Reduced ability to fight infection	Increased incidence of pressure ulcers
Reduced strength and mobility	Increased incidence of blood clots
Increased risk of falls	Reduced ability to respond to treatment
Poor wound healing	Prolonged recovery and hospital stay
Apathy or depression	Reduced cardio-respiratory function
Altered gastrointestinal function	Death

(Saunders & Smith, 2010; NICE, 2012; BAPEN, 2016)

Hospital patients identified as at risk of malnutrition may be referred for assessment by a dietitian and a ‘food first’ strategy planned. This can be through increased snacks, small meals, and fortified food and drinks (Barker *et al*, 2011; BAPEN, 2016). However, some patients are unable to meet their nutritional needs by oral consumption through incapacity or contraindication, and require nutrition via a feeding tube. Subject to the patient having a fully-functioning gastrointestinal tract, nutrients can be delivered

directly in to the stomach or small intestine through a tube. This can be the sole form of nutrition, or a supplement to reduced oral intake.

Feeding by tube is not a recent phenomenon, in fact this method has been used for centuries; there are reports of gastric feeding by tube via the nose or mouth since the 16th century (Cresci & Mellinger, 2006). In the past tubes were made of silver, leather, rubber and eel skin stretched over whale bone, with feeds of jelly, egg, sugar, milk, whiskey, beef-tea or wine passed into the tube through a funnel, bladder or syringe attached to the end of the tube (Harkness, 2002; Cresci & Mellinger, 2006).

Enteral nutrition methods and practices have significantly developed over the past 400 or more years. Nasogastric (NG) tubes are the most commonly used tube for modern enteral feeding, particularly for short-term feeding of two to four weeks. Figure 1.1 illustrates the position of an NG tube, passing through the nose, down the oesophagus and into the stomach. Fine bore NG tubes are preferred for patient comfort (Green & Jackson, 2006; ASPEN, 2009), and are typically made from polyurethane or silicone. The external diameter of NG tubes is measured by French gauge (FG) with 1 FG measuring ⅓ mm. Fine bore NG tubes of 6 to 8 FG measure approximately 2 to 2.7 mm external diameter, with the internal diameter of such fine bore tubes measuring much less, presenting a narrow lumen through which to administer enteral feed and medication.

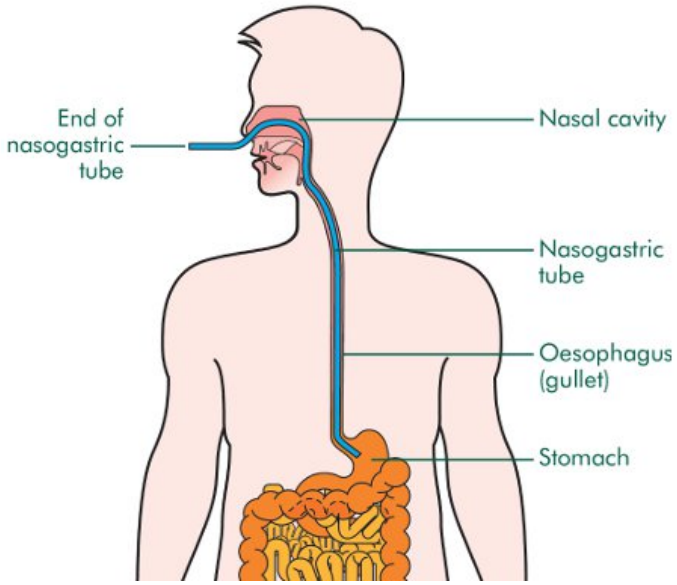


Figure 1.1: Position of an NG tube

The Figure shows the correct placement of an NG tube, indicating the gastric (stomach), oesophageal and nasal sections of the tube

The indications for NG feeding are numerous, some of which are noted in Table 1.2. Although not an exhaustive list, it is clear NG feeding is a relatively common intervention, and any risk to the patency of NG tubes through partial or complete blockage can potentially affect a large patient group.

Table 1.2: Indications for feeding via NG tube

Upper gastrointestinal obstruction	Unconscious, ventilated patient
Oesophageal stricture	Multiple sclerosis
Inflammatory bowel disease	Motor neurone disease
Short bowel syndrome	Liver disease
Head injury	Cystic fibrosis
Stroke	Renal disease
Dysphagia	Anorexia nervosa
Wound healing	Head/neck/oesophageal carcinoma

(Leibovitz *et al,* 2005; NICE, 2006; Lonergan *et al,* 2010)

Unfortunately, studies suggest approximately 35% of all NG tubes in use become blocked (Marcuard & Stegall, 1990; Bourgault *et al*, 2003). This is time consuming for nurses, with efforts to unblock them often proving unsuccessful, leading to tube replacement (Dandeles & Lodolce, 2011). Tube replacement can be a distressful procedure for the patient, and leads to increased demand on nursing resources. Combined with the expense of the new tube and potential radiography to confirm its correct position, NG tube blockages have the potential to add significantly to the cost of patient care (Scanlan & Frisch, 1992; Dandeles & Lodolce, 2011). Moreover, until correct placement of the tube is confirmed, the patient's nutrition, hydration and medication could be compromised, which can lead to delays in health recovery and prolonged hospital stays (Bergin *et al*, 2009).

Whilst a number of studies have focussed on strategies to unblock NG tubes (Wilson & Haynes-Johnson, 1987; Nicholson, 1987; Methany *et al*, 1988; Marcuard *et al*, 1989; Marcuard & Stegall, 1990; Mateo, 1994; Colagiovanni, 2000), a preliminary literature search revealed little research into optimal methods of maintaining NG tube patency. National and international bodies have summarised the available evidence and produced guidelines for practice (CREST, 2004; ASPEN, 2009). However, the research

evidence supporting each statement is generally limited or the statement is based on expert opinion. Essentially, to maintain tube patency practice guidelines suggest NG tubes used by adults should be flushed at regular intervals with water (CREST, 2004; NICE, 2006; PHT, 2013; NNNG, 2016). Table 1.3 outlines the key recommendations for the maintenance of NG tube patency noted by both local acute care hospital enteral feeding policy guidelines and national bodies (CREST, 2004; NICE, 2006; PHT, 2013; NNNG, 2016), with the rationale for each action clearly noted. However, one can appreciate from the table contents there is little notable difference between local and national recommendations, other than the addition of the use of carbonated water as an irrigant. It is clear the effectiveness of this intervention requires investigation, as prevention of tube blockage is preferable in terms of delay, patient distress and cost.

Table 1.3: Management of NG tube patency

Local recommendations	National recommendations	Rationale
Flush tube with 30 to 50 ml sterile or tap water before and after feed using a 50 ml enteral syringe	Flush tube with 10 to 50 ml sterile or carbonated or tap water before and after feed using a 50 ml enteral syringe	To reduce the risk of tube blockage
If patient is fluid restricted these flushing amounts may need to be reduced	Advised to follow local policy	To ensure fluid intake in 24 hour period does not exceed restriction limit
If continuous feed, flush every 4 to 6 hours	Advised to flush 'regularly' but does not state timings	To reduce the risk of tube blockage
Where possible medications should be given in liquid or dispersible form	As per local recommendations	To aid administration of medication via the NG tube
If feed is in progress, feed must be paused, and tube MUST be flushed with water prior to giving medications via the tube	Flush tube with a minimum of 10 ml water before and after medication administration using a 50 ml enteral syringe	To reduce the risk of feed/medication interactions and tube blockage
Medications to be given individually with a minimum 10 ml water flush in between	As per local recommendations	To reduce the risk of medication interactions and tube blockage
Flush tube with water at end of medications and prior to recommencing feed	As per local recommendations	To reduce the risk of tube blockage

(CREST, 2004; NICE, 2006; PHT, 2013; NNNG, 2016)

Previously other methods have been reported for unblocking NG tubes. The use of acidic beverages such as carbonated drinks or cranberry juice is considered

controversial in that such beverages may denature proteins within the feed formula, therefore essentially contributing to clogging (Wilson & Haynes-Johnson, 1987; Beckwith *et al*, 2004). Corpak Medsystems (2013) suggest sugars in such beverages may also contribute to NG tube blockage. Clog Zapper™ is a commercially available product which has shown efficacy in clearing formula-related blockages during a company-funded study of 17 blocked tubes (Corpak Medsystems, 2002; Beckwith *et al*, 2004). Whilst effective, this product does come at a cost of approximately £30 per single-use tube, which can make its use financially prohibitive. With the potential demand on financial and nursing resources blocked NG tubes brings, along with the added burden to patients, there is a clear need to identify and prevent the causes of NG tube blockage, and establish the optimal method of maintaining NG tube patency.

1.2 **Potential causes of NG tube blockage**

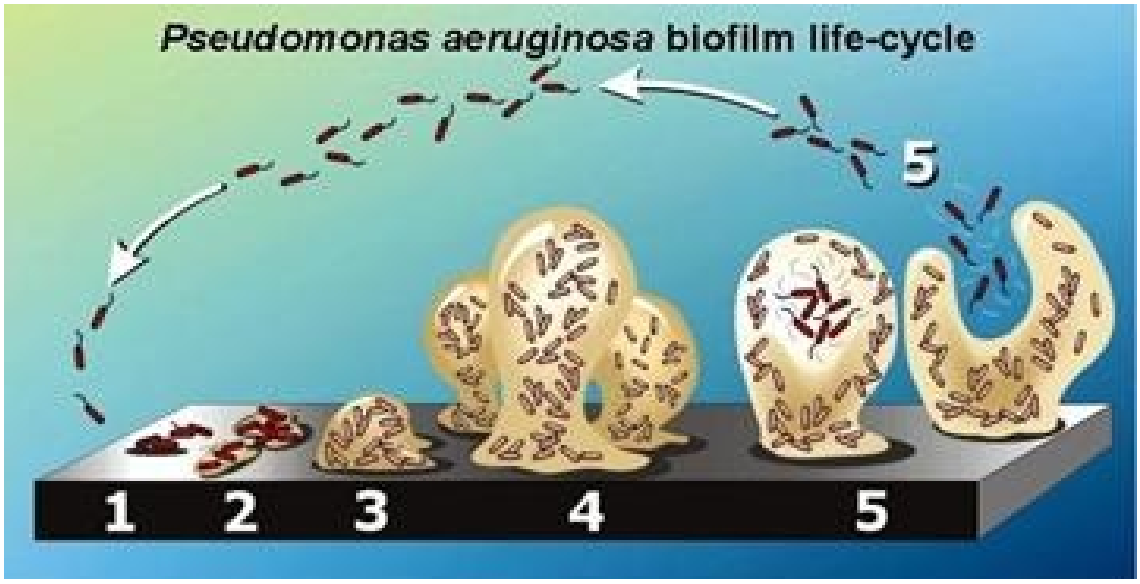
The possible causes of NG tube blockage appear numerous. Personal communication with local NHS Trust nutrition nurse specialists suggests they are often caused by medication and feed, which is a view supported in some part by published studies (Gaither *et al*, 2009; Lonergan *et al*, 2010; Dandeles & Lodolce, 2011). These studies indicate contributory factors may also include the size and material of the NG tube, the flow rate of enteral feed, potential residuals in stomach acid aspirate, and inadequate tube flushing.

Recent research indicates the flow of fluids through indwelling devices used in the healthcare setting can be affected by the presence of bacterial communities called biofilms (Drescher *et al*, 2013). Donlan and Costerton (2002) noted biofilms are present on medical devices such as urinary catheters, prosthetic heart valves, central venous catheters, intrauterine devices, and dental water lines, and Hurrell *et al* (2009a) reported the presence of biofilm within NG tubes used by neonatal patients.

1.3 **Biofilms**

Biofilms are biological systems with a high level of organisation where bacteria that have attached to a surface form structured coordinated communities, and are usually embedded in protective extracellular polymeric substance (EPS) produced by the

bacteria (Marshall, 1992; Davey & O'Toole, 2000). Current understanding states that there are five main phases to the biofilm life cycle: reversible attachment of the bacteria to a surface, irreversible attachment, maturation of the bacteria (first stage), maturation of the bacteria (second stage), and finally the detachment and dispersal of bacteria into the surrounding medium (Cunningham *et al*, 2008). During the reversible attachment phase, planktonic bacteria cells become attracted to the surface and attach themselves, and are able to move across this surface to form colonies with other cells. At the irreversible attachment phase, they become encased in exuded EPS. The maturation phases see the bacterial colonies increasing through multiplication and through 'catching' other planktonic bacteria in the EPS. The matrix formed by the EPS also causes other particles to become captured such as salts, organic matter and nutrients, adding to its mass and complexity. The final phase of dispersal sees planktonic cells released back into the surrounding media. It is thought this occurs to enable the cells to go on and develop new biofilm communities by attaching elsewhere, thus increasing the biofilm coverage of the material surface (Cunningham *et al*, 2008).



CBE (2003) (with permission)

This figure illustrates the biofilm lifecycle of *Pseudomonas aeruginosa* in five main stages: (1) reversible attachment, (2) irreversible attachment, (3) maturation 1st stage, (4) maturation 2nd stage, and (5) dispersal.

Figure 1.2: The life cycle of biofilm

Figure 1.2 illustrates the lifecycle of biofilm using the *Pseudomonas aeruginosa* model. This bacterium readily forms a biofilm, but not all bacteria do, and it may not reflect how biofilms appear in all cases. Our understanding of what biofilm is and how it appears, along with the types of bacteria that produce it, is constantly under review and no

definitive example yet exists. However, if bacteria are present within NG tubes used by adults, they are in a nutrient-rich environment held at body temperature providing the perfect conditions for them to flourish and form biofilm (Frank, 2001).

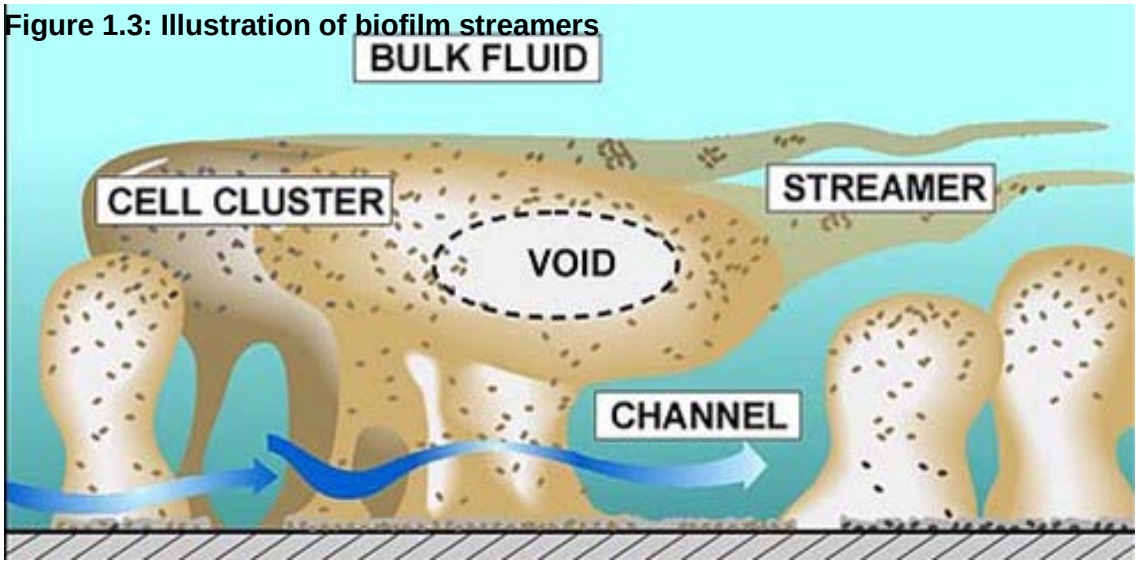
Previous published studies have indicated bacterial attachment and biofilm development were identified on the outside of NG tubes retrieved from adult patients (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005), and the inside of NG tubes retrieved from neonates (Hurrell *et al*, 2009a). *In vitro* studies have also demonstrated bacterial attachment and biofilm development on the surface of NG tube material (Hurrell *et al*, 2009b; Kim *et al*, 2006). Biofilm formation inside NG tubes retrieved from adult patients had not been reported, and the potential for biofilms to contribute to NG tube blockage had not been investigated.

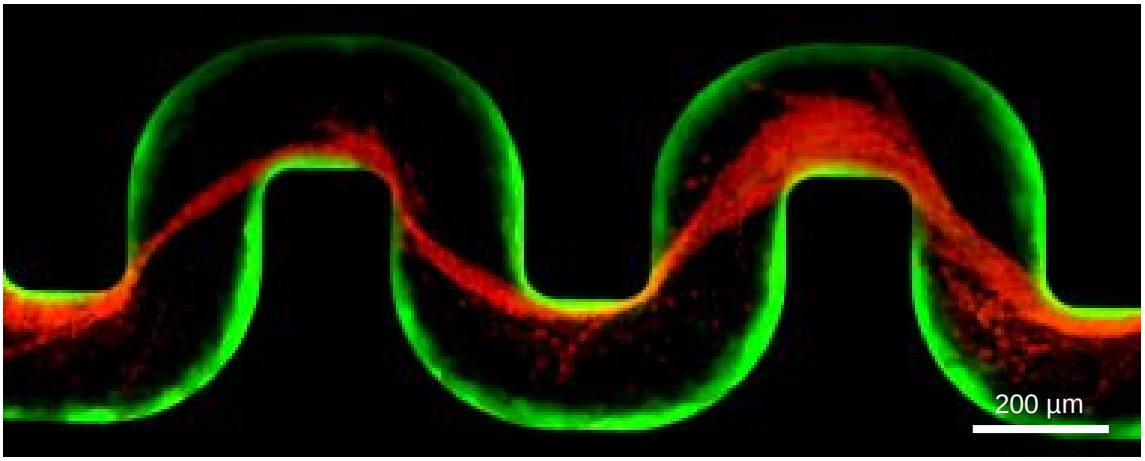
A study by Drescher *et al* (2013) has shown how biofilm development can severely alter the flow of fluids through filters, porous materials and medical stents following the formation of biofilm streamers. These are filamentous structures that extend away from the surface community of the biofilm (Figure 1.3), and have been observed bridging across gaps and fluid flow (Figure 1.4). These streamers in turn generate a sieve-like network which can trap planktonic bacteria, salts and organic matter flowing through, thus increasing its mass (Drescher *et al*, 2013).

CBE (1996) (with permission)

The figure shows a mature biofilm in a flowing environment. It demonstrates the filamentous structures known as biofilm streamers extending away from the surface community. These streamers can alter the flow of fluids through filters, porous materials and medical stents.

Figure 1.3: Illustration of biofilm streamers





Drescher *et al* (2013) (with permission)

The red staining indicates the biofilm streamers noted in Drescher *et al*'s (2013) research investigating the disruption these can cause to fluid flow through devices.

Figure 1.4: Biofilm streamers *in vitro*

1.4 The research question

As outlined earlier, maintaining the patency of NG tubes used for providing patients with nutrition, hydration and medication is paramount to ensuring they receive the optimal care they require and avoid unnecessary prolonged hospital stays. Biofilm is known to develop on indwelling devices, although the presence of biofilm within NG tubes used by adults, and the possibility of it forming an integral part of NG blockages, had not been investigated. The purpose of this thesis is to determine the presence of biofilm, and to investigate its development and distribution, on the inner surface of fine bore NG tubes used by adults. Moreover, to explore the nursing perspective of NG tube management and the maintenance of patency. Therefore, the main research question of this thesis is 'Does biofilm develop on the inner surface of fine bore NG tubes used by adults?' The sub questions and objectives are detailed in section 2.8 'Aims and objectives'.

1.5 Outline of the thesis

This thesis comprises a series of complimentary interlinked studies, employing both quantitative and qualitative paradigms, designed to answer the research question. Chapter 2 presents a review of the literature undertaken to establish what is already

known regarding biofilm in connection with NG tubes. The knowledge gained through completing the literature review process underpinned the design of the subsequent laboratory investigations. Chapters 3 to 8 describe a series of laboratory studies investigating the presence and nature of biofilm development within both sterile and used NG tubes. Undertaking laboratory studies enabled variables such as time, temperature and nutrient availability to be controlled, thus allowing features of biofilm development to be investigated in detail. The time course of bacterial attachment and biofilm development was studied, along with the effect bacteria can have on the pH of enteral feed. Sterile water and tap water flushes were compared in flow-model studies to establish whether significant levels of bacteria were introduced to NG tubes by tap water during flushing, and which irrigant was most effective in preventing or reducing bacterial attachment.

Although data gained through the *in vitro* studies were valuable, it was important to determine what was happening inside NG tubes used by adults in the acute clinical setting. The study reported in Chapter 8 was undertaken to determine whether certain patient variables could influence biofilm presence within NG tubes which were removed as part of the patients' normal care.

Once the laboratory studies were complete, the final study in this thesis was undertaken to gain the nursing perspective of NG tube management. This was achieved through one-to-one interviews with nurses in which they discuss their beliefs and practices, and is reported in Chapter 9. The final discussion chapter then draws together the findings from each of the seven studies, discussing the strengths and limitations of the thesis and interpreting the results, with plans for future work outlined.

Chapter 2: Literature review

2.1 Introduction

Before undertaking any research, a detailed search of available literature should be undertaken to identify previous published studies and literature that are similar or identical to the proposed study (Polit & Beck, 2010a; Beecroft *et al*, 2015). This chapter presents a review of the literature relating to the laboratory-based studies reported in Chapters 3 to 8, and seeks to summarise the most relevant evidence regarding the subject of biofilm presence and development in relation to NG tubes. The literature review for the qualitative aspect of the research question regarding the nursing perspective of NG tube management and the maintenance of patency is reported in Chapter 9.

A structured approach to the literature review was taken in order to ensure a thorough search was conducted, and the most relevant literature identified. The literature was then appraised, and a synthesis of the findings was conducted, guided by themes that emerged from the reported studies, to establish the current understanding regarding biofilm development in relation to NG tubes.

2.2 Search strategy

The first step in developing an effective search strategy was to clearly articulate the focus of the literature that would be sought. The key research question was to determine the presence and investigate the development of biofilm on the inner surface of fine bore NG tubes used by adults. Preliminary searching at the early stages of developing the research question highlighted a distinct scarcity of relevant articles. Key researchers in the fields of biofilm and NG tubes were highlighted as Bill Costerton and Annette Anderton, however their research did not relate to the two subjects combined. Due to the lack of evidence relating to the research question, help was sought from a University librarian to ensure a suitably detailed search was being conducted.

Literature searching began in February 2012, and was repeated periodically up to October 2016 to ensure inclusion of more recently published papers. A comprehensive, structured approach to the literature search was employed, on the continuum between

a narrative review and a full systematic review. A narrative review would have been insubstantial, with no focussed research question or search strategy, and no clear method of appraisal or synthesis of literature (Aveyard, 2010). Conversely, conducting a full systematic review as a researcher working alone with limited resources would have been a substantial undertaking. Therefore, elements were incorporated into the structured approach, such as having a well-focused research question and search strategy, along with explicit synthesis of the literature (Aveyard, 2010).

Indwelling human medical devices such as catheters (venous and urinary), tracheostomy tubes, and prostheses are highly susceptible to harbouring bacteria. The role of biofilms in the contamination of these devices is well established, suggesting 60 % of all nosocomial (hospital-acquired) infections are due to biofilms (Leibovitz *et al*, 2005; Lima *et al*, 2011). As the focus of this research thesis was not the threat of infection biofilm may pose, but rather the potential for biofilm to develop within NG tubes used by adults, with a view to understanding its possible role in reduced tube patency, it was decided only papers relating to either bacterial attachment or biofilm development in combination with NG tubes, as opposed to other indwelling devices, would be considered.

To undertake a comprehensive literature search several sources were utilised, including electronic databases, ancestry searching, author searching, and hand searching. Two especially useful electronic databases for nurses are CINAHL (Cumulative Index to Nursing and Allied Health Literature) and MEDLINE (Medical Literature On-line) (Polit & Beck, 2010a). CINAHL covers references to virtually all English language nursing and allied health journals back to 1982, while MEDLINE is widely recognised as the premier source for bibliographic coverage of biomedical literature. It covers more than 5,000 medical, nursing and allied health journals published in approximately 70 countries, and has records dating back to the mid-1960s. Also important to nursing is the British Nursing Index (BNI), a leading database for the support of practice, education, and research for nurses and allied health carers in the UK. It provides references to literature in the most relevant nursing journals, and of nursing articles in selected medical, allied health, community and health management journals. Those journals covered are mainly published in the UK, although a selection of important international nursing titles is included (ProQuest, 2016). As the thesis links nursing practices to microbiology, the Web of Science ™ database was also searched. This provides access to literature from engineering, science, health, humanities, biological and biomedical science, physics

and computing (Thomson Reuters, 2016), subjects particularly relevant to the proposed thesis, and of the research question.

To ensure the search strategy identified relevant literature, a structured approach to framing the research question was adopted. The PICO model (Population/problem, Intervention/exposure, Comparator/control, and Outcome) works well for questions regarding health care. Once applied to the research question, a list of search terms can be compiled for each of the PICO elements (University of Oxford, Undated; Beecroft *et al*, 2015). Table 2.1 presents the search terms employed when using the databases. The free text, or keywords, were used on all databases, and were drawn from background reading into NG tube use and biofilm development. As preliminary searches had highlighted an apparent lack of relevant evidence relating to the thesis, and in particular the question linking biofilm with NG tubes, papers referring to bacterial attachment only, with no reference to biofilm formation, were also included.

MEDLINE uses MeSH terms (Medical Subject Headings) while CINAHL uses a similar system called suggested subject terms (Polit & Beck, 2010a). In Table 2.1 the MeSH terms indicated were those terms suggested by the MEDLINE thesaurus, and the suggested subject terms were those proposed by the CINAHL database. The Boolean operative 'OR' was employed to broaden the search to include either/all search terms in the identified papers. Conversely, the Boolean operative 'AND' to link the four PICO elements of the research question was dismissed as too restrictive in the circumstances, with initial searches producing no articles whatsoever. Truncation was used with many keywords to widen the search by including synonyms and alternative spellings, and are indicated in Table 2.1 with asterisks.

Table 2.1: Database search terms for the laboratory studies literature review

PICO	Free text or keyword	Boolean Operative	MeSH term or suggested subject term
Population or problem	Nasogastric Tube-fed Enteral Lumen	OR	Enteral nutrition Tube feeding Feeding tube Nasoenteral tubes Feeding tube irrigation Enteral tube irrigation Feeding tube care Enteral tube care
Intervention or exposure	Bacteri* Biofilm	OR	Biofilms Gram-positive bacteria Gram-negative bacteria Gram-negative anaerobic bacteria Gram-negative aerobic bacteria Bacteria, anaerobic Bacteria, aerobic Bacteria and fungal diseases
Comparison or control	Maintain* Maintenance Paten* Flush* Clear*	OR	Flushing
Outcomes	Obstruct* Occlu* Block* Clog* Sediment	OR	Occlusion Obstruction
The free text / keywords and MeSH terms / suggested subject terms used in the search for literature relating to the research question. The Boolean operative 'OR' was employed to broaden the search. Truncated terms are denoted with an asterisk			

Database searching using CINAHL, MEDLINE and BNI identified 134 articles containing the search terms, with the Web of Science™ database producing duplicates only. Other searching techniques were adopted to attempt to increase the number of papers found (Figure 2.1). The Cochrane Library (The Cochrane Collaboration, 2016) is a collection of summaries of research evidence, produced by 37,000 contributors from more than 130 countries, to enable health care workers and patients to make informed choices about treatment. Unfortunately, no relevant evidence was found. Hand searching of relevant nursing, microbiology and nutrition journals was conducted, by referring to their annual indexes or, where indexes were unavailable, their tables of contents. This method produced eight further articles for consideration.

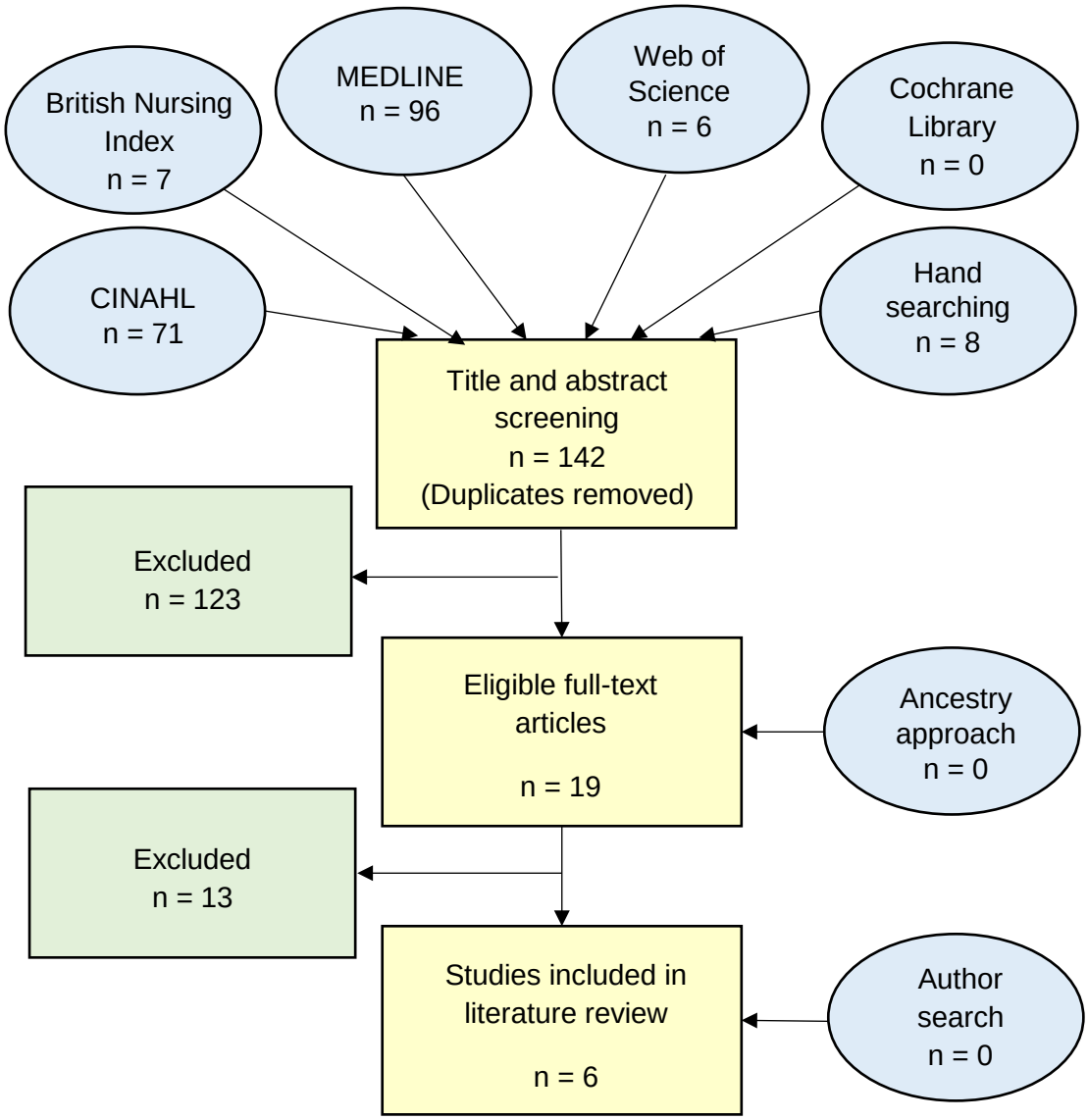


Figure 2.1: Flow diagram of literature search process

In order to identify the most relevant articles for the thesis, the titles and abstracts of the 142 original articles gathered by the database and hand searches were screened, after removal of 46 duplicates. When searching for evidence to inform practice or research, eligibility criteria are typically applied in the form of inclusion and exclusion criteria to refine and reduce the number of articles during screening, thus ensuring the most relevant evidence is identified. As the initial searches highlighted a lack of available evidence, the inclusion criteria were deliberately broad, with no exclusion criteria applied (Table 2.2). The majority of the articles identified (n = 123) were rejected due to little or no relevance to the research subject, or because they were duplicates. Each paper had a tenuous link through one or two elements of the research

question, and were most likely identified through the use of the Boolean operator 'OR'. However, as the Boolean operator 'AND' identified no articles it had been necessary to widen the search and sort through the subsequent findings.

Table 2.2: Inclusion and exclusion criteria for literature accessed

Inclusion criteria	Exclusion criteria
Human and animal studies	None
Adult (≥ 16 years) and Neonate/paediatric (< 16 years)	None
Biofilm formation and bacterial attachment	None
Both *in vitro* and *in vivo* studies	None
All languages	None
Inclusion criteria were deliberately broad due to the lack of literature on initial searches. There were no exclusion criteria set.	

With the remaining 19 articles an ancestry approach was undertaken, whereby the citations from studies noted within each article were used to track down earlier research on which the articles were based (Polit & Beck, 2010a). However, this method highlighted duplicates only. The 19 articles then underwent full text screening, and a further 13 articles were excluded as they were not considered relevant or of sufficient quality to support the research being undertaken. This resulted in six remaining articles, each presenting a study connecting the key subjects of bacteria or biofilm and NG tubes. At this point an author search was conducted, to establish whether the authors of the six identified articles had written other articles that could be beneficial to the literature review. Again, this search identified duplicates only.

2.3 Results

Through undertaking the structure searching process depicted in Figure 2.1, the following six articles were identified as relevant to the research study:

- Leibovitz *et al* (2003) '*Pseudomonas aeruginosa* and the oropharyngeal ecosystem of tube-fed patients'
- Leibovitz *et al* (2005) 'Biodynamics of biofilm formation on nasogastric tubes in elderly patients'

- Kim *et al* (2006) ‘Attachment of and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes’
- Hurrell *et al* (2009a) ‘Neonatal enteral feeding tubes as loci for colonisation by members of the *Enterobacteriaceae*’
- Hurrell *at al* (2009b) ‘Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii*, *Salmonella serovars* and other *Enterobacteriaceae*’
- Lima *et al* (2011) ‘The hydrophobicity and roughness of a nasoenteral tube surface influences the adhesion of a multi-drug resistant strain of *staphylococcus aureus*’

When considering the research undertaken in the six articles, each of them is concerned with the attachment of bacteria to, and biofilm formation on, NG tubes. Three studies focussed on the outer surface of the NG tubes that had been used by adults, and *in vitro* (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005; Kim *et al*, 2006), with the remaining studies (Hurrell *et al,* 2009a; Hurrell *et al,* 2009b; Lima *et al*, 2011) investigating bacterial attachment on the inner surface of NG tubes that had been used by neonates, and *in vitro*. This thesis looks to determine the presence and investigate the development of biofilm on the inner surface of fine bore NG tubes used by adults through both *in vivo* and *in vitro* investigation.

2.4 Appraisal of the identified studies

The six identified papers were subsequently critically appraised. Critical appraisal focuses on the validity, reliability and applicability of a study. It is considered a key skill in nursing, where ‘nursing ritual’ is justifiably challenged, avoiding an unquestioning adoption of new technologies or innovations (Rees *et al*, 2015). To assist with this process, checklists were utilised from Greenhalgh (2010) and the Critical Appraisal Skills Programme (CASP, 2013). Of the six papers, three were cohort studies (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005; Hurrell *et al*, 2009a) and three were experimental *in vitro* studies (Kim *et al*, 2006, Hurrell *et al*, 2009b; Lima *et al*, 2011). An overview of the fundamentals of the six papers are presented in Table 2.3.

Author(s), title, location, setting, and design	Research aim	Relevance	Sample	Laboratory methods	Results and conclusions
Leibovitz *et al* (2003) *Pseudomonas aeruginosa* and the oropharyngeal ecosystem of tube-fed patients **Israel** Cohort study followed by a Cross-sectional study	To confirm the incidence of *P. aeruginosa* colonisation in the oropharynx of NG tube fed older patients, and explore the possibility of biofilm formation on the outer surface of NG tubes	Primarily investigated the incidence of *P. aeruginosa* in the oropharynx of older adults, but also looks at bacteria and biofilm on outer surface of four used NG tubes	Hospitalised older adult patients: 53 NG tube fed patients and 50 orally fed controls. NG tubes were made from polyvinyl chloride (PVC)	Cultures from tongue dorsum and buccal mucosa spread on MacConkey agar, incubated at 35 °C for CFU counting. Samples of oropharyngeal section of NG tubes (x4) prepared for SEM analysis	*P. aeruginosa* identified in the oropharynx of 18 (34%) of NG tube fed participants, but none of the orally fed controls. Four NG tubes from the positive group were found to be positive for biofilm on the *outer surface*
Leibovitz *et al* (2005) Biodynamics of biofilm formation on nasogastric tubes in elderly patients **Israel** Prospective cohort study	To define the bio dynamics of biofilm on NG tubes by investigating the time relation between insertion of new NG tube and the formation of biofilm	Examined biofilm on the outer surface of NG tubes used by older adults	35 PVC NG tubes self- removed by hospitalised older adult patients within 7 days of insertion: 5 tubes obtained for each of the 7 days post-insertion	Samples of oropharyngeal section of NG tubes from days 1 and 2 (x10) prepared for scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Bacteria stained with Propidium Iodide	Day 1 = 3 out of 5 NG tubes had biofilm development. Day 2 = all 5 tubes had biofilm development. NG tubes from days 3 to 7 not tested as considered unnecessary in view of reported results
Kim *et al* (2006) Attachment of and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes **USA** *In vitro* experiment	To study the effects of temperature and nutrient availability on bacterial attachment to, and biofilm development on, NG tubes by *E. sakazakii*	Aimed at microbiologists in the food industry. Looks at bacteria attachment and biofilm development on the *outer surface* of unused NG tubes	5 cm sections of sterile PVC NG tube placed in test tube with inoculum	NG tube samples placed in 3 varying concentrations of nutrient, and incubated at either 12 or 25 °C.	Bacterial growth increased in line with the combination of temperature increase and nutrient availability, but results suggest neither factor causes increased bacterial attachment in isolation
Hurrell *et al* (2009a) Neonatal enteral feeding tubes as loci for colonisation by members of the *Enterobacteriaceae* **UK** Cohort study	To establish the presence of *E. sakazakii* and other *Enterobacteriaceae* within NG tubes, and the effect of the patient's feed regimen on this	Looks at bacteria and biofilm formation within NG tubes used by hospitalised neonates	129 PVC NG tubes obtained from two neonatal intensive care units: 10 from neonates receiving nothing orally	2 cm samples of used NG tubes vortexed in saline. 100 µl volumes of cell suspension plated on Violet red bile glucose agar and incubated at 37 °C. 1 cm sections of NG tube prepared for SEM analysis	Organisms found in 76% of sample, as biofilm and in residual feed in the lumen irrespective of feed regimen
Hurrell *et al* (2009b)	To evaluate the	Looks at bacteria and	5 x 1 cm lengths of each of	5 NG tube sections for each	Organisms and biofilm found

Author(s), title, location, setting, and design	Research aim	Relevance	Sample	Laboratory methods	Results and conclusions
Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii*, *Salmonella serovars* and other *Enterobacteriaceae* **UK** *In vitro* comparative observational study	properties of three types of NG tubing with regard to bacterial attachment and biofilm development	biofilm formation on the *outer surface* of unused NG tubes and silver-impregnated flexelene tubing	three types of tubing: PVC and polyurethane (PU) NG tubes, and silver-impregnated flexelene tubing, inoculated and incubated at 37°C. Full NG tubes inoculated and incubated at 37 °C	tubing/bacteria combination, incubated for 24 hours at 37 °C. Calculated biofilm load by automated impedance technique. Complete sterile NG tubes inoculated and incubated at 37 °C, with simulated feeding every 2 hrs. 1 cm Sections prepared for SEM analysis	on the outer surface of all 3 types of tubing, the highest incidence on the silver-impregnated flexelene tubing, suggesting it has no potential for antibacterial activity in this use
Lima *et al* (2011) The hydrophobicity and roughness of a Nasoenteral tube surface influences the adhesion of a multi-drug resistant strain of *Staphylococcus Aureus* **Brazil** Three inter-linked *in vitro* observational studies	To establish the hydrophobicity, roughness, microtopography, and bacterial adhesion properties of the internal surface of NG tubes made from PU and silicone	In particular, looks at bacterial attachment and biofilm development on the inside of NG tubes *in vitro*	12 flow models (6 PU and 6 silicone) to simulate NG feeding. Incubated at 37 °C. NG tubes were fragmented for scanning electron microscopy and CFU analysis of bacterial adhesion	Hydrophobicity determined via contact angle assessment. Topography assessed by atomic force microscopy. Flow model NG tubes sectioned for bacterial attachment analysis by SEM	No significant difference ($p > 0.05$) between PU and silicone NG tubes in terms of bacterial attachment

This table presents a summary of each of the six studies identified in the literature review. It provides an overview of the fundamentals of each study, detailing the aims, methodology and outcomes, as well as providing a quick reference guide throughout the thesis.

Table 2.3: Summary of identified studies

2.4.1 Synthesis of the identified studies

The key tasks of a literature review are to summarise and critically evaluate the literature in order to establish the current knowledge on a topic, and to reveal the themes deemed to be important (Polit & Beck, 2010a). A thematic review of the literature involves the detection of patterns and regularities, as well as inconsistencies (Polit & Beck, 2010a).

As noted earlier, knowledge regarding the presence of biofilm on indwelling devices such as catheters, tracheostomy tubes and prostheses is well established. However, limited research has been undertaken to investigate bacterial attachment and biofilm development on NG tubes. Those studies that have been published focus on the risk to health biofilm poses if present on the NG tube surface (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005; Kim *et al*, 2006), and on the inner surface of NG tubes (Hurrell *et al*, 2009a; Hurrell *et al*, 2009b; Lima *et al*, 2011). The main aim of this research study is to investigate biofilm presence and development within NG tubes used by adults, with a view to understanding if this may be integral to reduced tube patency through future investigation, notwithstanding the perceived risk to health biofilm may present. As such, little evidence is available that relates closely to this, and therefore an assessment of the current evidence base of the process of bacterial attachment to NG tubes has been undertaken.

2.4.2 Rationale for each of the identified studies

Each of the six studies identified were essentially commenced with a view to assessing the risk to human health bacteria may pose when attached to, or allowed to pass through, NG tubes. The studies were published between 2003 and 2011, and were undertaken in direct response to incidents that suggested risk to human health was a distinct possibility. Although each study is unique, together they build a picture of the predicament of bacterial attachment and biofilm development on NG tubes from an infection perspective.

Leibovitz *et al* (2003) undertook a cohort study of 53 older adult NG tube fed patients, with 50 orally fed controls, at an elderly-care hospital in Israel. Their study was completed following evidence of a high incidence of Gram-negative bacteria within the oropharynx of patients with NG tubes, noting this mainly affected the increasing population of older patients. They highlighted *Pseudomonas aeruginosa* has a predilection for wet sites and the potential to exploit NG tubes to create a thriving

habitat, thus creating a potential reservoir of infection in long-term-care facilities for an already immunocompromised patient group. The authors set out to confirm the bacterium's presence, its antibiotic susceptibility, and investigate the possibility of biofilm formation on the outer surface of NG tubes. Following on from this study, Leibovitz *et al* (2005) built on the knowledge previously gained to define the biodynamics of biofilm formation on NG tubes. They collected 35 NG tubes that had inappropriately been removed by the patients themselves within the first seven days of placement, five for each day, and assessed the biofilm presence on the outer surface. They investigated the time relation between insertion of a new NG tube and the formation of biofilm. Again, this research was undertaken using NG tubes removed from older adult patients in a hospital setting. Leibovitz and colleagues suggested these patients could become reservoirs of resistant pathogens due to the antibiotic pressure they are subjected to as a result of frequent clinical infections. And as older adult long-term care patients are often transferred to other health care environments, they may serve as vectors of resistant organisms, with the authors concluding that biofilm formation on NG tubes can undoubtedly have serious clinical implications.

Kim *et al's* (2006) objectives were to determine the growth characteristics of *Enterobacter sakazakii*, a food-borne pathogen capable of causing meningitis, sepsis, bacteraemia, and necrotizing enterocolitis in pre-term neonates and immunocompromised adults. Like Leibovitz *et al* (2003) and Leibovitz *et al* (2005) they were concerned about the risks bacteria could pose to the health of NG tube-fed older adults. And like Hurrell *et al* (2009a), they were also concerned about the risks to the neonate patient group. Their research was conducted in a controlled laboratory setting using sterile NG tubes, with their results intended to inform microbiologists in the food industry. *E. sakazakii* had been previously found in powdered infant formula and milk powder, fresh food produce, and within food processing plants, and therefore Kim *et al* (2006) set out to study the effect of temperature and nutrient availability on bacterial attachment and biofilm formation on NG tubes and stainless steel surfaces. They believed these materials could act as a vehicle for *E. sakazakii* infection in infants and immunocompromised adults.

Similar to Kim *et al* (2006), Hurrell *et al* (2009a) undertook their cohort study as a result of the attention directed at the microbiological safety of powdered infant milk, which was found to have caused infections in neonates through *E. sakazakii* and *Salmonella* contamination (Forsythe, 2005; Caubilla-Barron *et al*, 2007). They collected 129 used NG tubes from two neonatal intensive care units, along with a questionnaire on each

patient's feed regimen. Their objective was to establish the presence of *E.sakazakii* and other *Enterobacteriaceae* within the inner surface of NG tubes, rather than the outer surface as in previous studies. They were particularly interested in the effect the patients' feed regimens had on this. In addition, Hurrell *et al* (2009b) investigated whether the material the NG tube was manufactured from had an impact on biofilm growth. Their laboratory based observational study determined the extent to which 29 strains of *Cronobacter sakazakii*, *Salmonella* serovars, other *Enterobacteriaceae* and *Acinetobacter* spp. adhered to and developed on NG tubes composed of polyvinyl chloride and polyurethane. The study also included silver-impregnated flexelene tubing which was expected to demonstrate antibacterial activity. They state that since NG tubes are held at body temperature, can be in place for more than 48 hours, and contain nutrients through patient feeds, it is reasonable to anticipate that bacteria will multiply in the tube and contaminate subsequent feeds. They discuss how, as the biofilm ages, the cells become detached in clumps, and may be protected from the stomach acidity due to capsule formation, thus constituting a risk to neonatal health. Further research may be needed to establish the basis for this statement. However, if this is correct, one would imagine this could be comparable to immunocompromised adults, which account for many NG tube users.

In Lima *et al's* (2011) laboratory-based observational study, the objective was to characterize the microtopography, hydrophobicity and resistance to biofilm formation of NG tubes made of different polymers. This was in reaction to the frequent incidence of chronic infections experienced by patients with indwelling devices such as catheters, prosthesis and NG tubes, caused by the microbe *Staphylococcus aureus*. Lima and colleagues noted these devices are often composed of polymers that can potentially support bacterial colonization, which often occurs rapidly within the first 24 hours, as was demonstrated by Leibovitz *et al* (2005). More troubling, and similar to Leibovitz *et al's* (2005) comments, is that antibiotic-resistant bacteria that have adhered to these devices could be transferred to other devices through direct or indirect contact with the contaminated device, causing infection to spread rapidly among patients, and potentially through different health care environments.

2.4.3 Study design

Both Leibovitz *et al* (2003) and Leibovitz *et al* (2005) conducted cohort studies, investigating the presence of *P. aeruginosa* in NG tube-fed patients. Leibovitz *et al* (2003) recruited 53 NG tube-fed older adult patients across four wards in a hospital setting in Israel, and also recruited 50 orally-fed patients from the same wards as a

control group. The patients' tongue dorsum and buccal mucosa were swabbed to obtain bacterial cultures. These were laboratory prepared and incubated at body temperature, from which Gram-negative bacteria, including *P. aeruginosa*, were identified. Used NG tubes from four of the 18 patients found positive for *P. aeruginosa* were obtained, and the oropharyngeal section was prepared for microscopy. Each NG tube was found to have *P. aeruginosa* biofilm formation attached to the outside. In their 2005 study, Leibovitz and colleagues obtained used NG tubes from four wards of a hospital caring for older adults, although do not state if it was the same one as in the 2003 study. Their sample consisted of 35 NG tubes that patients had self-removed in the first seven days of insertion. Five NG tubes were obtained for each of the seven days' period of insertion, but the authors do not state over what time period these were collected. Leibovitz *et al* (2005) clearly state the method of preparation of the NG tubes and the analysis of the samples in their methods section. Microscopy using a scanning electron microscope and a confocal laser scanning microscope revealed three of the five tubes from day one insertion demonstrated biofilm development, suggesting biofilm starts to form within 24 hours of insertion. Of the day two sample, biofilm was apparent on all five tubes. As this was such a strong positive result, Leibovitz *et al* (2005) did not complete the microscopy on the remaining NG tubes from day three through to day seven, as it was assumed they would all have biofilm on them. However, with such a small sample each day, it would perhaps have been beneficial to have completed the investigation of each tube to confirm the findings. The authors state theirs was the first report of this phenomenon based on *in vivo* observations in humans, and were consistent with previous animal studies involving endotracheal tubes.

Similar to Leibovitz and colleagues, Hurrell *et al* (2009a) conducted a cohort study investigating the presence of bacteria in NG tubes used *in vivo*, although their sample was recruited from the neonatal age group who, similar to older adults, often require feeding via NG tube to supplement or replace oral intake. Hurrell *et al* (2009a) collected 129 used NG tubes from two neonatal intensive care units (n = 25, n = 104), where119 of the neonates were fed a selection of natural and reconstituted formula milks, and ten neonates were nil by mouth when their NG tubes were removed, and thus regarded as the control group. The authors investigated the effects of feed regimen on the levels of bacteria attached to the NG tubes but, unlike Leibovitz *et al* (2003) and Leibovitz *et al* (2005), they were examining the internal surface of the NG tube, as opposed to the outer surface. Similar to the planned investigation into biofilm presence and development on the inner surface of NG tubes used by adults. In Hurrell *et al's* (2009a) study, the NG tubes were prepared for microscopy by cutting into 1 cm sections, then

cutting longitudinally to expose the inner surface. They were assessed using a scanning electron microscope. *Enterobacteriaceae* were isolated in 76% of the samples from all feed regimens. Remarkably, it was noted that 81% of the NG tubes received from neonates undergoing feeding with sterile ready-to-feed formulas in tamper-proof packaging contained *Enterobacteriaceae* within the tube, suggesting bacteria were being introduced to the feeding set from an external source.

Unlike the three studies noted above, the remaining evidence relevant to this literature review derives from studies undertaken within a laboratory environment (Kim *et al,* 2006; Hurrell *et al,* 2009b; Lima *et al*, 2011). In response to infant formula contamination outbreaks, Kim *et al* (2006) used *E. sakazakii* cultivated in infant formula to inoculate 5 cm sections of sterile NG tubes, to establish the effect of temperature and nutrient availability on biofilm formation. These were placed in test tubes with nutrients, and stored at either 12 or 25 °C for up to 10 days. The samples were regularly checked over the 10 day period, with the results suggesting nutrient depletion did not cause great decreases in the population of bacteria attached to NG tubes stored at 12 °C. The authors considered it was not the surrounding medium or incubation temperature alone, but rather a combination of medium, temperature and other factors that influence biofilm formation by *E. sakazakii*. However, bacterial attachment was higher at 25 °C than 12 °C, thus emphasising the importance of temperature control in the infant formula and produce processing industries.

Similar to Kim *et al* (2006), Hurrell *et al* (2009b) conducted their research following concerns for the microbiological safety of infant formula. They investigated whether the material NG tubes are made of can help prevent or reduce the attachment of bacteria and formation of biofilm. They inoculated polyvinyl chloride (PVC), polyurethane (PU) and silver-impregnated flexelene tubing with bacteria isolated from powdered infant formula and dairy products. The inoculated tubing was incubated for 24 hours at 37 °C to simulate body temperature. *E. sakazakii* was identified on each of the three types of tubing, with the highest level recorded on the silver-impregnated flexelene tubing, suggesting it achieved limited or no antibacterial activity, and would therefore not appear appropriate for producing NG tubes. The authors deduced that it is plausible bacteria can attach *in vivo* to neonatal NG tubes, and grow to high cell densities.

The final study in the review, Lima *et al* (2011), was a series of three interlinked experiments also designed to investigate the benefits or consequences of NG tubes made from different materials, by examining the physiochemical properties of PU and silicone NG tubes. The tubes' hydrophobicity and microtopography were analysed

using a goniometer to test the contact angle, and microscopy methods were used to analyse the surface topography and roughness of the NG tube inner surface. Along with this, the adhesion levels of introduced *S. aureus* were assessed using a flow model set up to simulate an NG tube in use. This was incubated at 36 °C to simulate body temperature. After three days of simulated use, the flow models were dismantled, and the NG tubes were fragmented for analysis using scanning electron microscopy (SEM). Adhered bacteria were quantified through counting colony forming units (CFU). The study demonstrated no statistically significant difference in the two NG tube materials, with regard to bacterial adhesion. However, the authors were keen to point out that the *in vitro* data may not accurately reflect the bacterial adhesion process that occurs when NG tubes are in use in humans.

2.5 **Laboratory methods**

Each of the six studies identified employed laboratory techniques to determine the incidence of bacterial attachment to NG tubes to a greater or lesser extent. As such, a review of the laboratory methods used was undertaken to establish which may be beneficial to the laboratory investigations planned in this thesis in order to answer the research question.

2.5.1 Biofilm definition

Although each of the studies investigated bacterial attachment and biofilm formation on NG tubes, their individual descriptions of biofilm differed. Leibovitz *et al* (2003) state biofilms serve as a culture media for bacteria and contribute to the development of antibiotic resistance, whereas the Leibovitz *et al* (2005) provides further description that biofilms are biological systems where bacteria form structured coordinated communities, which is more in keeping with the established Costerton description (Costerton *et al*, 1995). They go on to suggest the ecological advantages of existing in a biofilm for bacteria include protection from hostile environments, and increased nutrient availability. Kim *et al* (2006) also state that bacteria in a biofilm formation are known to have enhanced resistance to environmental stresses, and in particular they provide protection against sanitizers. The remaining studies do not give a definition of biofilm (Hurrell *et al*, 2009a; Hurrell *et al*, 2009b; Kim *et al*, 2011). However, each was

published in a microbiology journal where such knowledge would seemingly be known by its readers, and therefore a description would not be considered essential.

2.5.2 Tubing material

Five of the studies identified investigated bacterial attachment to PVC NG tubes. In addition, Lima *et al* (2011) investigated PU and silicone NG tubes, whilst Hurrell *et al* (2009b) investigated PU and silver-impregnated flexelene tubing for its expected antibacterial activity. Rather surprisingly, across all the bacterial species investigated, Hurrell and colleagues found the level of biofilm formation on the silver-impregnated tubing was generally higher than on the PVC and PU NG tubes. Consequently, silver-impregnated tubing would not appear to be a means of preventing bacterial attachment and biofilm formation on NG tubes. In Lima *et al's* (2011) investigation comparing the properties of PU and silicone NG tubes in relation to bacterial attachment, they concluded there was no statistically significant difference between hydrophilic PU tubes and hydrophobic silicone tubes, although they did note the PU surface was rougher than the silicone surface, which may have counteracted any benefit of its hydrophilic properties. The type of NG tube widely used in NHS hospitals for short term enteral feeding is the PU Corflo® 8 FG, 92 cm long, with an external diameter of 2.8 mm, and surface area of 162 cm^2 (Corpak MedSystems, UK). In order for the results of the subsequent laboratory studies in this thesis to be generalisable to the UK population, these were used throughout.

2.5.3 Bacterial strains

Leibovitz *et al* (2003) and Leibovitz *et al* (2005) were able to isolate *Pseudomonas aeruginosa* in the oropharynx of NG fed older adult patients in an elderly-care hospital, and were able to demonstrate these bacteria were also attaching to the NG tube at the oropharynx junction, whilst Lima *et al* (2011) used *Staphylococcus aureus* they had previously isolated from the NG tube of one ICU patient at the authors' local hospital. Hurrell *et al* (2009a) isolated *Escherichia coli* in 29 % of their sample. In addition, Hurrell *et al* (2009b) used 29 strains of bacteria including *Cr. Sakazakii*, *Salmonella* serovars, *Enterobacter cloacae*, *E. coli*, *K. pneumonia,* and *Acinetobacter* they had isolated from powdered infant formula, dairy products, and discarded NG tubes from a neonatal ICU. Whilst Kim *et al* (2006) used *E. sakazakii* strains isolated from food and an environmental source for both their attachment study and biofilm formation study.

For this thesis, two pathogens were chosen for inoculating NG tube samples in the interlinked laboratory studies, *E. coli* and *P. aeruginosa.* Both of these pathogens were used in the identified published studies. They form part of the human microbiota, and are usually present in the hospital environment. *E. coli* is part of the normal flora of the gut, and *P. aeruginosa* can be found in the nasopharynx and oropharynx of humans. Both pathogens are known producers of biofilm (Costerton *et al*, 1995; Solseng *et al*, 2008), making them particularly suitable for this study.

2.5.4 NG tube sample: preparation and technique

For their biofilm studies, both Leibovitz *et al* (2003) and Leibovitz *et al* (2005) removed samples of unknown length from the oropharyngeal section of the NG tubes retrieved from older adult patients. Information regarding how these samples were obtained and prepared is very limited. Microscopic analysis, using the methods outlined in the microscopy method section below, was undertaken to assess the presence of biofilm.

The remaining studies provide more detailed descriptions of their sample selection and preparation (Kim *et al*, 2006; Hurrell *et al*, 2009a; Hurrell *et al*, 2009b; Lima *et al*, 2011). Kim *et al* (2006) cut 5 cm lengths of feeding tube with a sterile blade, and heat-sealed each end. These samples were immersed in 70 % ethanol for 10 minutes to disinfect the surface. Each sample was placed in a 15 ml test tube, and 10 ml of prepared bacterial culture added. These were incubated at either 12 or 37 °C for 4 hours, after which the NG sections were removed using sterile forceps, washed with sterile distilled water, and placed in 50 ml centrifuge tubes containing 30 ml sterile phosphate buffered saline with 3 g of glass beads to facilitate the removal of adherent cells from the surface of the NG tube sections. These were vortexed for one minute, and suspensions serially diluted in 0.1 % peptone water and surface plated in duplicate on tryptone soya agar (TSA), incubated at 37 °C for 24 hours in preparation for CFU counting.

Hurrell *et al* (2009a) collected used NG tubes from neonatal patients, placed them into sealed bags, and refrigerated them at 5 °C for up to 24 hours until analysis. The outside of the tubes were then sterilised using isopropyl alcohol to remove any oral-pharyngeal flora contamination, which would help ensure any organisms detected were deemed to originate from the inside of the tubes. Aseptic techniques were used to cut 2 cm lengths from the NG tube. These were placed in test tubes containing 5 ml volumes of sterile saline, and underwent a series of alternating vortexing and ultra-sonicating. The saline was decanted off and centrifuged at 2400 rpm for 10 minutes. The supernatant was discarded, and the bacterial pellet resuspended in 1 ml sterile saline.

This suspension was serially diluted, and 100 µl aliquots plated on to Violet Red Bile Glucose Agar, and incubated at 37 °C for 48 hours in preparation for CFU counting.

For the first part of their study, Hurrell *et al* (2009b) chose to replicate hospital feeding practices for neonates by flushing an inoculated PVC NG tube with sterile infant formula every two hours. This tube was incubated at 37 °C for 48 hours. 1 cm lengths of the tube were cut longitudinally to reveal the inner surface, and these were prepared for microscopy as detailed in the microscopy method section below. For the second part of their study, 1 cm lengths of tubing (PU, PVC, and silver-impregnated flexelene) were sterilised using 70 % ethanol, and placed in 10 ml volumes of sterile infant formula. These were inoculated with an overnight culture, and incubated at 37 °C for 24 hours. The NG tube samples were removed and washed in 10 ml of sterile saline, and placed in to sterile impedance tubes containing 2 ml volumes of Brain Heart Infusion Broth (BHI, Merck) and placed in a Rapid Automated Bacterial Impedance instrument (RABIT ^{TM}, Don Whitley Scientific Ltd, UK) for bacterial biofilm quantification.

Similar to Hurrell *et al* (2009b), Lima *et al* (2011) investigated the ability of bacteria to attach to NG tubes made from PU and silicone by simulating NG tubes in use, using a feeding system flow model within the laboratory setting. Inoculated feed volumes were passed into the NG tubes at set intervals throughout each day (8:00, 11:00, 14:00, 17:00, 20:00, and 23:00 hours), and after three days the NG tubes were fragmented for analysis. Three 10 cm lengths of tube were removed from the beginning, middle and end of each tube. These were gently rinsed with a syringe of 0.6 ml of 2 % sodium citrate solution to remove planktonic cells, then vigorously rinsed with 10 ml of the same solution to remove adherent cells. Aliquots of the collected solution (0.1 ml) were plated in duplicate on to agar and incubated at 37 °C for 24 hours in preparation for CFU counting. Shorter sections of 0.4 cm were also removed from the inoculated NG tubes, and prepared for microscopy as detailed in the relevant section.

2.5.5 Bacteria and biofilm detection and quantification

The method of counting bacterial CFUs by plating aliquots containing bacteria onto TSA Petri dishes was the preferred method of bacterial quantification used by the studies apart from Leibovitz *et al* (2003), who used blood and MacConkey agar for identifying the bacterium present in the oropharynx of NG tube fed older adult patients, and Leibovitz *et al* (2005), who used microscopy for direct biofilm detection.

Two methods of applied CFU counting were demonstrated by Hurrell *et al* (2009a) and Hurrell *et al* (2009b), with the former displaying results as CFU per millilitre of residual liquid tested, and the later indicating Log_{10} CFU per centimetre $^{-1}$ of NG tube.

Hurrell *et al* (2009b) also used the Rapid Automated Bacterial Impedance Technique (RABIT ^{TM}, Don Whitley Scientific Ltd, UK) to determine the biofilm cell density measurement, with the impedance calibration curves for each bacterial strain calculated using CFU counts of aliquots plated on to TSA and incubated at 37 °C for 24 hours.

Five of the identified studies (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005; Hurrell *et al*, 2009a; Hurrell *et al*, 2009b; Lima *et al*, 2011) also used microscopy to directly visualise the presence, morphology, and extent of biofilm on their samples. This will be expanded on in the next section.

2.5.6 Microscopy method

The overall picture gained from the identified papers is that the preferred method of microscopy was scanning electron microscopy (SEM). This particular method was used in each of the studies, with the exception of Kim *et al* (2006), who investigated and calculated the bacteria present on their samples of NG tubes and stainless steel coupons through CFU counts only.

Leibovitz *et al* (2003) obtained samples of the oropharyngeal section of used NG tubes, which they fixed overnight with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), dehydrated using increasing concentrations of ethanol, then coated with gold ready for SEM examination using a Jeol-840A scanning electron microscope (JEOL USA, Peabody, MA). Leibovitz *et al* (2005) used the same microscopy equipment and method of sample preparation, and in each case detailed images were produced of representative biofilms attached to the surface of the NG tube samples. In addition to this method, Leibovitz *et al* (2005) also used confocal laser scanning microscopy (CLSM) (Zeiss LSM 410 system) to view the morphology of the attached bacteria. For examination under CLSM, the samples were fixed with formaldehyde, washed with sterile distilled water (ddH_2O), and stained with Propidium Iodide (PI) bacterial stain (Life Technologies, 2012). Detailed microphotographs were produced demonstrating the sequential biofilm formation on the NG tubes from day one to day seven of patient placement.

Both Hurrell *et al* (2009a) and Hurrell *et al* (2009b) used a Stereoscan S250 Mark III SEM (Cambridge Instruments, Somerville, MA) to examine the inner surface of sample NG tubes used by neonates, and of the inner surface of PVC, PU and silver-impregnated flexelene tubing inoculated in the laboratory. Their method of sample preparation was similar to previous studies in that they were washed in phosphate buffer, fixed in 1% osmium tetroxide, dehydrated through a series of alcohols up to 100% alcohol, and coated with gold. Detailed images were produced demonstrating bacterial attachment to the tubing material.

Lima *et al* (2011) used a LEO 1430 VP SEM (Zeiss, Cambridge) to visualise their samples of inoculated NG tube cut from the beginning, middle and end of the length of tube. These samples were segmented into four pieces by horizontal and transversal cuts in the centre of the test samples. The images produced show *S. aureus* attached to the inner surface of the silicone and PU tube samples, whilst SEM images of sterile NG tubing demonstrated the irregularities in the surface such as fissures, orifices and protuberances. Lima *et al* (2011) also used atomic force microscopy (VEECO Instruments Inc.) to further investigate the PU and silicone NG tube surfaces. This method of microscopy produced detailed three dimensional images of the surface topography, again highlighting the fissures and elevations present in the tube material. It demonstrated the silicone tube had a smoother surface than the PU tube samples, with Lima and colleagues suggesting the surface roughness is relevant to bacterial adhesion and biofilm formation, creating a larger surface area for bacterial colonisation, and protection from shear forces.

2.5.7 Time course of attachment

Not all of the studies were concerned with establishing the time course of bacterial attachment as a primary aim. For example, Leibovitz *et al* (2003) had found *P. aeruginosa* was present in the oropharynx of NG tube fed older adult patients, and had set about to establish whether this bacteria had also attached to the oropharynx section of the NG tube. These tubes were not examined until two to four weeks after patient placement. Likewise, Hurrell *et al* (2009a) investigated whether the enteral feed regimen of neonatal patients had an effect on the bacterial attachment on the inner surface of their NG tubes. The majority of the 129 NG tubes they collected for the study over an 11 month period were removed between 6 and 48 hours of patient placement, and little relevance was placed on duration of placement and levels of bacterial attachment. Lima *et al* (2011) also paid little relevance to the time course of bacterial attachment, as their study was designed to investigate the effects the tubing material

and surface topography can have on the levels of bacterial attachment. Samples of NG tubes were examined after three days of inoculation and incubation.

Three of the identified studies did investigate the time course of attachment. Both Leibovitz *et al* (2005) and Kim *et al* (2006) established bacterial attachment and biofilm development was present at 24 hours. Kim and colleagues set out to investigate the effects of nutrient availability and temperature on the prevalence of bacterial attachment and biofilm formation on both stainless steel coupons and NG tubes, with the enteral food preparation industry in mind. Leibovitz *et al* (2005) designed their study to complement the Leibovitz *et al* (2003) study which had established *P. aeruginosa* was present as biofilm on the oropharyngeal section of NG tubes used by older adult patients. In the later study, the authors set out to establish how soon biofilm developed by examining NG tubes self-removed by the patients between one and seven days. They were able to demonstrate 60 % of the tubes at day one were positive for biofilm, with 100 % positive at day two.

Hurrell *et al* (2009b) inoculated sterile NG tubes to investigate the possibility of bacteria passing into the stomachs of neonate patients with subsequent feeds. To simulate hospital practices, they inoculated PVC NG tubes with *Cr. Sakazakii*, incubated them at 37 °C, and flushed them every two hours. Air was initially used at each point to flush out the tube contents, which were collected for analysis, and this was followed by flushing with sterile infant formula. Using this method, Hurrell and colleagues were able to demonstrate the attached bacteria subsequently inoculated the fresh infant formula, which could result in bacteria being flushed into the compromised neonate patient stomach. The authors proposed this could occur after just two hours of exposure, but did not directly visualise the biofilm present on the NG tubes until after 24 hours of incubation.

2.5.8 Statistics

In order to effectively design the laboratory studies to answer the research question, it was important to consider all aspects of planning, including how the data collected for each study were to be analysed. The expectation was that relevant literature assessed in the review may indicate suitable tests for use in similar studies.

Statistical tests were used in four of the identified studies to assist with analysing the data and reaching a conclusion. Leibovitz *et al* (2003) used a Chi Squared test for their comparative study, between the control group and sample group, by analysing

categorical data of whether or not the older adult patients in each group were positive for Gram-negative bacteria in the oropharynges. Kim *et al* (2006) states they used Fisher's Least Significant Difference test for their study, to determine whether the attachment of bacteria, and biofilm formation, on NG tube sections and stainless steel coupons was significantly affected by temperature or nutrient availability. However, this type of test would usually follow a one- or two-way ANOVA, where it is used to determine whether there are any significant differences between the means of two or more independent groups, but no mention is made of this having taken place.

Hurrell *et al* (2009a) clearly state they used a one-way ANOVA to test whether their neonate patients' ages affected bacterial colonisation levels on the NG tubes within a feed group. When all data were pooled, the neonates' ages and duration of NG tube placement did have a significant effect on colonisation levels, with the maximum CFU counts recorded at 48 hours of NG tube placement. Following on from the one-way ANOVA where $p = <0.05$, comparisons between group means were made using Fisher's Protected Least Significant Difference *post-hoc* test to analyse colonisation of the NG tubes against the patients' feed regimens, with the nil by mouth cohort as the control group. Hurrell *et al* (2009a) completed their statistical analyses using a Chi Squared test to analyse the distribution of bacterial species between the tubes retrieved from the nil by mouth group and the remaining used NG tubes.

In the most recent study, Lima *et al* (2011) state they conducted 'variance analyses'. They compared the *S. aureus* colonisation of PU and silicone NG tubes, and for this one would anticipate they used a one-way ANOVA to test whether the means of the groups were different or equal. However, before statistical analysis of the planned laboratory studies was completed, advice was sought from the University statistician.

2.5.9 Outcomes

In their study of 53 older adult NG tube fed patients, Leibovitz *et al* (2003) found 34 (64 %) were positive for Gram-negative bacteria in the oropharynx, of which 18 (60 % of the Gram-negative group) tested positive for *P. aeruginosa*. Of the four NG tubes retrieved from patients in this group, all four tubes were found to have *P. aeruginosa* biofilm present on the outer surface, at the oropharynx section. Leibovitz and colleagues concluded that biofilms present on NG tubes may play a role in the persistent colonisation of patients' oropharynges, and may interfere with its eradication by antibiotics. Similarly, Leibovitz *et al* (2005) considered the risks of biofilm attached to NG tubes in the same patient cohort, and set about establishing the time frame for

bacterial colonisation. They concluded that bacteria are attached and organised in a sessile antibiotic-resistant biofilm structure on the outer surface of NG tubes within 24 hours of patient placement.

The issue of optimal conditions for biofilm development was considered by Kim *et al* (2006) in their study of the effect of temperature and nutrient availability on bacterial attachment and biofilm development on NG tubes and stainless steel coupons. Their findings demonstrated a significantly higher number of attached bacteria on NG tube samples at 25 rather than 12 °C, and that there were significantly higher bacteria attached to NG tube samples that were immersed in infant formula broth than lettuce juice broth, at both 12 and 25 °C. The authors concluded that it is not only nutrient availability or temperature alone that affects bacterial colonisation levels on both NG tubes and stainless steel coupons, but rather a combination of both factors. They emphasised the important of temperature control in infant formula and produce processing industries to prevent bacterial colonisation.

Hurrell *et al* (2009a) investigated whether neonates' feed regimens affected the level of bacterial attachment to the inner surface of NG tubes. They were able to demonstrate the feed regimen did not affect the levels of bacterial colonisation of NG tubes, and concluded that NG tubes in neonates act as a loci for bacterial colonisation by numerous opportunistic pathogens, particularly within the *Enterobacteriaceae* family, and as such are an important risk factor with regard to neonatal infections. Following this finding, Hurrell *et al* (2009b) set about testing three different materials for NG tubing, to establish which material would better prevent bacterial colonisation. Following their investigations into PU, PVC and silver-impregnated flexelene tubing, they concluded that the highest bacterial counts were found on the flexelene tubing, thus suggesting the silver-impregnation of this material provided none of the anticipated antibacterial activity.

The effect the material NG tubes are made of can have on bacterial attachment levels was also investigated by Lima *et al* (2011), along with the tube surface topography and material hydrophobicity. They found no significant difference between the hydrophilic PU tubes and the hydrophobic silicone tubes tested, and concluded that bacterial adhesion to NG tubes must depend on other factors, such as surface roughness.

2.5.10 Recommended future work

Both studies by Leibovitz *et al* (2003) and Leibovitz *et al* (2005) were conducted at a single elderly-care facility in Israel, and as such the results are limited in their generalisability. In the earlier study, Leibovitz and colleagues suggest similar studies could be conducted at other long-term care facilities in order to provide further evidence, and lead to the results becoming more applicable to the general population. In the later study it is suggested that new ways should be established for preventing, delaying or reducing bacterial attachment and biofilm formation on NG tubes as they are held in such a critical position.

To expand on the knowledge gained in their study, Kim *et al* (2006) recommend further studies are conducted to establish the effect of sanitizers and disinfectants used in the infant formula and produce processing industries may have on *E. sakazakii*, and also in formula reconstitution and feeding areas.

The studies by Hurrel *et al* (2009a) and Hurrell *et al* (2009b) were conducted principally with the neonate patient in mind. In particular, the earlier study reports that the practice of prolonged placement of NG tubes in neonates needs to be considered in view of the risk opportunistic pathogens may pose, and the latter study stating that the material NG tubes are produced from needs investigating further to reduce neonatal exposure to pathogens attached to their NG tubes.

2.6 Summary of findings

This review was undertaken to identify the most relevant literature available to support the proposed research aim of determining the presence and development of biofilm on the inner surface of fine bore NG tubes used by adults. It quickly became evident limited research has been published relating to bacteria and NG tubes, highlighting the need for increased research in this area. Due to the lack of evidence, the eligibility criteria for the studies to be included in the review were purposefully unrestrictive in order to highlight those studies even loosely connected to the research topic. Using this search strategy and subsequent screening methods, six studies were considered relevant to the aim of this thesis.

Each of the studies identified were undertaken in response to incidents that suggested risk to human health was possible as a result of bacteria coming into contact with NG

tubes. Although this essentially differs from the research aim of exploring the presence of biofilm within NG tubes used by adults, with a view to prospective research exploring links with NG tube blockage, the methodologies of the identified studies combined to provide a supportive framework for the individual studies within this thesis. Similar to the planned studies, the identified papers report on both *in vivo* and *in vitro* studies, and are equally divided into *in vivo* hospital-based cohort studies with elements of laboratory investigation, and entirely laboratory-based *in vitro* observational research.

The cohort study design is considered the best design for studying causal relationships, with controls used in order to rule out competing explanations for observed effects (Polit & Beck, 2010d). Attrition rates can be high if conducted over a prolonged period (Polit & Beck, 2010d), although this was not evident in the identified studies. Hurrell *et al* (2009a) conducted their study into bacteria and biofilm within the NG tubes of hospitalised neonates over two hospital sites, thus achieving more generalisable results than perhaps both Leibovitz *et al* (2003) and Leibovitz *et al* (2005), who conducted their studies at a single site older adult hospital, thus potentially limiting their range of relevance.

The three *in vitro* studies by Kim *et al* (2006), Hurrell *et al* (2009b) and Lima *et al* (2011) were conducted entirely within laboratories which enabled the researchers to control the variables of time, temperature and nutrient availability in order to undertake detailed investigation of bacterial biodynamics and NG tubing material properties. This is considered the best design for testing hypotheses of cause-and-effect relationships, and can yield the highest quality evidence regarding the effects of an intervention (Polit & Beck, 2010d). Entirely laboratory-based investigation also avoids the ethical dilemma of researching devices such as NG tubes whilst in use by patients, potentially leading to greater in-depth investigation of variables than could be achieved with an *in vivo* study.

A particular strength of each of the six studies identified is the clear presentation of the laboratory materials and methods used to achieve each of the study aims. These detailed explanations will prove informative to a novice researcher and will support the robust development of the planned laboratory studies. In particular, quantifying bacteria through CFU counts, bacterial culture preparation, enteral tube preparation for inoculation and microscopy, flow model apparatus design and use, incubation times and temperature, and bacteria removal using vortexing methods.

The studies identify biofilm as a biological system where bacteria form structured coordinated communities, and state bacteria within a biofilm are protected from environmental stresses, gain increased nutrient availability, and may contribute to the development of antibiotic resistance. The biodynamics of bacteria in relation to enteral tubes have been investigated, demonstrating biofilm is developed at 24 hours of patient placement, and that bacterial colonisation is affected by a combination of nutrient availability and temperature providing optimal conditions for growth, rather than either in isolation. Strains of bacteria used within the studies include *Staphylococcus aureus*, *Escherichia coli*, *Cr. Sakazakii*, *Salmonella* serovars, *Enterobacter cloacae*, *K. pneumonia*, and *Acinetobacter.*

Preparation of the NG tube samples used for investigation varied only marginally between the studies. Aseptic techniques were employed throughout, with sterile forceps used to handle the tubes, and sterile blades to cut the sample sections of tube. The tubes were disinfected using alcohol washes, such at 70 % ethanol, and cut to lengths of 1 to 10 cm. Some sections were also cut longitudinally to reveal the inner surface, and one study analysed sections cut from the beginning, middle and end of the NG tubes. Bacterial cultures were introduced to NG tubes either directly through flushing with inoculated feed volumes, or by inoculating feed volumes into which NG tube sections were placed. Four of the identified studies investigated PVC tubes, with only two considering PU tubes, along with silicone and silver-impregnated tubing. As PU NG tubes are commonly used within UK health care, they will be used in the planned studies. How applicable the results of the identified papers will be to PU tubing will become evident as the planned studies unfold.

Incubation of inoculated NG tube samples was generally completed at 37 °C to replicate body temperature. Bacteria was removed for analysis by first washing samples with sterile distilled water, then vortexing in phosphate buffered saline with glass beads, or by ultra-sonication. Once removed, the re-suspended bacteria was serially diluted and surface-plated onto TSA, incubated for 24 to 48 hours at 37 °C, ready for CFU counting.

Direct biofilm detection methods were also employed using microscopy to visualise the presence, morphology, and extent of biofilm development on the NG tube samples. The favoured method of microscopy was SEM, which was used in five of the six studies. In addition, CLSM and atomic force microscopy methods were used to produce detailed three-dimensional images of the tube surface topography. Although these methods of microscopy were relevant to the requirements of the identified

studies at the time, they are not planned for use in this thesis. EDIC microscopy enables direct visualisation of hydrated biofilm on opaque, curved surfaces, making it particularly appropriate for the planned studies.

Outcomes of the six studies were varied. Leibovitz *et al* (2003) and Leibovitz *et al* (2005) established that *P. aeruginosa* present in the oropharynx of older adult patients can attach to an NG tube's outer surface and develop biofilm within 24 hours of introduction. Kim *et al* (2006) study established that bacterial colonisation of NG tubes *in vitro* is affected by a combination of both temperature and nutritional availability, as opposed to either individually. Overall, the outcomes of the studies demonstrated bacteria can attach to and thrive on NG tubes *in vivo* and *in vitro*. Hurrell *et al's* (2009b) investigation into the material NG tubes are manufactured from concluded silver-impregnated flexelene tubing provided no anticipated antibacterial activity, and Lima *et al* (2011) stated there was no statistically significant difference in levels of bacterial colonisation between inoculated PU and silicone NG tube samples.

Future work suggested by the studies reviewed include establishing ways of preventing, delaying or reducing bacterial attachment and biofilm formation on NG tubes, investigating the practice of prolonged placement of NG tubes in neonates, establishing the effect of sanitizers and disinfectants used in the infant formula and produce processing industries, and investigating the material NG tubes are manufactured from to reduce user exposure from pathogens.

2.7 **Gaps in the research**

Through undertaking the literature review, it is clear the studies identified as investigating bacteria in connection with NG tubes were concerned with the potential infection risk bacterial colonisation of such a device may pose to those people who need them. The main difference with the current thesis is that the overarching purpose is to establish whether bacterial colonisation, and in particular biofilm, exists within NG tubes used by adults, and what factors may influence its development. Although the purpose and expected outcomes differ, the knowledge gained from previous research, and the methods used, support and inform the current research.

The first laboratory study in the series of inter-linked studies looked to establish the presence and distribution of biofilm by examining the inner surface of used NG tubes

removed from adult patients in the course of their normal care. Once biofilm presence in NG tubes used by adults was established, the next step was to understand how soon bacteria attach to the NG tube material and develop biofilm. The identified studies indicate biofilm is present at 24 hours from the introduction of bacteria. Further investigation in this thesis looks closely at the first 24 hour period, to establish a more specific time frame for bacterial attachment and biofilm development to occur.

Many tubes are considered through anecdotal evidence to block as a direct result of the enteral feed they were designed to carry. Further investigation into the possible influence the presence of bacteria in NG tubes can have on the enteral feed passing through them is investigated in this thesis, when the pH levels of feed samples are monitored after inoculation with bacteria.

The recommended procedure of flushing NG tubes with water to maintain patency is not covered in either of the identified studies, but these were primarily concerned with infection risk. However, flushing is widely considered the optimal method of maintaining the patency of NG tubes and as such should be considered in this thesis. Therefore the effect of flushing with either sterile water or tap water on biofilm development within NG tubes is investigated in detail, along with the alternative option of not flushing.

Another aspect that required investigation was that of the effect of patient variables on biofilm development. Hurrell *et al* (2009a) established that their neonate patients' feed regimen did not affect bacterial colonisation of their NG tubes, but that their age and the duration of tube placement did have a statistically significant impact on bacterial colonisation levels. Also, Leibovitz *et al* (2005) established biofilm was present on the outer surface of all their participants' NG tubes after 24 hours of placement. Therefore, a study within this thesis set out to establish whether adult patient variables of age, gender, feed regimen, duration of tube placement, and medications administered via NG tube had the potential to affect the levels of bacterial attachment and biofilm development within their NG tubes.

2.8 **Aims and objectives**

The primary aim of this thesis was to determine the presence of biofilm, and investigate its development, on the inner surface of NG tubes used by adults. The secondary aim was to explore the nursing perspective of NG tube management and the maintenance of tube patency, which will be covered in Chapter 9.

The specific objectives of the primary aim were to:

- Undertake a review of the literature to establish what is already known
- Incorporate relevant and appropriate materials and methods used in the identified studies into the planned research studies
- Establish the presence and distribution of biofilm within NG tubes used by adults
- Establish the time frame for bacterial attachment and biofilm development on polyurethane NG tubes
- Determine the influence biofilm can have on its environment by investigating the pH of enteral feed inoculated with bacteria over a 24 hour period
- Compare bacterial attachment and biofilm development on the inner surface of sterile NG tubes flushed with either sterile water or tap water
- Compare bacterial attachment and biofilm development on the inner surface of inoculated NG tubes flushed with either sterile water or tap water, along with the control option of not flushing
- Investigate the presence and nature of substances, including biofilm and medication residue, within NG tubes retrieved from adult patients
- Explore the potential for patient variables including gender, age, duration of tube placement, medications given via the NG tube and feed regimen to contribute to biofilm development

Each objective is realised in the laboratory studies reported in Chapters 3 to 8, with the initial objective of a literature review having been completed in this chapter.

2.9 **Conclusion**

The main aim of this literature review was to identify relevant evidence relating to bacterial attachment or biofilm development in connection with NG tubes. The main theme of the identified evidence was that of the potential infection risk bacterial attachment to a device located in such a critical position could pose to those who need them. The structured search strategy employed for the review enabled the most relevant literature to be identified. The investigations undertaken, and the laboratory methods used throughout each of the identified studies, have been explored to provide a framework for the subsequent laboratory studies in this thesis. They have indicated the gaps present in the literature that will help to answer the research question

investigating the presence and development of biofilm on the inner surface of fine bore NG tubes used by adults.

Chapter 3: A preliminary investigation to establish the presence and distribution of biofilm within NG tubes used by adults

3.1 Introduction

The last chapter discussed previous research investigating the potential of bacteria to attach to, and develop biofilm on, NG tubes. Studies have established biofilm presence on the outer surface of NG tubes used by adults (Leibovitz *et al*, 2003; Leibovitz *et al* 2005) and on the inner surface of NG tubes used by neonates (Hurrell *et al*, 2009a). Moreover, *in vitro* studies have examined the ability of bacteria to attach to different types of NG tube material, namely PVC, PU, and silver-impregnated flexelene tubing (Kim *et al*, 2006; Hurrell *et al*, 2009b). These studies investigated the effect of time, temperature and nutrient availability on biofilm development.

This chapter presents a study designed to build upon previous published research by determining the presence and distribution of biofilm on the inner surface of polyurethane NG tubes used by adults. The findings of this study would inform the design of the subsequent laboratory studies. This study also provided an opportunity to develop the laboratory methods used in the series of laboratory studies presented in this thesis. The choice of methods were based on those described in previous studies (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005; Kim *et al*, 2006; Hurrell *et al*, 2009a; Hurrell *et al*, 2009b; Lima *et al*, 2011), and those developed in the laboratory in which this study took place. Methods of removing bacteria from NG tubes, quantifying the bacteria removed, and identification of bacterial load by bacterial staining and microscopy are discussed, along with preparation of bacterial culture, and encouraging bacterial growth.

3.2 Aims and objectives

The aim of this initial study was to establish the presence and distribution of biofilm on the inner surface of NG tubes used by adults.

The specific objectives were to:

- Establish the presence of bacteria and biofilm development on the inner surface of NG tubes used by adults through preparation and examination under episcopic differential interference contrast / epifluorescence (EDIC/EF) microscopy
- Establish effective laboratory techniques to remove biofilm from the inner surface of the NG tubes for further investigation
- Establish total culturable bacteria counts for 1 cm^2 sections of NG tube

3.3 Ethics

The study was considered to be outside of Research Ethics Committee and Research and Development criteria for approval, and was therefore conducted using two NG tubes used by adults, obtained from a local acute University Hospital Trust under proof-of-concept/mode-of-action arrangements.

3.4 Collection of used NG tubes

Two used tubes were collected from the local University Hospital Trust and transported to the University Category 2 Biological Sciences laboratory using a triple packaging system. This was in accordance with the University's protocol for transportation of Category B (UN3373) biological and clinical material (HSE, 2011). The used NG tubes were placed in individual sealable plastic bags. These were placed within a larger sealed bag, and into an insulated specimen bag with appropriate UN3373 labelling (VWR, 2013). The specimen bag was sealed using a tamper-proof security seal, and transported directly to the laboratory, where the NG tubes were removed for analysis.

3.5 Materials and method

This section describes the materials and method common to the five laboratory studies presented in this thesis. Additional materials and methods used in subsequent studies are described in the relevant chapters.

As little was known regarding the types of bacteria that may be present in the used tubes, investigations were undertaken to establish whether any potential bacteria

present grew in aerobic or anaerobic conditions; the findings of which would influence the methods used in the thesis.

The study took place within a Containment Level 2 laboratory (Centre for Biological Sciences, University of Southampton), in line with the Advisory Committee on Dangerous Pathogens and the World Health Organisation guidelines and techniques (ACDP, 2001; WHO, 2004). A risk assessment was undertaken of the laboratory materials and methods to be used, to ensure the correct precautions were adhered to throughout each of the laboratory-based studies (Appendix A). Laboratory methods were developed and refined during the early phases of the study to achieve effectual preparation of NG tube sections, successful removal of bacteria and biofilm from the NG tube sections, appropriate staining of the bacteria, and episcopic differential interference contrast/epifluorescence (EDIC/EF) microscopy.

3.5.1 Sterile distilled water (ddH_2O)

Sterile distilled water (ddH_2O) was used for suspending bacteria removed from the NG tubes, serial dilution of the bacterial suspensions, and for washing planktonic cells clear from NG tube samples prior to bacterial staining and/or microscopy. To prepare, the required volume of distilled water was poured directly into an appropriate laboratory glass bottle and autoclaved at 121 °C for 15 minutes. The resulting ddH_2O was sealed and stored at room temperature. Fresh stocks of ddH_2O were prepared for each experiment.

3.5.2 Ethanol 70 % (v/v) spray

Ethanol 70 % (v/v) spray was used as a disinfectant throughout the laboratory studies. Each NG tube was sprayed with ethanol 70 % (v/v) before preparation of the tube sections, to ensure bacteria from the outer surface of the tubes did not contaminate the study findings. The spray contains 70 % laboratory grade ethanol (Pfizer Ltd, 2012) and 30 % ddH_2O.

3.5.3 Tryptone Soya Agar

Tryptone soya agar (TSA) is a general purpose casein soya bean digest agar used for supporting the growth of a wide variety of microorganisms (Oxoid Ltd, 2013a, Oxoid Ltd, 2016a), suitable for the cultivation of both aerobes and anaerobes.

Powdered TSA (for constituents refer to Appendix B) was used and prepared according to manufacturer's instructions (30 g into 1 litre of dH_2O). This was autoclaved at 121 °C for 15 minutes and left to cool to 50 °C prior to pouring into sterile Petri dishes. Agar plates were stored at room temperature prior to use.

3.5.4 BacLight™ Live/Dead® bacterial viability stain

The BacLight™ Live/Dead® bacterial viability stain (Molecular Probes, Invitrogen, UK) consists of two components; SYTO 9 (Life Technologies, 2013) and Propidium Iodide.

The BacLight™ Live/Dead® solution was prepared using 1.5 µl SYTO 9 and 1.5 µl PI, along with 1 ml ddH_2O, in line with manufacturer's instructions. This was vortexed for 30 seconds to ensure all components became homogenised. As the BacLight staining solution is light sensitive, it was covered and stored in the dark at room temperature until required. If longer-term storage was required (more than a few hours), it was kept at 4 °C to ensure it remained stable.

3.5.5 Preparation of NG tube sections

The type of NG tube retrieved for this study was Corflo® 8 FG polyurethane NG tubes (Corpak MedSystems, UK). They were subsequently used in all the laboratory studies to ensure consistency. This type of tube is widely used in NHS hospitals for short term enteral feeding.

Each used NG tube was removed from its packaging using forceps sterilised with 70 % (v/v) ethanol spray. The outer surface of each tube was sprayed with 70 % (v/v) ethanol spray, removing bacteria that could contaminate the investigation of the NG tube inner surface.

The Corflo® NG tubes have centimetre markings from the distal end along their length for the purpose of measuring tube placement into a patient, and position checking prior to each use. These markings conveniently enabled specified 1 cm sections to be removed for study analysis. Sections were taken at 1, 2 and 3 cm, 40, 41 and 42 cm and 60, 61 and 62 cm of each of the used NG tubes, to represent the gastric (stomach) section of each tube, the oesophageal section, and the nasal section.

To remove the sections for examination, the tubes were held firmly with forceps, and cut through at the required measurement using fine-point scissors previously autoclaved at 121 °C and further sterilised with 70 % (v/v) ethanol spray. The 1 cm

sections were then cut longitudinally using the forceps and fine-point scissors to form open half-sections, revealing the NG tube inner surface.

Using forceps to hold the NG tube half-sections, liquid content was removed from the tube lumen by dabbing on to absorbent paper, and gently rinsing in ddH_2O for 5 seconds to help remove any further residue and planktonic cells. One half of each section was placed into a Petri dish, with the inner surface facing uppermost ready for direct microscopy. The other half was used for indirect analysis by re-suspending any attached bacteria and biofilm (Refer to 3.5.7).

3.5.6 Preparation of NG tubes for microscopy

For EDIC-only microscopy, the NG half tube sections in Petri dishes required no further preparation and could be examined directly using long working distance objectives. For comparison purposes, a new packaged sterile Corflo® medical-grade polyurethane NG tube similar to the retrieved used tubes was aseptically prepared in a BSC. Three 1 cm half sections were placed into Petri dishes ready for EDIC microscopy, which examined the surface topography of the NG inner surface, to compare with the images achieved of the used NG tubes.

To visualise attached bacteria, the BacLight ^{TM} Live/Dead® bacterial viability staining solution (prepared as described in section 3.5.4) was applied to label live and dead cells. A small amount of this solution was run along the inner surface of each section of NG tube ensuring full coverage. The sections were placed in the dark at room temperature for 20 minutes.

Following this incubation, each section was gently rinsed to remove excess BacLight by immersing briefly into ddH_2O, and dried by dabbing lightly onto absorbent paper. The sections were placed back into their Petri dishes and kept in the dark to air-dry for a minimum of 30 minutes.

Each section was then viewed at magnification x 1000. Under EDIC illumination, three representative images chosen at random of each section were captured at x 1000 to demonstrate the bacterial/biofilm load viewed. Under EF illumination, three representative images were again chosen at random along the length of each NG tube, using the green and red filters as appropriate, to highlight the live (green, SYTO 9 stained) and dead (red, PI stained) bacteria, with the aim of quantifying the levels of live and dead bacteria present.

3.5.7 Removing bacteria and biofilm from NG tube sections

Initially a swabbing method was used to remove the bacteria and biofilm from the inner surface of the NG tube using a sterile cotton swab lightly drawn across the surface, and rinsed in a Universal container holding 10 ml ddH_2O. However, this did not prove effective and staining of the NG tube sections with BacLight after swabbing showed a significant level of bacterial attachment remained. As the swabbing method appeared ineffective at removing the bacteria sufficiently, a vortexing method was employed, as used by Kim *et al* (2006) and Hurrell *et al* (2009a).

Each NG tube half section was placed into separate Universals, along with approximately 20 autoclaved glass beads and 10 ml of ddH_2O. This was vortexed at high speed for 30 seconds to ensure bacteria and biofilm were removed from the NG tube inner surface, and suspended in the ddH_2O. This suspension was used to establish total culturable bacteria count.

3.5.8 Plating aliquots of bacteria onto TSA

To ensure rigorous colony forming unit (CFU) counts, the TSA plates were prepared in triplicate, with a mean count recorded. A 50 µl aliquot of each suspension was pipetted onto each corresponding agar plate, and spread across the TSA surface using an L-shaped spreader. For aerobic incubation, plates were placed into a suitable container, upside down to avoid condensation building up on the TSA surface. For anaerobic incubation, plates were placed into an anaerobic jar along with a pre-prepared AnaeroGen^{TM} 2.5L anaerobic gas generating sachet (Oxoid, UK) to remove oxygen from within the jar. All plates were incubated at 37 °C overnight to represent the approximate body temperature the NG tube would normally be held at.

3.5.9 Counting Colony Forming Units

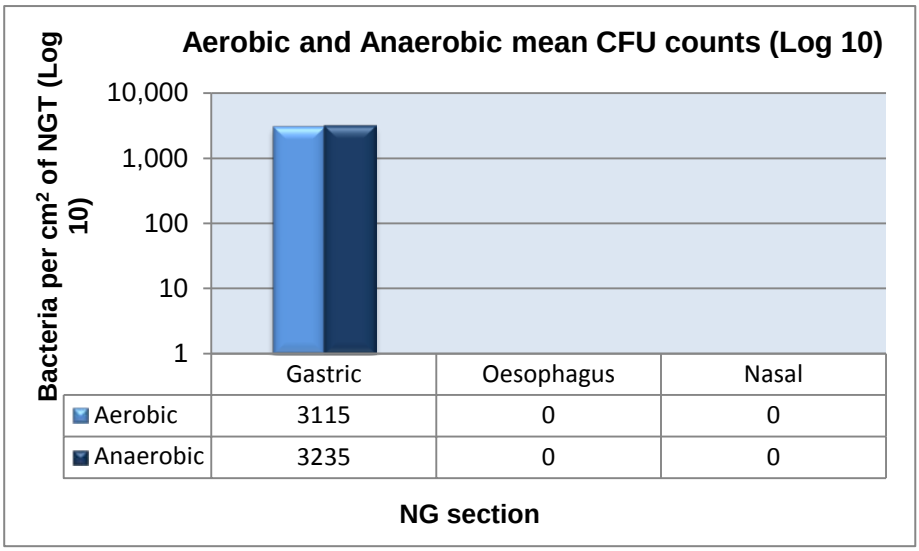
Following an overnight period of incubation at 37 °C for a minimum of 15 hours, the TSA plates were removed and colonies counted. Each separate colony was counted as one bacterium. The number of colonies for each plate was recorded, with a mean figure noted for each triplicate.

3.5.10 Preparation of NG tube stock culture

The bacteria cultured from the re-suspended biofilm retrieved from one of the two NG tubes investigated in the preliminary study was isolated and placed on Protect beads in glycerol stocks (Thermo Fisher, UK) for long-term storage at - 20 ˚C and - 80 °C. These were used to produce an inoculum for use in subsequent studies.

3.6 **Results**

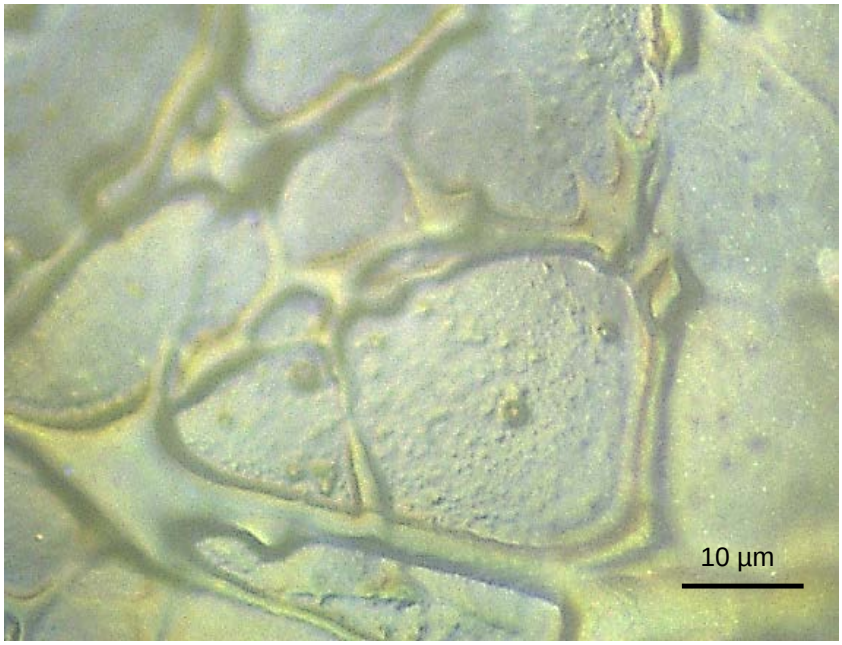
The results of the CFU counts for both aerobic and anaerobic growth conditions are demonstrated in Figure 3.1. The morphology of each colony was noted to be cream/yellow in colour, with a well-defined outer edge. The CFU counts were calculated to show the mean number of bacteria per cm^2 of NG tube. The limit of detection was 240 CFU/cm^2 of NG tube. As indicated at the gastric section of the NG tubes, similar numbers of bacterial density were reached under aerobic and anaerobic growth conditions, with a mean of 3115 bacteria per cm^2 of NG tube calculated aerobically, and 3235 per cm^2 anaerobically, a difference of 3.85 %. In contrast to the gastric section results, the oesophageal and nasal sections of the NG tubes produced no viable colonies either aerobically or anaerobically.



A comparison of CFU counts for aerobic and anaerobic conditions taken from three sections of used NG tube: gastric, oesophagus, and nasal. The counts suggesting there is minimal difference in either environment for the growth of the isolated bacteria

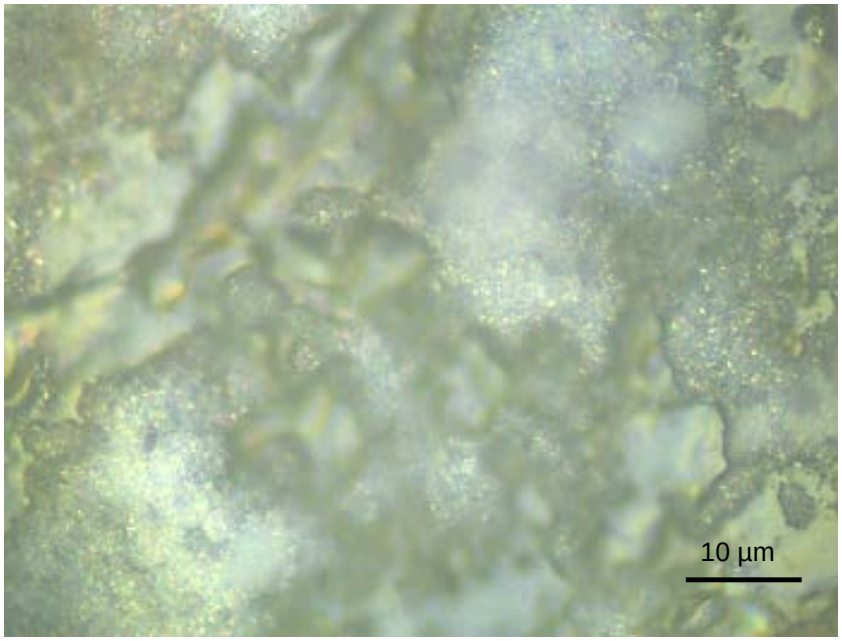
Figure 3.1: Aerobic and anaerobic mean CFU counts (Log 10)

EDIC microscopy showed significantly detailed NG tube inner surface structure along with an additional substance which appeared to be a dense amorphous material suggestive of biofilm and deposits. Figure 3.2 and Figure 3.3 show images of NG tube sections visualised under EDIC illumination at a magnification of x 1000, with a 10 µm scale shown. Taken for comparison purposes, Figure 3.2 is of the inner surface of an unused sterile NG tube, and clearly shows the undulating surface with numerous depressions and crevices presenting potential sites for planktonic bacteria to attach; in contrast Figure 3.3 shows a section from one of the used NG tubes at the gastric (stomach) area of the tube, and shows colonies of microorganisms grouped in a pattern strongly suggestive of biofilm distribution, with grouped colonies of bacteria forming the larger apparently raised structures, with relatively clear channels between allowing nutrients to pass through and be utilised by the bacteria.



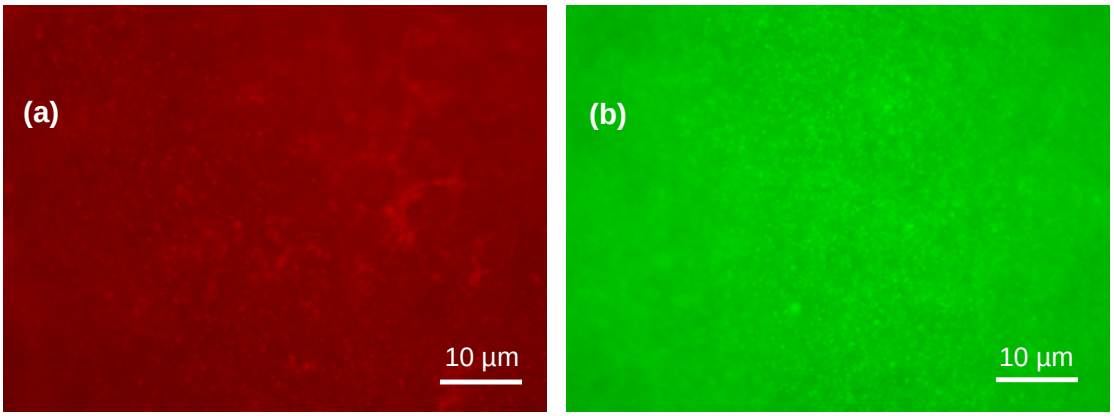
The inner surface of a sterile NG tube visualised under EDIC illumination clearly showing the undulating nature of the tube surface structure (mag x 1000). The scale bar represents 10 µm.

Figure 3.2: Image of a sterile NG tube inner surface under EDIC illumination



The inner surface of a used NG tube (gastric section) visualised under EDIC illumination (mag x 1000) suggestive of biofilm presence on the surface. The scale bar represents 10 µm.

Figure 3.4 shows further exemplar images of an NG tube section viewed using EF illumination. As seen in both (a) with Propidium Iodide staining, and (b) SYTO 9 staining, repeated bacterial staining demonstrated too great an autofluoresence to



Images of the effects of BacLight Live/Dead bacterial stain of used NG tube sections (mag x 1000). The autofluorescence of (a) Propidium Iodide and (b) SYTO 9 is too great to be an effective technique for estimating levels of attached bacteria and biofilm development. The scale bar represents 10 µm

Figure 3.4: Propidium Iodide and SYTO 9 bacterial stains

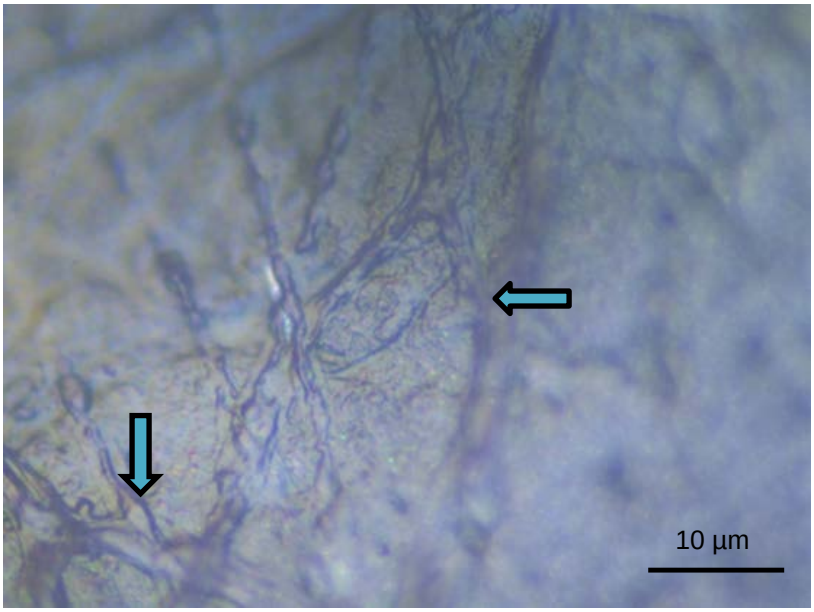
allow for a realistic estimate of bacterial cells attached to the NG tube. This could have been caused by potential autofluoresence of the NG tube or the enteral feed.

Figure 3.5 and Figure 3.6 show images taken of the same section of a used NG tube viewed under both EDIC and EF illumination. The EDIC image clearly demonstrates the presence of channels, depressions and crevices also noted in Figure 3.6. However, when viewed using EF illumination, the presence of bacteria becomes more evident, with the live bacteria illuminating green (in the FITC channel), and the dead bacteria illuminating red (in the TRITC channel).

Figure 3.3: Image of a used NG tube inner surface under EDIC illumination

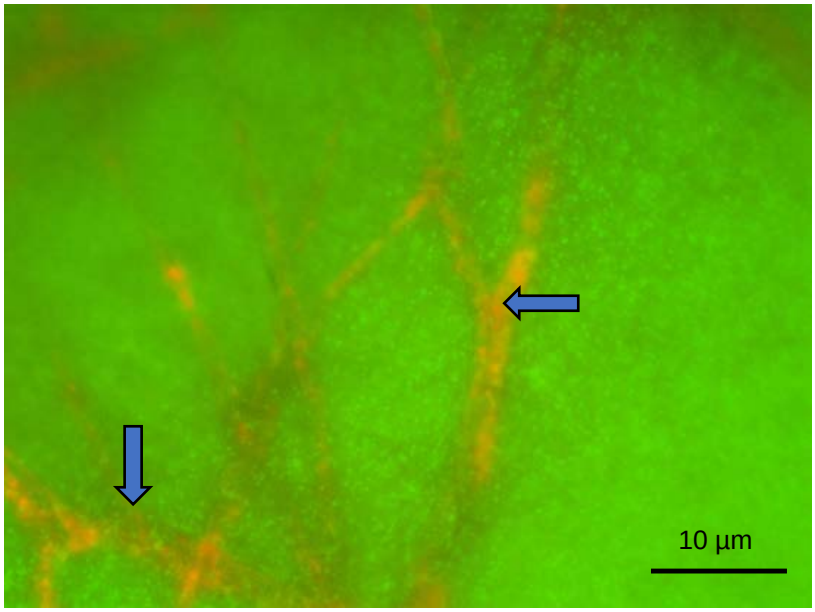
The inner surface of a section of used NG tube visualised under EDIC illumination (mag x 1000), clearly demonstrating the channels and crevices present, as noted earlier in the sterile NG tube image (Figure 3.2). The scale bar represents 10 µm.

Figure 3.5: Image of a used NG tube inner surface under EDIC illumination



3.7 Discussion

The aim of this study was to establish the presence and distribution of biofilm on the



The same section of used NG tube visualised under EF illumination (mag x 1000), showing the contrast of red (dead, PI) and green (live, SYTO 9) staining of the bacteria present achieved by using BacLight bacterial viability stain. The scale bar represents 10 µm.

Figure 3.6: Image of a used NG tube inner surface under EF illumination

inner surface of NG tubes used by adults. A key objective of the study was to develop suitable laboratory methods to effectively examine, remove and quantify attached bacteria and biofilm from the NG tube inner surface.

This study demonstrated the presence of bacteria and the evident development of biofilm on the inner surface of two tubes retrieved from adult patients at an acute care hospital. A review of published research on biofilm development on NG tubes suggests that this is the first time this has been reported. The study also enabled the most effective laboratory methods to be employed for examining, removing and quantifying attached bacteria and biofilm. The EDIC and EF microscopy methods enabled detailed visualisation of the NG tube inner surface and the bacteria present, with levels of bacterial colonisation successfully quantified through CFU counting.

Previous research into bacterial attachment on NG tubes (discussed in Chapter 2) has established biofilm presence on the outer surface of NG tubes used in an older-adult

health care setting (Leibovitz *et al*, 2005), and on the outer surface of NG tubes *in vitro* (Kim *et al*, 2006; Hurrell *et al*, 2009b). The presence of biofilms within NG tubes has been shown *in vitro* (Lima *et al*, 2011), and in tubes retrieved from neonates (Hurrell *et al*, 2009a). This study has contributed to the evidence base by demonstrating biofilm presence on the inner surface of NG tubes used by adults.

An important aspect of this study was to develop the laboratory methods and test the materials used. The NG tube samples were prepared from 1 cm lengths, cut longitudinally to expose the inner surface, similar to those sampled in Hurrell *et al* (2009b). The tube samples were taken from three sections of the NG tube length, as was similar to Lima *et al* (2011), who took sections from the beginning, middle and end of the NG tube. In this study, the samples taken coincided with the gastric, oesophageal, and nasal sections of the NG tube. This was considered appropriate to gain an indication of whether bacteria were more prolific in certain sections of the tube relating to human anatomical areas, establishing whether the environment in each area differs, with some potentially more suitable to supporting bacterial growth than others. It was also anticipated that this method could provide information regarding the potential source of bacteria introduction, by retrograde contamination from the stomach (Beattie & Anderton, 1998), or by opportunistic introduction at the feed attachment point. The results of the CFU counts indicate that bacteria was only present in the samples taken from the gastric section. As there is no available evidence accompanying the NG tube of bacteria entering the proximal end during its use, the assumption is that the bacteria originated from the patient's stomach. This would support Beattie and Anderton's (1998) statement that bacteria can enter feed reservoirs through retrograde contamination by removal of the NG tube stylet and by the practice of obtaining gastric aspirates to check correct tube position. However, it should also be noted that other published studies have indicated the possibility of alternative external sources of bacterial contamination, such as *P. aeruginosa* found in the oropharynx of older adult patients (Leibovitz *et al*, 2003) and *Cronobacter spp.* (*Enterobacter sakazakii*) and *Salmonella serovars* found in powdered infant formula (Hurrell *et al*, 2009b). The level of bacterial colonisation of each of the three anatomical points is further investigated in subsequent studies to establish a definitive answer.

Removal of bacteria from the prepared NG tube sections was initially done using a swabbing method, but bacterial staining after swabbing revealed significant levels of bacteria were left attached to the inner surface. This may have been due to the nature of the NG tube surface, which microscopy has shown to be markedly undulating, with

deep crevices where bacteria can potentially accumulate (Lima *et al*, 2011). Both Kim *et al* (2006) and Hurrell *et al* (2009a) used a vortexing method to remove bacteria from their samples, with Kim *et al* (2006) also using glass beads. Therefore, the vortexing method using glass beads to help remove the attached bacteria was employed throughout the laboratory studies in preference to swabbing.

The use of ddH_2O for suspending the removed bacteria was successful. This has other laboratory uses; it was used by Leibovitz *et al* (2005) for washing NG tube samples, prior to bacterial staining, and Kim *et al* (2006) used it for serial dilutions. The use of 70 % (v/v) ethanol for disinfecting laboratory equipment and the outer surfaces of NG tubes prior to sample preparation was effective. Both Kim *et al* (2006) and Hurrell *et al* (2009b) used 70 % ethanol as a disinfectant for their NG tube samples prior to inoculation with bacteria for their studies, whilst Leibovitz *et al* (2005) and Hurrell *et al* (2009a) used increasing concentrations of ethanol, up to 100 %, to dehydrate their NG tube samples for SEM.

The estimated bacteria count for the study was reached using the standard surface plate spread technique (Sutton, 2006), with 50 µl aliquots of re-suspended bacteria spread on TSA plates in triplicate and each colony of bacteria counted as one CFU. For the purposes of the study it was simply necessary to indicate there was a bacterial presence within the NG tube, and for this purpose counting CFUs was successful. By initially incubating the removed bacteria aerobically and anaerobically, the study was able to demonstrate the results were similar for each method, allowing further studies to be undertaken using the simpler aerobic method only. TSA is a general purpose agar used for supporting the growth of a wide variety of microorganisms, suitable for the cultivation of both aerobes and anaerobes, which was particularly advantageous as little was known regarding the bacteria that may have been present in the NG tube samples. Kim *et al* (2006) used TSA for sub-culturing representative colony types for identification using phenotypic profiles, whilst Hurrell *et al* (2009a) used it in a similar way to this preliminary study, to support and quantify CFUs.

Scanning Electron Microscopy (SEM) was used in the majority of literature review studies (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005; Hurrell *et al*, 2009a; Hurrell *et al*, 2009b; Lima *et al*, 2011) to observe bacteria and biofilm. Unfortunately this method of microscopy requires dehydration of the sample which may cause biofilm structures, stabilised by hydrated extra polymeric substances (EPS), to collapse. SEM often involves samples being held in a low vacuum environment which can also lead to biofilm structure collapse and destruction. Scanning confocal laser microscopy (SCLM)

can allow excellent three dimensional views of biofilm, but it has difficulty scanning a curved surface such as an NG tube and requires samples to be fluorescent. Traditional microscopy involves a light source beneath the subject which, when viewed from above through the microscopy lens, would form an image through the subject. However, with the advancement of EDIC microscopy, it is possible to view opaque objects such as NG tubes by applying a light source from above, enabling the analysis of hydrated biofilms on solid and curved surfaces with no need for coverslips and oil, thus increasing the knowledge of biofilm structure and function significantly (Keevil, 2003). A Nikon Eclipse E800 EDIC/EF microscope (Best Scientific, UK) was successfully used in this study, permitting direct visualisation of biofilms and surface materials by using high magnification and long working distance objectives with episcopic DIC illumination (Keevil, 2003), and was adopted for use in subsequent studies.

The use of BacLight^{TM} Live/Dead bacterial stain was not entirely successful. SYTO 9 is a small molecule which targets DNA and can cross intact cell membranes resulting in green fluorescence of live cells on microscopy. Propidium Iodide, which was also used by Leibovitz *et al* (2005), in contrast, is a much larger molecule which can only enter cells with a disrupted membrane. It then also binds to DNA and excludes SYTO 9, resulting in red fluorescence on microscopy. This is a commonly used staining system (Gião *et al*, 2009) that can localise viable and non-viable bacteria populations on microscopy, and was employed for this purpose. However, the extreme autofluoresence experienced from some tube samples on microscopy under EF illumination prohibited the counting of individual bacteria, and was considered not suitable for that purpose. It is possible that the Propidium Iodide had stained any dead bacteria still present following the sterilisation process during the manufacture of the NG tube. But this would not explain the results of the SYTO 9 staining, which should only indicate live bacteria. In view of these findings, the method of using BacLight bacterial stain on the inner surface of the NG tube sections was stopped, and viewing the inner surface under EDIC illumination to directly visualise the surface was adopted. The SYTO 9 component was used in isolation for staining live bacteria in later studies within this thesis.

A limitation of the study was that it was completed using only two NG tubes, retrieved from adults as part of their normal care. The bacterial colonisation identified in each of the tubes may not be indicative of all NG tubes used by adults. Also, as the tubes were obtained under proof-of-concept/mode of action arrangements, no information regarding their use, the feed regimen, medications, or patient details could be

examined. The outcomes of the microscopy and bacterial counts methods demonstrated the presence of bacteria within the tubes, but there is a limitation to the knowledge gained without further understanding the events which may have led to the bacteria being introduced. This is further explored in the study presented in Chapter 8, where a number of NG tubes obtained from adult patients in an acute care hospital, with ethics and NHS approval, were investigated for biofilm presence and distribution in relation to individual patient factors including duration of placement, feed regimen, and medications given.

3.8 **Conclusion**

This study was designed to be a preliminary investigation, the results of which have established the presence and distribution of biofilm within the two NG tubes used by adults, obtained for the study. This builds upon the findings of previous published studies investigating the presence of biofilm on the outer surface of NG tubes used by adults (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005), and on the inner surface of NG tubes used by neonates (Hurrell *et al*, 2009a).

It provided an opportunity to determine effective laboratory techniques to assist with removing bacteria and biofilm from NG tubes to quantify the total culturable bacteria count, and has demonstrated effective use of innovative EDIC/EF microscopy to capture images of the inner surface of the NG tube, supporting the hypothesis that bacteria attach to the NG tube inner surface and develop biofilm. Bacteria from one of the NG tubes obtained for the study was successfully isolated for use in later studies.

The next chapter will describe a study undertaken to better understand bacterial attachment and biofilm development on NG tube material by investigating the time frame at which each occurs.

Chapter 4: Bacterial attachment and biofilm development observed over 24 hours

4.1 Introduction

The previous study demonstrated the presence of bacteria and biofilm within polyurethane NG tubes retrieved from adults in the course of their normal clinical care. As the first study, it provided an opportunity in which the laboratory materials and methods utilised were evaluated and refined to facilitate assessment of biofilm development in the subsequent studies, which go on to explore factors that may influence the development of biofilm.

This chapter presents a study undertaken to determine the speed of bacterial attachment and biofilm development following the introduction of bacteria to sections of sterile NG tube under laboratory conditions. Leibovitz *et al* (2005), in their research of NG tubes as vectors for infection in a hospital environment, established that 60% of the tubes retrieved for their study were positive for biofilm on the outer surface after 24 hours of patient placement, and 100% were found to be positive within 48 hours of patient placement. Hurrell *et al's'* (2009b) laboratory based study also investigated the time frame of bacterial attachment to two types of NG tube used by neonates, PVC and PU, along with silver-impregnated flexelene tubing. This was in response to international concern over the microbiological safety of powdered infant formula following repeated outbreaks of *Cronobacter spp.* (*Enterobacter sakazakii*) and *Salmonella serovars* infections causing bacteraemia and meningitis in neonates. Routes of neonatal infections via powdered infant formula required investigating, particularly in relation to NG tube feeding, where the tube can be in place for more than 48 hours and is held at body temperature, thus providing suitable conditions to support bacterial growth. Hurrell *et al* (2009b) were able to confirm biofilm development on NG tubes after 24 hours of being incubated with inoculated infant formula.

With this in mind, and with the new knowledge gained from the preliminary study, a time frame study was designed to establish the speed of bacterial attachment and biofilm development within the first 24 hours of the introduction of bacteria to sterile sections of NG tube under controlled laboratory conditions. The hypothesis was that there would be a significant increase in the bacterial colonisation over the time period studied.

The laboratory methods adopted and developed during the preliminary study were used for this time frame study, with new methods introduced as required. This study was designed to investigate and quantify the levels of bacterial attachment to, and biofilm development on, NG tubes at set time frames of 15, 30, 60, 120, 240 and 1440 minutes (24 hours), with controls set at zero minutes.

4.2 Aims and objectives

The aim of this study was to establish the time frame for bacterial attachment and biofilm development on polyurethane NG tubes.

The specific objectives were to:

- Inoculate sterile NG tube sections with bacteria and place them in an environment to promote bacterial growth
- Establish the time frame of the bacteria to adhere to the NG tube surface and form biofilm *in vitro*
- Quantify levels of bacteria attached to the NG tube surface at set time points using CFU and CE counts and EDIC/EF microscopy

4.3 Materials and method

Taking ethical considerations into account, it would not have been feasible to obtain NG tubes used by adults throughout the first 24 hours of placement. Leibovitz *et al* (2005) obtained the used NG tubes for their study, at the time points of 24 hours and 48 hours post placement, through the tubes being self-removed by the patients. For this study, a laboratory based investigation was developed using an observational design. By undertaking the research in a laboratory setting, greater control of variables could be achieved, such as time, temperature, and nutrient availability. It also allowed for the controlled introduction of selected pathogens into new sections of NG tube, sterilised during the manufacturing process. Kim *et al* (2006) completed their research in a laboratory setting, and successfully demonstrated bacterial growth increased on the outer surface of NG tubes in line with temperature increase and nutrient availability.

The materials and methods employed in the study shown in Chapter 3 were used in this study. In addition, the following materials were used.

4.3.1 Pathogens used for inoculation

Two pathogens were used to inoculate the sections of NG tubes; *Escherichia coli* (non VT O157 NCTC12900) and *Pseudomonas aeruginosa* (PA01). *Both E. coli* and *Pseudomonas* pathogens were used in previous published studies reviewed in Chapter 2 (Hurrell *et al*, 2009a; Hurrell *et al*, 2009b). They form part of the human microbiota and are usually present in the hospital environment (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005). *E. coli* is part of the normal flora of the gut, and *P. aeruginosa* is found in the oropharynx and nasopharynx of humans. They have been found on medical equipment such as urinary catheters and in water systems (Donlan & Costerton, 2002; Gião *et al*, 2011), and both are producers of biofilm (Costerton *et al*, 1995; Solseng *et al*, 2008), making them particularly suitable for this study.

In order to safely use these pathogens in the study, a risk assessment was completed (Appendix A) in line with the Advisory Committee on Dangerous Pathogens (HSE, 2013) and the World Health Organisation (2004) guidelines, and in conjunction with The Control of Substances Hazardous to Health Regulations (COSHH) (2002). COSHH specifies four containment levels for activities which involve working with biological agents, which correspond to the classification of biological agents into Hazard Groups 1 to 4 (Appendix C). *E. coli* and *P. aeruginosa* both belong to Hazard Group 2, and therefore a Containment Level 2 laboratory provided a suitable environment for the study.

4.3.2 Tryptone Soya Broth

Tryptone soya broth (TSB) was used to support the growth of the stock bacteria. It is a highly nutritious and versatile casein soya bean digest broth medium used for the growth of bacteria and fungi (Oxoid Ltd, 2013b; Oxoid Ltd, 2016b). Powdered TSB (for constituents refer to Appendix D) was used and prepared in line with the manufacturer's instructions (30 g into 1 litre of ddH_2O). This was autoclaved at 121 °C for 15 minutes, left to cool, and stored at room temperature until required.

4.3.3 Growth of pathogens/overnight culture

The *E. coli* stock culture was stored at -20 °C on Protect beads in glycerol stocks (Thermo Fisher, UK), and the *P. aeruginosa* stock culture was stored at -80 °C in glycerol. Fresh culture was prepared for each experiment. The experiment was repeated eleven times with *E. coli*, and three times with *P. aeruginosa*.

A 20 ml volume of TSB was placed into a Universal for each bacterium. A Protect bead was removed from the *E. coli* container and placed into the prepared Universal; to introduce the *P. aeruginosa*, a scraping of the stock culture in glycerol was taken and placed into its prepared Universal. The two Universals were then incubated at 37 °C overnight (minimum 15 hours).

4.3.4 Enteral feed

The medium used to promote bacterial growth was an enteral feed solution. The solution was Fresubin® Original enteral feed (Fresenius Kabi UK Ltd, 2011). This was used in the laboratory studies to recreate nutrient availability during NG tube use. This solution was chosen as it is regularly used in hospital and community environments for enteral feeding via NG tube. It is supplied sterile, in sealed plastic pouches, ready for use. In order to ensure the sterility of the feed was maintained whilst it was stored for the laboratory studies, it was transferred to a laboratory bottle which had been sterilised by autoclaving at 121 °C for 15 minutes. Once cool, the feed was decanted into it directly from the pouch, by disinfecting the pouch with 70 % (v/v) ethanol spray and cutting a small opening in the top of the pouch, using scissors also sterilised with 70 % (v/v) ethanol spray. The feed was stored in the fridge at 4 °C until required, to reduce the possibility of contamination. For each study, a control test of the feed was undertaken by plating 50 µl aliquots on to TSA in triplicate and incubating at 37 °C for a minimum of 15 hours. Using this method, the sterility of each of the enteral feeds used for the laboratory studies was demonstrated to have been maintained throughout their use.

4.3.5 Inoculum preparation

In order to introduce the *E. coli* and *P. aeruginosa* pathogens to the NG tube surface, an appropriate inoculum was prepared. Using four microfuge tubes for each of the *E. coli* and *P. aeruginosa* pathogens, 1 ml of the overnight culture (4.3.3) was placed into each. The tubes were placed in a centrifuge set at 7.5 K rpm for 10 minutes to produce a pellet of bacteria. The excess supernatant was discarded, and 1 ml of enteral feed medium (Fresubin® Original enteral feed: Fresenius Kabi UK Ltd) was added to dislodge and mix with the bacteria pellet by drawing up and expelling as necessary. The contents of all four tubes were placed into one Universal to create the experimental inoculum.

4.3.6 NG tube sections

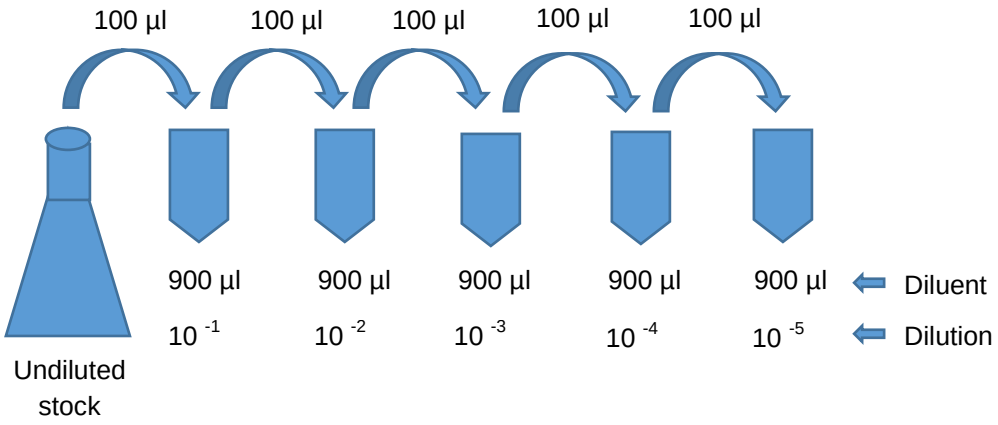
Corflo® 8 FG polyurethane NG tubes (Corpak MedSystems, UK) were used for this study, and are similar to those obtained in the preliminary study. To prepare the NG tube sections, a tube was removed from its packaging within a BSC and sprayed with 70% (v/v) ethanol to remove the possibility of any contaminants. Using scissors that had been autoclaved at 121 °C and further sterilised using 70% (v/v) ethanol spray, seven 1cm sections of sterile NG tube were cut. Each 1 cm section was further cut longitudinally to expose the inner surface.

4.3.7 Containment of inoculated samples

Six-well microtiter plates (Sigma-Aldrich®, UK) were used for the containment, inoculation and incubation of the NG tube sections. These have been used successfully in a previous study exploring bacterial species in drinking water biofilms (Gião *et al*, 2011), in which uPVC coupons were placed in six-well plates with inoculum.

4.3.8 Serial dilutions

To enable accurate and reproducible counting of colonies on TSA, an upper limit CFU/plate count needed to be set to increase the prospect of an accurate count. Breed and Dotterrer's (1916) paper looking at the acceptable limits of colony forming units, based on triplicate plating of serial dilutions, estimated the lower to upper limit of 50-200 CFU/plate. As the undiluted inoculum concentration was too high to achieve this target limit, it was necessary to perform serial dilutions of the inoculum on an ad hoc basis, dependant on the counts experienced at each stage of the study (CBS, undated).



The diagram demonstrates the action of serial dilution of undiluted bacterial stock culture by 10 fold at each dilution, from 10^{0} to 10^{-5}

Figure 4.1: Serial dilution of undiluted stock culture

To prepare serial dilutions, 900 µl of ddH_2O (3.5.1) was placed into the number of microfuge tubes required for each of the time frame serial dilutions, e.g. if a serial dilution of 10^{-11} is required, eleven tubes would be required, labelled 10^{-1} through to 10^{-11} (An example is shown in Figure 4.1). A 100 µl aliquot of the undiluted suspension was added to the 10^{-1} tube, mixing as it was expelled. Then, 100 µl of this solution was taken and placed into the 10^{-2} tube. This process was continued until all the serial dilutions required were complete.

4.3.9 DAPI DNA stain

To label bacteria, DAPI (4´, 6´-diamidino-2-phenylindole dihydrochloride) (Sigma-Aldrich®, 2015) was used. DAPI is a cell permeable, fluorescent dye that binds to bacterial cell DNA, staining it blue, thus allowing visualisation under epifluorescence microscopy.

A stock solution was prepared using 1 mg DAPI powder to 1ml ddH_2O in accordance with the manufacturer's instructions, and stored at -20 °C in the dark. Although the preliminary study used SYTO 9 bacterial stain to good effect, DAPI was selected in addition for comparison purposes. The results of the bacterial stains used in this study would determine which one would be most effective for use in the later studies.

4.3.10 R2B low-nutrient broth

R2B is a laboratory-prepared low-nutrient broth used to gently encourage bacteria to grow. When bacteria enter a viable but non-culturable (VBNC) state due to external stressors such as temperature extremes or low nutrient availability, TSB is considered inappropriate as it is too nutrient-rich to encourage such bacteria to multiply. To prepare 500 ml of R2B, 500 ml of distilled water was placed into a suitable laboratory bottle, along with the following constituents (Table 4.1):

Table 4.1: R2B constituents

Yeast extract	0.250 g
Proteose Peptone	0.250 g
Casein Hydrolysate (Cas Amino Acid)	0.250 g
Glucose	0.250 g
Starch	0.250 g
Di-potassium phosphate (K_2HPO_4)	0.150 g
Magnesium Sulphate	0.012 g
Sodium Pyruvate	0.150 g

This was autoclaved at 121 °C for 15 minutes, allowed to cool, sealed and stored at 4 °C until required.

4.3.11 Inoculating the NG tube samples

Frozen stock was used for each of the pathogens; the method or preparation of inoculum was similar for both pathogens. Bacteria culture was prepared (4.3.3) using either a Protect bead (*E. coli*) or a glycerol scraping (*P. aeruginosa*), and incubated at 37 °C overnight (minimum 15 hours). Each culture was used to prepare an inoculum (4.3.5). Using a packaged sterile NG tube, a total of 14 half-sections of NG tube were prepared (4.3.6). Of these, 12 were placed in a six-well microtiter plate, two sections into each well. Two sections were retained for the baseline control at zero minutes: one in a lidded Petri dish, one in a Universal. Each well was labelled with a time point of 15, 30, 60, 120, 240 or 1440 minutes. The study followed a similar methodology as described by Gião *et al* (2011), in which uPVC coupons were inoculated and incubated in six-well microtiter plates, and removed at set intervals to establish a time frame of biofilm development.

Due to the dimensions of the wells, 3 ml of enteral feed was added to each well of the six-well microtiter plate, thus ensuring each of the NG tube sections were fully immersed. To this, 100 µl of the prepared inoculum was added. This amount could be altered up or down in volume if the results warranted it. The six-well plate was placed within a plastic lidded box, and incubated at 37 °C.

4.3.12 Removal of NG sections from six-well plate for analysis

Seven Universals and seven Petri dishes were labelled with each time frame of 0, 15, 30, 60, 120, 240, and 1440 minutes. 10 ml ddH_2O and approximately 20 autoclaved glass beads of 2 mm diameter (Merck, UK) were added to each Universal. At each time frame, both of the appropriate sections of NG tube were removed from the relevant well. Excess feed/inoculum mix was removed from the NG tube sections onto absorbent paper by dabbing gently, followed by gentle rinsing in ddH_2O for approximately 5 seconds to help remove the remaining feed residue and any planktonic cells.

One section of NG tube for each time frame was placed into a Petri dish, with inside surface facing uppermost; the other into the corresponding Universal. This process was repeated for each of the time frames.

4.3.13 Preparation of NG sections for microscopy

After air drying for 30 minutes, the NG tube sections in Petri dishes required no further preparation and could be examined directly via EDIC microscopy using long working distance objectives.

4.3.14 Removing bacteria and biofilm from NG tube sections

Each Universal containing an NG tube section was vortexed at high speed for 30 seconds to ensure bacteria and biofilm were removed from the sample and suspended in ddH_2O. This suspension was used to establish total culturable bacteria count.

4.3.15 Plating aliquots of bacteria onto TSA

Serial dilutions as noted in Table 4.2 were completed. A 50 µl aliquot of each suspension was pipetted onto each corresponding agar plate, and spread across the TSA surface using an L-shaped spreader (3.5.8). All plates were incubated at 37 °C

overnight to represent the approximate body temperature the NG tube would normally be held at. TSA plates were prepared in triplicate, with a mean count recorded.

To ensure sterility of the enteral feed medium throughout each of the repeated experiments, controls of 50 µl samples were also plated onto TSA in triplicate. To assess the concentration of the inoculum, 50 µl samples of inoculum were plated onto TSA, following the serial dilutions noted in Table 4.2. The plates were incubated at 37 °C overnight (minimum 15 hours). The following day, the plates were counted.

The serial dilutions noted in Table 4.2 and Table 4.3 were chosen based on the bacteria counts achieved, and were reassessed after each individual experiment. These could be altered up or down as considered necessary to enable accurate CFU counting.

Table 4.2: Serial dilutions used for the time frame study

Serial dilutions of tube samples used in study to enable CFU counting:						
0 mins	Undiluted					
15 mins	Undiluted	10^{-1}				
30 mins	Undiluted	10^{-1}				
1 hour	Undiluted	10^{-1}	10^{-2}			
2 hours		10^{-1}	10^{-2}	10^{-3}		
4 hours			10^{-2}	10^{-3}	10^{-4}	
24 hours				10^{-3}	10^{-4}	10^{-5}

This table indicates the serial dilutions found to be necessary to enable CFU counting on 50 mm TSA plates during the time frame study

Table 4.3: Controls of feed and inoculum

Serial dilutions of controls used in study:				
Feed medium	Undiluted			
Inoculum		10^{-6}	10^{-7}	10^{-8}

To ensure sterility of the feed medium, and to estimate the strength of the inoculum, controls of 50 µl aliquots were plated onto TSA as per the table

4.3.16 Counting colony forming units

Following an overnight period of incubation at 37 °C for a minimum of 15 hours, the TSA plates were removed and colonies counted. The number of colonies for each plate was recorded, with a mean figure noted for each triplicate.

4.3.17 Using stains to assess bacterial colonisation

The preliminary study reported in Chapter 3 assessed the use of BacLight Live/Dead bacterial stain, and found the SYTO 9 component effective in staining live bacteria. However, there were difficulties with autofluoresence on repeated experiments, which prevented accurate recording of the number of bacteria present. For this study, DAPI DNA stain was considered alongside SYTO 9 as an alternative for assessing bacterial colonisation of the NG tube samples.

Using a microfuge tube for each time frame, 2 µl (2 µg/ml final concentration) of DAPI and 1.5 µl of SYTO 9 were added, along with 1 ml of the relevant suspended sample from the corresponding Universal. These were vortexed for 10 seconds to allow for the sample and bacterial stains to homogenise, then placed in the dark for 15 minutes to allow the stain to develop.

After 15 minutes, and using vacuum filtration equipment, the contents of each of the tubes were individually filtered through new 0.2 µm pore size polycarbonate filter membranes. Nuclepore® (Whatman, UK) filter membranes were used throughout the subsequent laboratory studies. Each filter membrane was placed onto a microscopy slide and allowed to air dry for approximately 5 minutes. Once dry, each membrane had a drop of non-fluorescent immersion oil (Fluka, UK) added, and a cover slip applied, before observation under a Nikon Eclipse E800 EDIC/EF microscope (Best Scientific, UK).

To prepare the slides for microscopy, a drop of non-fluorescent immersion oil was added to each cover slip, for microscopy using an oil-immersion objective lens. These lenses are designed for use with refractive index matching oil, which must fill the gap between the front element and the object, giving greater resolution at high magnification (Best Scientific, UK). Using the oil-immersion objective lens (x 100) and EF illumination, along with the green and blue filters corresponding to the SYTO 9 and DAPI bacterial stains, the stained bacteria were viewed. The number of bacteria were determined in each microscope eyepiece field of view (Darcan *et al*, 2009). As the

bacteria cells were homogenously distributed, 20 fields of view on each membrane were chosen at random, and the number of cells in each field of view recorded.

4.3.18 Cell elongation

CFU counting can only detect those cells able to form colonies under the conditions of the test, such as the incubation media, the temperature, time, and oxygen conditions (Sutton*,* 2006). However, it is important to appreciate not all viable bacteria may be detected in this way, leading to discrepancies in bacteria counts. This is due to some bacteria being in a viable but non-culturable (VBNC) state, which is a survival strategy undertaken by many bacteria in response to adverse environmental conditions (Gião *et al*, 2011). Such conditions include extreme temperatures, nutrient starvation, oxygen availability, and marked changes in pH. The bacteria still maintain a metabolism, although much reduced, and are still virulent, capable of causing infections in animals and plants (Fakruddin*,* 2013). Examples of bacteria that can enter the VBNC state include *E. coli, P. aeruginosa, Helicobacter pylori,* and *Legionella pneumophila.* In view of this VBNC state, and the fact that many of the bacteria noted to enter this state are clinically significant, a further method of bacteria assessment was introduced.

Direct viable counting (DVC) (Juhna *et al*, 2007) through cell elongation (CE) is a technique where VBNC bacteria are encouraged to grow and multiply in a low nutrient broth such as laboratory-prepared R2B, rather than the stronger TSB which may cause stressed bacteria to remain in a VBNC state. To prevent the bacteria separating as they multiply an antibiotic, Pipemidic Acid (Sigma-Aldrich®, 2013), is added, which inhibits bacterial DNA polymerisation and supercoiling, and chromosome fragmentation. Therefore, as the bacteria multiply and form new bacteria, they cannot break away, causing a ‘chain’ of bacteria to form. Each bacteria chain is significantly easier to identify, but is still counted as one bacterium for the purposes of the study, as each chain originated from one bacterium removed from an NG tube section.

Taking a universal for each of the seven time frames, 5 ml of R2B, 4 ml of ddH_2O, and 1 ml of the corresponding undiluted suspended bacteria sample for each time frame were added, along with 100 µl of 1 mg/1 ml Pipemidic Acid. The universals were placed in a plastic lidded box, and gently incubated at 22 °C overnight**.** The following day, the bacteria were stained using SYTO 9 to assist with identifying them on microscopy. Using a microfuge tube for each time frame, 1.5 µl of SYTO 9 along with 1 ml of the relevant sample were added. These were vortexed for 10 seconds to allow for the sample and bacterial stain to homogenise, then placed in a dark space for 15 minutes

to allow the bacterial stain to develop. Once developed, the stained samples were filtered through 0.2 µm pore size polycarbonate filter membranes ready for microscopy.

4.3.19 Statistical analysis

Statistics advice was sought from the University Statistics Department. The study was analysed using SPSS software version 22.0 (SPSS, Chicago, IL, USA). The first analysis was that of establishing whether there was an association between the length of time the NG tube samples were inoculated for, and the level of bacteria attached. The hypothesis was that there is a significant increase in the CFU and CE counts over the time period studied. Shapiro-Wilk was used to test for normality of distribution due to the small sample sizes, and confirmed that not all variables were normally distributed. Therefore, the non-parametric Spearman's Rank-Order Correlation test was used to measure the strength and direction of the association between the two continuous variables of time inoculated and bacteria count. This was repeated for each of the pathogens used, and the bacteria counting methods of CFU and CE.

To further enhance the understanding of the relationship between time inoculated and bacterial colonisation, analyses were also undertaken to determine whether there is greater colonisation occurring in the period after 60 minutes compared with the period up to, and including, 60 minutes. A repeated-measures within-subjects design with one independent variable of two levels (time pre and post 60 minutes) was undertaken using the Paired-Samples t-test. However, significant outliers were noted in the data, and the distribution of differences of the CFU/CE counts between the two groups was not normally distributed. Therefore, the non-parametric Wilcoxon Signed-Rank test was used. The histogram produced showed a bivariate distribution. Rather than transform the data, which would have altered the ranking of differences and can lead to interpretational issues, the alternative Paired-Samples Sign test was used.

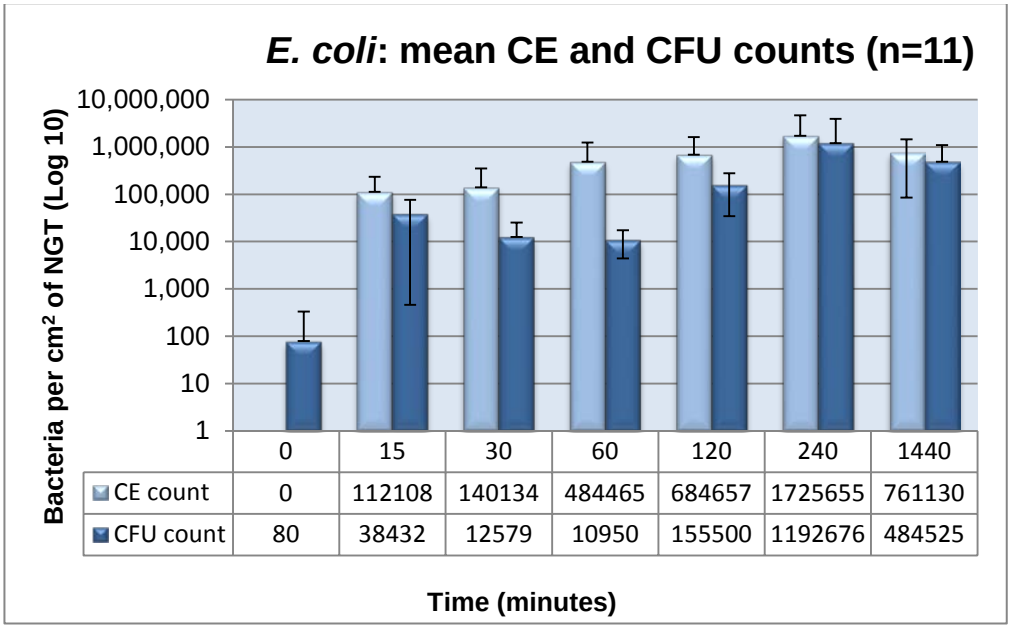
4.4 **Results**

Figure 4.2 and Figure 4.3 present charts displaying the mean CE and CFU results of both the *E. coli* and *P. aeruginosa* experiments. The *x* axis displays the timed intervals from the baseline 0 minutes through to the final 1440 minutes; the *y* axis indicates the number of bacteria calculated per cm^2 of NG tube (Log 10). These charts clearly show bacteria are attached within 15 minutes, following the introduction of the inoculum. The

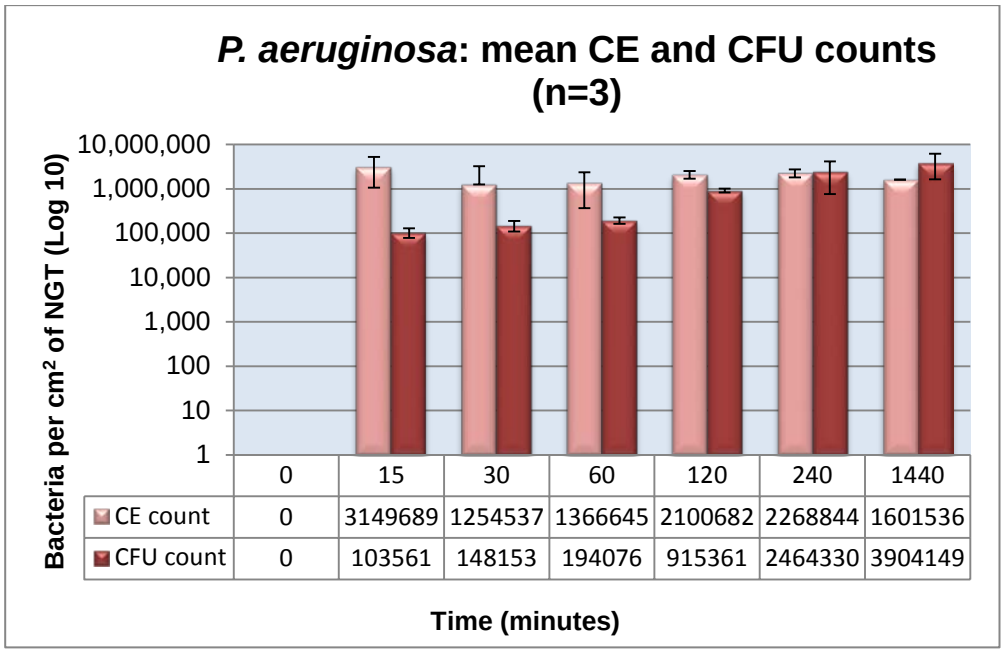
baseline measurement showed a low count of 80 CFU in one of the 11 *E. coli* experiments. This was likely to be due to contamination at the point of plating the sample onto TSA, which is supported by the fact the CE count for the same time frame remained at zero following filtration and microscopy. No further evidence of contamination was identified.

The mean CE and CFU counts for *E. coli* clearly indicate bacterial attachment at 15 minutes from inoculation. There is a trend towards increased numbers up to the 24 hour time frame, when levels begin to decrease

Figure 4.2: *E. coli* mean CE and CFU counts (Log 10)



	0	15	30	60	120	240	1440
CE count	0	112108	140134	484465	684657	1725655	761130
CFU count	80	38432	12579	10950	155500	1192676	484525



The *E. coli* results (Figure 4.2) show a higher CE count at each time frame compared

The mean CE and CFU counts for *P. aeruginosa* also indicate bacterial attachment at 15 minutes. There is a trend of increased mean CFU count to time frame, but the mean CE count remains reasonably consistent

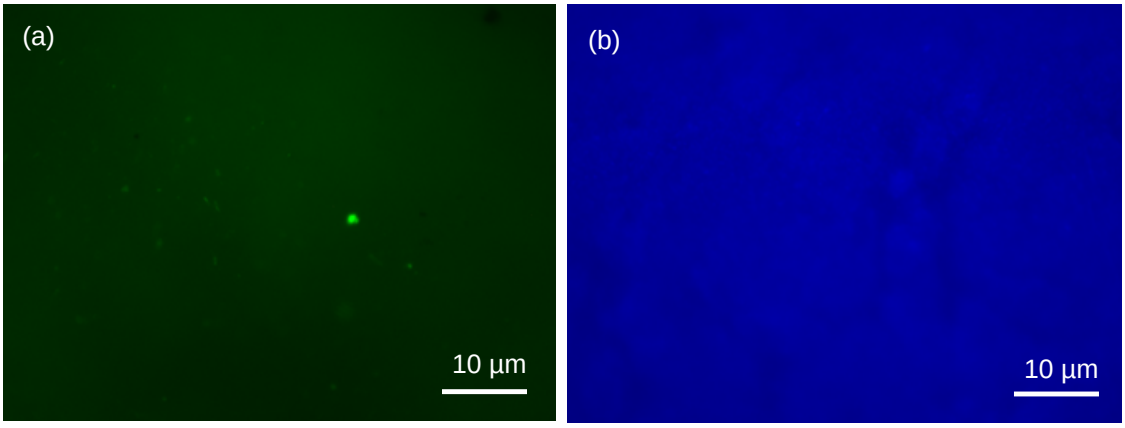
Figure 4.3: *P. aeruginosa* mean CE and CFU counts (Log 10)

with the CFU count over the course of the experiment. This higher CE count at each time frame compared with the CFU count is to be expected due to the presence of viable but non-culturable (VBNC) cells which would not be included in CFU counts, but may be included in CE counts. This can be demonstrated as early as 15 minutes as seen by the reduction in countable numbers of CE to CFU in both the *E. coli* and *P. aeruginosa* inocula (Figure 4.2 and Figure 4.3); with a 66 % decrease in *E. coli*, and a substantial 97 % decrease in *P. aeruginosa.* The decrease in numbers remains reasonably consistent throughout the *E. coli* experiments, although narrows to 31 % drop at 240 minutes and 36 % decrease at 1440 minutes. The *P. aeruginosa* (Figure 4.3) results initially follow a similar course, but then show an unexpected increase at 240 and 1440 minutes of CFU to CE cells of 8.62 % and an extraordinary 143.78 % respectively.

The overall *E. coli* CFU counts are noted to range within a two Log difference from 15 to 1440 minutes and the CE count a one Log difference, whilst the *P. aeruginosa* CFU

results are noted to range within a 1.5 Log difference, and the CE count approximately a half Log difference.

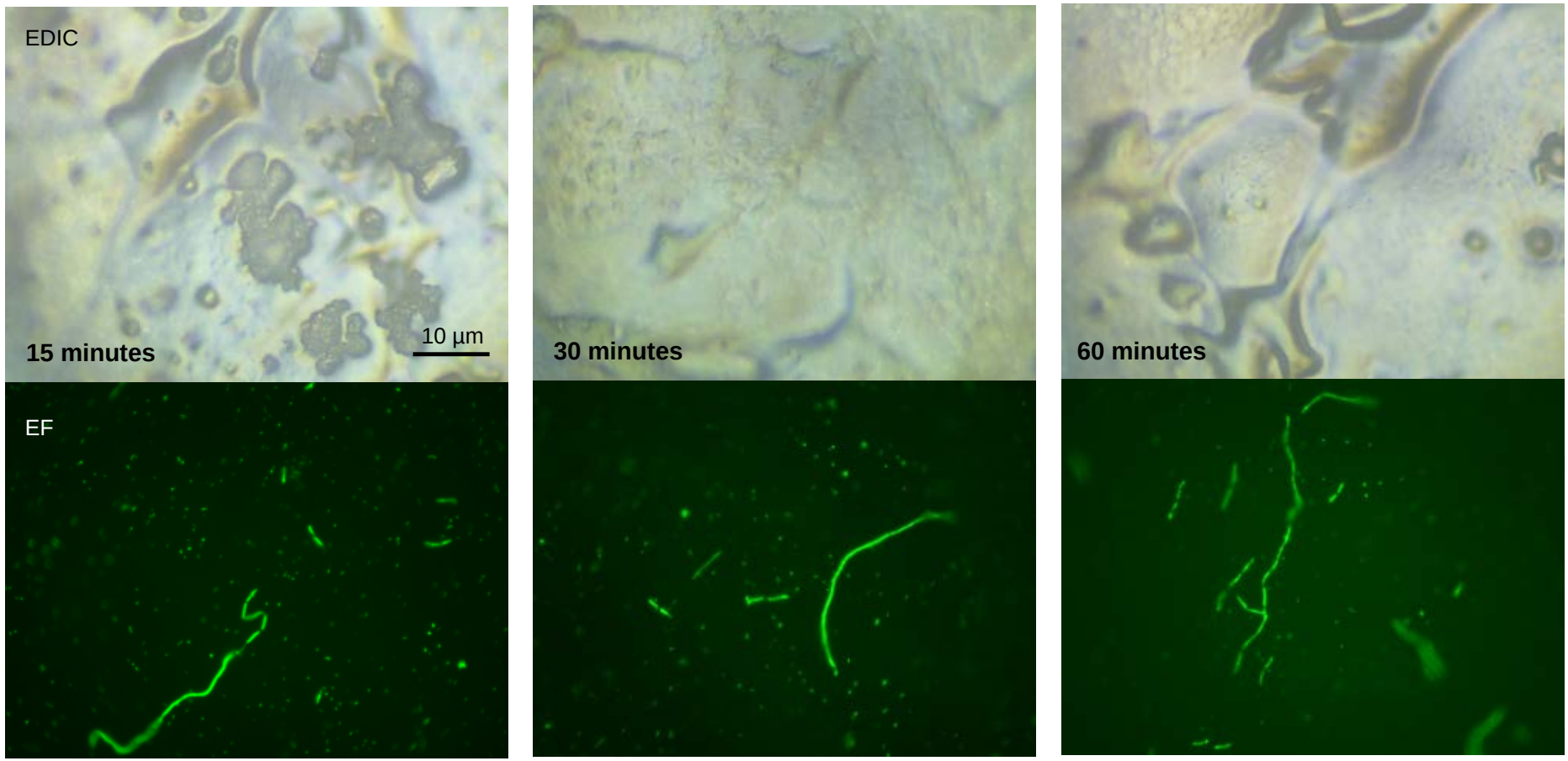
Figure 4.4 presents two images of filter membranes viewed using EF illumination at a magnification of x 1000 via oil-immersion lens. In the staining and filtration of suspended bacteria removed from the NG tube sections, SYTO 9 appears to have stained a bacterium, as seen in (a). However, DAPI did not prove effective (b), and did not facilitate the identification and subsequent counting of individual bacteria cells.



Images of filter membranes under EF illumination (mag x 1000) indicate (a) the effect of SYTO 9 bacterial stain, and (b) the effect of DAPI bacterial stain, with DAPI proving inconclusive for the purposes of assisting cell count. The scale bars represent 10 µm

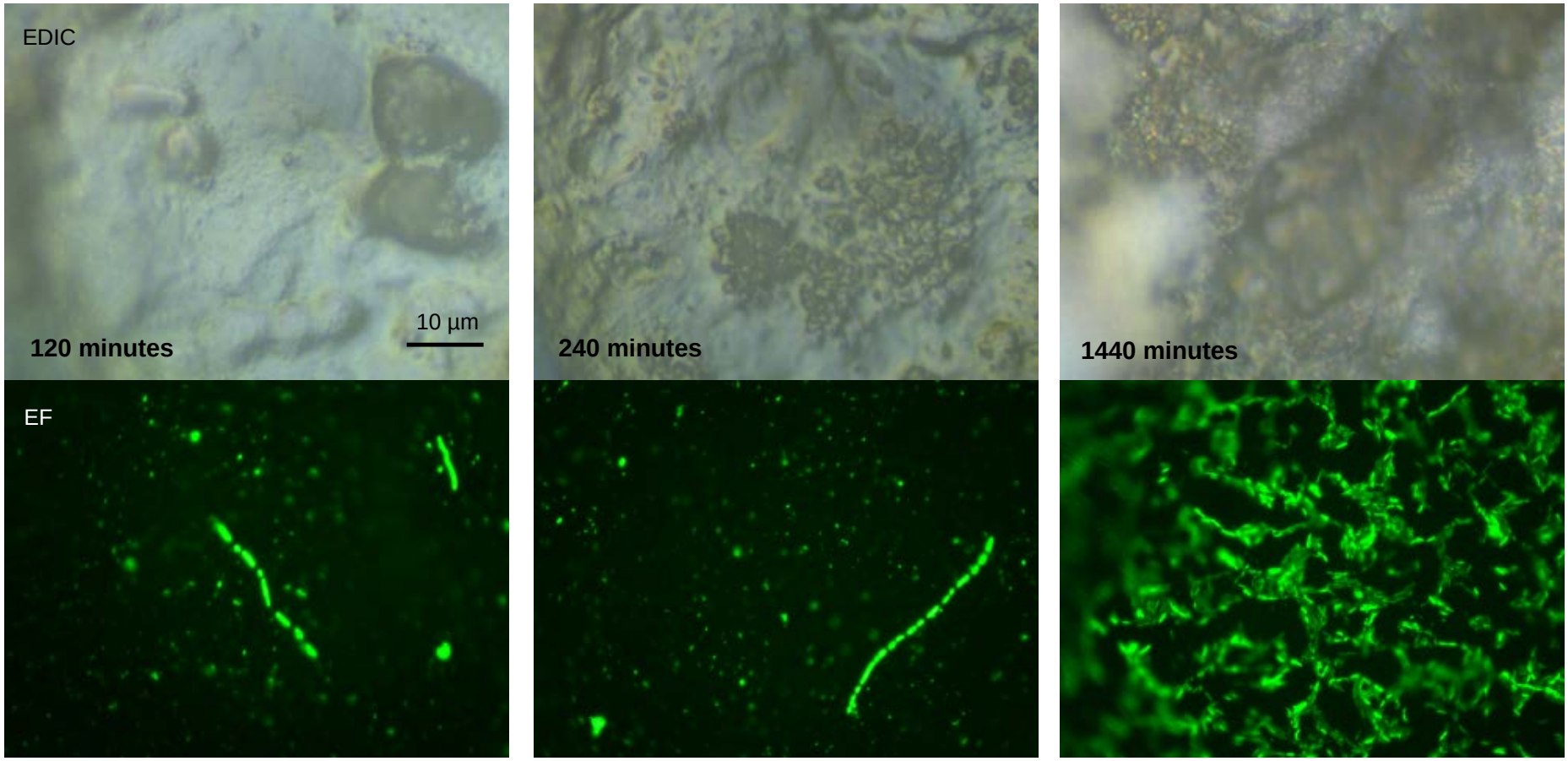
Figure 4.4: Using SYTO 9 and DAPI bacterial stains

Figure 4.5 and Figure 4.6 present exemplar images from each of the time frames from 15 minutes to 1440 minutes, for the *E. coli* pathogen. At 15 minutes, there appears to be a light coating over the surface of the NG tube sample. This coating is what remained attached to the surface following washing with ddH_2O to remove feed residue. The epifluorescence illumination image of the Nuclepore membrane clearly shows the presence of viable bacteria cells, with defined elongated cells present. The 30 minute image demonstrates a thickening of the coating, further supported by the increase in elongated cells. At 60 minutes, the beginnings of what appears to be bacterial colonies are evident, with clusters of cells attaching to the inner surface of the NG tube. The images at 120 and 240 minutes show similar findings, again supported by the clear views of elongated cells on epifluorescence illumination. At 1440 minutes the bacteria colonies appear established, and apparent biofilm formation can be seen as pale patches of extracellular polymeric substance (EPS) over the colonies. As can be clearly seen from the epifluorescence illumination image, there is now an abundance of elongated cells present.



Exemplar images of *E. coli* colonisation at 15 to 60 minutes, using EDIC illumination (mag x 1000) for NG tube inner surface images, and EF illumination (mag x 1000) via oil-immersion lens to capture cell elongation images from the filter membranes

Figure 4.5: NG tube inner surface and filter membranes under EDIC and EF illumination (15 to 60 minutes)



Exemplar images of *E. coli* colonisation at 120 to 1440 minutes, using EDIC illumination (mag x 1000) for NG tube inner surface images, and EF illumination (mag x 1000) via oil-immersion lens to capture cell elongation images from the filter membranes

Figure 4.6: NG tube inner surface and filter membranes under EDIC and EF illumination (120 to 1440 minutes)

Preliminary statistical analysis showed the relationship between the duration of time the NG tube samples were inoculated for, and the level of bacterial colonisation, was monotonic in each case, as assessed by visual inspection of scatterplots. There was a strong positive correlation between the duration of time inoculated and bacterial colonisation for the *E. coli* CE and CFU counts, and the *P. aeruginosa* CFU count:

- *E. coli* CFU – r_s (68) = .64, p < .0005
- *E. coli* CE – r_s (68) = .674, p < .0005
- *P. aeruginosa* CFU – r_s (19) = .957, p < .0005

These results show a statistically significant relationship between duration of time inoculated and bacterial colonisation, and therefore the null hypothesis can be rejected, and the alternative hypothesis accepted; there is a significant increase in the CFU and CE counts over the time period studied. The analysis of the *P. aeruginosa* CE count also showed a positive relationship, but this was weak and not statistically significant, r_s (19) = .327, p > 0.05.

For the *E. coli* pathogen, 30 matched pairs were used to assess the difference between post-60 minute to pre-60 minute bacterial colonisation. Data are medians unless otherwise stated. Continuity correction was applied with the Paired-Samples Sign test as the sample was greater than 25. Of the 30 pairs, post-60 minutes elicited an increase in CFU (CE) count in 25 (26) pairs, whereas a reduction in CFU (CE) count was observed in 5 (4) pairs, no ties noted. Overall, the CFU (CE) count was greater post-60 minutes at 171,048 (560,538) than pre-60 minutes at 11, 601 (100,096), a statistically significant increase in the median of the differences of 164,765 (420,404), Z = 3.469 (3.834), p = .0005 (.0005).

For the *P. aeruginosa* pathogen, nine matched pairs were assessed to compare the difference between the two timed periods. An Exact Sign test was used as there were fewer than 25 matched pairs. Of the nine matched pairs of the study, an increase in CFU count was observed in all cases. The post-60 minutes median was 1,383,024 compared with the pre-60 minutes median of 156,006, a statistically significant increase in the median of the differences of 1,172,975, p = .004. Therefore, the null hypothesis can be rejected: there is a significant increase in bacteria colonisation post-60 minutes of inoculation. However, for the *P. aeruginosa* CE count results, there were five positive differences, and four negative differences, no ties noted. As no statistically significant difference was seen (p = 1.0), the null hypothesis must be accepted.

4.5 **Discussion**

The aim of this study was to determine the speed of bacterial attachment and biofilm development following the introduction of bacteria to sections of sterile polyurethane NG tube. The hypothesis was that there would be a significant increase in bacterial colonisation of the NG tube sections over the time period studied. The pathogens used for this purpose were *E. coli* and *P. aeruginosa*, both known biofilm formers. Inoculation was performed under laboratory conditions. The objectives were to quantify bacterial attachment on the surface of the NG tube sections, and to determine the presence of biofilm on the inner surface of the NG tube sections, by examining the sections at specified time points. Both Leibovitz *et al* (2005) and Hurrell *et al* (2009b) had previously demonstrated biofilm is present on NG tubes at 24 hours *in vivo* and *in vitro* respectively. Therefore specified times leading up to the 24 hour (1440 minutes) point were selected for this study, with NG tube sections examined at 15, 30, 60, 120, 240 and 1440 minutes, and controls at 0 minutes.

It is clear both *E. coli* and *P. aeruginosa* are attaching to the NG tube samples as rapidly as 15 minutes following inoculation under controlled laboratory conditions. There was a statistically significant increase in bacterial colonisation per cm^2 of NG tube section over the course of the 24 hour period studied, demonstrated by *E. coli* CFU and CE results, and *P. aeruginosa* CFU results. The CE results for *P. aeruginosa* were also positive for an increase in bacterial count following statistical analysis, although this was not statistically significant. When considering the rate of bacterial attachment pre- and post-60 minutes of introduction, the CFU and CE results for *E. coli*, and the CFU results for *P. aeruginosa*, showed a significant increase in bacterial count per cm^2 of NG tube post-60 minutes compared with pre-60 minutes. The bacterial attachment appears to be exponential, and could be indicative of not only planktonic bacteria attaching, but attached bacteria multiplying. The *P. aeruginosa* CE results were not significantly greater post-60 minutes.

A literature review of research into biofilm development on NG tubing suggests this is the first time bacterial attachment has been reported in this way, in the period up to 24 hours. Therefore the study has contributed to the evidence base by demonstrating bacteria attach within 15 minutes of introduction, the bacteria count increases exponentially post-60 minutes of introduction, and biofilm is established at 24 hours. This adds to the knowledge gained through previously published investigation (Leibovitz *et al,* 2005; Hurrell *et al*, 2009b). Hospital-placed NG tubes are typically *in situ* for two to four weeks, and this can be considerably longer in the community

setting. With this in mind, and with the knowledge gained in undertaking this study, a further study is reported in Chapter 8 in which a number of used NG tubes were obtained from adults in an acute hospital setting. These tubes were investigated for biofilm presence and distribution in relation to several factors which may influence biofilm development, one of which is duration of placement.

The materials and methods that proved successful in the preliminary study (Chapter 3) were employed here, along with additional materials and methods as required. Modifications were made as necessary to achieve the aim of the study. It would have been unethical to have retrieved used NG tubes from patients at set time points to coincide with the requirements of the study, irrespective of the patients' care needs. Therefore, the study design was laboratory based, using new packaged sterile NG tubes. Six-well microtiter plates were successfully used for the containment, inoculation and incubation of the NG tube sections throughout the study. This method allowed for the six separate time point samples of each experiment to be kept together, within a similar environment simultaneously, subject to the same variables associated with environmental conditions. They had been used effectively in a previous published study exploring bacterial species in drinking water biofilms (Gião *et al*, 2011), in which uPVC coupons were placed in 6-well plates with inoculum, and retrieved at set time points.

The pathogens used, *E. coli* and *P. aeruginosa*, were chosen as they form part of the human microbiota and are usually present in the hospital environment (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005), with *E. coli* part of the normal flora of the gut, and *P. aeruginosa* found in the nasopharynx and oropharynx of humans. Both are known to be producers of biofilm (Costerton *et al*, 1995, Solseng *et al*, 2008), which proved especially beneficial for this study. TSB was used in the study to support the growth of the pathogens for use in the inoculum, and was used by Kim *et al* (2006) to good effect to support the growth of *E. sakazakii* used in their investigation into bacterial attachment on enteral tubes. Although monospecies contamination is unlikely in a health care environment, both pathogens were chosen for their biofilm-forming properties and their relevance to health care. A limitation of examining single species biofilms would be that they may not develop as effectively as multi species biofilms, where planktonic bacteria of differing properties add to the biofilm mass during the maturation phase. The resulting multi species biofilm may be more resistant to environmental factors such as temperature and nutrient availability. The inoculum concentration for both pathogens was feasibly greater than would occur naturally in a hospital environment. However, the studies within this thesis are essentially concerned with exploring the action of bacterial attachment and biofilm development, as well as

investigating the affect biofilm can have on its environment. As a result, the concentration levels of the inocula used were not considered focal to the outcomes of the studies.

In the staining and filtration of suspended bacteria removed from the NG tube sections, SYTO 9 was found to be effective although DAPI was not, and did not allow the identification of individual bacteria. As a result, DAPI DNA staining was withdrawn from use in the study. However, the method of direct counting of bacteria needed refining to ensure all viable cells were included. Cell elongation was introduced as a method to assist in assessing bacterial colonisation by including VBNC bacteria through direct counting. An alternative to the nutrient-rich TSB was required for the cell elongation method, for gently encouraging the growth of VBNC bacteria that had entered that state due to stressors such as extremes of temperature or nutrient availability. The laboratory-prepared low-nutrient broth R2B was introduced for this purpose and proved effective, with clear elongated cells observed on repeated EF images throughout the study.

The aim of this study was to ascertain the time frame for bacterial attachment and biofilm development on polyurethane NG tubes. The first 24 hours were to be considered in detail, with time points of 15, 30, 60, 120, 240 and 1440 minutes, and a control, or baseline, measurement at 0 minutes. If the results of the study had demonstrated no biofilm was present prior to the 240 minutes time point, the experiments would have been rerun with additional time frames between 240 and 1440 minutes to gain further insight into the bacterial attachment process. As bacteria were attaching at the 15 minute time point for both *E. coli* and *P. aeruginosa*, additional time points were considered unnecessary.

The preliminary study in Chapter 3 demonstrated the benefit of EDIC/EF microscopy for visualising the inner surface of NG tubes under EDIC illumination, and the effect of staining bacteria to aid identification under EF illumination. Therefore, EDIC/EF microscopy was also successfully employed throughout this study. The images captured at the set time points provided visual evidence that matched the findings of both the CFU and CE bacterial counts. In particular, the images of the NG tube sections under EDIC illumination at 1440 minutes repeatedly indicate the presence of biofilm on the NG tube inner surface, with the EF illumination images of the SYTO 9-stained elongated bacteria supporting this, displaying an abundance of elongated bacteria removed from the NG tube surface.

Evidence of contamination was noted in a 0 minute control in one of the 11 *E. coli* experiments. However, this was particularly low at 80 CFU per cm^2 of NG tube, and appears to have occurred at the point of plating an aliquot onto TSA. This was supported by the CE count remaining at zero for the same control. No further evidence of contamination was noted throughout the study. Furthermore, to ensure sterility of the feed medium used in the both the *E. coli* and *P. aeruginosa* experiments, controls of 50 µl aliquots were plated onto TSA in triplicate for each of the 14 separate experiments. Each enteral feed control was found to be devoid of bacteria.

A limitation of this study was the fact that the feed and bacteria within the six-well plates during incubation remained static. In a clinical environment, feed flows through NG tubes at approximately 100 ml per hour, along with opportunistic planktonic bacteria, which could influence the process of bacterial attachment and biofilm formation. To help address this issue, a later study in this thesis considers the use of sterile water and tap water flushes by using a flow model to recreate the NG tube in use, with feed passing through the tubes at a regular rate, and flushes completed at timed intervals. The flow model is similar in parts to that used by Lima *et al* (2011), who noted in their investigation into bacterial attachment to NG tubes that it is important to realise that the *in vitro* data may not accurately reflect the bacterial adhesion process that occurs when a nasoenteral tube is utilised by humans. Although, the findings of this study at the 24 hour point do agree with those of Leibovitz *et al* (2003), which was conducted *in vivo*. However, given the ethical dilemma of conducting such research *in vivo*, the limitations of the flow model study design are acceptable for the purposes of examining factors that may influence biofilm development.

A limitation of the CE counting method via EF illumination is that only 20 random fields of view were studied. Including additional fields of view would undoubtedly achieve a more accurate result. But as there are more than 66,000 potential fields of view per Nuclepore membrane filtration area, as calculated using microscopy graticules, the increase in count required would have to be considerable to be effective, and beyond the scope and time restrictions of this thesis. To help overcome this limitation, the sample of suspended bacteria was vortexed prior to filtration, to ensure the bacterial cells were homogenised in the suspension liquid. Also, the 20 fields of view were chosen at random to ensure an unbiased outcome of the CE calculation.

The *E. coli* experiment was repeated, in triplicate, 11 times but the *P. aeruginosa* experiment was repeated only 3 times. If repeated, both pathogens would be used an equal number of times, to enable greater clarity when comparing the results of one to

those of the other. A future study could take this into consideration, and for enhanced investigation could also include a third inoculum, that of the NG stock culture isolated in the preliminary study in Chapter 3.

4.6 **Conclusion**

This study aimed to ascertain the time frame for bacterial attachment and biofilm development on polyurethane NG tubes, using the two pathogens *E. coli* and *P. aeruginosa*. The study proves bacterial attachment occurs within 15 minutes of introduction to NG tubes *in vitro*, and biofilm development is evident at the 24 hour time point on all samples. The bacteria count significantly increases over the 24 hour time period studied, with an exponential increase in the period after 60 minutes of inoculation, supporting the hypothesis that there would be a significant increase in bacterial colonisation of the NG tube sections over the time period studied. Therefore the study has contributed to the evidence base, adding to the knowledge gained through previously published investigation (Leibovitz *et al,* 2005; Hurrell *et al*, 2009b).

The next chapter reports a study designed to examine the effect bacteria can have on its local environment, by recording the pH level of enteral feed inoculated with bacteria and monitored over a 24 hour period.

Chapter 5: Examining the influence of bacteria on the pH of enteral feed

5.1 Introduction

The first study in this thesis established the presence of biofilm on the inner surface of NG tubes used by adults through the use of advanced microscopy techniques and bacteria quantification. The second study set out to establish the time taken for bacteria to attach to NG tubes and develop biofilm under laboratory conditions. This was shown to have occurred at 15 minutes after bacteria introduction, with established biofilm present at the 24 hour time point.

An observation during the second study was made of samples of inocula incubated overnight at 37 °C. These were regularly found to have thickened to a yoghurt-like consistency (Figure 5.1). In contrast, controls consisting of feed without the introduction of bacteria remained liquid.

Three Universals are shown, each containing thickened enteral feed that was apparent after overnight storage of feed containing bacteria

Figure 5.1: Universals containing feed and bacteria

One of the potential causes of the thickening of the feed could be a change in pH as a result of bacterial action. In a study on dental biofilms, Von Ohle *et al* (2010) noted biofilm bacteria can cause extreme modifications to the local environment through metabolic activity, causing it to become highly acidic. Feed formula has been shown to coagulate when exposed to an acidic environment, as found in research studies into the effects of gastric acid on the casein protein component of the feed (Gaither *et al* 2009; Marcus *et al,* 2010). This study set out to investigate whether bacteria potentially found on the inner surface of NG tubes can have a similar effect on enteral feed. If it did, a similar process may be occurring *in vivo*, potentially impacting on the patency of NG tubes.

5.2 Aims and objectives

The aim of this study was to investigate the effect bacteria potentially present within NG tubes used by adults could have on its local environment by monitoring the pH level of inoculated enteral feed samples.

The specific objectives were to:

- Inoculate samples of sterile enteral feed with *E. coli, P. aeruginosa* or bacteria isolated from a used NG tube, and place them in an environment to promote bacterial growth
- Establish the pH of the enteral feed at 0, 15, 30, 60, 120, 240, 360 and 1440 minute time frames, following exposure to *E. coli, P. aeruginosa* or bacteria isolated from a used NG tube

5.3 Materials and method

E. coli (non VT O157 NCTC12900) and *P. aeruginosa* (PA01) as used in the time frame study were used to inoculate the enteral feed samples, along with the bacteria cultured from one of the used NG tubes retrieved for use in the preliminary study. The unknown, and potentially multiple, bacteria isolated from the used NG tube was introduced at this point to examine the similarities and differences in the results achieved with the known, and singular, *E. coli* and *P. aeruginosa* bacteria, and to give a more accurate picture of what may be happening *in vivo*. Each of the three stock

bacteria was cultured overnight in TSB in line with the method in Chapter 4 (4.3.3). Using these overnight cultures, inocula were prepared (4.3.5) ready for use.

A 3 ml volume of Fresubin® Original enteral feed (Fresenius Kabi UK Ltd) was measured into sterile Universals; eight for each stock bacteria, and one set aside as a control to calibrate the pH probe between readings. A 100 µl inoculum of each of the three bacterial cultures (*E. coli, P. aeruginosa* and the mixed culture isolated from the used NG tube in the first study) was added to each of the eight Universals particular to that bacteria. The time frames of 0, 15, 30, 60, 120, 240, 360 and 1440 minutes were used, similar to those used in Study 2, with the addition of 360 minutes.

A Jenway 3510 pH Meter (Jenway, UK) was used for this study. To prepare the probe for use, it was washed with running distilled water. The probe was then placed into the control Universal to establish a baseline pH for the sterile feed medium used. Once a reading was gained, the probe was removed and washed again with running distilled water, and then placed in the zero minute Universal of the first bacteria to measure the pH. This was repeated for each of the three bacteria, with washing of the probe and recalibration between each measurement. To store, the probe was placed into a holding bottle containing pH 4 buffer. The Universals for the remaining time frames were incubated at 37 °C.

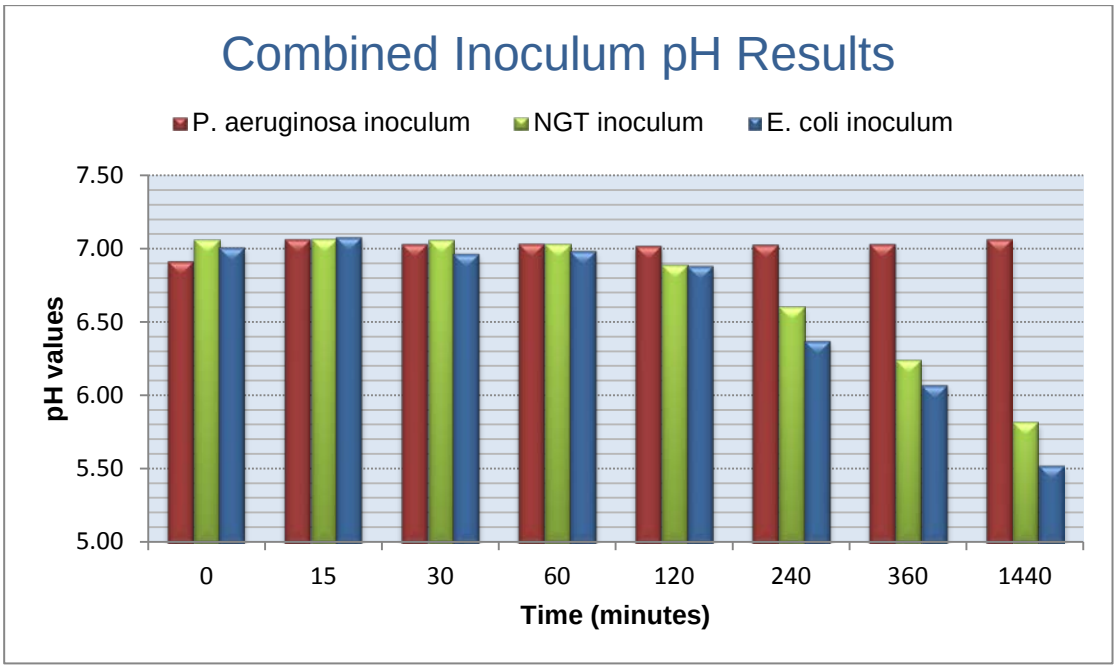
At each time frame of 15, 30, 60, 120, 240, 360 and 1440 minutes, the corresponding three Universals were removed from the incubator, and a pH reading was taken for each sample. To prevent cross-contamination, and achieve an accurate pH reading, it was important to ensure the probe was washed with running distilled water after each measurement, calibrated using the sterile feed, washed again before testing the next sample, and washed a final time before placing it back into the holding bottle. The experiment was repeated three times for each of the three bacteria cultures.

5.3.1 Statistical analysis

The study was analysed using SPSS software version 22.0 (SPSS, Chicago, IL, USA). A Spearman's rank-order correlation test was completed to assess the relationship between the time the samples were incubated and the pH measurements.

5.4 **Results**

The results of the pH measurements taken of the three different bacteria is displayed in Figure 5.2. They indicate the pH of the samples containing *P. aeruginosa* (shown in red) remained relatively stable, ranging from 6.92 to 7.07 (mean 7.03), throughout the 24 hour time period studied. In contrast, the pH of the samples containing *E. coli* (shown in blue) and NG tube isolate (shown in green) follow a remarkably similar pattern. The pH was reduced, thus increasing acidity, 120 minutes from exposure onward. The *E. coli* samples ranged from pH 7.08 to 5.53, and the NG tube isolate from 7.07 to 5.82, with both sets of readings falling from the 120 minute point at a similar rate. Each of the *E. coli* and NG tube isolate inoculated feed samples began to thicken at the 240 minutes time point, and were entirely thickened at the 1440 minutes (24 hour) time point (18 samples in total). In contrast, all the feed samples containing *P. aeruginosa* remained liquid throughout the 24 hour period studied. These results show there were clear differences in the effect of the bacteria on the pH of the enteral feed.



The three samples of inoculated feed had a similar pH reading up to 60 minutes following inoculation. After 120 minutes the *P. aeruginosa* inocula remains relatively constant over the 24 hours of the experiment. The NG tube and *E. coli* inocula pH readings fall from the 120 minute point at a similar rate.

Figure 5.2: pH testing results comparing three inocula

On statistical analysis, a scatterplot showed the relationship between the duration of incubation and the pH measurements to be monotonic. There was a strong negative correlation between time and pH measurement; as time increases, pH level decreases, r_s (22) = -.968, p = .0005.

5.5 **Discussion**

This study examined the effect of three different inocula on the pH of enteral feed over a period of 24 hours. The results clearly show a statistically significant (p = .0005) increase in acidity in the *E. coli* and NG tube isolate inoculated samples, but this is not noted in the *P. aeruginosa* inoculated samples. All The *E. coli* and NG tube isolate inoculated feed samples were found to thicken from the 240 minutes time point onward, although the *P. aeruginosa* inoculated feed samples remained fluid throughout. Moreover, controls of feed that did not contain bacteria were also subject to the same environmental conditions, and similarly remained fluid. The increase in acidity and the thickening of feed samples suggest the *E. coli* and NG tube bacteria could be triggering a fermentation process using the glucose substrate available in the enteral feed formula. Table 5.1 displays the nutritional composition of the Fresubin® Original enteral feed used in the study, containing 1g/100ml of sugars, of which 0.27g/100ml is glucose.

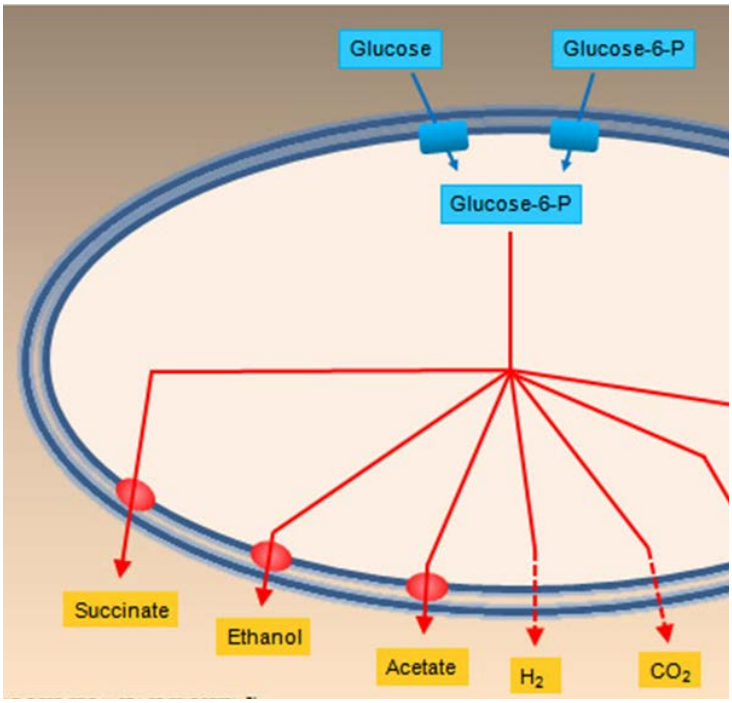
Table 5.1: Nutritional composition of enteral feed used in the studies

NUTRITIONAL COMPOSITION OF ENTERAL FEED	Per 100 ml	Per 1500 ml
ENERGY kcal (kJ)	100 (420)	1500 (6300)
PROTEIN g	3.8	57
CARBOHYDRATE g	13.8	207
Of which sugars g	1	15
Of which lactose g	</=0.02	</=0.03
TYPICAL CARBOHYDRATE PROFILE		
Glucose	0.27	4.05
Maltose	0.95	14.25
Lactose	0.02	0.3
Polysaccharides	12.52	187.8
FAT g	3.4	51
Of which saturated fatty acids g	0.3	4.5
Of which monounsaturated fatty acids g	2.1	31.5
Of which polyunsaturated fatty acids g	1	15
Of which EPA and DHA g	0.03	0.45
FIBRE g	0	0
WATER ml	84	1260
OSMOLARITY mosmol/l	220	
OSMOLARITY mosmol/kg H_2O	265	

Fresenius-Kabi (2016)

Table 5.1 displays the nutritional composition of the Fresubin® Original enteral feed used in the study, containing 1g/100ml of sugars, of which 0.27g/100ml is glucose. The *E. coli* and NG tube inocula could be using the glucose contained in the feed, triggering a fermentation process and thus producing mixed acid by-products.

Glycolysis, a central metabolic pathway in most living systems, results in the metabolism and conversion of glucose molecules into pyruvate and ATP (adenosine triphosphate). Pyruvate is subsequently converted into one or more end products including lactate, ethanol, acetate, carbon dioxide and hydrogen gas by one or more alternative pathways. Specifically, *E. coli* performs a sugar-based 'mixed-acid' fermentation, as shown in Figure 5.3. The mixed acid pathway produces alternative end products, and in variable amounts, such as succinate, ethanol, acetate, hydrogen, carbon dioxide, formate and lactate (Gunsalus & Schroder, 2014).



Gunsalus & Schroder (2014) (With permission)

The sugar-based fermentation process of *E. coli*, which produces mixed acids, causing the environmental pH level to decrease.

Figure 5.3: The *E. coli* 'mixed-acid' fermentation pathway

If the *E. coli* introduced to the enteral feed samples is metabolising the fermentable substrate glucose found in the feed there is every possibility it is producing the acids noted, thus increasing the acidity of the enteral feed with a resulting reduction in pH. This is a similar finding to Von Ohle *et al's* (2010) research into dental biofilm, who noted how biofilm bacteria can cause extreme modifications to the local environment, causing it to become highly acidic through metabolic activity. The inoculum concentration used in the study may have been greater than that which occurs naturally in the health care environment. However, if lower concentrations had been used, the 20 minute doubling time of the *E. coli* pathogen at optimal temperatures would quickly reach maximum numbers, with bacteria multiplying exponentially to the stationery phase of the growth curve, regardless of the starting values (Sekse *et al*, 2012; Amrita, 2017).

Anecdotal evidence given by nurses caring for patients with NG tubes has noted the enteral feed itself as a potential cause of NG tube blockages. Frequent comments regarding the regularity of tube blockages occurring where only enteral feed and water is placed in the tubes have been made, and in particular where flushing of the tubes is

delayed once a feed has finished. It may be that bacteria within NG tubes are causing the pH level to lower as a result of fermentation, and thus the feed is thickening inside a tube with a lumen diameter of less than 3 mm. This could certainly lead to tube blockage.

Conversely, respiration is the preferred metabolism for *P. aeruginosa*, gaining its energy by transferring electrons from glucose to oxygen, and not producing the acidic end products seen in the fermentation process (UK Standards for Microbiology Investigations, Public Health England, 2014). This would explain the consistent pH readings noted throughout the 24 hour period studied (Figure 5.2).

The study was undertaken *in vitro*, allowing the control of known variables. However, it is possible the results are relevant to the management of NG tubes in the health care environment. Components of the feed passing through the NG tube could be metabolised by bacteria attached to the tube's inner surface. If the bacteria are fermenters, the fermentation process could take place. This will require further investigation following the findings of a study described in Chapter 8, which looks at the inner surface and contents of NG tubes obtained from adult patients in the acute hospital ward environment.

5.6 **Conclusion**

This study was designed to investigate how bacteria that could potentially be present within NG tubes can affect its local environment. To achieve this aim, the pH of inoculated feed samples was recorded at regular intervals of 15, 30, 60, 120, 240, 360 and 1440 minutes. The three inocula used contained *E. coli, P. aeruginosa* and the bacteria isolated from a used NG tube.

The results demonstrated *E. coli* and the NG tube isolate follow a similar pattern, reducing the pH level of the enteral feed samples markedly over the 24 hour period studied, and causing the feed samples to coagulate. Conversely, *P. aeruginosa* had little effect on the pH of the feed, and the feed samples remained fluid throughout. *E. coli* is known to perform sugar-based mixed-acid fermentation, producing acidic end products. Conversely, the preferred metabolism of *P. aeruginosa* is respiration, which does not produce the acidic end products evident in the fermentation process. The findings of this study suggest the presence of biofilm on the inner surface of NG tubes used by adults could therefore be responsible for the coagulation of the enteral feed

within it, with the potential of leading to tube blockage. This theory requires further investigation.

The next chapter reports a study undertaken to investigate the effect flushing of NG tubes with water has on the development of biofilm within the tubes. In particular, it looks to establish the most effective type of flush in terms of prevention of bacterial attachment and biofilm development by comparing tap water and sterile water flushes.

Chapter 6: Comparing sterile water with tap water flushes on the levels of bacterial attachment and biofilm development in sterile NG tubes

6.1 Introduction

The previous chapter established a link between bacteria potentially found in NG tubes and the progressive drop in pH, and subsequent thickening, of enteral formula. Consequently there appears to be the potential for the fluid nature of enteral feed to change as a result of bacterial influence on the environment of the tube. Publications concerning the management of NG tubes frequently suggest inadequate or missed flushing of NG tubes promptly after a feed has been stopped can lead to rapid tube blockage (Gaither *et al*, 2009; Dandeles & Lodolce, 2011). Recommendations to avoid blockage of NG tubes in adults include routine flushing with 30 to 50 ml of water between feeds and medication. This practice is undertaken in line with local policy guidelines (PHT, 2013). National and international guidelines also outline this as best practice (CREST, 2004; ASPEN, 2009). However, studies supporting these guidelines are limited, with recommendations concerning tube flushing based on expert opinion rather than empirical research.

A further issue is that areas of practice have differing views concerning the use of either sterile water or tap water to irrigate NG tubes. National practice guidelines recommend freshly drawn tap water is used to flush NG tubes used by patients who are not immunocompromised (NICE, 2012). However, anecdotal nursing evidence received from colleagues at different NHS Trusts through discussion forums and in person indicates sterile water is routinely used to flush NG tubes, regardless of the patient's immunological status. Freshly drawn tap water is the more economical method, although tap water is known to contain microorganisms which have the potential to contaminate the NG tube inner environment (Szewzyk *et al*, 2000; Berry *et al*, 2006; Gião *et al*, 2011). The fluid used to flush the tube may remain in the tube for up to 24 hours depending on the frequency of flushing, and microorganisms contained in the water may multiply and rapidly develop biofilm. Sterile water may reduce the development of biofilm within NG tubes. However, there is a lack of evidence to support the use of either freshly drawn tap water or sterile water to flush NG tubes. In

light of this, investigation was required to establish whether there are any significant benefits to using sterile water flushes in preference to tap water flushes in the management of NG tubes.

Two interlinked studies were undertaken to investigate the effect of sterile water and tap water flushes on bacterial attachment and biofilm development in both sterile and inoculated NG tubes. The first study, presented in this chapter, was designed to test the hypothesis that flushing sterile NG tubes with sterile water compared with tap water leads to reduced levels of bacteria introduced to the NG tube, and therefore reduced bacterial attachment and biofilm formation on the inside surface. The study was undertaken *in vitro*, enabling greater control of possible variables such as time, temperature, and bacterial introduction. The second of the two interlinked studies is presented in Chapter 7.

6.2 **Aims and objectives**

The aim of this study was to compare bacterial attachment and biofilm development on the inner surface of sterile NG tubes flushed with either sterile water or tap water.

The specific objectives were to:

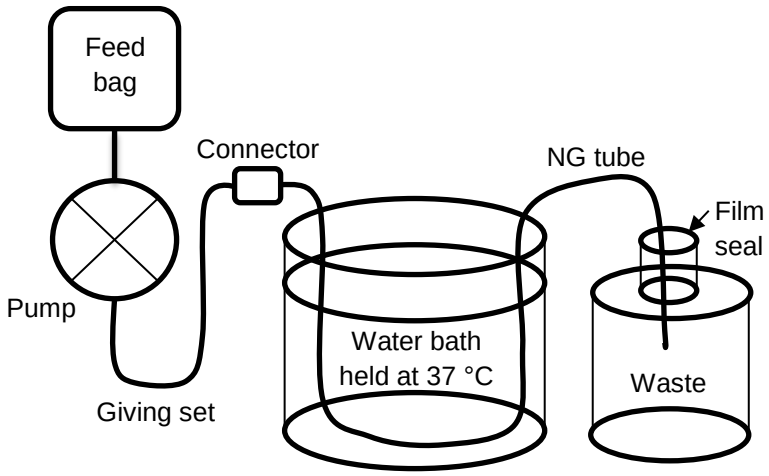
- Develop a laboratory flow model to recreate an environment for NG tubes similar to an *in vivo* setting in terms of temperature and enteral feed delivery
- Inoculate two sterile NG tubes concurrently with either sterile water or tap water, and place them in an environment to promote bacterial growth
- Inoculate one sterile NG tube with NG tube bacteria isolate, and place it in an environment to promote bacterial growth, to act as a control to ensure the apparatus supports bacterial growth
- Flush the three NG tubes at specified intervals with 30 ml of either sterile water, tap water or air
- Quantify levels of bacteria using CFU and CE counts and EDIC/EF microscopy

6.3 Materials and method

Laboratory materials and methods used in the three previous studies were utilised, with new methods introduced as required.

6.3.1 Laboratory flow model

The flow model developed for the study was based on that described by Lima *et al* (2011). A closed system comprising of ready-to-hang 1000 ml bags of enteral feed (Fresubin Original enteral feed) attached to an APPLIX pump set EasyBag giving set (Fresenius Kabi UK Ltd), passed through APPLIX Smart enteral feed pumps (Fresenius Kabi UK Ltd). The giving set connected to an NG tube (Corflo® 8 FG medical grade polyurethane NG tube) via the feed administration port, and the NG tube was placed into the water bath held at 37 °C by a thermostatically-controlled hot plate induction heater. The distal end of the tube was placed into a waste collection bottle, with cotton wool placed in the neck of the bottle to hold the tube in position, and a Parafilm M® all-purpose laboratory film (Bemis Co. Inc.) seal to prevent content spillage. The apparatus set up for the flow model is shown in Figure 6.1.



Schematic diagram of the flow model apparatus set up, displaying the feed passing through the pump and giving set, on through the NG tube in the water bath held at 37 °C, and into the waste container.

Figure 6.1: Flow model apparatus schematic

Sterile water used for flushing was ddH_2O. The tap water used for flushing was taken from a laboratory tap and stored in a sterile glass bottle at the beginning of each

experiment. Sterile 50 ml enteral-specific syringes were used for introducing the flushes. Each experiment was set up with three pumps and three NG tubes, passing through the same water bath to ensure each of the NG tubes were subject to similar environmental conditions.

The flow model described above was used to test three situations: sterile water flushes of sterile NG tubes, tap water flushes of sterile NG tubes, and NG tubes inoculated with bacteria isolated from used NG tubes to test the flow model equipment provided a suitable environment to support bacterial growth. Each situation was tested using the procedure described below seven times.

6.3.2 Tube preparation

Inoculated NG tubes were used to demonstrate the flow model apparatus provided a suitable environment for bacterial attachment and biofilm development. A bacterial culture was prepared overnight (minimum 15 hours) using the NG tube stock culture isolated in the preliminary study reported in Chapter 3. Using the resulting culture and the stored sterile enteral feed, an inoculum was prepared. This produced 4 ml of inoculum.

Three sterile NG tubes were placed into a BSC and removed from their packaging. The distal end of each NG tube was placed into a separate waste collection bottle. Cotton wool was used to form a plug to fill the neck of the bottle, whilst also helping to keep the NG tube in position. This was sealed over with laboratory film to prevent accidental spillage of the bottle contents which could come to contain harmful bacteria.

Approximately 2.5 ml of the NG tube inoculum was passed into one of the sterile NG tubes through the access port of the tube, ensuring any excess was dispelled into the attached bottle, indicating the NG tube was full of the inoculum. The NG tube access port was closed using the attached stopper to retain its contents, and prevent any cross-contamination. This procedure was repeated with the remaining two tubes, adding tap water freshly collected from the laboratory tap to one, and laboratory-prepared sterile water to the other.

6.3.3 Method

All three tubes and their waste bottles were taken to the water bath apparatus. Each of the three NG tubes were placed into the water, with the access port above the waterline and taped into place, and the distal end of the tube in its waste bottle

positioned close to the water bath, ensuring the majority of the NG tube was submerged.

The NG tubes were kept at a constant 37 °C temperature to replicate the body temperature the tubes would be subject to *in vivo*. This was achieved using a water bath and an induction heater. The water bath was filled with warm tap water until almost full and then placed onto a heat pad connected to an induction heater. A thermometer attached to the heater was placed into the water to monitor the temperature. With the regulator set at 37 °C, the heat pad raised the water temperature up to the required setting, ready for use. The top of the water bath was covered with foil to act as a heat insulator, and to ensure the tubes remain submerged and not floating on the surface where the temperature may not be as accurately controlled. The tubes were left for one hour to allow time for bacteria within the inocula to attach to the NG tube inner surface.

After one hour, each NG tube was connected to an enteral feed giving set, which in turn was passed through a feed pump and attached to a bag of enteral feed as depicted in the flow model schematic (Figure 6.1). The feed pump rate was set at 100 ml per hour to replicate the standard enteral feed rate in the clinical environment. The feed passed through the NG tubes and collected in the waste bottles for a period of four hours.

At four hours, the pumps were paused to enable each NG tube to be flushed through the access port. For the sterile water and tap water NG tubes, sterile enteral syringes containing 30 ml of sterile water and tap water respectively were used. For the tube containing the NG tube inoculum, 30 ml of air was used instead of water to replicate a flush, and avoid introducing further bacteria throughout the study. The feed pumps were restarted for a further four hours.

Following a further four hours, the NG tubes required flushing again as detailed above. Once flushed, the enteral feed giving sets were detached, and the access port of each NG tube sealed with its stopper. The NG tubes and waste bottles were removed from the water bath apparatus and placed back into the BSC.

Starting with the sterile water flushed NG tube, as this one was least likely to introduce bacteria into the BSC environment and risk contamination of the following tubes, the distal end of the tube was carefully removed from its waste bottle. The tube was sprayed with 70 % (v/v) ethanol, and allowed to dry. Three 1 cm NG tube sections were prepared from 22, 50, and 80 cm to represent the gastric, oesophageal and nasal

sections respectively. These particular measurements correspond with the tube's position in the water bath rather than the usual measurements associated with human anatomy. Bacteria attached to the NG tube inner surface were removed by vortexing. This process was repeated for the tap water and NG tube inoculum tubes.

Volumes of 50 µl aliquots for each of the vortexed samples were plated in triplicate onto TSA and incubated at 37 °C overnight (minimum 15 hours) ready for CFU counting. The serial dilutions noted in Table 6.1 for the NG tube inoculum tube were used. Undiluted samples for the tap water tube, the sterile water tube, and inoculated tube samples of the laboratory prepared sterile water (ddH_2O), the feed medium, and the NG tube inoculum were also plated onto TSA. 1 ml of each of the tube samples were taken to prepare cell elongation samples for incubation, staining using SYTO 9 bacterial stain, filtration and microscopy. The serial dilutions noted in Table 6.1 were chosen based on bacteria counts achieved, and were assessed after each individual experiment. These can be altered up or down as considered necessary to enable accurate CFU counting.

Table 6.1: Serial dilutions required for study

Serial dilutions of tube samples to enable CFU counting:						
ddH_2O	Undiluted					
Tap H_2O	Undiluted					
NGT inoc.	10^{-4}	10^{-5}	10^{-6}			
Controls of materials used						
ddH_2O	Undiluted					
Feed	Undiluted					
NGT inoc.				10^{-7}	10^{-8}	10^{-9}

This table indicates the serial dilutions found to be necessary to enable CFU counting on 50 mm TSA plates.

6.3.4 Statistical analysis

The study was analysed using SPSS software version 22.0 (SPSS, Chicago, IL, USA) through two analyses. The first was planned to establish whether CFU and CE counts achieved at the three anatomical points of the NG tubes, the gastric, oesophageal and nasal sections, were significantly higher at the nasal end, which would suggest bacteria attach to the point nearest introduction, a theory that could be tested in later studies. A Kruskal-Wallis H test was conducted to determine if there were differences in bacterial

colonisation at the three separate points (each n = 7). Values are mean ranks unless otherwise stated.

The second analysis looked to establish whether tap water introduces a significant level of bacteria to NG tubes when used for flushing, as opposed to sterile water. A Kruskal-Wallis H test was conducted to determine if there were differences in bacterial colonisation between the NG tubes flushed with sterile water (n = 7) or tap water (n = 7), and the inoculated tube (n = 7). A post hoc analyses was conducted using Dunn's (1964) procedure for pairwise comparisons, and a Bonferonni correction for multiple comparisons.

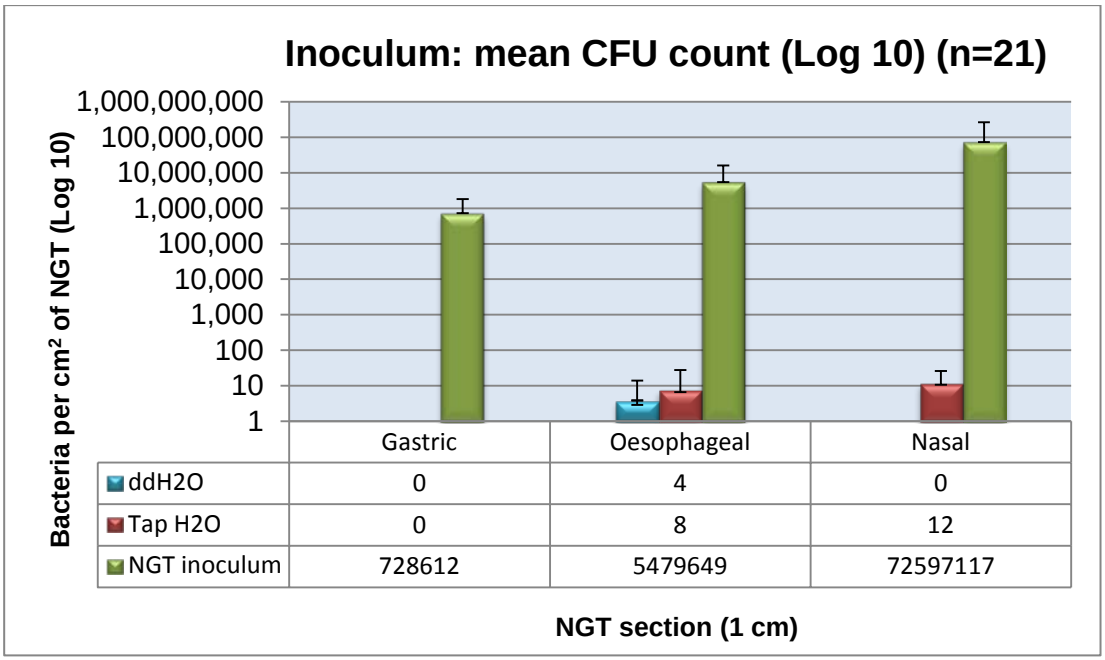
6.4 **Results**

Figure 6.2 and Figure 6.3 display the mean CFU and mean CE counts of the 21 sets of NG tube sections for each of the NG tube conditions investigated, 3 sections from each individual NG tube at the nasal, oesophageal and gastric sections; the experiment was repeated seven times. Figure 6.2 shows a substantial number of bacteria introduced to the inoculated NG tubes using the NG tube isolate inoculum have attached to the NG tube inner surface. Conversely, the mean CFU count for both sterile water (ddH_2O) and tap water were consistently low, with both at zero at the gastric end, 4 and 8 at the oesophageal section, and zero and 12 at the nasal end respectively. For the tap water tubes, there is a slight increase in mean CFU count as it nears the nasal end of the NG tube, which is the point of inoculum introduction. This is also the case for the NG tube inoculum tubes, but not for the sterile water (ddH_2O) tubes.

Cell elongation indicated high numbers of bacteria were present in both the sterile water (ddH_2O) and tap water tubes compared with the mean CFU counts (Figure 6.3). However, this increase was not evident in the inoculated NG tubes, with a drop of approximately 2 Log CFU to CE count at each section. The total mean CE counts over the three sections of NG tube for each inoculum are approximately 2.9×10^4 for sterile water (ddH_2O), 7.1×10^4 for tap water, and 3.5×10^5 for NG tube inoculum. The difference in mean CE count for sterile water (ddH_2O) to tap water was 147 %. Therefore, there are approximately 2.5 times more bacteria remaining in the NG tubes after tap water inoculation than after sterile water (ddH_2O) inoculation.

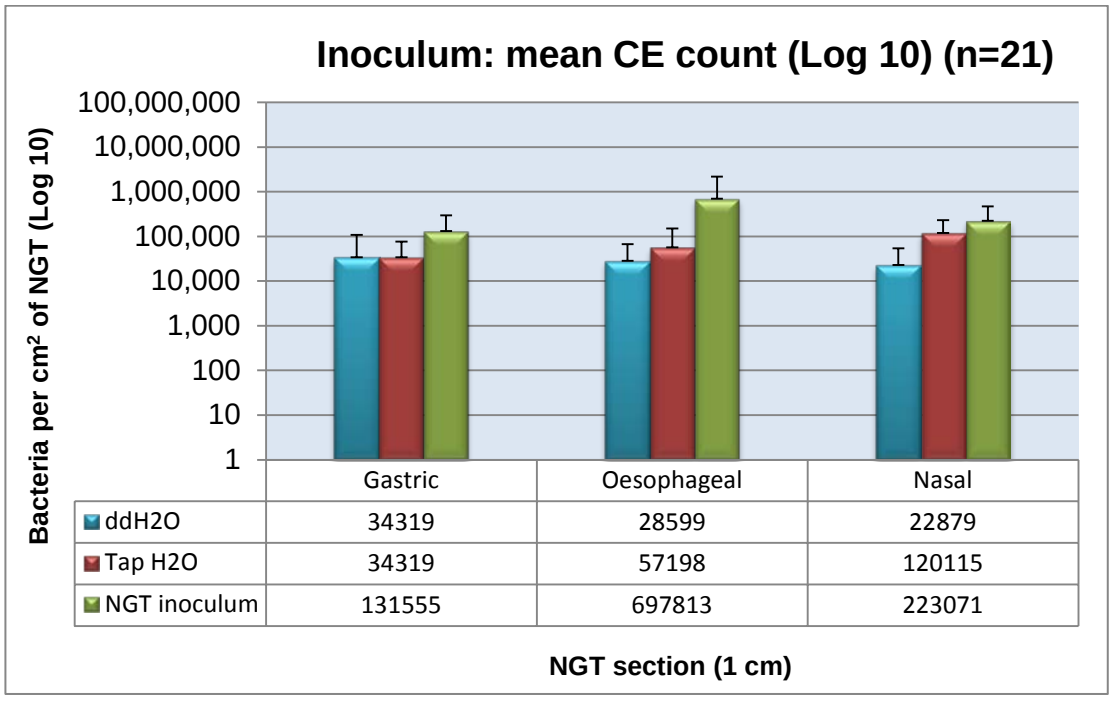
The mean CFU counts for each of the sterile water and tap water inocula are minimal, whilst the NGT inoculum control indicates significant levels of bacteria have attached.

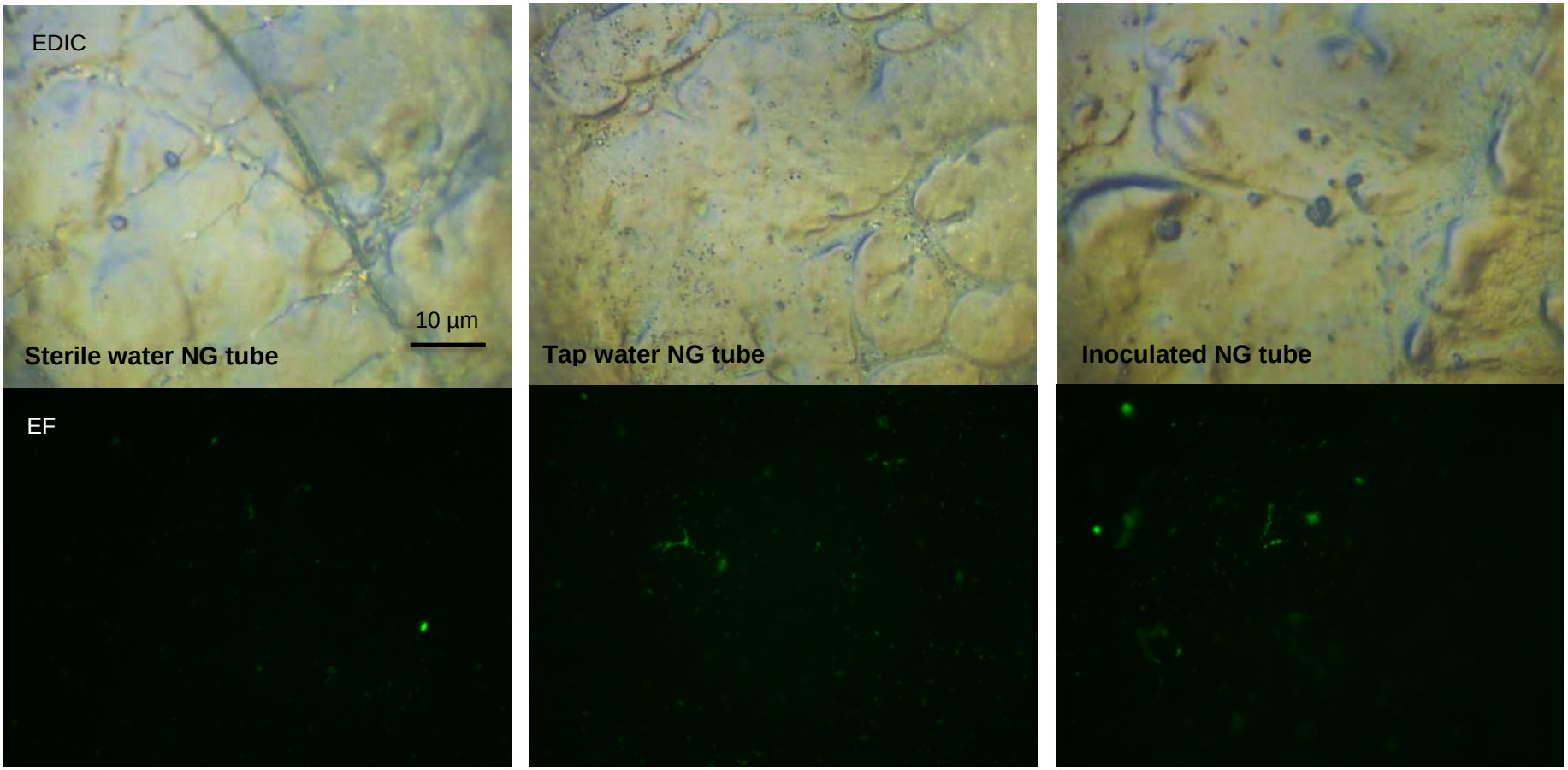
Figure 6.2: Sterile water (ddH_2O), tap water and NG tube inoculum mean CFU count (Log 10)



The mean CE counts for the sterile water and tap water inocula are similar within 1 log throughout, with the NG tube inoculum control indicating higher levels of bacterial attachment.

Figure 6.3: Sterile water (ddH_2O), tap water and NG tube inoculum mean CE count (Log 10)





Exemplar images of each NG tube condition tested, using EDIC illumination (mag x 1000) for NG tube inner surface images, and EF illumination (mag x 1000) via oil-immersion lens to capture cell elongation images from the filter membranes.

Figure 6.4: NG tube inner surfaces and filter membranes under EDIC and EF illumination

The EDIC and EF exemplar images for the sterile and tap water flushes, along with the inoculated NG tube, are shown in Figure 6.4, indicating the qualitative evidence to support the mean CFU and CE counts. Both the sterile and tap water flushed NG tube images appear to show little difference regarding the level of potential bacteria, which would support the findings of the mean CFU and CE counts. The images taken from the inoculated tube also support the CFU and CE counts, suggesting bacteria attached to the NG tube inner surface, confirming the flow model apparatus has provided a suitable environment to promote bacterial growth.

The first of two statistical analyses looked to establish differences in the levels of bacteria attached at each of the three anatomical points along the length of the NG tube. Distributions of CFU and CE were not similar for all groups, as assessed by visual inspection of boxplots. The differences in CFU and CE counts were not statistically significant between groups, as follows:

- sterile water CFU X^2 (2) 2.000, p = .368
- tap water CFU X^2 (2) 3.619, p = .164
- inoculated CFU X^2 (2) .007, p = .996
- sterile water CE X^2 (2) .253, p = .881
- tap water CE X^2 (2) 2.581, p = .275
- inoculated CE X^2 (2) .524, p = .770

Therefore, there was no statistically significant difference in the bacterial colonisation at either of the three anatomical points.

The second analysis looked to establish whether tap water introduces a significant level of bacteria to NG tubes when used for flushing, as opposed to sterile water. A Kruskal-Wallis H test was conducted to determine if there were differences in bacterial colonisation between the NG tubes flushed with sterile water (n = 7) or tap water (n = 7), and the inoculated tube (n = 7). Distributions of CFU and CE counts were not similar for all groups, as assessed by visual inspection of boxplots. The distribution of CE counts were not statistically significantly different between tubes, X^2 (2) = 5.799, p = .055. However, the distribution of CFU counts were statistically significantly different between tubes, X^2 (2) = 15.576, p = .0005. Subsequently, pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferonni correction for multiple comparisons. This post hoc analysis revealed statistically significant differences in CFU counts between the sterile water tubes (5.93) and the inoculated tubes (18.00)

(p = .0005), and the tap water tubes (9.07) and the inoculated tubes (p = .015), but not between the sterile water and the tap water tubes (p = .966). Therefore, based on the findings of this study, tap water flushes do not introduce statistically significant levels of bacteria to NG tubes, as compared with sterile water flushes.

6.5 Discussion

The primary aim of this study was to compare the levels of bacterial attachment and biofilm development on the inner surface of sterile NG tubes flushed with sterile water or tap water. This was undertaken in response to the practice of flushing NG tubes with sterile water in preference to tap water, and sought to establish evidence of tap water introducing significant levels of bacteria to NG tubes on routine flushing. No empirical studies have been identified to date that have investigated this hypothesis. Tap water is known to contain bacteria (Environment Agency, 2010), but whether a significant amount is introduced to the tubes on flushing required investigation.

The mean CFU and mean CE results for the NG tubes inoculated using NG tube isolate obtained in the preliminary study reported in Chapter 3 demonstrated the flow model with water bath apparatus arrangement worked effectively through the substantial attachment of bacteria to the NG tube inner surface. This was consistent throughout each of the seven separate experiments, the amalgamated results of which are displayed in Figure 6.2 and Figure 6.3. It is clear when comparing the mean CE counts to the mean CFU counts for both the sterile and tap water flushes that the numbers of VBNC cells included in the CE counts were much higher, with the CFU results depicting almost no culturable cells from the sterile water and tap water flushed tubes at either of the three anatomical sections, and yet both flushes produced a 4 to 5 log increase in mean CE counts at each section. With such a contrast in bacterial count between the CFU and CE values, one concern would be that the R2B low nutrient broth used to culture the elongated cells could be contaminated with unsolicited bacteria. However, the results of the study reported in Chapter 7 using the same batch of R2B demonstrate much closer values for both CFU and CE at each of the three anatomical points, suggesting there was no contamination of the R2B. The number of bacteria attached to the inner surface of the NG tubes after introducing either option of sterile water or tap water were not found to be statistically significant, suggesting there is no benefit to using sterile water to flush NG tubes in preference to tap water when considering the bacterial colonisation of NG tubes.

As is noted from the mean CFU count, the levels of bacterial attachment were higher the nearer the section tested was to the source of inoculation, although not statistically significant in this data set. However, as it is known the inoculum was introduced at the nasal end, this theory is one that can be further tested to see if this same pattern is repeated in a later study, which looks at used NG tubes obtained from adult patients in an acute hospital setting. If it is repeated, one would strongly consider the bacteria are being introduced during the management and care of the NG tube, including attaching and detaching enteral feed giving sets, introducing medication, and flushing with sterile or tap water. However, both ends of the NG tube have the potential for bacteria to enter, and if the opposite outcome occurs it is possible the bacteria are migrating up the NG tube from the patient's stomach, as discussed in Chapter 3. Previous research has shown bacteria are capable of effectively growing against the flow of a current, so this is not an impossible concept (Hall-Stoodley *et al*, 2004).

6.6 **Conclusion**

This study was designed to investigate the effect of flushing NG tubes with sterile water or tap water on levels of bacterial colonisation. The flow model apparatus designed for the study effectively recreated the NG tube in patient use within a laboratory environment, and demonstrated it successfully supported bacterial growth through the use of inoculated NG tubes.

The study confirmed freshly drawn tap water did not introduce a significant level of bacteria into NG tubes when considering the levels of bacteria attached to the inner surface of NG tubes, as compared with sterile water. The study also determined no statistically significant difference in bacterial colonisation at three separate points associated with the nasal, oesophageal, and gastric anatomical sections of the NG tube design.

The next chapter describes the second study using the flow model apparatus, which investigates the efficacy of sterile and tap water flushes on the bacterial attachment and biofilm development within inoculated NG tubes, and also investigates how these flushes compare to the option of not flushing NG tubes as part of their routine management.

Chapter 7: Comparing sterile water with tap water flushes for the prevention of bacterial attachment and biofilm development in inoculated NG tubes

7.1 Introduction

The study reported in the previous chapter demonstrated tap water did not introduce a statistically significant level of bacteria into sterile NG tubes when used as a flush, compared with sterile water flushes. It also demonstrated the successful use of the flow model apparatus designed to recreate the NG tube in patient use, within a laboratory environment.

As noted in the previous chapter, flushing NG tubes with water is the recommended practice to maintain patency of the tubes, and delayed flushing can lead to rapid tube blockage. However, the evidence to support this practice is limited or based on expert opinion rather than empirical evidence. This chapter describes the second of the two laboratory studies that used the flow model apparatus to investigate the use of sterile and tap water flushes. The study investigated the efficacy of the intervention of sterile and tap water flushes on the bacterial attachment and biofilm development within NG tubes inoculated with bacteria, and compared the outcomes with the control of not flushing NG tubes at all.

7.2 Aims and objectives

The aim of the study presented in this chapter was to investigate the effect of the flushing options of sterile water and tap water, along with the control option of not flushing, on the levels of bacterial attachment and biofilm development within inoculated NG tubes.

The specific objectives were to:

- Inoculate three sterile NG tubes concurrently with NG tube bacteria isolate
- Using the flow model apparatus, place the NG tubes in an environment to promote bacterial growth

- Flush the NG tubes at specified intervals with 30 ml of either sterile water or tap water, or no flush
- Quantify levels of bacterial attachment using CFU and CE counts and EDIC/EF microscopy

7.3 Materials and method

Laboratory materials and methods used in the four previous studies were utilised, with new methods introduced as required.

7.3.1 Laboratory flow model

The flow model developed and used in the previous study was also used here (6.3.1), as it successfully established it can support the growth of bacteria within NG tubes. The apparatus was set up identically to that in the previous study. Corflo® 8 FG polyurethane NG tubes (Corpak MedSystems, UK) and Fresubin Original enteral feed (Fresenius Kabi UK Ltd) in 1000 ml ready-to-hang bags were used, along with APPLIX Smart enteral feed pumps (Fresenius Kabi UK Ltd) for controlled-rate pumping of the enteral feed through the NG tubes via an APPLIX pump set EasyBag giving set (Fresenius Kabi UK Ltd).

7.3.2 Tube preparation

Three new packaged NG tubes were placed into a BSC, and removed from their packaging. The distal end of each NG tube was placed into a separate waste bottle. Cotton wool was used to form a plug to fill the neck of the bottle, whilst also helping to keep the NG tube in position. This was sealed over with laboratory film to prevent accidental spillage of the bottle contents which could come to contain harmful bacteria.

A bacterial culture was prepared overnight (minimum 15 hours) using the NG tube stock culture isolated in the preliminary study reported in Chapter 3. Using the resulting culture and the stored sterile enteral feed, an inoculum was prepared. Approximately 2.5 ml of the NG tube inoculum was passed into each of the sterile NG tubes through the access port of the tubes, ensuring any excess was dispelled into the attached bottle, indicating the NG tube was full of the inoculum. The NG tube access port was closed using the attached stopper to retain its contents.

7.3.3 Method

All three tubes and their waste bottles were taken to the water bath apparatus. Each of the three NG tubes were placed into the water, with the access port above the waterline and taped into place, and the distal end of the tube in its waste bottle positioned close to the water bath, ensuring the majority of the NG tube was submerged. The tubes were left for one hour to allow time for bacteria within the inocula to attach to the NG tube inner surface.

After one hour, two of the NG tubes were flushed using a new sterile 50 ml enteral syringe for each with 30 ml of sterile water (ddH_2O) in one tube, 30 ml of freshly drawn tap water in the second tube. The third tube required no flush. Enteral feed giving sets were attached to each NG tube, and enteral feed was passed through the tubes at 100 ml per hour.

After four hours, two of the NG tubes were flushed again with either sterile or tap water. No flush was required for the third tube. The enteral feed was passed through the NG tubes for another four hours, at which point the pumps were stopped. Two of the NG tubes were flushed with sterile or tap water as before, and the no-flush tube was flushed with 30 ml of air to expel excess feed from the tube. All three NG tubes were then removed from the water bath apparatus and placed into a BSC.

Starting with the sterile water flushed NG tube, three 1 cm sections were prepared from 22, 50, and 80 cm to represent the gastric, oesophageal and nasal sections respectively. Bacteria introduced to the NG tube were removed by vortexing. This process was repeated for the tap water flushed NG tube and the no-flush tube. Once all tube sections were prepared, 50 µl aliquots of the samples of suspended bacteria were plated onto TSA in preparation for CFU counting. Serial dilutions noted in Table 7.1 for each of the tubes were used, and control samples of the laboratory prepared sterile water (ddH_2O), the feed medium, and the NG tube inoculum were also plated onto TSA. Volumes of 1 ml of each suspended bacteria sample were used to prepare cell elongation samples, using SYTO 9 bacterial stain, subsequent filtration through Nucleopore membranes, and microscopy.

Table 7.1: Serial dilutions required for study

Serial dilutions of tube samples to enable CFU counting:						
ddH_2O flush	10^{-1}	10^{-2}	10^{-3}	10^{-4}		
Tap H_2O flush	10^{-1}	10^{-2}	10^{-3}	10^{-4}		
No flush			10^{-3}	10^{-4}	10^{-5}	10^{-6}
Controls of materials used						
ddH_2O	Undiluted					
Feed	Undiluted					
NGT inoc.				10^{-7}	10^{-8}	10^{-9}

This table indicates the serial dilutions found to be necessary to enable CFU counting on 50 mm TSA plates.

7.3.4 Statistical analysis

The study was analysed using SPSS software version 22.0 (SPSS, Chicago, IL, USA) through two analyses. The first was planned to establish whether CFU and CE counts achieved at the three anatomical points of the NG tubes, the gastric, oesophageal and nasal sections, were significantly higher at any specified point. A Kruskal-Wallis H test was conducted to determine if there were differences in bacterial colonisation at the three separate points (each n = 5). Values are mean ranks unless otherwise stated.

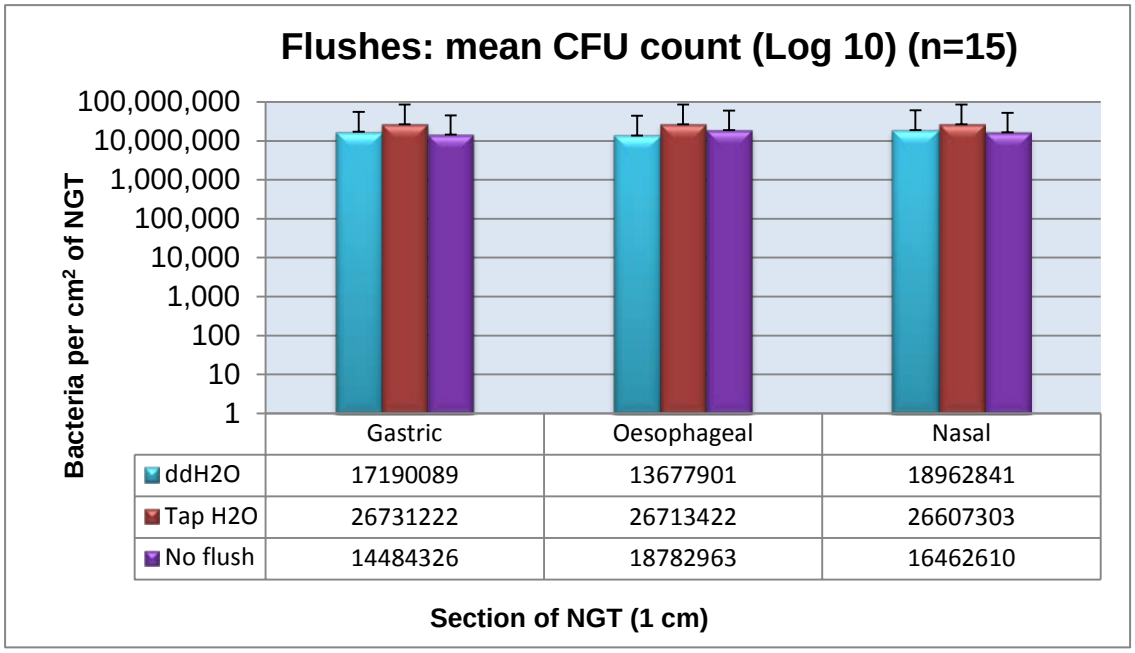
The second analysis was undertaken to compare the efficacy of using sterile water or tap water to flush inoculated tubes, to the alternative of not flushing, by quantifying the bacterial colonisation of the tubes. The data failed the assumption of normality and has outliers, therefore a Kruskal-Wallis H test was conducted.

7.4 Results

The experiment was repeated on five separate occasions, producing a total of 15 sets of NG tube results. Figure 7.1 and Figure 7.2 indicate the mean CFU and mean CE counts respectively. The mean CFU counts for each of the three types of flush are remarkably similar, and range within less than 1 log difference, with the tap water flush results slightly greater than both the sterile water (ddH_2O) and no-flush results.

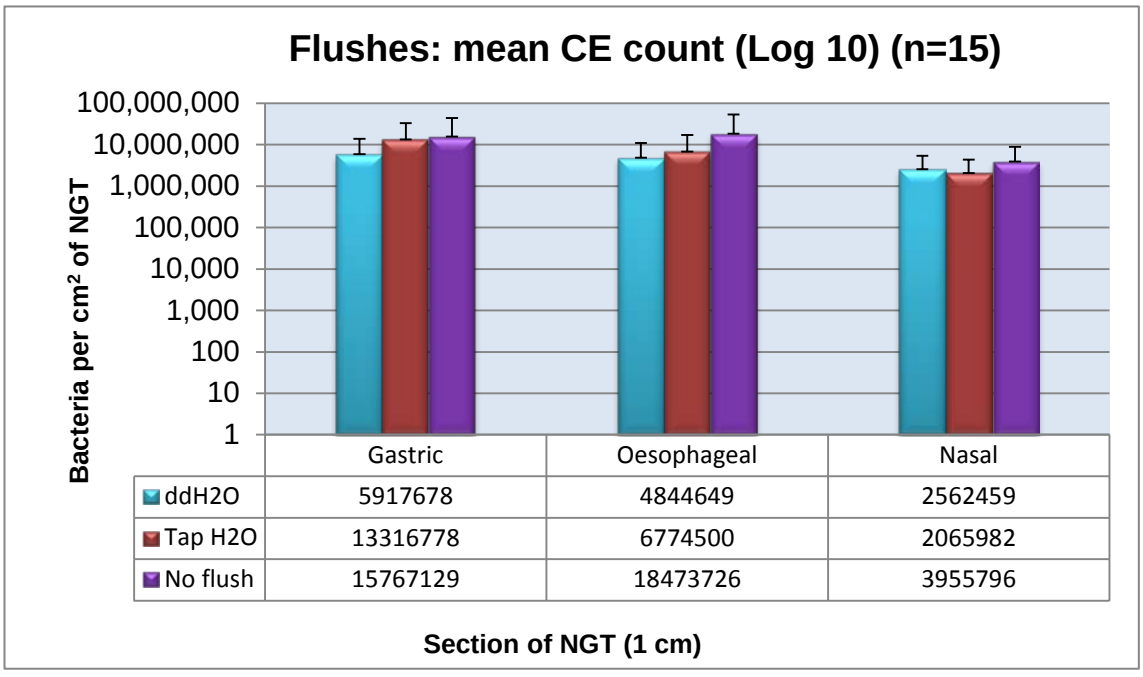
The mean CE counts again indicate similar levels of attached bacteria were noted for each of the three types of flush. The no-flush option produced the higher counts of the

three flushes but, similar to the mean CFU counts, all three ranged within a 1 log difference.



The mean CFU counts for each of the three types of flush are remarkably similar, with the tap water flush results the highest of the three.

Figure 7.1: Sterile water (ddH_2O), tap water and no-flush mean CFU count (Log 10)

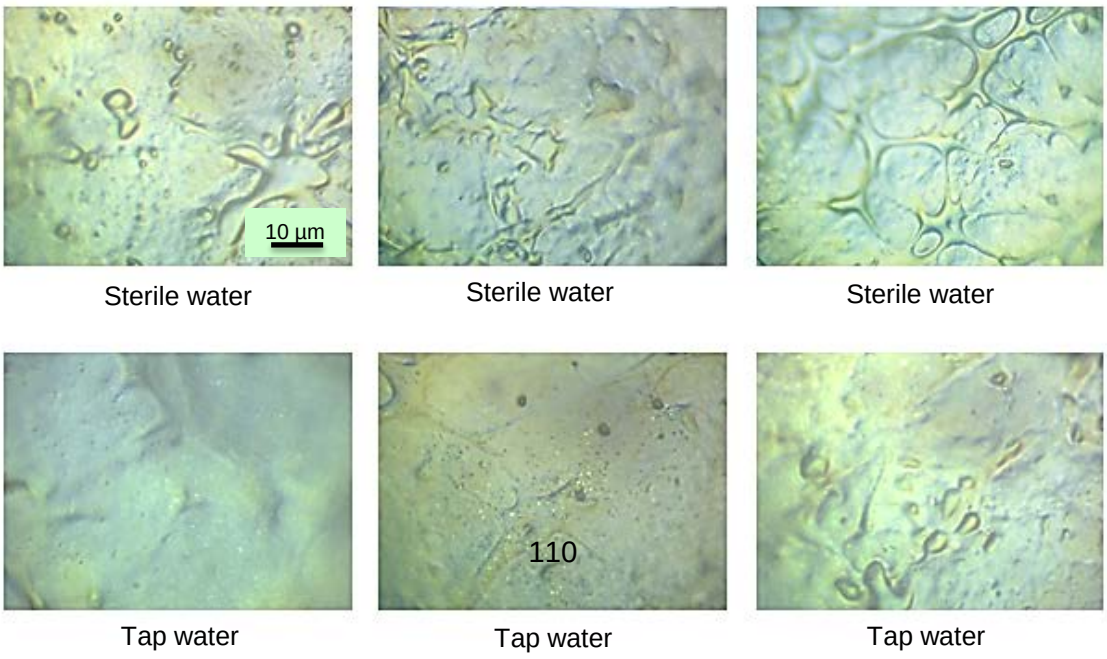


The mean CFU counts for all three types of flush are within a 1 log range, with the no-flush option producing the highest counts.

Figure 7.2: Sterile water (ddH_2O), tap water and no-flush mean CE count (Log 10)

Images of NG tube inner surfaces for each of the three types of flush. The Tap water flush samples appear slightly smoother than the sterile water samples, possibly due to the slight difference in bacteria count, supported by the mean CFU and CE results. The no-flush option appears markedly smoother, which would be supported by the higher mean counts in CE alone.

Figure 7.3: NG tube inner surface images under EDIC



The EDIC exemplar images for each type of flush shown in Figure 7.3 indicate the qualitative evidence to support the mean CFU and CE counts. The sterile water flush images appear to have less of a coating of potential bacteria on the surface, as opposed to the tap water flush images. These in turn are less than the surfaces depicted in the no-flush option images, which appear to show a smooth dense covering of potential bacteria. This is supported in part by the mean CE count results, when compared with the sterile water and tap water results.

The first statistical analysis showed distributions of CFU and CE counts were not similar for all sections of NG tube, as assessed by visual inspection of boxplots. All cases showed no significant difference at either of the three anatomical points, with significance levels as follows:

- sterile water CFU X^2 (2)2.240, p = .326
- tap water CFU X^2 (2)3.852, p = .146
- control CFU X^2 (2)1.460, p = .482
- sterile water CE X^2 (2) .195, p = .907
- tap water CE X^2 (2) .316, p = .854
- control CE X^2 (2) .180, p = .914

The second analysis showed distributions of CFU and CE counts were not similar for all tubes, as assessed by visual inspection of boxplots. The distribution of CFU and CE counts were not statistically significantly different between the three flush types:

- CFU X^2 (2)2.780, p = .249
- CE X^2 (2) .740, p = .691

7.5 **Discussion**

The aim of this study was to investigate the effect of three flushing options of sterile water, tap water, and not flushing at all, on the levels of bacterial attachment and biofilm development within inoculated NG tubes. This was completed in support of the study reported in Chapter 6 which concluded that tap water used for flushing the NG

tubes examined did not introduce statistically significant levels of bacteria when compared with sterile water flushes. Both studies combined to investigate the efficacy of flushing NG tubes with sterile water in preference to tap water, as this is a contentious issue with nurses responsible for the care of patients with NG tubes. No empirical studies have been identified to date that have investigated this subject. Anecdotal evidence in the clinical setting, and on discussion forums such as that of the National Nurses Nutrition Group, show ongoing debate and disagreement as to the benefits of one over the other, with some hospitals specifying the practice of using sterile water in preference to tap water, and others extoling the use of tap water (PHT, 2013).

In this, the second of the two flow model apparatus studies, all three NG tube flushing options of sterile water, tap water and no flush, achieved remarkably similar mean CFU counts. This suggests that there is no discernible difference in either of the flush options. However, the images in Figure 7.3 show the no-flush option appears to leave a film of bacteria on the inner surface. This image was captured after the NG tube section had been washed with ddH_2O as part of the process of preparation for microscopy. With this in mind, any enteral feed residue should have been removed in much the same way as the sterile water and tap water flush images would suggest and support. Therefore, although the mean CE and CFU counts are not significantly greater for the no-flush option, it would appear that flushing with either type of water is more effective than not flushing at all.

As with the previous laboratory studies, statistical analysis was undertaken to establish whether bacterial colonisation was more prevalent at a specified anatomical point of the NG tubes used in the study. No statistically significant difference was noted at either point, which supports the earlier analysis noted in Chapter 6, confirming there is no statistically significant outcome suggesting bacteria colonise the section of the NG tube nearest to the introduced inoculum.

The sterile water and tap water flush results do not suggest one is particularly more effective than the other. The tap water flush results are slightly higher than the sterile water flush results throughout, except for the mean CE count at the nasal section. Therefore there would appear to be little benefit to routinely using sterile water flushes in preference to tap water flushes in the management of NG tubes. When factoring in the additional cost of obtaining bottled sterile water in the hospital setting, when tap water is readily available and convenient to use, it would seem the efficacy of tap water flushes is supported for this purpose. However, it is also accepted that it remains

appropriate to use sterile water to flush NG tubes placed in immunocompromised patients.

A limitation of this study is that, although the same inoculum was introduced to each of the NG tubes used in the study, there is no way of knowing the levels of bacteria that attached to the inoculated tubes prior to flushing would have been similar between each of the three NG tubes. This uncertainty was reduced by ensuring the NG tubes were inoculated at the same time, in the same surroundings, with the same inoculum, and incubated in the same water bath. Repeating the experiment five times will also have reduced the risk of chance findings. However, it would be beneficial to repeat the experiments in greater numbers to assess this possibility further, but it is beyond the scope of this PhD thesis.

7.6 **Conclusion**

This study was designed to investigate the use of sterile water and tap water to flush NG tubes, compared with the option of not flushing, on the level of bacterial colonisation within the NG tubes studied. It successfully employed the flow model apparatus to recreate the NG tube in use *in vivo.*

The study demonstrated no statistically significant difference in bacterial colonisation of the three anatomical sections of NG tube, and was able to determine no statistically significant advantage of using sterile water in preference to tap water to flush NG tubes, in terms of bacterial attachment and biofilm development. However, it also found not flushing the tube achieved similar results.

The next chapter reports a study undertaken to gain further knowledge regarding the potential influences on biofilm development. The study examined tubes obtained from adult patients in an acute care hospital, and considered the effect variables such as medication, feed regimen and duration of placement could have on the bacterial colonisation of NG tubes.

Chapter 8: Investigating the effect of patient variables on biofilm development within NG tubes used by adults in an acute care hospital

8.1 Introduction

The previous five chapters have presented laboratory studies designed to investigate the presence and development of biofilm within NG tubes used by adults. These studies have established bacteria is attached to the NG tube material at the 15 minute time point, with biofilm developing within 24 hours. The pH of enteral feed can significantly drop in response to the presence of bacteria that may be attached to the NG tube inner surface, causing the feed to thicken. Moreover, flushing of NG tubes with water as a preventative measure against tube blockage can be completed as effectively with tap water as it is with sterile water in terms of bacterial attachment and biofilm development within the data sets studied. These findings have provided the evidence base to progress to an investigation of biofilm development within NG tubes used *in vivo.*

This chapter presents the final study in the series of interlinked laboratory studies, and amalgamates the knowledge gained through the previous laboratory studies, to investigate whether specified patient variables can effect biofilm development within NG tubes. Hurrell *et al* (2009a) found patients' age had a statistically significant effect on mean bacterial counts in their study of NG tubes used by neonates, but found no such statistical difference in connection with the patients' feed regimen.

Little is known regarding specific factors affecting biofilm development in NG tubes, and certainly none identified in tubes used by adults, therefore the aim of this study was to broadly identify any. Used NG tubes were collected from patients at an acute care hospital along with details regarding the tubes' use and specific patient demographics. In addition to this, medication administered via NG tube for each patient was considered to establish whether certain medications affect the rate of biofilm development, or whether particles of medication would be evident within the biofilm matrix. Bacteria from each tube were also isolated to produce inocula, which were used to investigate the pH level of inoculated enteral feed at specified time points over a 24 hour period.

8.2 Aims and objectives

The study aim was to investigate the presence and nature of substances, including biofilm and medication residue, within NG tubes retrieved from adult patients. It also set out to explore the potential for patient variables including gender, age, duration of tube placement, medications given via the NG tube and feed regimen to contribute to biofilm development.

The specific objectives were to:

- Gain ethical approval to recruit patients from four wards of an acute care NHS hospital Trust
- Identify patients who have NG tubes and the capacity to provide valid informed consent within four wards of an acute care NHS hospital Trust
- Obtain the used NG tubes from participants once removed as part of their normal clinical care, or self-removed
- Prepare and examine NG tubes for the presence and nature of substances, including biofilm and medication residue, using EDIC microscopy
- Establish total bacteria count and total culturable bacteria count using CFU, CE and microscopy methods
- Identify patient variables correlated with biofilm development and possible tube blockage
- Establish the pH of enteral feed samples at 0, 15, 30, 60, 120, 240, 360 and 1440 minutes time frames, following exposure to bacteria isolated from each used NG tube

8.3 Ethics

Not only did the study call for used NG tubes to be collected from patients within a hospital environment, but it also required access to the patients' medical records to obtain specific details regarding each patient's demographics and details of the use of their NG tube. Therefore, before the research study could commence, ethical approval was required. This can be complex and time-consuming, and is always required for research involving human participants (Gelling, 2015). As the study was part of a PhD thesis, and was to be conducted in an NHS hospital with patient involvement, ethical approval was sought from the academic institution, the host NHS hospital Trust

Research and Development department, and through a Research Ethics Committee (REC).

Faculty ethical approval was sought from the University of Southampton through Ethics Research Governance Online (ERGO, 2014), a centralised online system designed to facilitate the process of gaining ethical, governance and insurance approval for research studies (Identification number: 11999). They provided sponsorship for the study, along with professional indemnity and clinical investigation insurance to meet potential liabilities; if a patient were to be harmed as a result of participating in the study, they may have grounds for legal action for compensation against the University of Southampton as the sponsors of the study. Approval was received in November 2013.

As access to patient identifiable information was required by persons outside the patients' normal health care teams, the study was assessed as also requiring REC approval. A REC is a committee of volunteer professional and lay people whose aim is to ensure protection of the safety, rights, dignity and well-being of research participants (NPSA, 2010; Gelling, 2015). Applications for REC consideration for ethical approval are made using the Integrated Research Application System (IRAS) at *www.myresearchproject.org.uk*, where a Project Filter of key questions is completed in order to generate the appropriate application forms for the proposed research study (IRAS Project ID: 132039). For those new to research, the web site provides a comprehensive training manual to assist with each stage of the research ethics process using the IRAS system.

The most recent version of all the documents intended for use in the study were attached to the application. These included the peer-reviewed research proposal, a risk assessment regarding the laboratory work (Appendix A), an advertisement Ward Poster (Appendix E), a Participant Information Sheet (Appendix F), a Participant Consent form (Appendix G), and a Data Record form (Appendix H). Also, curricula vitae for the research and supervisory team were provided to ensure the correct experience, qualifications and expertise was in place to conduct the study. The study was classed as a Non-Clinical Trial of an Investigational Medicinal Product (Non-CTIMP), and would not look to store or use any material from the living, which would be subject to strict guidelines under the Human Tissue Act (DH, 2004). Any human cells existing on the outside of the NG tubes on removal were incidental, with a procedure in place to remove them using 70 % ethanol (v/v). A REC meeting was arranged and held

in March 2014 (Reference: 14/SC/0111), attended by the research and supervisory team, where initial approval was received.

When completing the application form for the REC, the IRAS system also generated an application for the host NHS hospital Trust Research and Development office, through use of an integrated dataset (NPSA, 2010) (Application reference: PHT/2014/30). The host NHS Trust Research and Development department requested an amendment to the process of gaining the participants' informed consent in June 2014. This was to discuss the PIS with the patient before their NG tube was removed rather than the planned stage of after the tube was removed. After discussing the PIS with the patients, and following a 24 hour cooling-off period, valid informed consent would be sought, and a notification of the patients' intent to participate in the study by donating their used NG tube on removal (Appendix I), along with supporting information, would be placed in their nursing notes. Following advice from the REC, a substantial amendment to the research study proposal to reflect this change was submitted in July 2014. REC approval regarding the substantial amendment was received in August 2014. The host NHS Trust Research and Development department were then in a position to grant approval to start the study, which was received in September 2014.

8.4 Materials and method

So far in this thesis, the studies have all been laboratory based. Although a large element of this study was also conducted in the laboratory using many of the materials and methods outlined in previous studies, it also involved direct patient contact in the clinical environment. Once appropriate ethical approval was in place, recruitment of the study participants began.

8.4.1 Sample

As there was very little research identified in the area of biofilm development in connection with NG tubes, a formal calculation of sample size was not completed. Instead, a convenience sample of 50 tubes was specified, based on Leibovitz *et al*'s (2003) study of 53 NG tube fed older adults, investigating *P. aeruginosa* colonisation in the oropharynx and as developed biofilm on the outer surface of NG tubes. A sample of 50 NG tubes for the current study was considered sufficient to broadly identify factors which may affect biofilm development on the inner surface of the tubes.

Four wards within an acute NHS hospital Trust were identified as regularly caring for patients with NG tubes; Stroke, Head and Neck, Upper Gastrointestinal, and Surgical High Care. By selecting four wards, it was considered the burden on the nursing staff to collect the used tubes for the study would be reduced. Also, as each of the wards had differing specialities, and there would be no crossover of nursing or medical staff, it was felt the NG tubes retrieved would come from a broader representation of the enteral feeding patient cohort.

The patients were not required to undergo any extra procedure for the study; their NG tubes were removed as part of their normal clinical care. There were no anticipated benefits to the patients who donated their used NG tubes, as any benefit of the research would be experienced by patients requiring NG tubes in the future. Conversely, there were also no foreseeable risks or burdens for the patient.

Those patients included in the study were aged 16 years or over, with a fine bore (6 to 8 FG) NG tube which had been used for feeding, and with the mental capacity to provide valid informed consent to participate in the study. Excluded were any patients under 16 years of age, those with NG tubes used for drainage purposes, and patients who did not have the mental capacity to provide consent (DH, 2005a). Without such capacity, the patient's medical doctor would have been called upon to provide appropriate consent, which would have added to the burden on the ward resources. If valid informed consent was received, after which the patient lost their capacity to consent within the study period, they would be withdrawn from the study along with any data collected. Non-English speaking patients were not approached, as to do so would have required an interpreter at cost, and could have added to the ward's burden. As a person's language should not have an effect on the contents of their NG tube, it was not felt excluding patients from this group would lead to a biased outcome.

8.4.2 Recruitment

A meeting was arranged to discuss the research study with the management team of each of the four wards, and a recruitment poster (Appendix E) advising the ward staff of the study was placed in each of the ward treatment rooms. This was considered by each ward manager to be an appropriate setting for the poster as most ward Nurses would have visited the treatment room during each shift. The poster provided information about the study, and advised Nurses to place used NG tubes from participating patients into individual Ziploc bags, attach a patient identification label,

and place in a plastic sealable box in the ward specimen fridge. The Ziploc bags and plastic boxes were provided for this purpose.

The wards were contacted regularly in person or by telephone to identify suitable patients with NG tubes who may consider participating in the study. The patients were approached in person to discuss the study and if they wished to consider taking part, a Participant Information Sheet was given to them.

8.4.3 Participant Information Sheet

The Participant Information Sheet (PIS) (Appendix F) provided an overview of the research study in lay terms. In particular, it outlined the purpose of the study, the reason the patient had been invited to participate, and the aspects of consent and confidentiality. It also provided details of the participant complaints procedure, along with insurance provision and details of the study investigator and sponsor. The PIS was discussed with the patients at their bedside for their convenience. However, a private room would have been sought if any patient had requested more privacy. The PIS was left with the patient, and a 24 hour cooling off period was observed for the patient to consider their participation in the study, and to talk to friends and relatives as required. After 24 hours, the patient would be approached to discuss receiving their valid informed consent.

8.4.4 Consent Form

Before patients' consent was sought, they were given the opportunity to discuss the PIS and study further and to ask any questions they had. Once ready, the Consent Form (Appendix G) was discussed. This consisted of a series of individual statements relating to the study, the data collection, and the data management. It also confirmed that consent to participate should be given voluntarily and could be withdrawn at any point throughout the study. The patient was asked to initial each individual statement, and then print and sign their name, and date them. This was countersigned by the researcher, and a copy of the completed Consent Form was given to the patient for their records, with a further copy placed in their medical notes, and the original filed in the Investigator Site File (8.4.10). To alert the ward Nurses that the patient had consented to donate their used NG tube to the study on its removal, a Notification of Patient's Intent to Participate in Research form (Appendix I) was attached to the front cover of their individual nursing notes.

Large font documents were available for any patient with visual impairment, if required. If patients were unable to sign the Consent Form due to poor vision or physical impairment, an independent witness was required to sign on their behalf as witness to their verbal informed consent. Also, if a patient appeared not to understand the informed consent process, or if they were assessed by the investigator as potentially not having mental capacity to consent, the consent process was stopped.

8.4.5 Qualifications

All research must be conducted in accordance with local, national and international policy and guidelines, including the principles of Good Clinical Practice (GCP) and the Research Governance Framework for Health and Social Care (DH, 2005b). All investigators conducting research in the NHS must possess a valid research passport or hold an honorary contract (research or clinical) with the relevant NHS hospital Trust. Therefore training was undertaken to gain the GCP certificate, a course was attended regarding obtaining participants' valid informed consent, and an honorary contract was set in place with the host NHS hospital Trust.

8.4.6 Tube collection

The ward staff were notified of the details of each patient who had consented to participate in the study through the Notification of Patient's Intent to Participate in Research form attached to their individual nursing notes. After their tubes were removed in the course of their normal care, or by self-removal, they were placed into individual Ziploc bags and then into a sealable plastic box in the ward specimen fridge. The wards were contacted regularly to enquire whether any tubes were waiting collection for transporting to the University laboratory, and this was completed as soon as possible. It was not anticipated significant biofilm development would occur once the tube had been removed from the patient (Wilks, 2013, per comm). Once outside the body any bacterial growth would slow due to the drop in temperature from body temperature to 4 °C in the fridge, at which temperature growth is greatly reduced (Ratkowsky *et al*, 1982).

8.4.7 Tube transport to laboratory

The used tubes were transported to the Centre for Biological Sciences at the University of Southampton by private car, in UN3733 packaging (VWR, 2013), in accordance with

the Centre's protocol for transportation of Category B (UN3733) biological and clinical material (HSE, 2011). Samples were doubled bagged in sealed Ziploc plastic bags and placed in an insulated pathology specimen transport bag (Versapak, UK) with appropriate UN3733 labelling (VWR, 2013), which was sealed using a tamper-proof security seal. The NG tubes were transported through the manned restricted access rear entrance of the Centre for Biological Sciences, with direct access to the laboratory, thus avoiding carrying the samples through public areas.

8.4.8 Data collection

At the time of tube collection from the wards, details of patient demographics, duration of tube placement, medication given, and feed regimen were collected. A Data Record Form (Appendix H) was used to collate the information from the patients' medical notes, and a unique code was allocated to each patient to anonymise their data before it was transported from the hospital. The unique codes were only identifiable to the investigator and supervisory team, thus maintaining confidentiality of the participants' personal information.

8.4.9 Data management

Patient information was stored in line with the University of Southampton guidelines on data management and storage of confidential data; documents recording personal data will be stored for 10 years at the Faculty of Health Sciences, and will be securely destroyed after 10 years. Paper documents were kept in a locked cabinet in a restricted access room at a Clinical Academic Research Facility of the University of Southampton, at the NHS hospital Trust where the tubes were collected. All information transported to the laboratory in hard copy form was anonymised, and all computer records stored on a password protected computer.

8.4.10 Investigator Site File

To assist with the management of the study, an Investigator Site File (ISF) was created as a central point of reference for all essential documents connected with the study (NIHR, 2016). As such, all versions of the study documents are contained within the ISF, with the most recent version of each document indicated, and earlier versions marked as superseded. Also contained in the ISF were the research study proposal, the investigator's CV and CGP certificate, the screening and enrolment log, and the original of each consent form completed by the study participants. Copies of Standard

Operating Procedures (SOPs) issued by the host NHS hospital Trust for recruiting and consenting participants, reporting adverse incidents, and maintaining an ISF, were included to ensure the study was conducted to the standards expected.

The ISF was kept in a locked cabinet in a restricted access room, alongside the hard copies of the participants' data. Access to the ISF was by the investigator and supervisory team only, with the host NHS hospital Trust advised of the location of the file. Maintaining an accurate ISF is essential should a monitoring or auditing assessment of the study be required.

8.4.11 Laboratory methods

As with the previous laboratory studies within this thesis, the NG tubes retrieved for this study were all Corflo® 6 - 8 FG polyurethane tubes (Corpak MedSystems, UK), a feeding tube widely used by the NHS. Preparing one NG tube at a time, each used tube was removed from its packaging using forceps sterilised with 70 % (v/v) ethanol spray. The outer surface of each tube was sprayed with 70 % (v/v) ethanol spray, removing bacteria and human cells that could contaminate the investigation of the NG tube inner surface. Using sterile equipment, 1 cm sections at the 2, 40 and 60 cm markings were taken, representing the gastric, oesophageal and nasal sections of the tube. This was similar to Lima *et al's* (2011) study, in which they removed sections at the beginning, middle and end of their flow-model NG tubes. Each 1 cm section was cut longitudinally to expose the inner surface. Each half section was gently rinsed in ddH_2O for 5 seconds to help remove planktonic bacteria and feed residue. One half was then placed into a Universal containing 10 ml of ddH_2O, to be used for analysis by resuspending any attached bacteria and biofilm, the other half placed with the inner surface facing uppermost in a Petri dish ready for direct microscopy.

The half sections of NG tube in the Petri dishes required no further preparation for direct visualisation using EDIC microscopy with long working distance objectives. Each section was viewed at magnification x 1000, and three representative images chosen at random of each section were captured to demonstrate the surface viewed. The half sections in Universals with 10 ml of ddH_2O had approximately 20 autoclaved glass beads added. These were vortexed at high speed for 30 seconds to ensure bacteria and biofilm were removed from the NG tube inner surface, and suspended in the ddH_2O. This suspension was used to establish the total culturable bacteria count by plating 50 µl aliquots onto TSA in triplicate. All plates were incubated at 37 °C overnight. After a minimum 15 hour period of incubation, the TSA plates were removed

and colonies counted. The number of colonies for each plate was recorded, with a mean figure noted for each triplicate.

The cell elongation method (4.3.18) was used to enable the direct viable counting of VBNC bacteria removed from the NG tube sections. Undiluted volumes of 1 ml of the bacteria suspension for each of the tubes were added to a Universal containing 5 ml of R2B, 4 ml of ddH_2O, and 100 µl of 1 mg/1 ml Pipemidic Acid. These were incubated at 22 °C overnight. The following day, the bacteria were stained using 1.5 µl SYTO 9 to 1 ml of each relevant sample, and filtered through 0.2 µm pore size polycarbonate filter membranes ready for EF microscopy. For calculating the CE count, 20 fields of view were chosen at random, with the number of elongated bacteria counted within each field. Exemplar images were captured for each filter.

8.4.12 pH testing

The materials and method described in Chapter 5 were utilised for this part of the study. Bacteria resuspended from each of the used NG tubes were placed on Protect beads in glycerol stocks (Thermo Fisher, UK), stored at - 20 ˚C, and used to produce inocula for investigating the effects bacteria from each of the NG tubes had on pH levels of sterile enteral feed. Cultures of bacteria from each of the NG tubes were prepared using one Protect bead in a Universal containing 20 ml TSB. These were incubated at 37 °C overnight (minimum 15 hours).

The following day, inocula were prepared using 4 ml of the bacteria culture, centrifuged for 10 minutes at 7.5 K rpm to produce a pellet of bacteria, and added to 4 ml of sterile enteral feed (Fresenius Kabi UK Ltd). A 100 µl volume of each of the inocula were added to Universals containing 3 ml of sterile enteral feed, with eight universals for each NG tube to denote the eight time points of investigation, along with a ninth Universal for each containing only enteral feed as a control. All Universals were placed in an incubator at 37 °C. As with the pH study in Chapter 5, a Jenway 3510 pH Meter (Jenway, UK) was used to measure the pH levels at the allotted time points of 0, 15, 30, 60, 120, 240, 360 and 1440 minutes.

8.4.13 Statistical analysis

Statistics advice was sought from the University Statistics Department. The study was analysed using SPSS software version 22.0 (SPSS, Chicago, IL, USA). The first analysis was to establish whether there was a significant difference between the levels

of bacteria identified in the gastric, oesophageal and nasal section of each of the 10 retrieved NG tubes. The hypothesis was that there was a significant difference, and the analysis was completed using a Kruskal-Wallis H-test. This test was also employed for the second analysis exploring whether there were statistical differences in the CE and CFU counts between each of the 10 used NG tubes. The third statistical analysis, also employing the Kruskal-Wallis H-test, was performed to establish whether the duration of tube placement, along with the patient's age and gender, had a bearing on the levels of bacteria quantified by CE and CFU counts.

8.5 Results

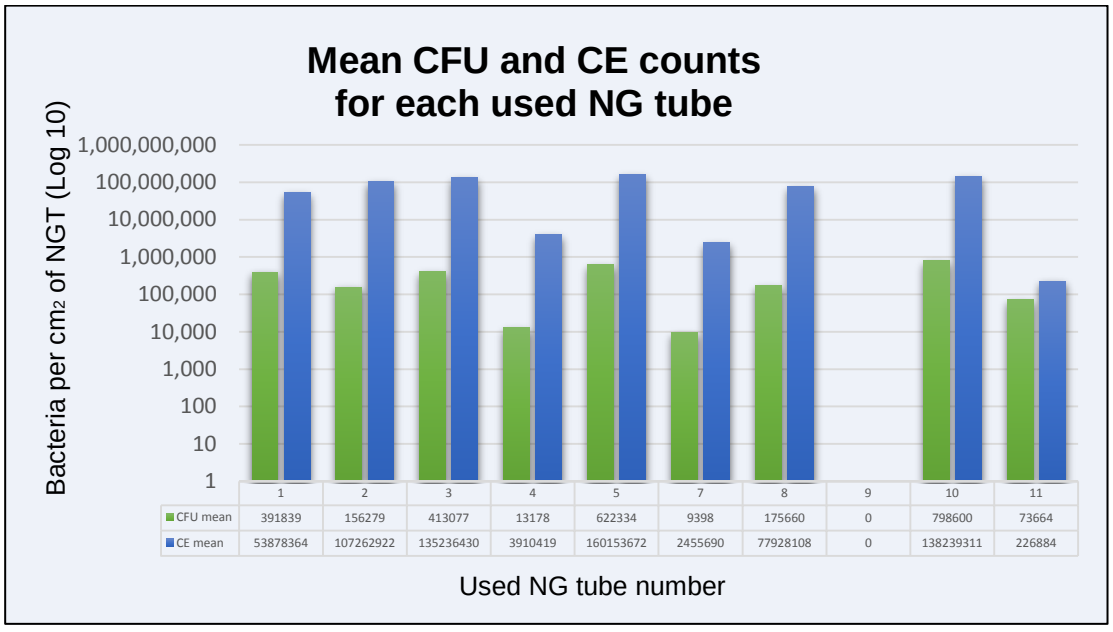
The original proposal was to use data from 50 retrieved NG tubes although this number was not achieved. This is further explained in the discussion section of this chapter. Eleven used NG tubes were retrieved for this study, collected between October 2014 and May 2015. Of the 11 tubes, six were from females, five from males, with ages ranging from 54 to 86 (mean age 69.5). All but one of the tubes remained patent at removal, and the duration of placement ranged from 3 to 22 days (mean 11.9 days). Tube 10 was removed from the patient at 11 days as it had become blocked, and was the only tube included in the study to have become blocked. Data generated by tube 6 were withdrawn from the study due to concerns by the investigator regarding the patient's ongoing capacity to consent for their tube to be used in the study. In total, data from 10 NG tubes were assessed.

8.5.1 Bacteria counts

The mean CFU and CE counts for each tube are presented (Figure 8.1). The *x* axis indicates the retrieved NG tube number; the *y* axis represents the number of bacteria attached per cm^2 of NG tube (Log 10). The mean CE count is notably higher than the mean CFU count for eight of the tubes, with tube 11 indicating a marginal difference, and tube 9 indicating no bacteria were identified using either method of detection. The mean CFU and CE counts range between a 2 Log and 3 Log difference respectively, discounting the zero CFU and CE counts for tube 9.

Mean CFU and CE figures for the 10 retrieved NG tubes. Mean CFU values range within a 2 Log difference; mean CE values within a 3 Log difference. No bacteria were identified for tube 9

Figure 8.1: Mean CFU and CE counts (Log 10) for each used NG tube



8.5.2 pH levels

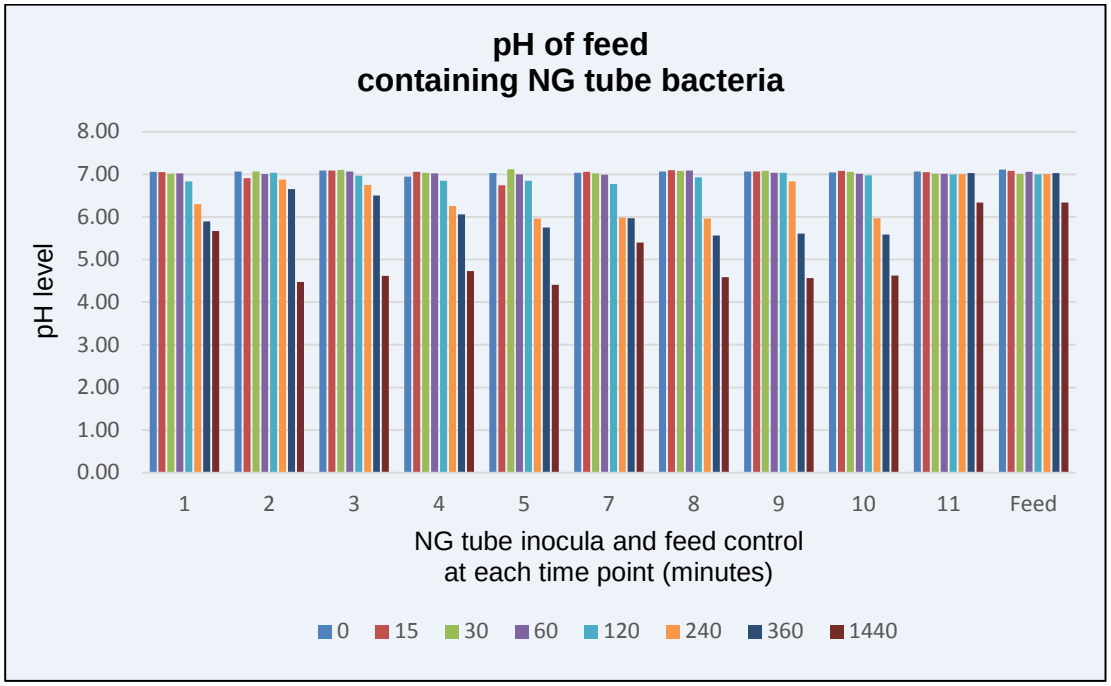
The pH measurements of enteral feed following the introduction of bacteria from each of the 10 retrieved NG tubes at each of the time points of the study are presented in Figure 8.2. The *x* axis indicates the pH level of the feed; the *y* axis shows the number

of the tube the bacteria was isolated from, and the eight separate time points as indicated in the legend.

The pH levels of the enteral feed samples containing bacteria from each of the 10 tubes all demonstrated an increase in acidity. In six groups, the acidity increased from the 120 minute time point following introduction of an inoculum, and three further groups increased acidity at 240 minutes. The remaining group increased acidity at 1440 minutes, in a similar pattern to the enteral feed control group to the right of Figure 8.2. The pH of the inoculated feed over the 24 hour period studied ranged from 7.09 (mean 7.05) to 4.41 (mean 4.94). The feed control remained relatively stable, recorded at 360 minutes as 7.03, but dropped to 6.34 over the next 18 hours. Two of the inoculated feed samples began to show signs of thickening at 240 minutes (8 and 9), with samples set firm at 360 minutes (8, 9, 10 and 11). All samples were set firm at 1440 minutes, except the feed control sample, which remained liquid throughout.

The pH measurements for inoculated enteral feed samples for each of the 10 retrieved tubes are indicated. Measurements were taken at 0, 15, 30, 60, 120, 240, 360 and 1440 minutes.

Figure 8.2: pH levels of inoculated feed samples



8.5.3 Patient variables

A summary of the patient variables recorded at the time of used NG tube collection are displayed in Table 8.1. Medications prescribed for the participants in the study are shown in Table 8.2. In the medications table, similar medications have been arranged into groups, and allocated a number (1 – 22). In the patient variables table, the corresponding number is indicated for any medication that was given via NG tube.

On consideration of the patient variables in relation to the level of bacterial colonisation, the patient's age and gender do not demonstrate a pattern of increased or decreased CFU or CE counts. Similarly, the duration of placement does not appear to be connected to the level of bacterial colonisation, with irregular CFU and CE counts noted. Medication was passed down seven of the NG tubes as part of the patients' normal clinical care, with three NG tubes receiving no medication. Of these, one NG tube which had been used for enteral feed only (tube 10) was found to be blocked after 12 days of use.

NG tube	Gender	Age	Days	Is tube patent?	Enteral feed (Fresubin)	Medication given via NG tube (groups)	Mean CE per cm^2 of NG tube	Mean CFU per cm^2 of NG tube
01	Female	58	5	Yes	Original	None	53,878,364	391,839
02	Female	72	3	Yes	Original	1,4,6,7,13,14	107,262,922	156,279
03	Male	79	13	Yes	Original, Energy, 2 Kcal, 5 Kcal	1,4,7,6,11,14	135,236,430	413,077
04	Female	73	3	Yes	Original	13,15	3,910,419	13,178
05	Male	86	9	Yes	Original, Original Fibre, Energy	7,14,18	160,153,672	622,334
07	Female	69	8	Yes	Original Fibre	4,6,7,8,11,12,14, 16,17,18,19	2,455,690	9,398
08	Male	62	22	Yes	Original Fibre, Energy, Energy Fibre, 2 Kcal, 5 Kcal	1,2,3,4,6,7,14,21	77,928,108	175,660
09	Male	70	11	Yes	Energy, Energy Fibre, Original Fibre, Energy HP	4,8,16	0	0
10	Female	54	12	Blocked	Energy	None	138,239,311	798,600
11	Female	72	6	Yes	Energy Fibre, Energy HP,	None	226,884	73,664

Details of the patient variables for each of the 10 study participants, along with the mean CFU and CE counts from their retrieved NG tubes.

Table 8.1: Patient variables

Table 8.2: Medications prescribed for participants of the study

Drug	Family	Group No.
AdCal-D_3	Vitamin supplement	1
Calcichew-D_3	Vitamin supplement	
Forceval soluble	Vitamin supplement	
Pabrinex I & II	Vitamin supplement	
Sanatogen	Vitamin supplement	
Thiamine	Vitamin supplement	
Vigranon B	Vitamin supplement	
Amitriptyline	Antidepressant - tricyclic	2
Sertraline	Selective serotonin re-uptake inhibitor	
Amlodipine	Calcium-channel blocker	3
Aspirin	Analgesic – non-opioid	4
Paracetamol	Analgesic – non-opioid	
Pregabalin	Analgesic – non-opioid	
Morphine sulphate MR	Analgesic – opioid	5
Atenolol	Beta-adrenoceptor blocker	6
Bisopralol	Beta-adrenoceptor blocker	
Atorvastatin	Lipid regulator	7
Pravastatin	Lipid regulator	
Clarithromycin	Antibiotic	8
Trimethoprim	Antibiotic	
Cyclizine	Antihistamine	9
Enoxaparin	Anticoagulant - parenteral	10
Ferrous fumarate	Iron supplement	11
Folic acid	Iron supplement	
Furosemide	Diuretic	12
Spironolactone	Aldosterone antagonist	
Gliclazide	Antidiabetic	13
Metformin	Antidiabetic	
Lansoprazole	Proton pump inhibitor	14
Omeprazole	Proton pump inhibitor	
Levetiracetam	Antiepileptic	15
Levothyroxine	Thyroid hormone	16
Prednisolone	Corticosteroid	17
Ramipril	Angiotensin-converting enzyme (ACE) inhibitor	18
Salazopyrin	Aminosalicylate	19
Sando K	Potassium replacement	20
SandoPhos	Phosphate replacement	21
Zopiclone	Hypnotic	22

Medications prescribed for participants of the study as part of their normal clinical care. Similar medications are grouped together, with a number allocated for each group. The corresponding number appears in the patient variables table for each medication given via NG tube.

8.5.4 Microscopic analysis

Nine images were captured during EDIC microscopic analysis; three images of each of the three 1 cm sections of each NG tube, chosen at random. Exemplar images were captured during EF microscopic analysis of the filter membranes for each NG tube. One exemplar EDIC and EF image for each of the 10 NG tubes are displayed here, with individual comments noted beneath.

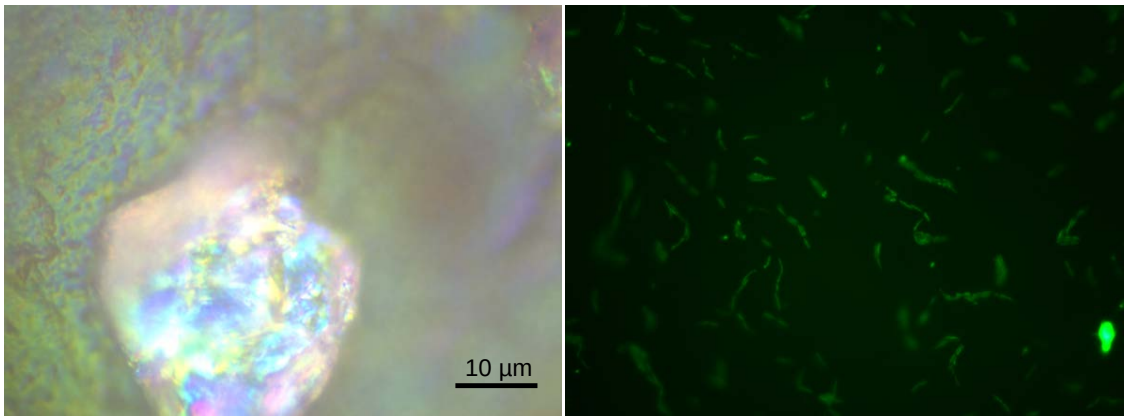


Figure 8.3: NG tube 01 - EDIC and EF images

This tube was from a 58 year old female. It had been in place for five days, and remained patent. The patient had received Fresubin Original enteral feed and no medication via her NG tube. EDIC microscopy showed repeated images of apparent crystals attached to the NG tube inner surface. EF microscopy captured images of elongated bacteria.

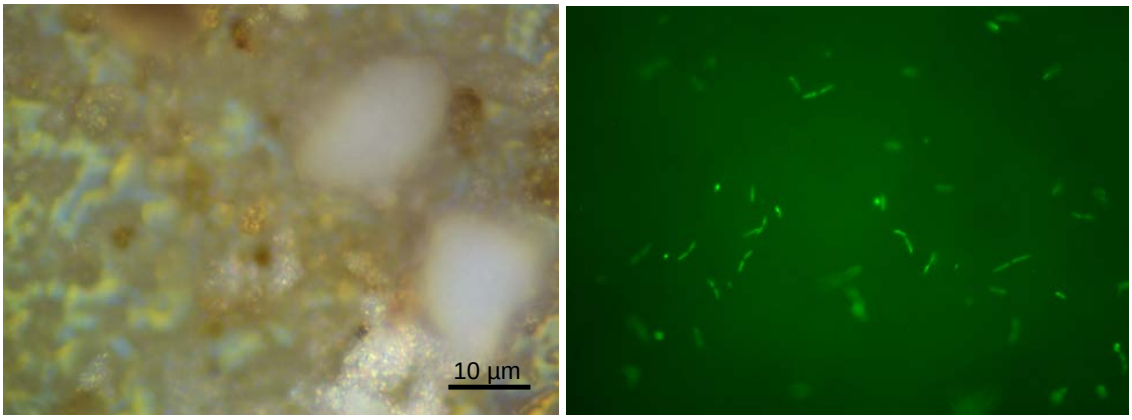


Figure 8.4: NG tube 02 - EDIC and EF images

This tube was from a 72 year old female. It had been in place for three days, and remained patent. The patient had received Fresubin Original enteral feed along with medications from six groups via her NG tube. The EDIC image appears to indicate

biofilm development to the right of the image, with bacterial colonies seen throughout. The EF image has captured elongated bacteria.

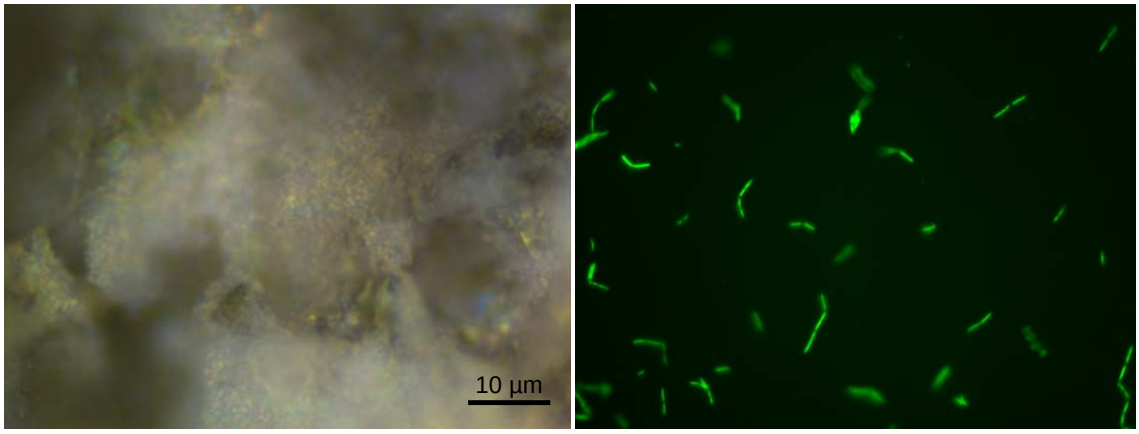


Figure 8.5: NG tube 03 - EDIC and EF images

This tube was from a 79 year old male. It had been in place for 13 days, and remained patent. The patient had received Fresubin Original, Energy, 2 Kcal and 5 Kcal enteral feed along with medications from six groups via his NG tube. EDIC microscopy indicated prolific biofilm development. EF microscopy has captured images of elongated bacteria. This tube had a high mean CE count.

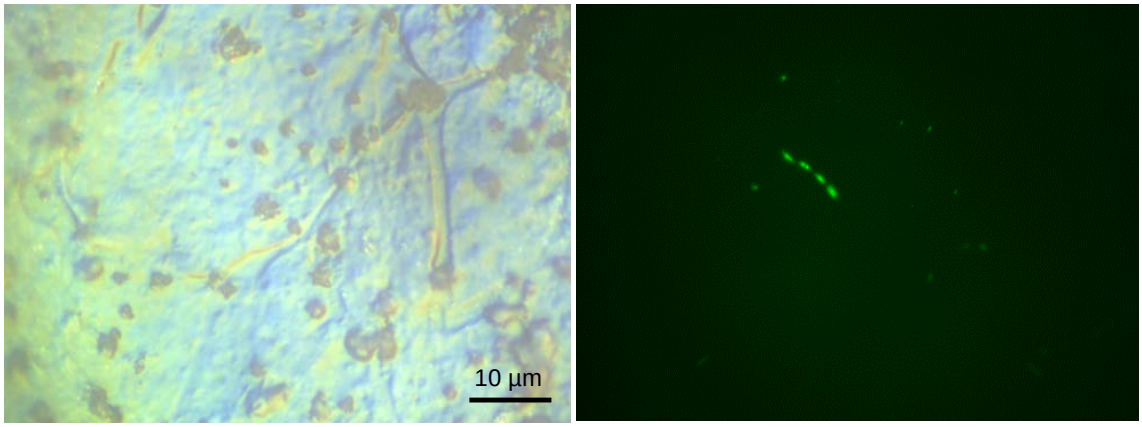


Figure 8.6: NG tube 04 - EDIC and EF images

This tube was from a 73 year old female. It had been in place for three days, and remained patent. The patient had received Fresubin Original enteral feed and medications from two groups via her NG tube. The EDIC images show bacterial colonies scattered across the NG tube inner surface, supported by the EF image, although the CFU and CE mean counts were relatively low.

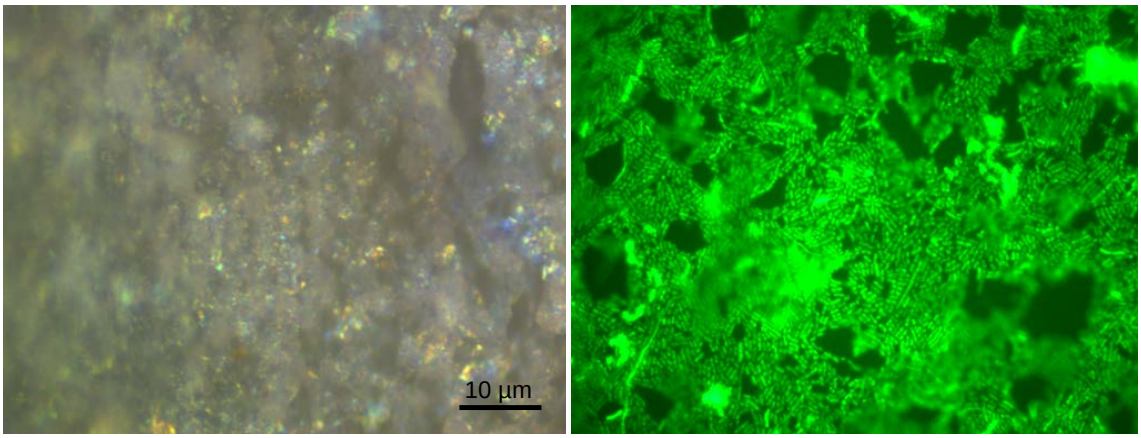


Figure 8.7: NG tube 05 - EDIC and EF images

This tube was from an 86 year old male, the oldest of the participants. It had been in place for nine days, and remained patent. The patient had received Fresubin Original and Original Fibre enteral feed along with medications from three groups via his NG tube. The EDIC image indicates biofilm development, with the EF image demonstrating prolific bacterial presence. This NG tube had the highest mean CE count, and the second highest mean CFU count of the study.

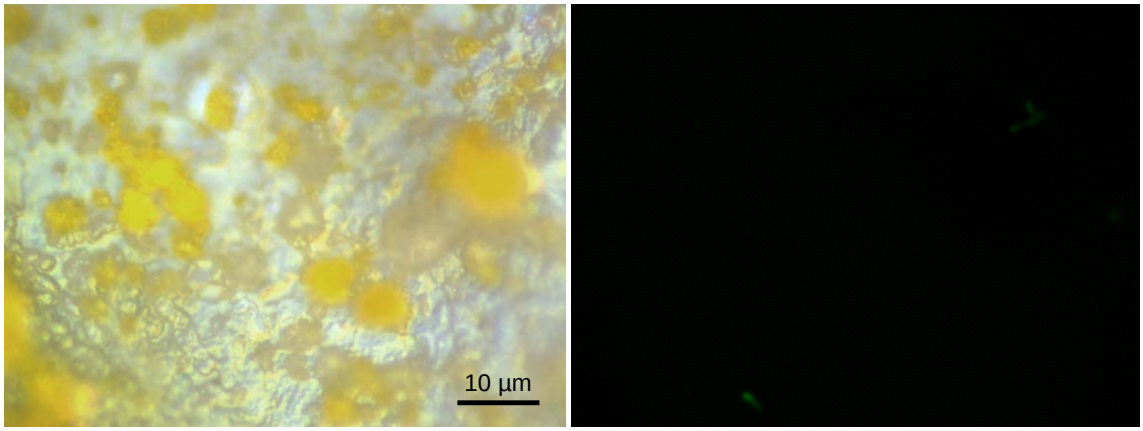


Figure 8.8: NG tube 07 - EDIC and EF images

This tube was from a 69 year old female. It had been in place for eight days, and remained patent. The patient had received Fresubin Original Fibre enteral feed along with 12 different medications from 11 groups via her NG tube. The mean CFU and CE counts were low, supported by the EF image. EDIC microscopy showed repeated images of an unusual surface pattern and colour. The filters used during microscopy were maintained throughout the study observations.

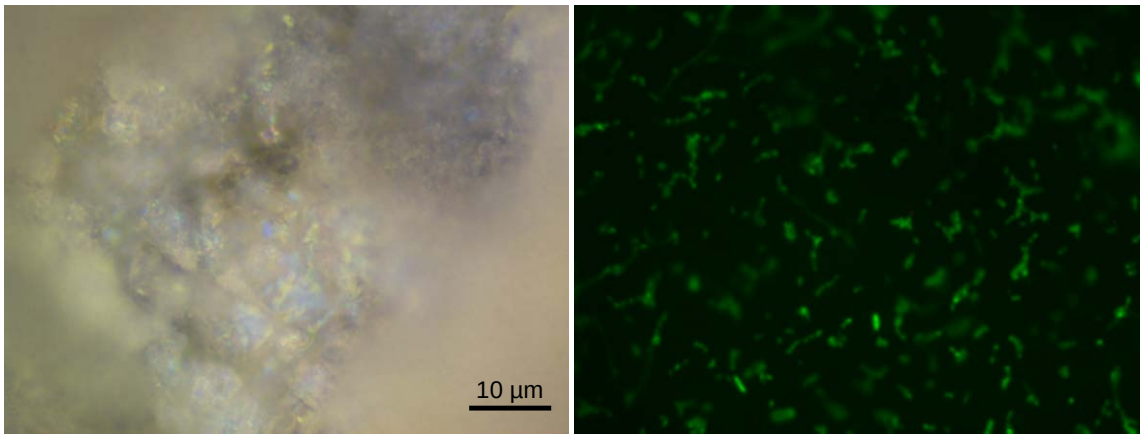


Figure 8.9: NG tube 08 - EDIC and EF images

This tube was from a 62 year old male. It had been in place for the longest period in the study, 22 days, and remained patent. The patient had received Fresubin Original Fibre, Energy, Energy Fibre, 2 Kcal and 5 Kcal enteral feed along with medications from eight groups via his NG tube. The EDIC image indicates prolific biofilm development, supported by the EF image of elongated bacteria.

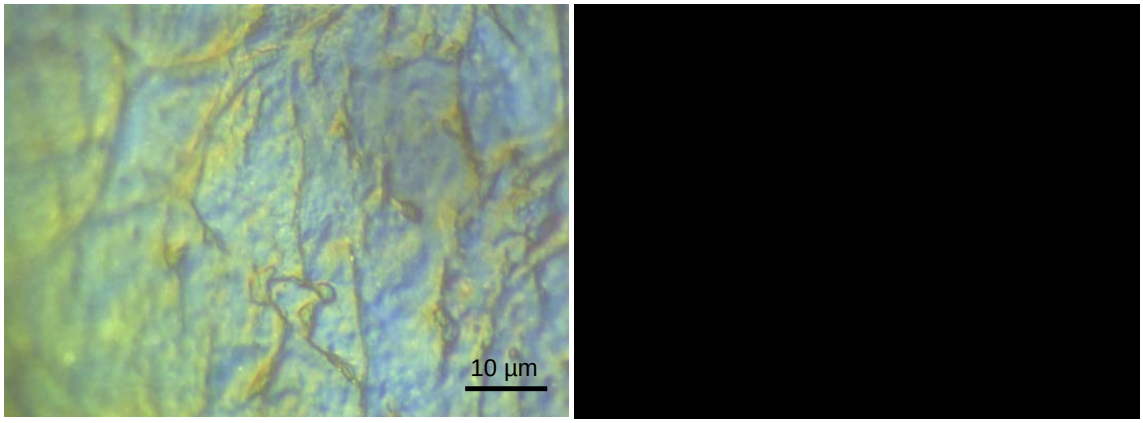


Figure 8.10: NG tube 09 - EDIC and EF images

This tube was from a 70 year old male. It had been in place for 11 days, and remained patent. The patient had received Fresubin Energy, Energy Fibre, Original Fibre and Energy High Protein enteral feed along with medications from three groups via his NG tube. The EDIC and EF images do not appear to indicate bacterial presence, supported by both the mean CFU and CE counts. Conversely, a culture was achieved for the pH testing element of the study.

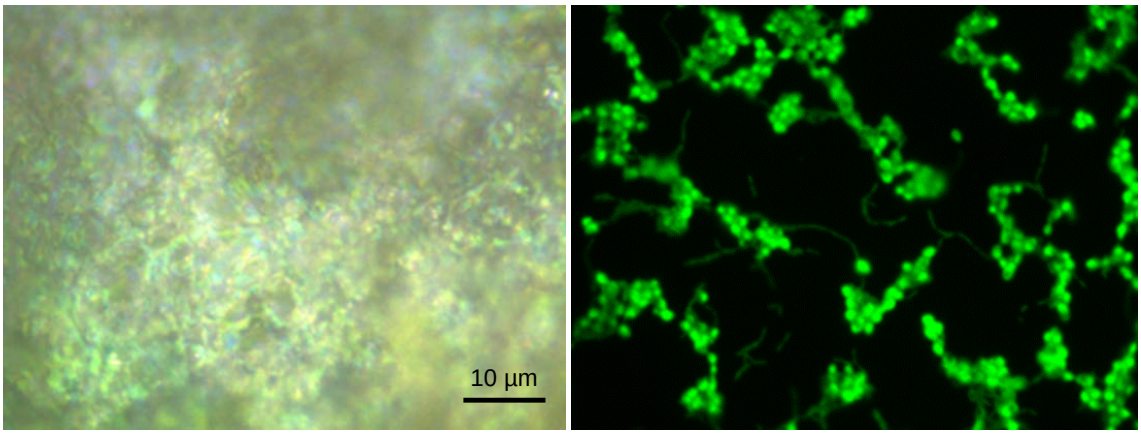


Figure 8.11: NG tube 10 - EDIC and EF images

This tube was from a 54 year old female, the youngest of the study participants. It had been in place for 12 days, and was the only NG tube in the study to have become blocked before its removal. The patient had received only Fresubin Energy via her NG tube, and had taken all medications orally. The EDIC image indicates bacterial colonies and biofilm development throughout the image. The EF image demonstrates an abundance of elongated cells. The NG tube had the highest mean CFU count, and the second highest mean CE count.

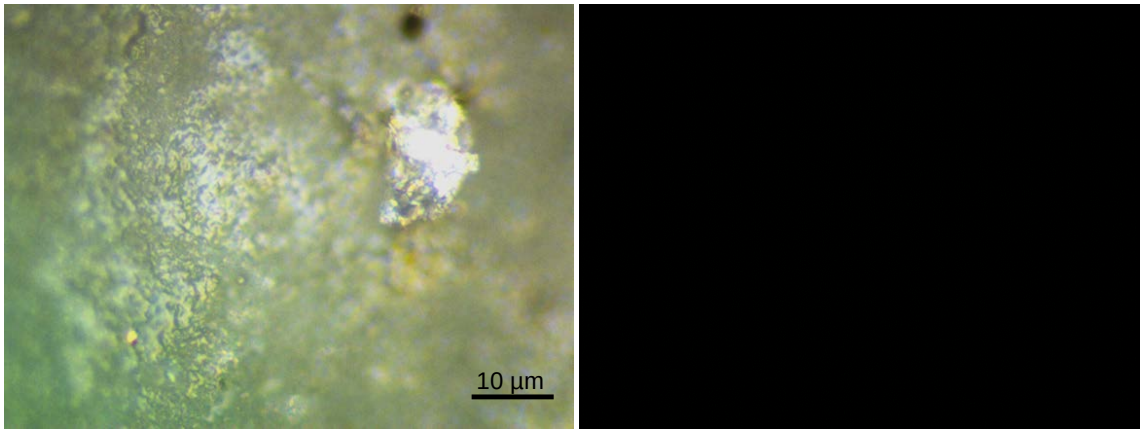


Figure 8.12: NG tube 11 - EDIC and EF images

This tube was from a 72 year old female. It had been in place for six days, and remained patent. The patient had received Fresubin Energy Fibre and Energy High Protein enteral feed via her NG tube, but had received no medication. EDIC microscopy showed repeated images of apparent crystals attached to the NG tube inner surface. EF microscopy captured no images of elongated bacteria, supported by the low mean CFU and CE counts.

Statistical analyses of the levels of bacterial colonisation reported were undertaken. The first statistical analysis to establish whether there was a difference in the levels of bacteria attached to each of the three anatomical areas of the used NG tubes. Visual inspection of box plots confirmed the distributions of CE and CFU counts were not similar for all groups, and in each case $p > 0.05$. Therefore the null hypothesis was retained that no significant difference was noted in CE and CFU counts between the three sections of NG tube in each case.

A Kruskal-Wallis H test was run to determine if there were differences in bacterial colonisation between the 10 NG tubes. Distributions of CE and CFU counts were not similar for all tubes, as assessed by visual inspection of boxplots. The distribution of both the CE and CFU counts were not statistically significantly different between the tubes, where X^2 (9) = 9.000 and p = .437 for both CE and CFU.

For the third analysis, the recorded variables of age, gender and duration of NG tube placement were tested. Neither appeared to have a bearing on the bacterial colonisation of NG tubes. This was established by assessing for monotonic relationships on visual inspection of scatter plots for each variable, of which none were noted. As the assumption of a monotonic relationship was violated, no suitable test was available to analyse the findings further.

8.6 **Discussion**

This study aimed to investigate the potential for specified patient variables to influence levels of biofilm development within NG tubes used by adults in an acute hospital environment, whilst also exploring the presence and nature of substances found within the tubes. To achieve this aim, the study examined the inner surface of NG tubes retrieved from adult patients, established the mean bacterial colonisation within the used tubes through CFU and CE counts, and further investigated the effect bacteria removed from the inner surface of the NG tubes can have on the pH of enteral feed, using materials and methods refined during the earlier laboratory studies in this interlinked series

Ethical approval was sought and received from the University of Southampton, a REC, and the host NHS hospital Trust. This proved to be a lengthy process, particularly because a substantial amendment was required by the host NHS hospital Trust which entailed a re-application to the REC. However, the whole issue of obtaining the correct

ethical approval from the relevant bodies was a valuable learning experience and has provided an opportunity to develop useful skills and knowledge for conducting future research.

The previous laboratory studies in this thesis had enabled variables to be controlled, and inocula were used of potentially greater concentration than would be expected in a normal health care environment. This is compared with the current study where NG tubes were used *in vivo* and no control was administered over the levels of potential bacterial colonisation, apart from the normal precautions practiced by the nurses caring for the tubes. With this in mind, the CFU and CE counts achieved from the used NG tubes would be expected to be lower than those of the inoculated tubes examined in the earlier studies. Whilst the CFU levels were lower (mean 294,881 compared with 10,931,934) the CE levels were much higher (mean 75,476,867 compared with 3,452,234), indicating that the concentrations of the inocula used in the previous studies were not necessarily incomparable to the levels found in the health care environment.

The results of the study show the patient variables of age, gender and duration of NG tube placement have not effected the CE and CFU counts reported within the NG tubes studied, as supported by statistical analyses. This differs from Hurrell *et al's* (2009a) study which suggested the neonate participants' ages had a statistically significant effect on the mean bacterial count noted within their used NG tubes. Although statistically there was no correlation in the current study, it should be noted the highest mean CE and CFU counts were seen in the tubes from both the oldest (86 years) and youngest (54 years) participants in the study.

Similar to Hurrell *et al* (2009a), the patients' feed regimen did not demonstrate an effect on the mean bacterial counts recorded. The patient variable of medication received via the NG tube was also considered; the inner surface of the NG tubes were examined for evidence of medication residue, in response to anecdotal evidence and published literature suggesting medication may cause blockages (Gaither *et al*, 2009; Lonergan *et al*, 2010; Dandeles & Lodolce, 2011). No such findings were noted in the seven NG tubes that had been used for administering medication. But of the three tubes that did not carry medication, crystal-like structures were noted repeatedly in two (tubes 01 and 11), and one had completely blocked (tube 10). As the crystal-like structures could not have been medication, the hypothesis is they are deposits of feed, potentially precipitated salts. The NG tube that blocked was found to contain enteral feed and

bacteria, thus providing evidence to support the theory that bacteria may be integral to NG tube blockage, through its effect on enteral feed.

The pH testing aspect of the study proved effective and produced a remarkably similar response to the study in Chapter 5. That study noted enteral feed inoculated with *E. coli* or bacteria isolated from a used NG tube increases in acidity from the 120 minutes time point, demonstrating a strong negative correlation between time and pH measurement ($p = .0005$) over the 24 hour period studied. In this study, the pH of the feed controls were noted to remain stable for the first 360 minutes, then drop to a mean of 6.34 by the 24 hour (1440 minutes) time point. The mean pH of the inoculated feed samples for each of the used tubes dropped to 4.94, therefore the feed control pH was considerably less acidic. Enteral feed is hermetically sealed in sterile ready-to-hang bags. Once opened it is no longer considered sterile, despite the careful handling processes. The hypothesis is that the small pH drop was due to exposure to unsolicited bacteria, or due to the effect the environment had on the sample feed.

The laboratory materials and methods employed for this study had been refined during the earlier laboratory studies in Chapters 3 to 7, and were used successfully here to achieve the aim of the study. Clear images were seen of the NG tube inner surface via EDIC microscopy, and of the CE filter membranes via EF microscopy. And bacteria counts using CE and CFU methods of determination achieved a similar pattern of results to previous studies, with CE counts higher than CFU counts due to the inclusion of VBNC bacteria, but the difference between individual tubes was not statistically significant ($p = .437$). Moreover, no statistically significant difference in CE and CFU counts between the three anatomical points of nasal, oesophageal and gastric sections of the NG tubes were reported. This indicates it is not possible to establish which end of the NG tube the bacteria entered, and thus determine the potential source of the bacteria. This is in keeping with the findings of the *in vitro* studies.

The patient consenting process worked well, and did not impact on the ward resources. One patient gave his consent to participate, as he was noted as having capacity to provide valid informed consent. On re-visiting the patient at a later date, his ongoing capacity was questionable, and therefore the results for his NG tube (tube 06) were withdrawn in line with the study protocol and the expectations of ethical approval.

Not all aspects of the study were successful; tube 09 produced CE and CFU counts of zero. However, a culture of bacteria was achieved from this NG tube for the pH experiment in the study, and the results were similar to those demonstrated by other tubes. The NG tube was the only one processed in the laboratory on that particular

day, with a fresh batch of TSA used for plating the suspended bacteria, but this would not account for the lack of CE count. On EF microscopy, 20 fields of view were chosen at random, and no elongated cells were seen. An explanation could be that the bacterial colonisation was too low to detect using these methods. Conceivably, the culture grown for the pH test was more successful as it used nutrient rich TSB, rather than the CE preparation that used low nutrient R2B.

The results of this study add to those of the earlier laboratory studies. The findings of bacterial attachment and biofilm development in NG tubes used by adults noted in Chapter 3 are supported here. Bacterial attachment and biofilm development within 24 hours of exposure was noted in Chapter 4, and certainly the results of this study would suggest that remains the case *in vivo*, with the tubes in place even for the shortest duration of three days (tubes 02 and 04) demonstrating established bacterial colonisation. The pH testing of enteral feed samples inoculated with bacteria isolated from each of the NG tubes demonstrated remarkably similar results to the pH testing study in Chapter 5, supporting further the hypothesis that this effect could be happening within NG tubes *in vivo*.

There were limitations experienced with the study. The original proposal had been to recruit 50 patients to participate in the study, a figure decided upon in relation to Leibovitz *et al's* (2003) study in which 53 NG tube fed older adult patients had been recruited to investigate bacterial attachment and biofilm development on the outer surface of NG tubes. The four wards selected to collect used NG tubes from for the current study were identified as caring for a number of patients with a need for enteral nutrition. The stroke ward in particular used NG tubes for the majority of their patients, and had experienced regular blockage problems (Ward Manager, 2013, per comm). Unfortunately, due to the strict directive regarding patients' capacity to provide valid informed consent stated by the host NHS hospital Trust Research and Development department, it proved very difficult to access this source of NG tubes for the study.

Although only 10 NG tubes were eventually used in the study, 20 patients had discussed the study and received and read the PIS. Of these, 17 gave their valid informed consent to participate. Unfortunately, six NG tubes were removed and destroyed in error by nursing staff, and one was withdrawn due to investigator concerns regarding the patient's capacity to consent. However, the 10 tubes used came from three of the four separate wards with differing specialities, encompassing patients with wide ranging medical conditions. The patients were both male and female, and ranged in age from 54 to 86 (mean 69.5), with duration of NG tube

placement from 3 to 22 days. Seven tubes were used to administer a variety of medications, and all 10 were used for enteral feeding. One tube had become blocked, providing the opportunity to investigate inside the tube for indications of what may have caused the blockage.

The nature of the laboratory based studies reported within this thesis have enabled the known variables for each study to be controlled to ensure the results of each can be attributed to the intervention undertaken. However, this study has examined NG tubes used *in vivo.* Although certain specified patient variables were documented, not all of the patient nuances can be accounted for when considering NG tubes used in a real world situation. As such, the study results can only provide a snap shot of NG tube use and management within the acute care hospital environment, and begin to provide a framework for future research seeking to investigate factors contributing to NG tube blockage.

This study has provided an opportunity to investigate the effect of patient variables on biofilm development within NG tubes, to explore the nature of deposits within the tubes, and to examine the effect bacteria from the tubes can have on the pH of enteral feed. The results achieved suggest bacteria attached within NG tubes do play a role in NG tube blockages, most likely through the effect on the pH of enteral feed, causing it to thicken. However, 10 tubes is a small sample to base such a statement on. This study could be considered a pilot study for a more directed study with a larger sample.

The statistical analyses indicate there is no correlation between the patient variables of age, gender, and duration of tube placement, whereas this study further discounts links with feed regimen and medication. Therefore, a future research study investigating NG tubes used by adults in an acute hospital environment could avoid requesting personal information from the patients' medical notes, thus potentially circumventing the strict guidelines regarding patient consent that prevented this study reaching its desired sample of 50 used NG tubes.

8.7 **Conclusion**

This study was designed to investigate the potential effect of specific patient variables on the levels of bacterial colonisation within NG tubes used by adults in an acute hospital environment. It also sought to explore the presence and nature of substances

in each of the tubes, and to establish whether bacteria within the tubes could affect the pH of enteral feed.

In order to gain access to the essential supporting personal information regarding patient variables, full ethics approval was sought resulting in assessment by a Research Ethics Committee, which provided a valuable learning opportunity for planned future research.

Laboratory methods tested and refined in the previous *in vitro* studies were successfully employed in this study. The results found no statistically significant relationship between the patient variables of age, gender, feed regimen, and duration of NG tube placement, with the levels of bacterial colonisation found on the inner surface of the tubes. It also found no correlation between medication passed through the NG tubes and potential blockage. A significant outcome of this study has been the effect the bacteria isolated from each NG tube has had on the pH of enteral feed, clearly supporting the findings of the pH testing study reported in Chapter 5. This potential cause of NG tube blockage requires further investigation.

8.8 Summary of laboratory studies

This chapter marks the completion of the laboratory studies designed to investigate the presence of bacteria and biofilm development within NG tubes used by adults. The first study established bacteria and biofilm were indeed present within NG tubes used by adults, and the speed of bacterial attachment and biofilm development were the foci of the following study, which demonstrated bacteria were attached at the 15 minute time point after introduction, and biofilm development was evident at the 24 hour time point. Furthermore, the presence of bacteria was found to influence the acidity of enteral feed, leading to a thickening of the feed.

Flushing is considered an effective way of maintaining the patency of NG tubes, with water as the preferred and recommended irrigant. The laboratory study investigating the effect of flushing with water on bacterial attachment and biofilm development supported this approach of NG tube management, and could demonstrate no significant benefit to flushing with sterile water in preference to tap water. The final laboratory study investigated the effect of patient variables on bacterial attachment and biofilm development in polyurethane NG tubes retrieved from adult patients at an acute

care hospital. The study found no effect of age, gender, feed regimen, medication or duration of tube placement on the levels of bacteria isolated from the tubes. Moreover, pH testing using bacteria isolated from each tube indicated an increase in acidity of enteral feed which earlier investigation had demonstrated can cause the feed to thicken. Throughout the laboratory studies bacterial colonisation of three anatomical points were considered, with the intention of establishing whether bacteria colonised a specific area in relation to the end of the tube through which bacteria were introduced. The results do not demonstrate a significant outcome to support this concept.

The next chapter describes a qualitative study undertaken to establish the nursing perspective of NG tube management by exploring the beliefs and reported practices of nurses caring for patients with NG tubes placed at an acute care hospital.

Chapter 9: A qualitative study of acute care nurses' beliefs and reported practices regarding NG tube management

9.1 Introduction

The management of NG tubes that have been placed in clinical practice by nurses requires decision making which is informed by research evidence, clinical expertise, patient preference and available resources. In order to ensure nurses provide high-quality care to their patients, it is essential that they respond proactively to developments in nursing and health care delivery. The previous studies have focussed on the generation of research evidence in a laboratory setting. With research generating new knowledge, nursing research in particular can help to inform nursing practice by translating empirically-derived knowledge into real-world applications (Polit & Beck, 2010b).

This PhD thesis has been undertaken as part of a Clinical Academic Doctoral Research Fellowship, comprising roles in both nursing and academia. Similar to the practitioner researcher role, the clinical academic role enables real-world enquiry, providing opportunities to identify gaps in nursing knowledge or practice. In particular, the findings of the laboratory studies could be used to present a case for enhancing patient care by informing practice development. Clinical academics are ideally placed to drive the adoption and spread of best practice, innovation and new technologies (DH, 2012). However, before this can be implemented, a greater understanding of current practice is required.

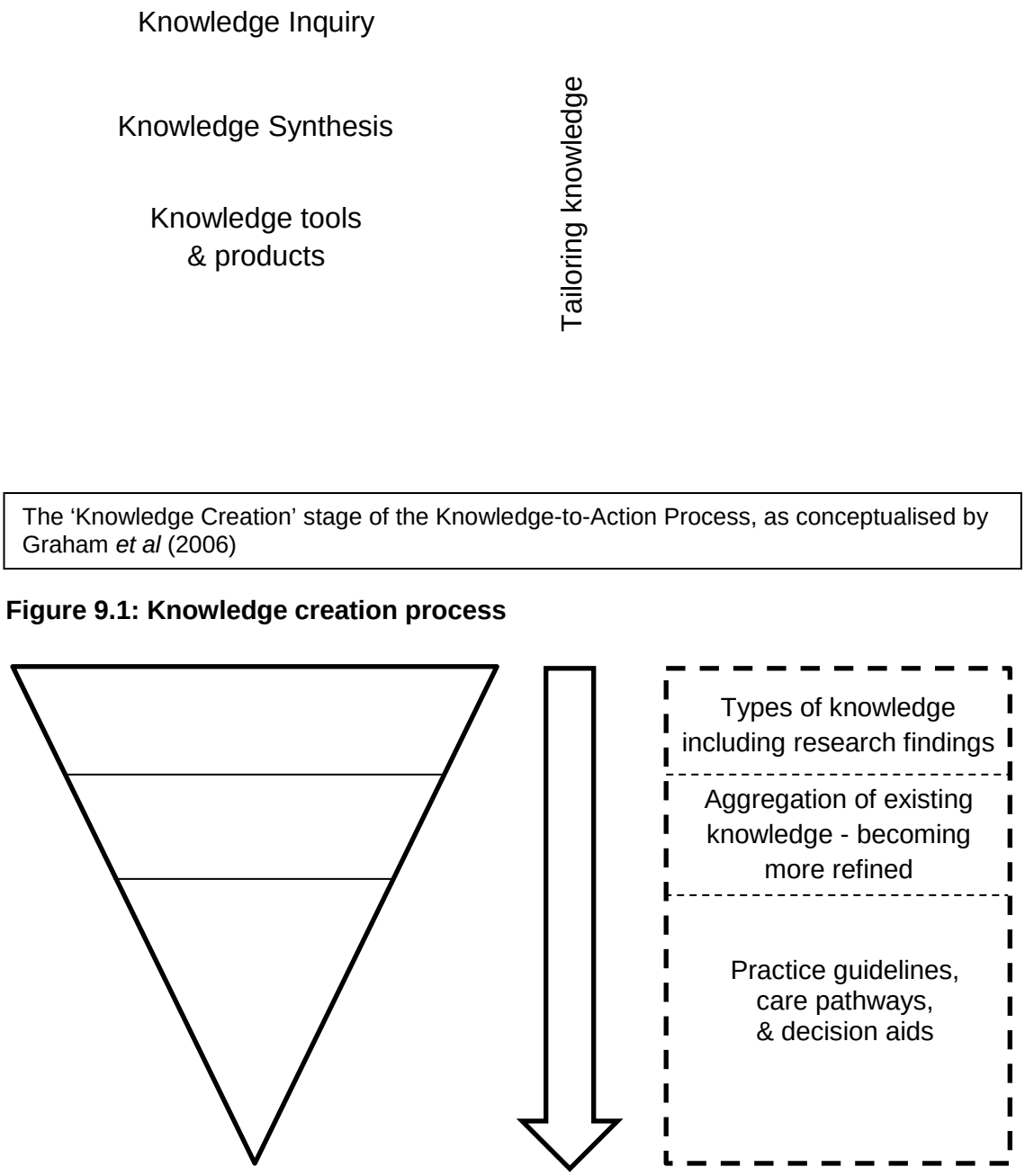
Investigation into how nurses manage NG tubes in the clinical environment and use their clinical expertise is important in order to understand clinical decision making concerning NG tube management. Enteral tube feeding guidelines are frequently developed by senior clinicians in hospital Trusts and by nutrition groups (ASPEN, 2009; BAPEN, 2016; NNNG, 2016), however the views of acute hospital ward nurses in the management of NG tubes has been little explored. This chapter presents a study that was undertaken to gain the nursing perspective of NG tube management through exploring nurses' beliefs and reported practices concerning their care of patients with NG tubes placed in clinical practice. For the purposes of the study, belief is defined as

the acceptance of something as true or real, a firmly held opinion, or a perspective; practice is defined as the actual application or implementation of an idea or method.

9.2 Knowledge translation

Before the potential benefits of any knowledge gained through research can be applied in practice with the intention of improving health outcomes, the findings of research studies need to be incorporated into an appropriate format, or tool, for translation to practice. Knowledge translation is the complex process of moving research findings into practice settings (Gerrish, 2015), and encompasses the whole of the research process including knowledge creation and knowledge application. It is a much broader concept than evidence-based practice, where practitioners use the best available evidence to inform what they actually do. Graham *et al* (2006) report that the interaction between researcher and practitioner in knowledge translation is collaborative and two-way.

Several frameworks to help guide the translation of research findings into practice exist, each helping to bridge the knowledge-to-action gap. One pathway, the Knowledge-to-Action Process, was conceptualized by Graham *et al* (2006), and consists of a funnel symbolising ‘knowledge creation’ (Figure 9.1), which is surrounded by a cyclical ‘action’ process. The knowledge creation process takes place in three stages. The first stage of knowledge inquiry draws different types of knowledge together, including research findings, tacit knowledge held by practitioners, and procedural knowledge derived from clinical expertise. These knowledge sources are reduced through the second stage, called knowledge synthesis, where the resulting aggregation is more refined. The final stage is the creation of knowledge tools and products, which include practice guidelines, care pathways and decision aids to facilitate implementation of the knowledge into practice. The cyclical action process surrounding the knowledge creation process is concerned with implementing change to practice and the subsequent evaluation of outcomes, and will not be covered at this stage.



The 'Knowledge Creation' stage of the Knowledge-to-Action Process, as conceptualised by Graham *et al* (2006)

Figure 9.1: Knowledge creation process

9.3 **Barriers to change**

Implementing change can experience potential barriers in the local context. In the translation of knowledge to practice, research findings that reflect closely current practice are more readily accepted and incorporated into everyday practice than findings that challenge existing practice and indicate the need for significant change (Gerrish, 2015).

Barriers to change include staff information and skills deficits, where nurses may not have sufficient knowledge concerning NG tube management and the rationale for their actions. There are also psychosocial barriers, including nurses' attitudes, beliefs, values and previous experience that affect practice and an individual's willingness to change (Gerrish, 2015). Other barriers include a lack of organisational support, poor quality of available evidence, and nurses not valuing research, with some nurses simply resistant to change (Polit & Beck, 2010b).

9.4 Literature search

Before research findings can be applied in practice, they need to be considered within the context of what is already known about the topic. It must be established whether the findings support or refute current knowledge, and whether they provide insights into practice that indicate the need for change (Gerrish, 2015).

To ensure the search strategy identified relevant literature to incorporate into the review, it was important to employ a structured approach to framing the research question. For questions relating to qualitative methodologies, the SPICE model (Setting, Perspective, Intervention, Comparison, Evaluation) presents a suitable alternative to the PICO model used in Chapter 2 (Beecroft *et al*, 2015). Using this model, the research question to be answered was 'What are the beliefs and reported practices of acute care nurses regarding NG tube management?'

A comprehensive search for published literature was undertaken. The databases CINAHL and MEDLINE were used as these are considered especially relevant for research focussed on nursing practice (Polit & Beck, 2010a). The database searches were periodically repeated between January 2015 and October 2016 to identify any new literature previously unavailable. Table 9.1 presents the free text, or keywords, employed when using the databases. These were derived from background reading into NG tube management, and were compiled to encompass each element of the SPICE model research question. Also indicated in the table are the relevant MeSH terms and suggested subject headings, along with the results achieved at each phase of searching. Similar to the literature searching process in Chapter 2, preliminary searches highlighted an apparent lack of relevant published literature. The Boolean operative 'OR' was employed to broaden the search to include either search term in the identified papers, and the Boolean operative 'AND' was used to link each of the five

elements of the search. Truncation was used with some of the keywords to widen the search by including synonyms and alternative spellings, and are indicated in Table 9.1 with asterisks.

Table 9.1: The search for evidence using the CINAHL and MEDLINE databases

Search #	Free text or Keyword	MeSH term or Suggested subject term	Results	Boolean 'OR'
1	Nurs*		656,413	656,413
2	Belief	Beliefs or attitudes Beliefs and practices Beliefs and values	1,358 1,532 541	3,400
3	Practic*	Practice Practice guideline Practice theory	722,370 0 157	722,370
4	Maintain* Maintenance Management		458,658 229,396 924,128	1,537,927
5	Nasogastric tube	Nasogastric tube Enteral nutrition Nasoenteral tube Feeding tube Feeding tube care Feeding tube irrigation	18,905	18,905
1+2+3+4+5	Searches combined with Boolean 'AND'			0
1+2+3+5				0
1+4+5				39 and 71

The free text/keywords and MeSH term/suggested subject terms used in the search for literature. The Boolean operative 'OR' was employed to broaden the search. Truncated terms are denoted with an asterisk

The initial searches seeking to include each of the five elements of the research question identified no literature. A further search excluding the management element also identified no literature. Therefore, a less restrictive search was completed to include literature only relating to nursing management of NG tubes. This search identified 39 published studies using CINAHL, and 71 studies using MEDLINE (Figure 9.2). There were 39 duplicates, and 71 original publications. The titles and

abstracts of each were screened to identify those studies most relevant to the research subject, with four studies selected for full text screening. All four were considered relevant. Ancestry searching of these papers, along with author searching, did not identify further relevant published literature.

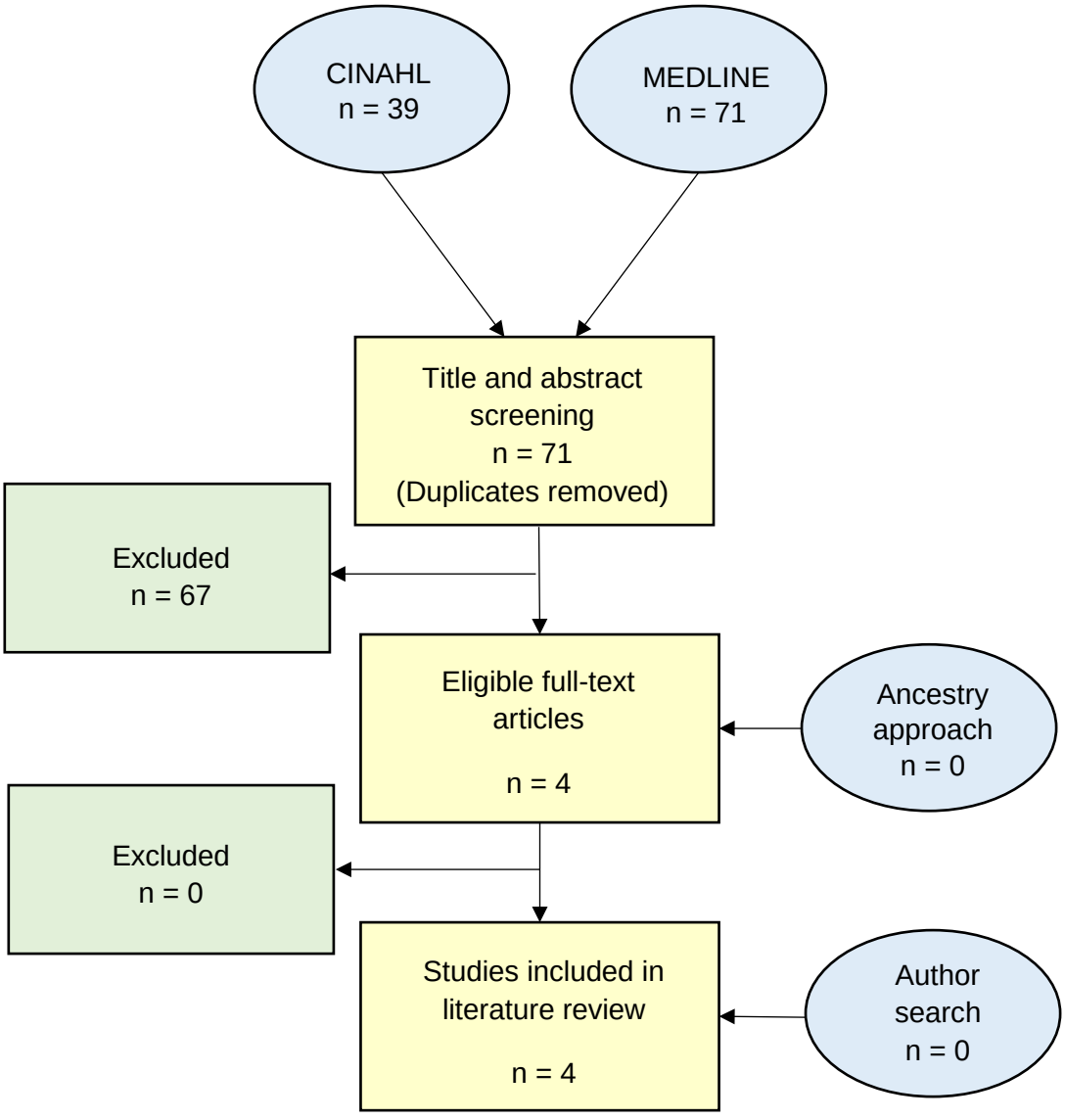


Figure 9.2: Flow diagram of literature search process

The four studies included in the literature review are:

- Mateo, M. (1996) Management of enteral tubes: nursing management of enteral tube feedings, *Heart & Lung: the Journal of Acute and Critical Care*, 25 (4), pp318-23
- Cannaby, A-M., Evans, L. and Freeman, A. (2002) Nursing care of patients with nasogastric feeding tubes, *British Journal of Nursing*, 11 (6), pp366-372
- Phillips, N. and Endacott, R. (2011) Medication administration via enteral tubes: a survey of nurses' practices, *Journal of Advanced Nursing*, 67 (12), pp2586-2592
- Al Kalaldeh, M., Watson, R. and Hayter, M. (2013) Jordanian nurses' knowledge and responsibility for enteral nutrition in the critically ill, *Nursing in Critical Care*, 20 (5), pp229-241

9.5 Synthesis of identified literature

Whilst the literature review identified only four studies relevant to the research question, each combine to provide an informative framework for the developing research study. Published in the USA, Jordan and Australia, as well as in the UK, they provide an insight into international enteral tube management. Each of the four studies reviewed describe research which set out to establish self-reported practices, experience and knowledge of nurses caring for patients requiring nutrition by enteral tube. Considerable variation in nursing practice was highlighted, including good and not-so-good practices such as using air auscultation to determine tube position, crushing enteric-coated medications, and using cola and fruit juices for flushing blocked tubes. Three studies included all types of enteral-access tubes, whilst the study by Cannaby *et al* (2002) solely focused on the views, opinions and experiences of nurses working with NG tubes.

Mateo's (1996) USA-based research paper regarding the nursing management of enteral tubes was published 20 years ago and describes nursing practice in the 1990s. Practice concerning NG tube management has evolved over the past 20 years with more focus on safe placement and methods to confirm NG tube position (NPSA, 2009). Likewise, Cannaby *et al's* (2002) paper describes UK practice over 15 years ago. The practice described in these older studies is of historical interest but it cannot be assumed that they reflect current practice, and thus were considered with this in mind. More recent practices with regard to enteral tube management are described in Phillips

and Endacott's (2011) Australia-based research paper and Al Kaladeh *et al's* (2013) paper regarding Jordanian nurses' related knowledge and responsibilities.

Each of the four studies employed a descriptive survey design, using questionnaires ranging from 35 questions (Cannaby *et al*, 2002) to 85 questions (Al Kalaldeh *et al*, 2013). Three of the studies used questionnaires entirely designed by the researchers (Mateo, 1996; Cannaby *et al*, 2002; Phillips and Endacott, 2011) whilst the fourth (Al Kaladeh *et al*, 2013) used a pre-prepared questionnaire that had been modified and added to in order to achieve the study objectives. Each questionnaire was designed and developed following extensive reviews of relevant literature, thus ensuring content validity with questions relating to nursing practice regarding detailed aspects of enteral tube management. Cannaby *et al* (2002), Phillips and Endacott (2011) and Al Kaladeh *et al* (2013) each conducted a pilot study to test the reliability and face validity of their questionnaires, whilst Mateo (1996) ensured face validity of her questionnaire through reviews undertaken by critical care and medical-surgical nurses.

Questionnaire surveys can be a cost-effective data collection method to administer by hand or alternatively by post or by email, which is particularly beneficial if the intended sample is geographically dispersed (Polit & Beck, 2010c). By self-completing questionnaires in their own time, respondents have more time to assess issues carefully before responding, and have a perceived privacy that may lead to a more honest response (Hasson *et al*, 2015). However, questionnaires can be limited in their length and complexity, as respondents cannot ask for clarification in the way they could if an interview was conducted (Hasson *et al*, 2015). This particular study design is often associated with poor response rates which can lead to bias, as respondents are rarely a random subset of the original sample (Polit & Beck, 2010c; Jones & Rattray, 2015). Alternatively, recruiting to face-to-face interviews can be more effective, and can provide the opportunity for nurses to project their own thoughts through the use of open questions and the chance to explore topics further (Hasson *et al*, 2015). One of the greatest advantages of interviews over questionnaire surveys is the flexibility of its format and structure, which can be adapted to fit the needs of a study (Tod, 2015).

A particular strength of the identified studies is the fact the combined sample of nurses come from a wide range of disciplines including critical care, acute medicine, surgical care, and nutritional nurse specialists, thus providing a wealth of knowledge and experience in enteral tube management. The questionnaires used in the studies mostly related to enteral tube management by nurses and incorporated topics such as flushing

of tubes, medication administration, restoring the patency of tubes, and the training and education of nurses involved in enteral tube management.

9.5.1 Flushing tubes

Sterile water and tap water were reported to be the most common choice for irrigation of enteral tubes (Mateo, 1996; Cannaby *et al*, 2002). In addition, Mateo (1996) reported the use of sterile normal saline. Nurses who responded to Mateo's (1996) study reported that they flushed enteral tubes regularly before feeding (29 %), after feeding (43 %), and every four hours (59 %). The most commonly used syringe size was 50 ml, particularly when NG tubes were flushed (Cannaby *et al*, 2002).

9.5.2 Medication administration

Medication has been reported to be a factor precipitating tube blockage. Almost all participants in the studies reviewed reported that they flushed enteral tubes after giving medication (Mateo, 1996; Phillips & Endacott, 2011; Al Kalaldeh *et al*, 2013), with a reported volume of 10 to 30 ml (Phillips & Endacott, 2011). However, less than half of all participants reported flushing enteral tubes before and between medications (Mateo, 1996; Phillips & Endacott, 2011). Medication may be associated with tube blockage because of particulate matter. Before administering medication, Phillips and Endacott (2011) reported 85 % of nurses reported that they usually crushed medication in a pestle and mortar, with 87 % diluting medication in water or normal saline before administration. In the same study, 34 % of nurses questioned considered it acceptable to crush enteric-coated medication.

Al Kalaldeh *et al* (2013) report that the majority of nurses included in their study considered that medications that can be given orally can also be crushed and administered along with the patients' feed. However, Al Kalaldeh *et al* (2013) outline that local advice is to administer medications separately after stopping the feed and flushing the feeding tube with at least 15 ml of water.

9.5.3 Unblocking tubes

The general consensus between the nurses in the studies was that irrigation is the best method of unblocking enteral tubes. The type of irrigation fluid reported to be used included carbonated beverages (cola, water), sterile water, dissolved papain (papaya

proteinase enzyme), pineapple juice, cranberry juice, hot water and tap water (Mateo, 1996; Cannaby *et al*, 2002; Phillips & Endacott, 2011). Methods of using a turbulent flushing or pumping technique and pulling back on the syringe plunger were described (Cannaby *et al*, 2002; Phillips & Endacott, 2011).

9.5.4 Checking tube position

The predominant method of checking tube position (89 % of respondents) in Cannaby *et al*'s (2002) UK-based study was by testing the pH level of gastric aspirates. Phillips and Endacott (2011) describe 63.5 % of Australian nurses report using pH to check tube position. However, the most common method reported by Australian nurses to check tube position was air auscultation (74.6 %). This method of tube placement check was also reported by 66.4 % of nurses in Al Kalaldeh *et al's* (2013) study undertaken in Jordan, where only 4 % of nurses reported using pH to test tube position. Air auscultation as a method to check tube position is no longer recommended in the UK (NPSA, 2009).

9.5.5 Education

Nutrition nurse specialists who responded to Cannaby *et al's* (2002) study stated that they provided NG tube education for nurses in their practice area. This was most commonly through informal sessions arranged between individuals (72 %), but they also provided one-to-one sessions along with workshops and seminars as required.

9.6 Aims and objectives

From the literature review it is clear the evidence regarding nurses' beliefs and practices in relation to NG tube management is wide-ranging, with the four studies included reporting considerable variation in the nurses' reported practices. Therefore the aim of this study was to explore acute care nurses' current beliefs and reported practices concerning the management of NG tubes.

The specific objectives were to:

- Explore the beliefs of Registered Nurses working in an acute care hospital concerning the management of NG tubes

- Explore the reported practices of Registered Nurses working in an acute care hospital concerning the management of NG tubes

9.7 Method

A qualitative methodology was employed to enable a deeper understanding of the views of nurses in a real-world context. One-to-one semi-structured interviews with Registered Nurses working in an acute care setting were undertaken and the interviews analysed to identify key themes arising. The results will detail the responses received, and these will be examined further in the discussion section of the chapter.

9.7.1 Sample

Qualitative research studies usually use non-probability sampling to identify participants that best illuminate the phenomena of interest (Hunt & Lathlean, 2015). This study sought to gain an understanding of the beliefs and practices of Registered Nurses responsible for NG tube management. For this study a purposive sampling approach was undertaken employing a homogenous strategy, with which a sample whose units share the same (or very similar) characteristics or traits (e.g. occupation) is identified (Lund, 2012). Using this approach, a sample of 20 Registered Nurses from acute care wards within a local NHS Hospital Trust was recruited, however the exact sample size would be determined during data collection. It was considered that approximately 20 participants would be required to reach data saturation based on previous studies utilising semi-structured interviews regarding nurses' beliefs and practices towards one aspect of nursing care in institutional settings (Sjöberg *et al,* 2015; Radzyminski & Clark Callister, 2015; Guillemin *et al,* 2015). It was not anticipated that practice would vary significantly across wards as nurses would be expected to adhere to the local hospital guidelines on enteral feeding

The eligibility criteria for participants included Registered Nurses who work on an acute ward at the local NHS hospital Trust, who care for patients receiving enteral nutrition through an NG tube, and who were available to take part in the research during the interview phase. Excluded were any non-permanent ward staff such as temporary agency nurses, and any ward staff that were not Registered Nurses.

9.7.2 Recruitment

The research study was advertised on four wards of an acute care hospital, following a meeting with each of the four ward managers at which verbal agreement was received to display a poster detailing the study (Appendix J). Potential participants were asked to contact the researcher using the contact details provided on the poster. Registered Nurses who expressed an interest in participating in the study were given a Participant Information Sheet by hand, and offered verbal information either in person or later via telephone. This enabled any questions that the participants had to be answered before they gave their consent to take part. If a potential participant agreed to take part in the study a date for the interview was arranged.

9.7.3 Participant Information Sheet

The Participant Information Sheet (PIS) (Appendix K) provided an overview of the research study, outlining the purpose of the study, the reason the Registered Nurse had been invited to participate, and the aspects of consent and confidentiality. It also provided details of the participant complaints procedure, along with insurance provision and details of the study investigator and sponsor. The PIS was handed to Registered Nurses who had expressed an interest in participating in the study, and was discussed in person or later by telephone if that was more convenient for the nurse. An appointment was then made for conducting the interview, whilst observing a minimum 24 hour cooling off period for the nurse to consider their participation in the study. The PIS would be discussed further at the interview appointment if the nurse required, after which their valid informed consent would be received before starting the interview.

9.7.4 Consent form

The PIS and Consent form (Appendix L) were discussed prior to the nurse consenting to take part in the study. The consent form consisted of a series of individual statements relating to the study, the data collection, and the data management. It also confirmed that consent to participate should be given voluntarily and could be withdrawn at any point throughout the study. The nurse would be asked to initial each individual statement, and then print and sign their name, and date. This was countersigned by the researcher, and a copy of the completed consent form was given to the nurse for their records. The original form was filed in an Investigator Site File.

9.7.5 Interviews

Interviews are used in both qualitative and quantitative research as a primary data collection method (Tod, 2015). Qualitative interviews allow for depth of focus on the participant, providing an opportunity for detailed investigation of the individual's perspective (Lewis & McNaughton Nicholls, 2014). A continuum of structure exists from completely structured to unstructured, with lesser structured interviews allowing for more in-depth and flexible questioning (Tod, 2015). Semi-structured interviews follow an interview guide of topics that need to be addressed, but with open-ended questions allowing the flexibility to follow issues raised by participants (Polit & Beck, 2010c). As this study aimed to gain the nursing perspective of NG tube management, it was considered semi-structured interviews would allow the participants to expand on their responses as they felt necessary, whilst ensuring known aspects of NG tube management were covered. These aspects included the participants' experience of NG tube management, believed causes of tube blockage, methods of maintaining tube patency, and any challenges they may have encountered. This method of data collection has been used successfully in published studies where insight into the clinical care perspectives of nurses and other health care professionals was sought (Sjöberg *et al,* 2015; Radzyminski & Clark Callister, 2015; Guillemin *et al,* 2015).

The participants were each interviewed once, at a time and place within the hospital buildings agreed in advance between the researcher and participant, to provide privacy and freedom from interruption. The interviews were anticipated to last approximately 30 minutes, although the interview rooms were booked for a period of 60 minutes to allow the participant extra time if required. Open questions were used to elicit the participants' beliefs and practices concerning NG tube management. The interviews were guided by a semi-structured topic guide (Appendix M) which facilitated exploration of the participants' beliefs and practices. Interviewing using qualitative, open-ended questions encouraged the participants to respond in their own words, allowing for rich in-depth data collection.

There are potential risks to qualitative research participants (Richards & Schwartz, 2002); these include anxiety and distress caused from taking part in the research, misrepresentation of the data provided by the participants, and identification of the participants in published papers. It was not anticipated that the interviews would cause anxiety and distress, but participants were informed that they could stop the interview at any time and advised to speak to their manager if required. The participants were

informed that confidentiality would be maintained at all times unless, during the course of the interview, information of a criminal nature or a perceived risk or threat to the researcher, the patients, or others was imparted. In this case the researcher, as a Registered Nurse, would be under obligation to inform the participant's ward manager.

Participants were advised they would not benefit directly from taking part in the research study, but that their participation had the potential to improve the service they provide in the management of NG tubes. At the end of each interview, the participants were offered a £20 Marks and Spencer voucher funded by the University of Southampton. This was to reimburse them for undertaking the interview in their own time. Interviews were not conducted during the participants' allocated shift times to ensure their participation did not impact on patient care.

9.7.6 Data collection

At the beginning of each interview, a Data Record Sheet (Appendix N) was completed with details of each participant, including length of time qualified, years of experience of NG tube management, and NHS Banding. Each participant was allocated a unique identifier code, and only anonymised data was used on any documentation available to view as part of the study thereafter. Also noted on the Data Record Sheet was the date the PIS was given, and the date the nurses' consent to interview was received. Interviews were recorded using a digital audio recorder.

To ensure confidentiality was maintained throughout the interview process, any mention of the participant's name during the audio recordings was replaced by pseudonyms at the transcription phase. If the participant mentioned work colleagues' names during the interview, they were also anonymised using pseudonyms.

9.7.7 Data management

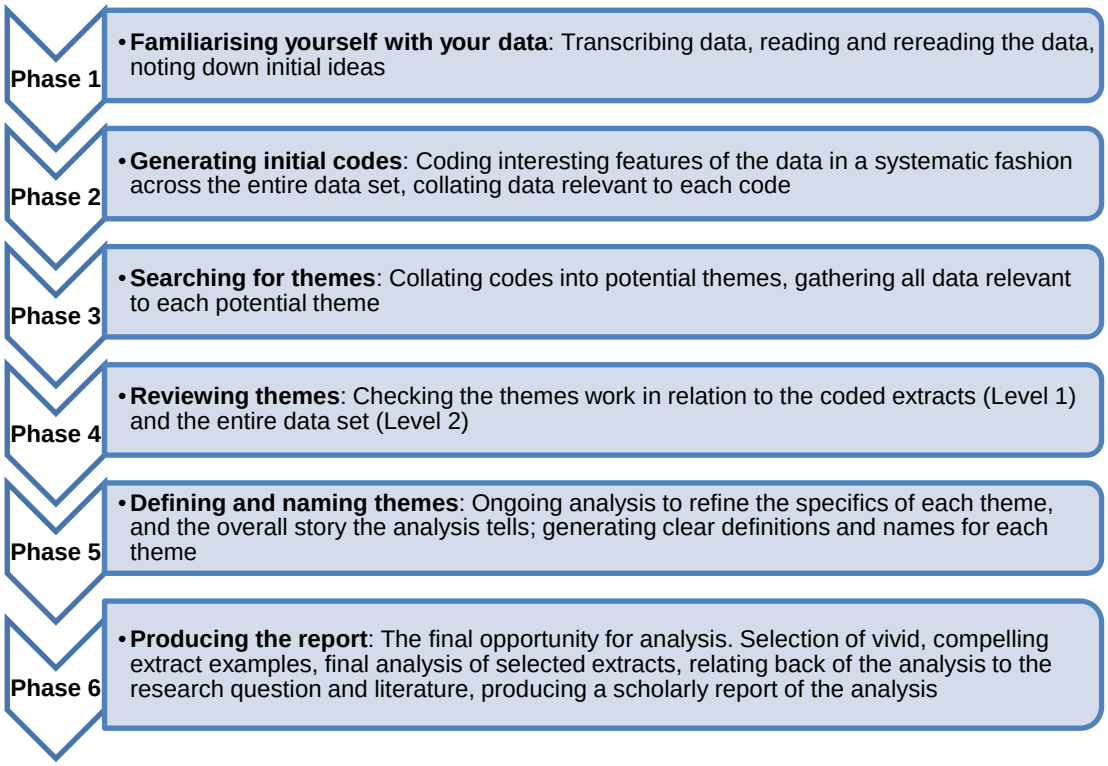
The audio recordings of the interviews were downloaded onto a password-protected computer owned by the University of Southampton and subsequently transcribed verbatim. Following transcription, the audio recordings were erased from the digital recorder.

Participant information and consent forms were stored in line with the University of Southampton guidelines on data management and storage of confidential data. Paper documents were kept in a locked cabinet in a restricted access room at a Clinical Academic Research Facility of the University of Southampton, at the NHS hospital

Trust where the interviews were conducted. All computer records were stored on a password protected computer. At the end of the study, documents recording personal data will be stored for 10 years at the Faculty of Health Sciences, University of Southampton, and then securely destroyed.

9.7.8 Data analysis

The interviews were transcribed verbatim from the audio recorder by the researcher rather than a professional transcriber. This provided the opportunity of familiarisation with the data from the outset, and for initial thoughts regarding codes to emerge. Once all the interviews were transcribed, the transcripts were read and re-read several times to enable the researcher to become immersed and intimately familiar with the content. Thematic analysis was used to study the findings of the interviews. As knowledge of nurses' beliefs and practices concerning NG tube management is limited, an inductive approach to the analysis was undertaken, with concepts allowed to emerge from the data rather than applying *a priori* concepts or hypotheses. Braun and Clarke (2006) state thematic analysis should be seen as a foundational method for qualitative analysis. For a novice researcher, the authors recommend it should be the first qualitative method of analysis learned, as it provides core skills that will be useful for



conducting other forms of qualitative analysis. To guide the data analysis, the six phases of thematic analysis outlined by Braun and Clarke (2006) were followed, as depicted in Figure 9.3.

Initial codes were assigned for salient constructs and expanded as necessary, and codes then compared across transcripts to identify convergence and divergence. The codes were then examined and collated to identify significant broader patterns of meaning. Data relevant to the emerging themes were then reviewed and the themes

Figure 9.3: The six phases of thematic analysis

refined. The scope and focus of each theme was then defined and the theme named. Quotations from participants were used where appropriate to illustrate the arguments of the researcher, and to give insight into the participants' experiences and their meanings and interpretations of the situation (Holloway & Wheeler, 2010).

9.7.9 Rigour

Use of an interview topic guide allowed for all participants to be asked questions relevant to achieving the research aim of exploring nurses' beliefs and practices concerning NG tube management. Adherence to the protocol also guided the systematic conduct of the study and allowed for transparency of the methods. Internal checks were undertaken regarding the accurate transcription of three of the audio recordings, and on the coding and interpretation of the data by another researcher.

9.7.10 Ethics

Health Sciences Faculty ethical approval was sought from the University of Southampton through the Ethics Research Governance Online system, as the study was part of a PhD thesis (Identification number: 14549). The university also provided sponsorship for the study, along with professional indemnity and clinical investigation insurance.

Approval was also required from the local NHS hospital Trust Research and Development department, as the study interviews were to be conducted at their site with Registered Nurses employed by the Trust. This was received in October 2015 (Application reference: PHT/2015/30 check). No further approval was required, as since 2012 research involving NHS staff no longer requires ethical review by an NHS Research Ethics Committee (Gelling, 2015).

9.7.11 Investigator Site File

An Investigator Site File (ISF) was created as a central point of reference for all essential documents connected with the study. All versions of the study documents are contained within the ISF, with the most recent version of each document indicated, and earlier versions marked as superseded. Also contained are the research study proposal, the researcher's CV and Good Clinical Practice certificate, the screening and enrolment log, and the original of each Consent Form completed by the study participants. Copies of Standard Operating Procedures (SOPs) issued by the local

NHS hospital Trust for recruiting and consenting participants, reporting adverse incidents, and maintaining an ISF, were included to ensure the study was conducted to the standards expected.

The ISF was kept in a locked cabinet in a restricted access room, alongside the source documents recording the participants' data. Access to the ISF was by the investigator and supervisory team only, with the local NHS hospital Trust advised of the location of the file. Maintaining an accurate ISF is essential should a monitoring or auditing assessment of the study be required.

9.7.12 Qualifications

All research must be conducted in accordance with local, national and international policy and guidelines, including the principles of Good Clinical Practice (GCP) and the Research Governance Framework for Health and Social Care (DH, 2005b). All investigators conducting research in the NHS must possess a valid research passport or hold an honorary contract (research or clinical) with the relevant NHS hospital Trust. Therefore training was undertaken to gain the GCP certificate, a course was attended regarding receiving participants' valid informed consent, and an honorary contract was set in place with the local NHS hospital Trust.

9.8 Results

The interviews were conducted throughout October to December 2015. Of the 20 participants, 19 were female, one was male. The interviews ranged in length from six minutes to 29 minutes (mean 12 minutes). Years of nursing experience ranged from 1 to 43 years (mean 17 years). Years of NG tube experience ranged from 1 to 26 years (mean 11 years). Nurses' NHS Bandings ranged from 5 to 7. Table 9.2 outlines the participants' demographic characteristics.

Table 9.2: Demographic characteristics of participants (n = 20)

Characteristic	Group	Responses
Gender	Female	19
	Male	1
Years of nursing experience	<1	0
	1-5	5
	6-10	2
	11-15	2
	>15	11
Years of NG tube experience	<1	0
	1-5	7
	6-10	4
	11-15	4
	>15	5
NHS Banding	Band 5	13
	Band 6	6
	Band 7	1

The 20 interviews were transcribed and analysed using a thematic analysis method, the codes and subsequent themes that emerged from the data are indicated in Table 9.3. Three main themes emerged of looking after the patient, using the NG tube, and stopping the NG tube blocking.

Table 9.3: Codes and themes that emerged from the transcribed interviews

Themes	Codes
Looking after the patient	▪ Keeping tubes in (bridles etc.), self-removing ▪ Conditions: dementia, confusion, stroke, dysphagia, post-surgery, fluid restricted, re-feeding syndrome, elderly, altered anatomy ▪ Patients' best interests, advocate ▪ Patient comfort ▪ Patient safety ▪ Patient perspective
Using the NG tube	▪ Lack of confidence ▪ Tube insertion ▪ Checking NG tube position – aspirate, measurement, X-ray ▪ Experience and training ▪ Feed regimen
Stopping the NG tube blocking	• Flushing and not flushing • Enteral syringe use • Medication preparation – liquid, tablets, crushing • Medication administration – nursing time (time poor), busy wards, inexperience, user error • Frequency and causes of NG tube blockages • Unblocking the tube – flushing, fizzy drinks, bicarbonate of soda, agitation ▪ Vigilance ▪ Precious tube

9.8.1 Theme 1: Looking after the patient

Once the NG tubes are placed into patients, the challenge described by many ward nurses was to ensure the tubes remain in situ. Nurses stated that some patients repeatedly removed the tube which they attributed to confusion:

> *"Some of the challenges are keeping the tubes in (…) patients who are confused pull their tubes out" [NUR03]*

This is particularly prevalent in older patients:

> *"We don't have it so much with the youngsters, it's always the elderly" [NUR17]*

One nurse talked about the consequence experienced by one of her patients after she repeatedly self-removed her NG tube:

> *"One that I'm looking after now is an elderly lady of 90 who doesn't like the NG tube in at all, but she can't swallow anything. So, um, we've been putting the*

NG tube down, and within two hours she pulls it back out again. Um, but she's an epileptic, and so we need to get her medication into her, which is crushable to put down the NG tube. And before we can give her her drugs, she pulls it out. And the other day, because she was late (with her medication) she had a fit" [NUR17]

The use of devices to prevent self-removal were rarely reported.

The participants in the study worked on wards where patients with a variety of conditions received care. People who had experienced a stroke were described as presenting a particular challenge for nurses, and often their cognition is altered as a direct result of their stroke. This can cause difficulty when nurses need to place an NG tube:

"It's difficult because patients come in with various understanding difficulties (…) each stroke is different (…) we try and make sure we explain really clearly what we need to do and why" [NUR11]

Whilst another nurse advised:

"When the patient's in initial acute stroke phase, they can be quite agitated, and not understanding what's going on with them, so those are more complicated patients with regard to their NG feeding" [NUR15]

Similar experiences are reported by nurses caring for patients with dementia:

"NG tubes and people with dementia, and everything gets quite difficult" [NUR09].

Patients on the head and neck ward present the nurses with a very different set of challenges. Due to having surgery, invasive tumours, or oesophageal pouches or strictures, many patients there have altered anatomies, which can lead to very difficult NG tube placements:

"This can be a challenge if the tumour is at the base of the tongue, or if the tumour is particularly large, it can be a problem, so we don't always get the NG tube down on the ward. Sometimes the doctors may need to look with a scope (endoscope), or they may have to be, um, radiologically placed, so under radio guidance" [NUR13]

Also, many patients who have undergone maxillofacial surgery require a period of NG tube feeding to allow for their post-operative recovery:

> *"We also have a lot of people who have major surgery, and because of where it is (maxillofacial) it's not advisable for them to eat so that the wounds heal on the inside" [NUR12]*

One condition that can affect patients on either of the acute care wards is re-feeding syndrome, which calls for nurses to adhere to a strict feed regimen planned specifically for the patient by the dietetics team:

> *"If they're below a certain weight, I think it's 38 kg, they've got to go on the re-feed regimen, and then, you know, they sort of gradually build them up to a higher rate, 'cos it starts on a very low rate if they're undernourished (…) gradually they have to have all the vitamins because the body can sort of go into some sort of crisis really, if it's rushed straight into them, if they've got a risk of re-feed" [NUR14]*

The nursing staff interviewed were acutely aware of the importance of effective management of NG tubes, and that patients were perhaps not always understanding of the need for an NG tube to be inserted, particularly if their health condition had caused cognitive impairment. As one nurse explained:

> *"You're trying to do what you do in their best interests" [NUR01]*

It was clear nurses did not want to subject their patients to any unnecessary discomfort, and this was avoided wherever possible:

> *"On the whole, I think, six-weekly you would be thinking 'Oh goodness, they may need their NG tube changing'. But if there's no trouble with the NG tube or getting aspirate or, um, we wouldn't tend to take it out and put someone through more pain by putting another one down, if everything's OK" [NUR13]*

There were also reported incidents of nurses being asked to place NG tubes in patients where they felt it was not in the patients' best interests or safety to have an NG tube, and have spoken out on the patients' behalf:

> *"We've had that recently where a doctor asked us to pass an NG tube, and we said 'No, not doing that'" [NUR12].*

The NG tube currently used on the wards of the hospital where the study was undertaken is the Corflo® polyurethane NG tube (Corpak MedSystems, UK). It is 92 cm long, with centimetre markings to assist with checking tube position, and has a water-activated lubricant on the tip to aid insertion. It is available in 6 or 8 FG, although 8 FG is the size of choice:

> *"We tend to use the 8 bore ones because the 6 tend to get blocked more often" [NUR12]*

The design of the tube incorporates a feed port and access port for irrigation, medication administration, and for obtaining gastric aspirate for pH testing to confirm the tube position prior to use. The nursing staff appear comfortable with the choice of tube:

> *"The tubes that we use are much better than the old ones (…) which are easier to look after and seem to last longer, and are a better quality material" [NUR18]*

In particular, the nurses commented on the suitability of the tube for their patients, with added patient comfort and tube durability:

> *"I think NG tubes have come a long way, along quite a lot. Um, I remember the old NG, the old NG tubes that we'd have to lubricate with, like, Aquagel and things like that. Oh it's just not pleasant for the patient, is it? I think, I think, you know, I think the new, the new NG tubes are so much better, and they're much more comfortable for the patients. And that, and that shows I think because, you know, the patients are more compliant with them, generally" [NUR15]*

Patient safety was an aspect of NG tube management commented on by several nurses in areas including tube placement, checking tube position, and longer-term NG tube management. The head and neck ward nurses commented of their patients' altered anatomies, with one explaining:

> *"You really have to know their history before you attempt to put a tube, or move a tube" [NUR08]*

Patient confusion due to stroke, dementia, or other condition can effect patient safety with regard to the management of NG tubes. One nurse talked about a patient who she was concerned about:

"She wouldn't have been safe going home with an NG tube, I think she would have been back here before too long" [NUR17]

Another nurse spoke of an initiative the hospital has introduced to reduce the number of X-rays patients are subjected to when confirming NG tube position. When placed, if the ward nurses cannot obtain a gastric aspirate to check pH levels to confirm the tube is in the correct position, the patient used to be booked for a chest X-ray. Now, with the initiative in place, the Nutrition Nurse Specialists are called to the ward to attempt to obtain an aspirate. They are often successful, which then prevents the patient having an X-ray:

"They've managed to reduce the number of X-rays done quite significantly since this was introduced" [NUR08]

Despite the many reasons patients need to be NG tube-fed, and the expertise and care the ward nurses and Nutrition Nurse Specialists provide with regard to NG tube management, perhaps an aspect that is sometimes overlooked is the patients' perspective. Many patients are not fully aware of the presence or purpose of their NG tube, and perhaps are not concerned with the aesthetics of it being taped in place. However, one nurse told of a patient that she had cared for who was acutely aware of the tube's presence, and forbid members of her family from visiting her in hospital:

"We had another lady, and she didn't want her grandchildren to come in, because she had this (NG tube) coming out of her nose, and she was quite a private lady, and she hated it, just because it was there and it's a sort of facial (thing) you know, like if you've got a tube here (abdomen) you can't see it, can you?" [NUR17]

9.8.2 Theme 2: Using the NG tube

Despite the wealth of knowledge and years of experience the participants demonstrated during the interviews, there was still some trepidation reported when it came to NG tube management:

"A lot of nurses don't like the placing of them, and they're scared of them" [NUR04]

The issue appeared to stem from nurses losing their skills during periods of reduced NG tube placement on their wards:

"But if they haven't passed any for, you know, the past couple of months, three months, that's a long time isn't it? They lose confidence" [NUR04]

Tube insertion was described by the nurses as adhering to local hospital guidelines which are written by the Nutrition Nurse Specialist team in line with manufacturer and national guidelines (Corpak MedSystems, UK; NPSA, 2009). The patient experience of the procedure was also considered:

"Just to remember how uncomfortable it can be on initial insertion, and how important it is to follow the guidelines when they go down, and to make sure if you can't get an aspirate the rules are to have an X-ray, but obviously the best way is to get aspirate, pH under 4.5, but if you can't then the patient does need an X-ray when it first goes down" [NUR13]

On the head and neck ward many patients have tumours which can create challenges when a patient requires an NG tube to be inserted, and it is not always possible to insert them following the standard guidelines. Sometimes they need to be inserted under direct vision by an endoscope, or even during surgery:

"A lot of the tumours are floor of mouth and the larynx, and some of them come in and they're quite bulky, so it's very difficult to pass the tube safely. So, on the round the doctors will bring the scope (endoscope), and they will try and pass it with the scope so they can see, um, but often, because of the patients we have, they can't swallow anyway either, so it's very difficult to pass the tubes. So, a lot of the patients that go for surgery, they will pass the tube in theatre" [NUR17]

However, the decision to place an NG tube using the endoscopic method is not taken lightly:

"When we do scopes (endoscopies) everyone hates them, because you gag don't you, so they really hate having NG tubes put down" [NUR17]

When interviewing the participants, it became evident there was a wealth of experience of caring for patients with NG tubes, with a collective duration of 217 years. The nurses were employed in different areas including surgical and medical specialties, caring for patients with a diverse range of health issues. One nurse had spent 26 years caring for patients receiving enteral nutrition through NG tubes, and now uses her experience in a specialist role:

"I've looked after patients with NG tubes the whole of my career" [NUR01]

Other nurses talked about how their wards were particularly used to managing NG tubes, as their patient cohort were often required to have an NG tube inserted. This was particularly evident on the acute stroke ward, where the majority of patients require NG tubes, as one nurse confirmed:

"You know, it is our bread and butter. We deal with NG tubes every day" [NUR15]

Training received by nurses in the management of NG tubes appeared to be mainly conducted on-the-job by more experienced nurses, rather than through any formal training pathway. One nurse stated:

"We tend to teach each other so, I think that the experienced ones would teach the um, you know, the newly qualified people, and people who come on the ward that are not used to NG tubes, so I think there's quite a lot of experience on the ward" [NUR12]

However, one participant indicated she did not feel the training she received was adequate:

"When I started taking care of them, I was like completely lost (…) they always explain to you how to get it in right or how you will use it and everything, but they never explain to you, like, how you need to take care of it" [NUR07]

One ward has embraced the concept of cascade training, whereby experienced nurses are assigned to inexperienced nurses, to train them in procedures undertaken on their ward. In turn, those nurses will train the next newly qualified cohort to be employed by the ward:

"We have enough experienced members of staff on the ward now that we're (…) cascade training" [NUR03]

This method of training was implemented following a marked turnover of ward staff which lead to several inexperienced nurses being employed at once, and has thus far proved an effective pathway.

The Nutrition Nurse Specialists are experienced in the management of enteral tubes, and are often available for individual nurse training. Although these are not formal

training sessions, the nurses who have received them have found them to be valuable sources of information:

> *"We often get the Nutrition Nurses up if we've got problems, to go through things with us, because they're on, on call, you know, they're really good" [NUR17]*

Remarkably, in an age of clinical research and evidence-based practice, one nurse commented on the concept of nurses' intuition when asked how she cared for NG tubes:

> *"Nurses' intuition I suppose, knowing what's right" [NUR10]*

Each patient being cared for on the acute wards who are receiving nutrition by NG tube must be assessed by the dietetics team, and a feed regimen tailored to the patients' needs planned. The nurses need to follow these plans, which can vary considerably between patients, and remain vigilant throughout the feed duration:

> *"We are guided by the Dieticians and their regimens, and often patients would perhaps be on two or three bags (of enteral feed) a day, so you're talking about 1500 ml of feed, and it wouldn't really go much more than 100/120 ml per hour. So that's quite a significant amount of hours' feed" [NUR15]*

9.8.3 Theme 3: Stopping the NG tube blocking

The nurses interviewed stated they considered flushing of NG tubes to be the most effective way of preventing them from blocking. The irrigant normally used was reported to be tap water, with the occasional use of sterile water in exceptional circumstances. This was justified by stating that patients would normally drink tap water:

> *"It can be tap water, 'cos it's still going straight into your stomach, so, you know, what you normally drink is fine. On occasions, however, you use sterile water because, if, especially if they're not having food, if they're just having drugs down, because you've got issues that there might be a problem somewhere, you just use sterile water just in case there's a risk of infection" [NUR16]*

However, a senior nurse advised:

"Here we say normal freshly-drawn tap water for flushes, not sterile water, and we do that throughout our enteral tube range. It's not any different for any of the different tubes, um, and we tend to say, you know, by flushing them we should be clearing enough debris out of the tubes for the tubes to be usable, because they obviously, the manufacturers make them so they're functioning tubes" [NUR18]

Some nurses outlined the importance of flushing the tube with water before and after feeding:

"The most important thing is flushing your tube before feed starts, and as soon as your feed finishes" [NUR01]

However, some nurses reported that NG tubes did not need flushing before a feed commenced, with those nurses only flushing with 20 to 50 ml of tap water after the feed has finished. Most nurses described flushing the tube using a syringe, however, some stated patients received water through the enteral feed pump for a set period following their enteral feed and the nurses considered this a 'long flush':

"They have their bag of feed, then they have the flush for so long, and then they have a rest" [NUR10]

Another nurse indicated she would also flush when stopping a feed to move a patient:

"If you're gonna stop them (feed) to wash a patient or do some moving and handling, flush them properly before you disconnect it, because, you know, it's just sitting around going hard in the tube otherwise, isn't it. I think that's the most important thing, to keep it patent anyway" [NUR11]

A nurse also pointed out that NG tubes should be flushed after obtaining gastric aspirate for pH testing:

"We also tell them to flush after they've got aspirate, you know, when they're getting their aspirate to check their NG tube" [NUR18]

By flushing the tube at this point, and residuals of feed or gastric contents should be flushed clear, thus reducing or preventing an interaction of acidic gastric aspirate and enteral feed. In this participant's opinion, the best way of maintaining the patency of NG tubes in use on acute hospital wards was summed up perfectly:

"Flush. Flushing, flushing, flushing, flushing. You can never flush too much!" [NUR12]

Most nurses indicated they would flush with between 10 to 30 ml of water before medication, 10 to 20 ml between each medication, and 10 to 30 ml after medication:

"So what we normally do, we say, is definite, we would flush beforehand, and we flush as soon as the food is finished. Um, we flush before we give a medicine, and, uh, you know, with my patients I tend to say 20 mls beforehand, flush 10 mls in between each medicine, and then at least 20 mls after you've done your group of medicines" [NUR18]

However, several other routines were reported. These included flushing with 50 ml of water before and after medication, but not between each medication, and flushing with 50 ml of water before medication, 20 ml between each medication, but with no final flush after the patient's medication has been administered. One nurse indicated she does not flush before or after giving medication, but does flush between each medication with 5 to 20 ml of water.

Medication given through NG tubes on the wards at the local NHS hospital Trust is completed using liquid medication where available:

"Syrups are so much better than crushing the tablets, if you, if you can get a syrup to put down the NG, it's better than tablets" [NUR17]

Even liquid medications can cause problems, and measures are taken to reduce the impact:

"Some of the antibiotics, the liquid antibiotics that they use, that we use as well, some of those are quite thick, like Metronidazole, that's quite thick, so, you just have to make sure you dilute it well" [NUR12]

If liquid medication is not available the nurses have to crush the tablets to be administered at the patients' bedsides using a pestle and mortar:

"Because they're the best thing for crushing tablets" [NUR18]

Not all tablets are crushable, though, and care is taken to avoid administration errors:

"If you crush your medicines adequately, and then let them mix (with water) properly they can be OK, but we have to check what ones can be crushed and what can't. Obviously slow release ones you can't crush. Um, but the pharmacist will check that every day, the medicines they're on" [NUR13]

One nurse reported that:

"From the medication side of things, I think that that has improved quite a lot. We can obtain a lot of liquid medication that we couldn't always get hold of. We would have to crush medication where we could, and also that brings in the risk of blockages of NG tubes. Of course we still have to crush certain tablets now that we can't get in liquid form, but they're very few and far between. Um, you know, I think, um, I think our, our medication supply is good with regards to that" [NUR15]

Sometimes liquid forms of medication are not available, and tablets need crushing, which nurses felt could be a cause of NG tubes blocking:

"It's usually if someone's on lots of medication, they may have to just be crushed where we can't get a liquid form, then they've not been flushed properly, that's when we get the blockages" [NUR13]

Blockage of NG tubes was considered a problem by many of the nurses interviewed:

"They're a meter long, and thin, so blocking is always going to be a problem" [NUR01]

Theories regarding the cause of NG tube blockage ranged from lack of correct flushing, medication residue, medication reactions, and feed solidifying:

"It's ensuring that when the feed, if their patient's having a feed, that the NG tube's flushed properly with water to keep the tube clear. Because the, um, feed can, like, solidify" [NUR19]

Another nurse controversially suggested the main cause of NG tube blockages is nurses themselves:

"We know that nurses are the ones that block the tubes, based on the fact that we have patients at home with NGs, and they very rarely block their tubes, and the X factor is that they don't have a nurse looking after it, they look after

their own. So they do it as they're taught to do it, by us, and they do it properly" [NUR18]

Competing priorities was considered an issue that could promote tube blockage:

"Our food finishes (…) we stop the pump because it's making a noise, and we say we'll be back in a minute. And a minute in hospital time can be anything from a minute to 2 to 3 hours. You know, we've all done it, not deliberately, but we went to get a syringe, and you've got called because there's an arrest, or you've got to get somebody to the toilet, or you have to answer the phone, or you've got to find something for the doctor or there's something else going on and it completely slips your mind. When you go back to flush the tube you've got the potential that it could be blocked especially if people have done that multiple times" [NUR18]

This opinion was supported by another nurse, who explained

"*The pump finishes, it's alarming, but the nurses are doing six thousand other things, and that's not a priority (…) it's not necessarily that they don't know, it's just that they don't get round to doing it, because they're prioritising their care" [NUR01]*

Essentially, nurses report that they endeavour to avoid blockages and maintain the patency of NG tubes in order to provide their patients with the means to receive nutrition, hydration and medication, and to save them the discomfort and inconvenience of undergoing tube replacement:

"No, it's not a rare occurrence at all. And we're all trying to make sure they don't block, because we then have to put another one down, which is uncomfortable for the patient" [NUR16]

To gain an overview of nurses' knowledge of biofilm and possibly its implications to health care, the question was asked "*Do you know what biofilm is*?" The majority of nurses interviewed did not know what it was, with comments such as:

"No, no, never heard of it" [NUR17]

Other nurses thought it may be some kind of wound dressing:

"Well I think it's a new dressing" [NUR15]

"Is it like Bioclusive?" [NUR19]

However, one nurse knew exactly what is was, and was able to relate it to NG tube management:

"Well, just from the point of view of, um, biofilms in PICC lines and Hickman lines, and things, so you get a build-up of, uh, on the inside of the tubes, and, uh, bacterial growth and all sorts of things, and we use things like TauroLock™ (Antimicrobial catheter lock system) and things in IV lines to try and help stop that coating on the inside of the tube, and kill bugs and things. But, I assume like, anything furs up 'cos we're putting stuff through a tube that you, you would get bacterial and a, a food build-up within the, within the tube itself. But then they're bugs that live in your system, so they're not necessarily harmful bugs, I mean that's how the whole sterile water, non-sterile water, debate goes along isn't it, you know, and acid barriers in stomachs and all that kind of stuff" [NUR18]

Restoring patency to blocked NG tubes was discussed during the interviews, and the nurses freely talked about the methods they had used or had heard about others using. A senior nurse stated:

"Our recommendations are, is get a 20 ml syringe and half fill it with warm water, obviously not boiling, just warm, and put it on the end of the tube (…) and then you're doing an agitated flush, you're doing it as a push-pull, so that you've got half air and half water in the syringe (…) We've tried to explain to them it's actually the, the agitated force, that by push and then pull, push pull, of that water and air in the syringe chips away at the blockage, to then all of a sudden you start getting murky water back in the tube, in the water, in the fluid, so you're starting to break down and you get debris. Then you can clear out your syringe and half fill it with some fresh water if you've got lots of debris, and sometimes you can get chunks of food or medicine out, and then all of a sudden it'll go, and then give it a nice big flush afterwards" [NUR18]

Unblocking of NG tubes was considered a difficult procedure for nurses on the wards, and several methods were employed to try to restore tube patency. These included the use of carbonated beverages, bicarbonate of soda, and fruit juices, which the senior nurses considered inappropriate:

"And then they go on and say 'well I've tried Coca Cola, I've tried bicarbonate and water, I've tried pineapple juice', of which all of those things just make a sticky mess of you and your patient and your tube, they don't actually do anything" [NUR18]

Coca Cola was mentioned several times during the interviews, as a possible irrigant for dislodging blockages:

"If they were blocked (…) we used Coke (cola) to unblock them" [NUR09]

"You just flush with the, what is it, coke?" [NUR20].

But one nurse advised this method was in fact no longer an option:

"Back in the old days, people would do things with fizzy drinks, and we're not allowed to do that anymore. Um, I don't think it was particularly effective anyway" [NUR11]

When caring for patients with NG tubes in situ, nurses have to be aware of their patients' movements to prevent blockages, as some have experienced patients stopping their own feeds to leave the ward:

"You have to be really alert and vigilant of what's going on with your patient if they're being NG fed. We have had incidences of people having their feed stopped and then the tube blocks" [NUR11]

Certainly the impression given by the participants during their interviews was one of genuine concern for optimal patient care, with a clear understanding of the role correct NG tube management has in this. One nurse skillfully yet simply summed up the feelings of many nurses when considering the value of an NG tube:

"It's a precious tube" [NUR18].

9.9 **Discussion**

This chapter has described a qualitative interview study undertaken at a local NHS acute care hospital. It was designed with the aim of gaining the current nursing perspective of NG tube management. The objectives were to explore the beliefs and

reported practices of nurses concerning the management of NG tubes on acute care wards. The literature search conducted at the start of the study indicated there was little published information available concerning nurses' beliefs and practices of NG tube management from the nursing perspective. Of the four studies identified, two were initially considered primarily of historical interest due to their year of publication (Mateo, 1996; Cannaby *et al*, 2002).

Nurses were recruited into the study from four specially selected wards using purposive sampling. The wards were selected due to the differing fields of practice they offered, providing acute care to patients with a number of conditions, both medical and surgical. The outcome was that the nurses recruited came from different backgrounds and specialities, which would indicate a more representative model of current NG tube management. The interviews were conducted between October and December 2015, and were stopped at the point of data saturation. This was considered to have been achieved when no new data or themes were emerging during the interview discussions, and required the full sample of 20 nurses to be interviewed (Mason, 2010; Fusch & Ness, 2015). A semi-structured approach to interviewing was taken using an interview guide to ensure topics noted in background reading were discussed. As the researcher was a novice interviewer, this tool proved very helpful in maintaining the flow and direction of the interviews. The longest interview took 29 minutes, the shortest 6 minutes (mean 12 minutes). The original plan had been to interview each nurse for 30 minutes. However, during the University ethics review, the interview guide was considered too lengthy and would have taken one hour to complete. The guide was therefore halved with the view that the interviews should take approximately 30 minutes to complete. Unfortunately they took much less, which can be attributed to the fact the researcher is a novice interviewer. Perhaps with experience the interviews could have been extended by using more prompt questions.

All interviews were fully completed with no breaks or stoppages, and all were audio recorded for later transcription. Transcription was completed by the researcher, which proved to be a lengthy and challenging process. A professional transcription service could have been employed to complete the process, however, the act of repeatedly listening to the audio recordings and typing the transcripts lead to a deeper understanding of the nurses' beliefs and reported practices than would have otherwise transpired. If another qualitative study is undertaken by the researcher, video rather than audio recording may be used. It was unpredicted how often nurses would gesture their meaning of words, and several times they had to be asked to say what they meant

for the benefit of the audio recording. Also, it may be easier to transcribe from videos when interviewing non-UK participants. This study included participants from Spain, Portugal and the Philippines, as well as the UK. Although not difficult to understand during animated conversation, it was sometimes challenging to understand from audio recordings alone.

Previous studies had investigated enteral tube management using a descriptive survey design with questionnaires ranging from 35 to 85 questions (Mateo, 1996; Cannaby *et al*, 2002; Phillips & Endacott, 2011; Al Kalaldeh *et al*, 2013). The method of confidential interviewing was selected for this current study to encourage nurses to speak freely and openly of their experiences of NG tube management. This helped to build a picture of current practice which included aspects of care that perhaps would not have been covered in a questionnaire. Many of the nurses' beliefs and reported practices were reassuring, and it was evident how much the nurses interviewed cared for their patients, acting as advocates for them and putting their best interests first. Patient safety and comfort were important factors to those taking part, particularly regarding NG tube insertion and replacement. One aspect of concern was the prevalence of patients self-removing their tubes. Nurses felt this was due to confusion as a result of experiencing a stroke or dementia. With a progressively ageing population in the UK, this may be set to become a more frequent issue.

With regard to checking NG tube position, the British-based study by Cannaby *et al* (2002) reported the majority of nurses questioned tested the pH level of gastric aspirate. This method is still recommended today, with NICE (2012) and NNNG (2016) stating a pH of 5.5 or lower must be recorded using pH paper. However, local guidelines recommend achieving a stricter pH level of 4.5 or lower, using CE marked pH indicator strips, at least once daily (PHT, 2013). The auscultation method of checking NG tube position is frequently used in Australia and Jordan (Phillips & Endacott, 2011; Al Kalaldeh *et al*, 2013). However, this method is no longer recommended for use in the UK due to safety concerns (NPSA, 2009).

There appears to have been little change to the provision of training and education regarding NG tube management in the past 14 years. Training and education was provided by Nutrition Nurse Specialists in Cannaby *et al's* (2002) study, which is similar to the reported training resource on today's wards. Indeed, NICE (2012) calls for Nutrition Nurse Specialists to provide optimal ward-based training for nurses. Modern wards also use cascade training to improve their skill base.

All nurses interviewed for this study acknowledged the importance of flushing although the reported frequency and amount of fluid used did vary. Tap water was the predominant irrigant, with occasional use of sterile water if a patient is at risk of infection. Most nurses flush before and after a feed, with 20 to 50 ml of water, which is similar to local guidelines, which suggests 30 to 50 ml of tap water before and after a feed and at least every 4 to 6 hours. Mateo (1996), the oldest of the literature review studies, reported their participants also flushed tubes before and after a feed and every 4 hours which would suggest, 20 years later, this method is still considered the most effective on today's wards. Local guidelines also recommend not using sterile water, as the pH is 4.5 which can lead to a false positive result when checking tube position (PHT, 2013).

Historically, medication was reported to be crushed in a pestle and mortar for preparation prior to administering through NG tubes (Phillips & Endacott, 2011). This was followed with a flush of 10 to 30 ml of water (Mateo, 1996; Phillips & Endacott, 2011; Al Kalaldeh *et al*, 2013), with some nurses flushing before and between medication administration. Currently, the pestle and mortar is still considered the most effective way of crushing medication provided in tablet form, where liquid alternatives are unavailable. Nurses reported flushing before (5 to 30 ml), between (10 to 20 ml) and after medications (10 to 30 ml) with tap water, which is in line with current local guidelines (PHT, 2015). Nurses reported medication as one of the main causes of NG tube blockage, with solidifying of enteral feed also mentioned as a possibility. User error was also noted, where nurses do not flush the NG tubes within the optimal time due to competing priorities on busy modern wards.

Irrigation of NG tubes was considered the best method of clearing a blockage (Mateo, 1996; Cannaby *et al*, 2002; Phillips & Endacott, 2011; Al Kalaldeh *et al*, 2013), and still is the method of choice today. Nurses have reported using a variety of irrigants including sterile water, carbonated water, hot water, tap water, dissolved papain, cola, bicarbonate of soda and fruit juices. Each agreed on the method of an agitated flush using a pumping action. Current local guidelines recommend 10 ml of tap water in a 20 ml syringe (PHT, 2015), with national guidelines recommending 15 to 30 ml warm water or carbonated water in a 60 ml syringe (NNNG, 2016). Drinks containing sugar or saccharin such as cola or fruit juices are not recommended for irrigation as they could precipitate further blockages (NNNG, 2016).

Whilst this study has expanded the understanding of NG tube management in the current and historical context, it has several limitations. The study design, interviews

and data analysis were conducted by the same practitioner. Being close or familiar with the research setting and its participants can lead to practitioner researchers anticipating results or actions or behaving in a way that may interfere with the natural course of a study (Kent, 2015). It was therefore imperative to remain as objective as possible throughout the research process. However, the data analysis was approached with knowledge gained through completing the interviews and transcribing the audio recordings, which could have led to certain *a priori* beliefs affecting the analysis. Measures were taken to reduce this effect as a random sample of transcribed interviews and associated audio recordings were assessed for accuracy and content by an independent researcher.

In essence, despite the Mateo (1996) and Cannaby *et al* (2002) studies initially being considered for their historical interest, it would appear there has been little significant change to UK practice regarding NG tube management since these studies were published. There have been changes to tube placement and checking of tube position in response to safety alerts (NPSA, 2009), but essentially the practice of using and caring for NG tubes has remained very similar. The findings of this research study, along with the enteral tube feeding guidelines produced both locally and nationally (ASPEN, 2009; PHT, 2013; PHT, 2015; BAPEN, 2016; NNNG, 2016), can become part of the knowledge inquiry stage of the Knowledge Creation Process (Graham *et al*, 2006), and be synthesised with the findings of the laboratory-based studies with a view to improving NG tube management further. It is believed that a greater understanding of the correct care for NG tubes and the rationale behind that care, along with a more uniform approach to NG tube management, will lead to a reduction in the number of tube blockages and associated complications.

9.10 **Conclusion**

This study sought to gain the nursing perspective of an aspect of nursing care through exploring nurses' beliefs and reported practices regarding NG tube management. It achieved its aim through the use of a qualitative design, conducting one-to-one semi-structured confidential interviews with acute hospital based nurses. The findings of the study support those of historical interest, and are presented using the nurses' own words through the use of quotations to illustrate and support each aspect. Essentially, little has changed in the past 20 years regarding using and caring for NG tubes,

although there have been improvements in the areas of NG tube placement and position checking in response to national patient safety alerts. The findings of this study can now be amalgamated with the findings of the laboratory-based studies with a view to improving NG tube management and the understanding of the rationale behind it.

Chapter 10: Discussion and Conclusion

10.1 Introduction

This concluding chapter initially reviews the context of enteral feeding by NG tube that underpinned the design of the studies in this thesis. It then draws together the findings and conclusions of each of the seven studies undertaken, with a review of the research questions posed. A critique of the studies undertaken and the limitations follows, along with an overview of the contribution made to the current body of knowledge and the implications for clinical practice. The chapter concludes with suggestions for future research and planned publications.

10.2 Background

Up to 34% of patients admitted to hospital are at risk of malnutrition, and in particular those patients with chronic progressive conditions such as dementia (BAPEN, 2016). The cost of malnutrition in the UK is estimated to be in excess of £19 billion and is set to rise further in response to the ageing population (NICE, 2012; NHS England, 2015; NIHR, 2015). In the clinical environment it is imperative patients receive optimal nutrition, hydration and medication in a timely manner. For patients who are unable to meet their nutritional intake orally, but who have a functioning digestive tract, NG tubes are often used for short-term enteral feeding either wholly or as a supplement to limited oral intake.

NG tubes are indicated for patients with a wide range of conditions. Therefore, any risk to the patency of NG tubes can potentially affect a large patient group. Previous research has suggested up to 35 % of all NG tubes in use become blocked (Marcuard & Stegall, 1990; Bourgault *et al*, 2003). In addition to the distress and discomfort the possibility of replacing NG tubes can cause patients, there are financial implications for the NHS in terms of nursing resources to attempt to unblock NG tubes, along with potential tube replacement and position determination costs. However, limited empirical evidence is available investigating the causes of NG tube blockages or the optimal method of maintaining their patency to prevent blockage occurring.

Biofilm can develop on and within indwelling devices such as medical stents and prostheses, and can interrupt the flow of fluid through tube lumen. Published research indicates biofilm can attach to NG tubes (Leibovitz *et al*, 2003; Leibovitz *et al*, 2005; Kim *et al*, 2006; Hurrell *et al*, 2009a; Hurrell *et al*, 2009b; Lima *et al*, 2011). However, biofilm development on the inner surface of NG tubes used by adults has not been investigated. It is important to investigate bacterial attachment and biofilm development in NG tubes used by adults, particularly to establish the presence of biofilm on the inner surface, in order to explore the potential for biofilm to be a trigger for NG tube blockage.

This thesis reports a series of interlinked laboratory based studies investigating the presence and distribution of biofilm in NG tubes used by adults, and an exploration of the beliefs and practices of registered nurses of NG tube management. The preliminary study of two NG tubes used by adults established the presence of biofilm on the inner surface of the tubes. The next study showed the bacterial attachment and biofilm development occurs rapidly in NG tubes over a 24-hour period. A subsequent study demonstrated bacterial attachment and biofilm development was reduced by flushing NG tubes with sterile water or tap water compared with a no-flush control. One study clearly showed the nature of enteral feed can be altered when bacteria is present within the NG tubes which could potentially lead to blockage. The findings from the *in vitro* studies showed bacterial attachment and biofilm development as possible mechanisms for NG tube blockage. The final laboratory study examining NG tubes removed from adult patients showed that bacterial attachment and biofilm development occurred *in vivo*. An exploration of the beliefs and practices of nurses concerning NG tube management showed that registered nurses consider tube blockage to be caused by medication and feed, and maintain patency by regularly flushing the tubes with water.

10.3 **Gaps in the research**

The literature review in Chapter 2 indicated that the published research associating bacteria with NG tubes is concerned with the potential infection risk bacterial colonisation might pose. There is a paucity of research investigating biofilm development within NG tubes used by adults. The studies reported in this thesis focus on establishing whether bacterial colonisation, and in particular biofilm, exists within NG tubes used by adults, and what factors may influence its development with a view to undertaking future research establishing a link between biofilm and NG tube

blockage. Although the purpose and expected outcomes of the studies critiqued in the literature review differ to the studies undertaken for this thesis, the knowledge gained from previous research, and the methods used, have supported and informed this research project. The literature review and other reading undertaken relating to bacterial presence in NG tubes to inform the thesis, enabled gaps within the research to be identified. The studies in this thesis were planned to address some of the gaps in evidence identified, and increase our understanding of the nature of bacterial attachment and biofilm development in NG tubes used by adults. In order to address some of the gaps in the literature it was necessary to undertake a series of studies with specific aims. The studies within this thesis aimed to:

- Establish the presence and distribution of biofilm within NG tubes used by adults
- Establish the time frame for bacterial attachment and biofilm development on polyurethane NG tubes
- Determine the influence biofilm can have on its environment by investigating the pH of enteral feed inoculated with bacteria over a 24 hour period
- Compare bacterial attachment and biofilm development on the inner surface of sterile NG tubes flushed with either sterile water or tap water
- Compare bacterial attachment and biofilm development on the inner surface of inoculated NG tubes flushed with either sterile water or tap water, along with the control option of not flushing
- Investigate the presence and nature of substances, including biofilm and medication residue, within NG tubes retrieved from adult patients
- Explore the potential for patient variables including gender, age, duration of tube placement, medications given via the NG tube and feed regimen to contribute to biofilm development
- Explore the nursing perspective of NG tube management and the maintenance of NG tube patency

The main research question of this thesis was ‘Does biofilm develop on the inner surface of fine bore NG tubes used by adults?’ The purpose of this thesis was to determine the presence and distribution of biofilm on the inner surface of fine bore NG tubes used by adults. To set the findings in the context of clinical practice one study

aimed to gain an understanding of the nursing perspective of NG tube management and the maintenance of patency in a ward environment.

10.4 **Methods Development**

Previously published studies regarding bacteria and NG tubes were primarily concerned with the infection risk bacteria could pose to patients fed by NG tube. The research undertaken for this thesis was concerned with the possibility of biofilm being present within NG tubes used by adults, and aimed to develop an understanding of the biodynamics of biofilm in connection with NG tubes, with a view to future research investigating its potential role in NG tube blockage. Throughout the laboratory studies, a key objective was to source and develop suitable laboratory methods to effectively examine, remove and quantify attached bacteria and biofilm from NG tubing. The initial studies were guided by the laboratory methods used in the studies identified in the literature review in Chapter 2, along with background reading into laboratory techniques. Throughout each of the separate laboratory studies the methods used were constantly reviewed and improved where necessary to achieve the specified aims and objectives, and to improve the quality of subsequent studies.

The first laboratory study sought to establish the presence of biofilm within NG tubes used by adults, for which an effective microscopy method was required. Rather than use the SEM or CLSM methods detailed in the literature review studies, which each require intensive sample preparation, the preferred method employed was EDIC microscopy. This was more appropriate to the aims and objectives of the study as it allows detailed visualisation of biofilms on opaque and curved surfaces, with minimal sample preparation, making it ideal for examining the inner surface of NG tubes. A review of the literature indicates this is the first incidence of using EDIC microscopy to visualise biofilm on NG tubing. Although SEM and CLSM achieve excellent detailed images, EDIC microscopy enables the study of biofilm in its hydrated state, providing the opportunity to gain realistic information and an understanding of its structure and its interaction with the surface of NG tube material.

An effective method for the removal of attached bacteria and biofilm from the used NG tubes obtained for the first study was required. This was initially attempted by swabbing the inner tube surface using cotton buds, which were then rinsed in sterile water to suspend the bacteria. However, on examination using bacterial staining it was evident

a significant quantity of bacteria remained attached to the NG tube. A more effective method was required, therefore a technique similar to that used by Kim et al (2006) and Hurrell et al (2009a) was employed whereby the NG tube samples were placed in ddH2O with glass beads and vortexed to suspend the bacteria. This method proved more effective than the swabbing method, and was therefore employed throughout the laboratory studies. Also used throughout the studies was the method of aerobic incubation of aliquots of suspended bacteria plated on to TSA, following investigation to compare the outcome of aerobic with anaerobic incubation of bacteria isolated from used NG tubes. This was completed as it was unknown what pathogens were present within NG tubes used by adults, and therefore whether these would multiply most effectively with or without oxygen. Aerobic incubation was found to be appropriate and was adopted. The bacterial colonies that formed on the incubated TSA plates were counted to give an estimate of the levels of bacteria present on the NG tube samples. The accuracy of the CFU method depends greatly on use of the correct plate spread technique. To reduce the effect of this limitation, each plate was repeated in triplicate, with the mean count for each set considered.

The next study set out to understand the biodynamics of biofilm by examining in detail bacterial attachment and biofilm development on NG tubes in the first 24 hours following inoculation. The literature review suggested this was the first time bacteria had been investigated in this context within this time period. The methods for this study included the use of six-well plates to contain each of the inoculated NG tube samples within similar environmental conditions, as had been successfully used by Gião et al (2011) in earlier biofilm research. A limitation of this was that the feed and bacteria remained static throughout the investigation, which did not reflect the realistic situation of an NG tube in use, with feed and potential planktonic bacteria passing through. This matter was later addressed in a study in which a flow model was used to replicate NG tubes in use.

During the time-frame study a further bacteria counting method was explored in addition to CFU counting, which compared the use of DAPI with SYTO 9 to highlight cells under EF microscopy for individual identification. Unfortunately, the autofluorescence produced was too great to identify accurately individual cells for counting, therefore this method was disregarded. However, a serious limitation of using the CFU method of counting in isolation is that only cells able to form colonies under the conditions of each study are included. Although CFU counting is an acceptable method to use, VBNC bacteria are rarely considered in microbiological assessments

and yet including them in the final counts could arguably give a more accurate account of the levels of colonisation, and thus provide a more thorough dataset. This was achieved by introducing direct viable counting through cell elongation. The elongated cells were stained with SYTO 9 and counted following filtration through Nuclepore membranes. This method has not been used alongside CFU counting in this context before, but has been used effectively in earlier research investigating biofilm within drinking water systems (Gião et al, 2011). When contrasted with the CFU counts, and the visual analyses achieved from the EDIC images of the NG tube samples, a clear picture of bacterial colonisation and biofilm development could be achieved for each time point studied.

Perhaps the most valuable study undertaken in terms of future research exploring how bacteria could potentially play a role in NG blockage was the pH study. Previous research has investigated the action of acid environments on enteral feed, such as gastric acid causing feed coagulation. The pH study in this thesis uniquely investigated whether bacteria that could be present within NG tubes can affect the pH of the enteral feed that would pass through it. By using E. coli, P. aeruginosa and the NG tube isolated bacteria to conduct this study it was possible to demonstrate clear differences in the outcome of the pH levels at set time periods dependent on which bacteria were present. The outcomes of the E. coli and NG tube isolate inoculated feed samples were very similar, with marked pH drop resulting in feed thickening from 240 minutes, while the P. aeruginosa inoculated and control feed samples remained fluid throughout. This study has provided the basis for future investigation aiming to establish the role bacteria may play in the blockage of NG tubes, through the action bacteria present in NG tubes can have on enteral feed.

The methods used up to this point in the thesis observed bacteria and enteral feed in a static state. However, in order to meet the need for more realistic studies regarding NG tube use, the method used to investigate the use of sterile water and tap water for flushing NG tubes employed a laboratory flow model. This was developed to recreate the NG tube in human use by passing feed and irrigants through NG tubes at similar times and flow rates as their in vivo use, and was based on the apparatus used by Lima et al (2011). In their laboratory study, Lima and colleagues investigated the ability of bacteria to attach to different types of NG tubing, whilst this study was the first to use a similar apparatus set up to investigate and compare sterile water with tap water flushes. Despite ongoing debate between nurses regarding the use of sterile water to flush NG tubes in preference to tap water, little investigation has been conducted to establish a definitive outcome.

The final study employed laboratory methods proven successful in the previous studies, and was able to confirm that there was no effect seen in bacterial colonisation of NG tubes in relation to the patient variables studied. The results of the pH testing aspect of this study were remarkably similar to those achieved in the first pH study, indicating this is a reliable method of investigation for use in future studies regarding the role bacteria may play in the blockage of NG tubes.

The use of in vitro laboratory studies within this thesis enabled known variables to be controlled throughout, ensuring the results achieved could be attributed to the intervention undertaken. One method used throughout the laboratory studies was that which looked at the bacterial count for three anatomical points of each complete NG tube studied. This was to establish whether bacteria, and thus biofilm, were greater proximally or distally to the point of introduction. The idea was that by establishing this, it would be possible to pinpoint the route of bacterial access in NG tubes used in vivo, with a view to reducing such access. Unfortunately, the results of each of the studies were inconclusive, and therefore this method of investigation would not be repeated in future planned research.

Throughout each of the laboratory studies within this thesis many methods have be employed in order to achieve the planned aims and objectives. What has been learned is that not all laboratory methods are effective in every situation, and a flexible and adaptable approach was needed to reach the desired end point. Planning is essential, but it can be counterproductive to rigidly adhere to those plans where they become impracticable. The laboratory methods used throughout the studies have combined to show biofilm is present on the inner surface of NG tubes used by adults, and more has been learned regarding factors that influence its development. This knowledge will be used in future investigation to establish whether biofilm is integral to NG tube blockage.

Below is a summary of the advances achieved in the methods used throughout this thesis:

- The use of advanced but non-destructive direct microscopy (EDIC) alongside CFU counts to develop a detailed picture of bacterial colonisation of NG tubes
- The use of cell elongation to understand and include VBNC bacteria populations alongside those growing in culture media
- The use of a flow model to replicate the administration of enteral feed and irrigation flushes through NG tubes

- The use of appropriate enteral feed medium to investigate the effect bacterial presence can have on the pH of feed as opposed to the use of regular bacterial culture media

10.5 Key findings of the studies

The opening study in this thesis established the presence and distribution of biofilm on the inner surface of fine bore NG tubes used by adults. The literature review reported in Chapter 2 suggested this is the first time that this has been reported. The study presented the opportunity to review and develop suitable laboratory methods to effectively examine, remove and quantify attached bacteria and biofilm from the NG tubes' inner surface. Laboratory methods from published studies reported in Chapter 2 guided those used in this study, along with the innovative EDIC/EF microscopy methods employed to directly visualise the inner surface of the used NG tube and the bacteria found to be present. The laboratory methods that were successful for this study were employed for the subsequent laboratory studies.

Previous research regarding bacterial attachment on NG tubes discussed in the literature review had established biofilm presence on the outer surface of NG tubes used by adults (Leibovitz *et al*, 2005), and on the outer surface of NG tubes *in vitro* (Kim *et al*, 2006; Hurrell *et al*, 2009b). Lima *et al* (2011) demonstrated the presence of biofilm in NG tubes *in vitro,* whilst Hurrell *et al* (2009a) noted biofilm in NG tubes retrieved from neonates. The opening study in this thesis demonstrated the presence and distribution of biofilm on the inner surface of fine bore NG tubes used by adults *in vivo*.

The second study established the time course of bacterial attachment and biofilm development on NG tube sections *in vitro*. Both Leibovitz *et al* (2005) and Hurrell *et al* (2009b) had previously demonstrated that biofilm is present on NG feeding tubes at the 24 hour time point *in vivo* and *in vitro* respectively. This study determined bacterial attachment and biofilm development over a 24 hour period, and consequently a greater understanding of the properties of bacterial attachment and biofilm development in NG tubes. Bacterial colonisation levels were quantified at specified time points within the first 24 hours following inoculation with either *E. coli* or *P. aeruginosa*. These pathogens were specifically selected as they form part of the human microbiota and are therefore present in the hospital environment (Leibovitz *et al*, 2003; Leibovitz *et al*,

2005), and both are known to be producers of biofilm (Costerton *et al*, 1995, Solseng *et al*, 2008).

The study demonstrated bacteria attached within 15 minutes of introduction, the mean bacteria count per cm^2 of NG tube increased exponentially after 60 minutes of introduction, and biofilm was established at 24 hours, adding to the findings published by Leibovitz *et al* (2005) and Hurrell *et al* (2009b). The literature review noted in Chapter 2 indicates that this is the first reported finding of bacterial attachment to NG tube material in the first 24-hour period following introduction of bacteria.

The next study aimed to establish whether bacteria attached to the inner surface of NG tubes used by adults could influence the pH of the enteral feed that passes through it. Inocula used in the earlier time frame study thickened to a yogurt-like consistency following incubation at 37 °C from as little as 240 minutes. Earlier published studies reported that feed formula coagulated when exposed to gastric acid (Gaither *et al* 2009; Marcus *et al,* 2010), whilst Von Ohle *et al's* (2010) research into dental biofilm established its ability to markedly increase the acidity of its local environment.

Samples of enteral feed inoculated with *E. coli, P. aeruginosa* or bacteria isolated from a used NG tube, incubated at 37 °C, and monitored at set times of 0, 15, 30, 60, 120, 240, 360 and 1440 minutes demonstrated a statistically significant ($p = .0005$) increase in acidity over the time period studied. Enteral feed was found to thicken from the 240 minute time point. The *P. aeruginosa* inoculated samples and control samples that were not inoculated all remained fluid and maintained relatively stable pH levels throughout.

The findings suggest the *E. coli* and NG tube isolate bacteria may trigger a fermentation process, specifically a mixed-acid fermentation, using the glucose substrate in the enteral feed through glycolysis. End products of this process include lactate, ethanol, acetate, carbon dioxide and hydrogen. Conversely, *P. aeruginosa*'s preferred metabolism is respiration, which produces none of the acidic end products seen in the fermentation process. This study demonstrated the influence bacteria found in NG tubes used by adults can have on enteral feed, and adds to the rationale supporting the frequent flushing of NG tubes with water after feed to prevent tube blockage (Gaither *et al*, 2009; Dandeles & Lodolce, 2011).

Chapter 6 reported a study investigating bacterial attachment and biofilm development on the inner surface of NG tubes flushed with either sterile water or tap water, using a

laboratory flow model apparatus designed to recreate the NG tube in use. This study was in response to the practice of flushing NG tubes used by adults with sterile water in preference to tap water, and sought to establish evidence of tap water introducing significant levels of bacteria to NG tubes on routine flushing. The study was able to confirm tap water did not introduce a statistically significant level of bacteria to the NG tubes over the nine-hour period of each study, by quantifying the bacterial colonisation of the inner surface of the tubes. This study indicated no benefit to using sterile water in preference to tap water to flush fine bore NG tubes in reduced bacterial attachment and biofilm development on the inner surface of NG tubes.

This work was extended to investigate the efficacy of flushing inoculated NG tubes with either sterile water or tap water, along with the option of not flushing, on the levels of bacterial colonisation of the inner surface of the NG tubes. Again, this was in response to the contentious issue among nurses of flushing NG tubes with sterile water in preference to tap water, with no empirical studies identified that could support this practice. Levels of bacteria were quantified using CE and CFU methods, following the nine-hour period of each study. Using these methods, no statistically significant difference appeared between the three flushing options. However, qualitative data gained from EDIC microscopy of the inner surface of the inoculated and flushed NG tubes indicated flushing with either water option was preferable to not flushing. Again, this study demonstrated no discernable benefit, in terms of reducing bacterial attachment and biofilm development, to using sterile water in preference to tap water to flush fine bore NG tubes.

The final laboratory study investigated 10 NG tubes removed from patients in the course of usual clinical practice. Earlier published research indicated medication passed through NG tubes could cause tube blockage (Gaither *et al*, 2009; Lonergan *et al*, 2010; Dandeles & Lodolce, 2011), and Hurrell *et al* (2009b) noted patient's age can have a statistically significant effect on mean bacterial counts in their study of NG tubes used by neonates.

This study demonstrated no statistically significant difference in bacterial counts in relation to patient age, gender, feed regimen or duration of tube placement. Each of the tubes was examined for substances including medication residue. Nothing of note was found in the seven tubes that had been used to administer medication. However, the three tubes that had not had medication passed through them each contained crystal-like structures which could have potentially been feed deposits. One of the three tubes

was reported to have been blocked, and was noted to contain feed and high levels of bacteria. Bacterial isolates from the tubes caused the pH level of enteral feed samples to increase in acidity, mirroring the pH study reported in Chapter 5, and supporting the finding that bacteria present on the inner surface of NG tubes could potentially influence the pH level of enteral feed causing coagulation.

During the laboratory studies, 1 cm sections from complete NG tubes were removed at the nasal, oesophageal and gastric anatomical points of the tubes, with the aim of establishing whether bacteria colonise a particular region of the tubes in response to which end the bacteria is introduced. An understanding of this would lead to the ability to state whether the bacteria found within used NG tubes retrieved from adults had entered from the patient's stomach, or whether it was introduced from the administration port end. By understanding the route of bacterial access to NG tubes, measures could potentially be taken to reduce bacterial colonisation. However, the studies identified no statistically significant results to reach a conclusion on this aspect.

The final study in this thesis looked to gain the nursing perspective of caring for NG tubes by exploring nurses' beliefs and reported practices of NG tube management to contextualise the laboratory findings and suggest how they can be used to inform practice. A review of relevant published literature regarding nurses' management of NG tubes showed reports of contemporary practice are limited, and what was available suggested considerable variation in nursing practices (Mateo, 1996; Cannaby *et al*, 2002; Phillips & Endacott, 2011; Al Kalaldeh *et al*, 2013). Three main themes arose when nurses were asked to discuss their practice and beliefs. Nurses associated NG tube management with "looking after the patient" and described their practice as "using the NG tube" and "stopping the NG tube blocking".

All nurses interviewed acknowledged the importance of flushing NG tubes and predominantly used tap water, with occasional use of sterile water with immunocompromised patients. The frequency and volume of the water flushes varied although not markedly from historical literature of 20 years ago, and all nurses interviewed understood the need for flushing when administering medications via NG tube. Flushing with water was noted to be the best method of clearing blocked tubes, with several nurses stating medication or solidified enteral feed was responsible for many of the blockages they had experienced. None of the nurses interviewed stated they believed bacteria plays a role in NG tube blockages. Essentially, little has changed

regarding NG tube management in the UK in the past 20 years, although there are improvements in the areas of tube placement and position checking before use in response to patient safety alerts.

10.6 Contribution to knowledge

The purpose of this thesis was to determine the presence of biofilm on the inner surface of fine bore NG tubes used by adults, and to investigate its development and distribution in detail. In support of this, it was important to gain an understanding of the nursing perspective of NG tube management and the maintenance of NG tube patency. Research sets out to find answers to questions, develop new theories and contribute to the evidence base of knowledge. By completing the interlinked laboratory studies and the qualitative interview study, this thesis has contributed to the evidence base of knowledge regarding bacterial attachment and biofilm development in connection with fine bore NG tubes used by adults by:

- Demonstrating the presence and distribution of biofilm on the inner surface of fine bore NG tubes used by adults
- Demonstrating bacteria are attached at 15 minutes following introduction, the mean bacteria count per cm^2 of NG tube increases exponentially after 60 minutes of introduction, and biofilm is established at 24 hours
- Demonstrating the influence bacteria found in NG tubes used by adults, and within the health care environment, can have on enteral feed, and adds to the rationale supporting the frequent flushing of NG tubes with water after feed to prevent tube blockage
- Indicating no discernable benefit to using sterile water in preference to tap water to flush fine bore NG tubes used by non-immunocompromised adults
- Establishing patient variables of age, gender, duration of tube placement, medications given via the NG tube and feed regimen do not influence the level of bacterial colonisation of the inner surface of fine bore NG tubes used by adults
- Establishing that little has changed regarding NG tube management in the UK in the past 20 years with regard to the maintenance of tube patency

10.7 Limitations of the research

This thesis marks the completion of a series of studies undertaken in order to establish an understanding of the bacterial colonisation of fine bore NG tubes used by adults. As such, it is the first study to investigate bacteria and biofilm in this context, and achieved the aim and objectives set out in Chapter 2. However, there have been limitations with the research.

Essentially, the body of research presented in this thesis consists of several small-scale studies completed by one researcher with limited resources as part of a PhD project. Each of the studies presented its own individual limitations, which were accounted for and improved where possible, to ensure the study design and outcomes achieved were robust. The laboratory studies would benefit from being repeated on a larger scale, with the studies reported in this thesis supporting them as feasibility studies. In order to gain the numbers required for larger scale investigation it may be necessary to undertake multi-site studies. An example of where this could be advantageous is the study presented in Chapter 8 investigating whether patient variables can influence the level of bacterial colonisation within NG tubes used by adults. This study aimed to recruit 50 patients but only achieved 10, markedly short of its target. This was due to the number of patients with NG tubes who were unable to provide valid informed consent due to a lack of mental capacity. This study could either be repeated at the same site without requesting personal patient information, and hence no longer requiring patients' valid informed consent, or it could be conducted in its original format at more than one site, thus increasing the opportunity to achieve greater recruitment success.

A disappointing limitation of the laboratory studies was that they were not able to determine whether the source of bacteria entering the NG tubes could be indicated by comparing mean bacterial counts for three anatomical points within the tubes. It was anticipated that bacterial colonisation would be greater closest to the point of introduction, but this proved inconclusive. This is clinically relevant as NG management is performed as a 'clean' procedure. If the source of bacteria could be established as having been introduced into the portal end of the tube, the research could inform infection prevention and control practices, and local and national guidelines on enteral feeding.

Limitations of the final qualitative study in this thesis include the fact that the study was designed, conducted and analysed by the same practitioner. This could have introduced an element of researcher bias. This effect was reduced by approaching the study objectively, and by arranging for a random sample of transcribed interviews and associated audio recordings to be assessed by an independent researcher, who also reviewed the analysis of the findings. This was the first qualitative study conducted by the researcher. A good deal of information was gained from the interviews with acute care nurses, although the duration of these were shorter than planned. This could have been helped by increasing the number of prompt questions used. This limitation has been recognised, and measures taken to improve future interview conduct.

10.8 Implications for clinical practice

This research was completed as part of a Clinical Academic Doctoral Fellowship, comprising roles in both nursing and academia. This role bridges the gap between nursing and academia, and helps to recognise and address research questions relevant to clinical practice, with a view to directly improving the care given to patients (NIHR, 2017).

Malnutrition is estimated to cost the NHS billions per year and is predicted to rise in response to an ageing population and increased incidence of progressive conditions such as cancer and dementia. NG tubes enable people who cannot to meet their nutritional needs to receive short-term enteral feeding, either wholly or as a supplement to oral intake. Any risk to the patency of these tubes can lead to delays in nutrition, hydration and medication, which in turn can lead to consequences such as delayed wound healing, lowered immunity response, and prolonged hospital stays.

This thesis marks the first step toward understanding the role biofilm may play in the reduced patency of NG tubes. The findings of the laboratory studies can be synthesised with the outcomes of the interview study with a view to improving NG tube management through a more uniformed approach. A greater understanding of the rationale behind NG tube management could be achieved by designing a training tool for nurses caring for NG tubes, which would aim to support the knowledge translation of characteristics of biofilm in relation to NG tubes.

Anecdotal evidence from nurses in clinical practice suggests medications passed through NG tubes are responsible for the majority of tube blockages. This research has suggested it is not just medications that cause tubes to block. The study of 10 used

tubes retrieved from adult patients at an acute care hospital demonstrated crystal-like deposits within two tubes not used for medication, and one tube used for feed alone was blocked. Conversely, the tubes used for administering up to 11 types of medication remained patent.

As was seen in the pH testing reported in the studies in Chapters 5 and 8, bacteria present in NG tubes could influence the acidity of its environment. The study of pH levels of inoculated enteral feed samples demonstrated levels dropped to ≤ 5.5 in 11 out of 13 individual experiments at the 24-hour time point. National guidelines suggest a pH of ≤ 5.5 on aspiration indicates the tube is positioned correctly in the stomach. However, these study findings suggest this may not always be the case, especially if the tube has not been flushed regularly. The study results were achieved in a laboratory setting with inoculated 3 ml samples of enteral feed. *In vivo,* the volume would be much less, and this may influence the timings of the drop in pH level. Further research is required in this area.

10.9 **Future research**

The literature reviews completed in Chapters 2 and 9 indicate there is little published literature relating to bacterial attachment and biofilm development in relation to NG tubes used by adults, and similarly limited research regarding optimal methods of NG tube management. Several gaps exist in the knowledge available, which this thesis set out to fulfil. In this respect, the thesis has helped to answer many of the points raised. However, in doing so, it has indicated further research in the area of NG tubes and biofilm would be valuable to progress the knowledge base. A number of studies are proposed:

- Determine the optimal flushing method for irrigating NG tubes – to include size of syringe, frequency of flushing, flush technique, volume of water for flushing
- Determine methods to reduce the entry of bacteria into NG tubes from both the gastric end via retrograde contamination and/or guidewire removal, or by the portal end through infection prevention and control practices
- Determine the role bacteria may play in NG tube blockage through in-depth pH testing of enteral feed, using a flow model apparatus with enteral feed passed into inoculated NG tubes, and tubes removed and prepared for pH testing of their contents at set time frames

- Professor C. Keevil at the University of Southampton has undertaken some interesting research into the use of copper for its anti-bacterial properties within health care environments. Further research could examine the use of copper-impregnated tubing to test its ability to restrict bacterial attachment and biofilm development, with a view to incorporating this material into NG tube production if successful (Noyce *et al*, 2005; Warnes *et al*, 2012)

10.10 **Presentations and planned publications**

Local, national and international presentations of the research in this thesis have been undertaken. The conferences attended are shown below, along with a list of planned publications.

Conference presentations:

- Post Graduate Researchers Conference, Southampton, 26 June 2013. Poster presentation: Biofilm and blockages: maintaining the patency of nasogastric feeding tubes
- Portsmouth Hospitals Trust Research and Innovation Conference, Portsmouth, 2 June 2015. Poster presentation: Maintaining the patency of nasogastric tubes in adults
- Post Graduate Researchers Conference, Southampton, 18 June 2015. Oral presentation: Investigating the role of bacteria in nasogastric tube blockages
- 2nd Digestive Disorders Federation Conference, London, 22-25 June 2015. Poster presentation: Investigating biofilm formation in the lumen of sterile nasogastric tubes
- National Nurses Nutrition Group Conference, Stratford-upon-Avon, 6-7 July 2015. Poster and oral presentation (Won 2nd prize): Investigating the role of bacteria in nasogastric tube blockages
- 37th ESPEN Congress, Lisbon, Portugal, 5-8 September 2015. Poster presentation: Nasogastric tube blockage: does biofilm play a role?
- Portsmouth Hospitals Trust Research and Innovation Conference, Portsmouth, 10 May 2017. Oral presentation: Investigating bacterial colonisation of nasogastric tubes used by adults

Planned publications:

- The presence and distribution of biofilm within nasogastric feeding tubes used by adults – Baker-Moffatt, Wilks, Green, Fader, Keevil
- Bacterial attachment and biofilm development in sterile NG tubes, and the effects of flushing with sterile water or tap water - Baker-Moffatt, Wilks, Fader, Keevil, Green
- Literature review – Baker-Moffatt
- The influence of bacteria on the pH of enteral feed – Baker-Moffatt, Wilks, Green
- Nurses' views of NG tube management – Baker-Moffatt, Green

10.11 **Conclusion**

This chapter has reviewed the background underpinning the design of the interlinked laboratory studies and the semi-structured interview study. It provides an overview of the findings from each of the studies completed, along with the contributions made to the current body of knowledge and the implications for clinical practice.

The research undertaken has achieved its aim of investigating the presence and distribution of biofilm within NG tubes used by adults, and of exploring the nursing perspective of NG tube management and the maintenance of patency, through their beliefs and reported practices. The literature review reported in Chapter 2 suggests this is the first time this has been investigated.

The plan going forward is to use the knowledge gained through completing each of the seven individual studies to investigate the potential role biofilm may play in the blockage of fine bore NG tubes used by adults.

Appendices

Appendix A: Risk Assessment for Microbiology Laboratory Work

Reasonably Foreseeable Hazards	Inherent Risk	X	Controls	Residual Risk	X
Working in multi-user ACDP Containment Level 2 laboratory	**Low**		All personnel to receive training in Containment Level work, have read and understood the Local Code of Practice (LCoP) and be supervised until deemed confident and competent in all procedures by senior member of staff. This includes wearing appropriate PPE (dedicated Howie style microbiological lab coat, safety glasses and nitrile gloves)	**Low**	X
	Med	X		**Med**	
	High			**High**	
Loss of bacterial material into the environment	**Low**		Use of PPE at all times. All tube preparations and manipulations will be completed in a Class II microbiological safety cabinet (MSC Class II) that has been prepared using 70% Ethanol. Any spillages will be decontaminated by soaking with 70% Ethanol and use of absorbent blue roll which is then disposed of via the GM/Clinical Waste Stream	**Low**	X
	Med	X		**Med**	
	High			**High**	
Personal contact with biological material	**Low**		Wear PPE at all times. Work only in an ACDP Class II laboratory and all material manipulations will be carried out in a MSC Class II. In case of skin or eye contact, flush area thoroughly (using hand-wash sink or emergency eye wash station) and follow procedures in LCoP	**Low**	X
	Med	X		**Med**	
	High			**High**	
Disposal of biological material	**Low**		Wear appropriate PPE. All material disposed of using GM/Clinical Waste Stream. Labelled waste containers (Disposafe jars and labelled bins) are used, with waste then being double-bagged in high temperature bags. These are loosely tied and autoclaved at 121 °C for 30 minutes (using ventilated autoclave) prior to off-site incineration. Refer to LCoP	**Low**	X
	Med	X		**Med**	
	High			**High**	
Bacterial material on non-disposable equipment (e.g. glass filter apparatus)	**Low**	X	Wear appropriate PPE. Equipment will be decontaminated, where possible, in a bench-top autoclave at 121 °C for 30 minutes. Non-autoclavable equipment will be surface decontaminated by wiping with 70% Ethanol	**Low**	X
	Med			**Med**	
	High			**High**	

Reasonably Foreseeable Hazards	Inherent Risk	X	Controls	Residual Risk	X
Preparation of sections of NG tubes – sharps risk	**Low**	**X**	Wear appropriate PPE. Work in MSC Class II. Use fine-tip scissors where possible and cut away from body while holding tube in forceps	**Low**	**X**
	Med			**Med**	
	High			**High**	
BacLight Live/Dead stain containing SYTO 9 and Propidium Iodide – potential irritants to skin, eyes and respiratory tract. May be harmful if swallowed	**Low**	**X**	Wear appropriate PPE. Only very small volumes are used (< 5 µl of each). If contact with skin or eyes follow emergency procedures detailed in LCoP (flush thoroughly or use emergency eye wash station)	**Low**	**X**
	Med			**Med**	
	High			**High**	
EDIC/EF microscopy – exposure to UV light	**Low**	**X**	Wear appropriate PPE. Only to be used by trained personnel. Use shutter to block UV light when not needed. Ensure UV shield is in place	**Low**	**X**
	Med			**Med**	
	High			**High**	
Use of autoclave – burn risk from high temperatures and steam under pressure. Electrical hazard. Contamination risk due to insufficient sterilisation	**Low**		Wear appropriate PPE, including face shield and heat resistant gloves. Only to be used by trained personnel. Annual P.A.T., insurance and validation testing. Allow contents to cool to °80C before opening. Use indicators to monitor sterilisation efficiency	**Low**	**X**
	Med	**X**		**Med**	
	High			**High**	

Reasonably Foreseeable Hazards	Inherent Risk	X	Controls	Residual Risk	X
Preparation of TSA solution from media powder – potential irritation on eye/skin contact, ingestion and inhalation (Contains Sodium Chloride)	**Low**	**X**	Wear appropriate PPE. Move powder container as little as possible. Measure powder quantity required with spatula, do NOT pour, to avoid generating airborne dust. In case of skin or eye contact, flush area thoroughly (using hand-wash sink or emergency eye wash station) and follow procedures in LCoP	**Low**	**X**
	Med			**Med**	
	High			**High**	
Use of 70% Ethanol spray – potential fire risk as liquid/vapour flammable. Potential irritation on eye/skin contact, ingestion and inhalation.	**Low**	**X**	Wear appropriate PPE. Eliminate sources of ignition. Use in well-ventilated area. Avoid breathing vapour. Avoid contact with eyes, skin, and clothing. In case of skin or eye contact, flush area thoroughly (using hand-wash sink or emergency eye wash station) and follow procedures in LCoP	**Low**	**X**
	Med			**Med**	
	High			**High**	
Preparation of microscope glass slides using glass coverslips – sharps risk	**Low**	**X**	Wear appropriate PPE. Care when handling glass slides and coverslips. Prepare slides at laboratory bench. Dispose of any damaged glass slides and coverslips into sharps bin only.	**Low**	**X**
	Med			**Med**	
	High			**High**	
Use of vortexing equipment	**Low**	**X**	Wear appropriate PPE. Only use electrical equipment which has been PAT tested. Ensure that samples are in tightly capped tubes.	**Low**	**X**
	Med			**Med**	
	High			**High**	

Reasonably Foreseeable Hazards	Inherent Risk	X	Controls	Residual Risk	X
Transport of NG tubes into laboratory fridge – loss of bacterial material to the environment	**Low**	**X**	Wear appropriate PPE. The triple packaging system will be used, in accordance with the Centre for Microbiological Sciences protocols for transportation of Category B (UN3373) biological and clinical material (HSE, 2011). Samples will be transported in individual Ziploc plastic bags, placed within a larger sealed bag, and then placed in a preparatory polystyrene specimen box sealed with tape and with appropriate UN3373 labelling. This will be opened carefully in the laboratory, and the bagged tubes placed straight into the allocated fridge	**Low**	**X**
	Med			**Med**	
	High			**High**	

Appendix B: Constituents of Tryptone Soya Agar

Formula	gm/litre
Pancreatic digest of casein	15.0
Enzymatic (contains papain) digest of soya bean	5.0
Sodium chloride	5.0
Agar	15.0
pH 7.3 ± 0.2 @ 25°C	

(Oxoid Ltd, 2016)

Appendix C: Control of Substances Hazardous to Health Regulations

The Control of Substances Hazardous to Health Regulations (2002) specifies four containment levels for activities which involve working with biological agents, which correspond to the classification of biological agents into Hazard Groups 1 to 4:

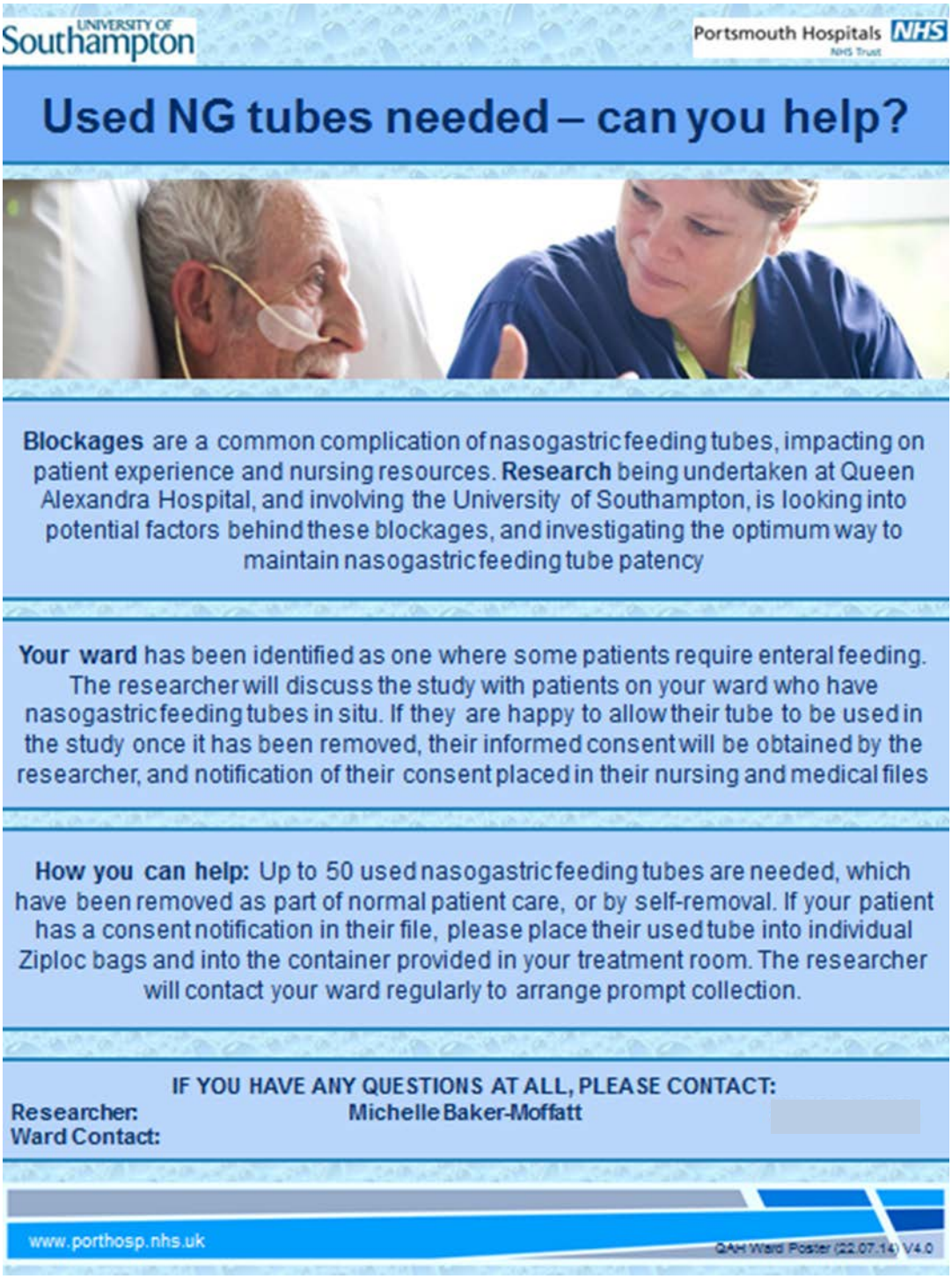
- Group 1 – Unlikely to cause human disease
- Group 2 – Can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available
- Group 3 – Can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available
- Group 4 – Causes severe human disease and is a serious hazard to employees; it is likely to spread to the community and there is usually no effective prophylaxis or treatment available

Appendix D: Constituents of Tryptone Soya Broth

Formula	gm/litre
Pancreatic digest of casein	17.0
Enzymatic digest of soya bean (contains papain)	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.5
Glucose	2.5
pH 7.3 ± 0.2 @ 25°C	

(Oxoid, 2016)

Appendix E: Recruitment ward poster - Patients



Appendix F: Participant information sheet - Patients

Participant Information Sheet

Investigating the presence and nature of substances, including bacteria, inside nasogastric tubes retrieved from adult patients

R&D Number: PHT/2014/30
REC Ref Number: 14/SC/0111

1. Invitation to participate
You have been invited to take part in a research study, which is looking at bacteria in nasogastric feeding tubes and identifying how best to keep the tubes free flowing. This study is being undertaken by Staff Nurse Michelle Baker-Moffatt as part of a PhD course at the University of Southampton. Before you decide to take part, it is important to understand why the research is taking place and what it will involve. Please take time to read this information sheet carefully, and discuss the study with others if you wish.

2. What is the purpose of the study?
The aim of the study is to identify what factors cause nasogastric feeding tubes to block, and to investigate ways of preventing these blockages

3. Why have I been invited?
You have been invited to take part in this study because you currently have a nasogastric feeding tube that will be removed at a future date as part of your normal care

4. Do I have to take part?
It is up to you to decide to join the study. The study will be explained to you, along with this information sheet. If you agree to take part, you will be asked to sign a consent form following a 24 hour cooling-off period. You are free to withdraw at any time, without giving a reason

5. What will happen to me if I take part?
When your tube is removed as part of your normal clinical care, it will be will be kept by the ward staff instead of being placed in the clinical waste, and will subsequently be used in laboratory-based investigations. To assist with the research, Staff Nurse Michelle Baker-Moffatt and another member of the research team will record the following information from your hospital notes: Age, gender, details of medication, feed regimen, and duration of nasogastric feeding tube placement. Your name and hospital number will also be obtained and stored separately, with only a given code used in the research

6. What are the possible benefits of taking part?
There is no direct benefit to you; the results from the study will help us to understand how nasogastric feeding tubes can be prevented from blocking.

However, if we are concerned by anything we see in the tube we will contact your consultant

7. Are there any risks involved?
There are no expected risks to you in taking part

8. Will my General Practitioner (GP) be informed?
It is not necessary for us to inform your GP of your participation in this study

9. What if there is a problem?
If you have a concern or a complaint about this study you should contact Martina Prude, Head of the Governance Office, at the Research Governance Office (Address: University of Southampton, Building 37, Highfield, Southampton, SO17 1BJ ; Tel: +44 (0)23 8059 5058;
Email: rgoinfo@soton.ac.uk). If you remain unhappy and wish to complain formally Martina can provide you with details of the University of Southampton Complaints Procedure

10. Will my participation be confidential?
All of the information collected about you during the study will be kept confidential.
Data will be collected on a paper form and stored in a locked filing cabinet. Relevant data will be entered onto a computer spread sheet which is protected by a password. Your name and personal details will not be entered onto the computer. The procedures for handling, processing, storing and destroying data relating to your participation in the study are compliant with the Data Protection Act 1998. In accordance with the University's regulations we are required to keep your data secure for 10 years

We will not pass any of your information on; however, for the purposes of monitoring research there is a possibility that the hospital's Research and Development department will audit the data collected

11. Are insurance provisions in place?
In the event that something does go wrong, and you are harmed during this research as a result of someone's negligence, then you may have grounds for a legal action for compensation against the University of Southampton, who are the sponsors for this study. You may however have to pay legal costs. The lead researcher, Staff Nurse Michelle Baker-Moffatt, is a doctoral student at the University of Southampton. The University of Southampton provides additional professional indemnity and clinical investigation insurance

12. What will happen to the results of the research study?
Your name will not be used within the results. The results will be presented to the ward staff and written up as part of an assignment. Anonymised findings will also be presented at conferences and meetings, and published in journals. It will not be possible to identify you from the results presented

13. Who is running the study?
The University of Southampton is acting as the sponsor for this study. This project is part of a PhD undertaken by Staff Nurse Michelle Baker-Moffatt. The

project is being supervised by Dr Sue Green and Dr Sandra Wilks at the University of Southampton

14. Who is organising and funding the research?
There is no funding available for this research. The University of Southampton is acting as the sponsor for this research. Portsmouth Hospitals Trust will not receive any funding if you participate in this research

15. Who has reviewed the study?
This research study has been reviewed by the South Central (Hampshire A) research ethics committee, who have given a favourable opinion. A research ethics committee is a group of independent people who review studies to ensure it is ethical to run them. They look to protect your interests. Portsmouth Hospital Trust Research Office has also approved the study

16. Where can I find out more about the study?
Should you require further information about this study, please contact Staff Nurse Michelle Baker-Moffatt

Tel:

Email: Michelle.Baker-Moffatt@porthosp.nhs.uk

Appendix G: Participant consent form - Patients

Consent Form

(*Version 1.4, 22.07.14)*

Study Title: Maintaining the patency of nasogastric feeding tubes

Researcher name: Staff Nurse Michelle Baker-Moffatt
Ethics reference: PHT/2014/30
Participant ID:

*Please **initial** the box(es) if you agree with the statement(s):*

I have read and understood the information sheet *(version 1.4)* and have had the opportunity to ask questions about the study	Initial
I agree to take part in this research study and agree for my data to be used for the purpose of this study	Initial
I understand that my medical consultant on the ward and other healthcare professionals involved in my care will be informed of my participation in the study	Initial
I understand my participation is voluntary and I may withdraw at any time without my legal rights or medical care being affected	Initial
I understand that relevant sections of my notes will be looked at by the researcher, and agree that responsible individuals from regulatory authorities or from the NHS Trust where it is relevant to me taking part in this research may have access to my medical records	Initial
I understand that information collected about me during my participation in this study will be stored on a password protected computer and that this information will only be used for the purpose of this study. All files containing any personal data will be made anonymous	Initial

Name of participant (print name): Date: Signature:

……..

Name of Researcher: Date: Signature:

MICHELLE BAKER-MOFFATT…….....

Appendix H: Data record form - Patients

Data Record Form

Tube Number		Date of:	Removal	
Ward			Collection	
Patient Code			Assessment	

Tube Patent?		Tube occluded?	
Consultant's permission obtained?			

Patient details

Age	
Gender	
Feed regimen	NG fed – see attached
Duration of Placement	

Details of ALL medication given

Medication	Dose	Frequency	Rx Form	NG Route?	Duration

Date	Feed	ml/hr	Hours	Total

Appendix I: Notification of patient's intent to participate

University of Southampton

Portsmouth Hospitals NHS
NHS Trust

NOTIFICATION OF PATIENT'S INTENT TO PARTICIPATE IN RESEARCH

Are you planning to remove this patient's NG tube?

If so, please don't bin it…… bag it!

If you have any queries, please contact SN Michelle Baker-Moffatt on

WARD TO COMPLETE	
Date of NG tube removal: …./…./….	**Attach patient label**

Appendix J: Recruitment ward poster – Nurses

Portsmouth Hospitals NHS
NHS Trust

Be part of a research study into NG tube maintenance

- Are you a nurse?
- Do you care for patients with NG tubes?
- Can you spare 60 minutes of your own time?

The purpose of the study is to gain an understanding of nurses' beliefs and practices concerning the maintenance of NG tube patency.

Interviews lasting up to 60 minutes will take place at Queen Alexandra Hospital. All interviews must take place in your own time.

As a gesture of good will, you will receive a Marks and Spencer voucher for £20 for attending an interview in your own time.

Please contact Michelle Baker-Moffatt SN on Michelle.Baker-Moffatt@porthosp.nhs.uk or call on for more information

ERGO Number: 14549 Poster take down date: 01/12/2015

Health Sciences
UNIVERSITY OF Southampton

Appendix K: Participant information sheet – Nurses

Participant Information Sheet

(Version 4.0, 28.07.2015)

Nurses' beliefs and practices concerning the maintenance of nasogastric tube patency: a qualitative study

Ethics reference: PHT/2015/81

1. Invitation to participate

You have been invited to take part in a research study because you are a Registered Nurse who cares for people who have nasogastric tubes as part of your clinical role. This study is being undertaken by Staff Nurse Michelle Baker-Moffatt as part of her PhD programme at the University of Southampton. Before you decide to take part, it is important to understand why the research is taking place and what it will involve. Please take time to read this information sheet carefully, and discuss the study with others if you wish.

2. What is the purpose of the study?

The purpose of the study is to identify what factors cause nasogastric feeding tubes to block, and to investigate ways of preventing these blockages.

3. Why have I been invited?

You have been invited to take part in this study because you are a member of a nursing team who cares for patients with nasogastric feeding tubes.

4. Do I have to take part?

It is up to you to decide whether you wish to take part in the study. If you agree to take part, you will be asked to sign a consent form following a minimum 24 hour period. You can change your mind and withdraw from the study without giving a reason at any time up until the writing up of the study.

5. What will happen to me if I take part?

The study will involve up to 20 participants who will all be interviewed separately. The interview will take approximately 60 minutes. If you choose to take part the researcher will organise a location for interview convenient to you on the hospital site. Before the interview she will explain to you what the study involves and you will be given the opportunity to ask any questions. If you would still like to take part the researcher will ask you to sign a consent form, and will note your name, contact details, and qualification details on a Data Record Sheet. The interviews will be recorded using a digital recorder, but notes may also be made throughout the interview to assist in transcribing the data. The interview will be conducted in confidence, and the findings will be anonymised.

6. What are the possible benefits of taking part?

There is no anticipated direct benefit to you. The results of the study will help us to gain an understanding of the knowledge held regarding the maintenance of NG feeding tubes. You will be offered a £20 Marks and Spencer voucher to compensate you for your time and inconvenience. The University of Southampton is providing the funding required for the vouchers.

7. Are there any risks involved?

There are no expected risks to you in taking part. If you find any aspect of the interview upsetting you are free to stop the interview if you do not wish to continue. If the interview upsets you, you will be advised to talk to your clinical manager.

8. What if there is a problem?

If you have a concern or a complaint about this study you should contact the Research Integrity and Governance Manager , Head of the Governance Office, at the Research Governance Office (Address: University of Southampton, Building 37, Highfield, Southampton, SO17 1BJ ; Tel: +44 (0)23 8059 5058; Email: rgoinfo@soton.ac.uk). If you remain unhappy and wish to complain formally, they can provide you with details of the University of Southampton Complaints Procedure.

9. Will my participation be confidential?

Participation will be confidential, within the boundaries of the NMC Code of Conduct (2015), and will be collected in line with the Data Protection Act and University Policy. If, however, any issues arise that may compromise patient care and safety the researcher is duty bound by the NMC Code of Conduct (2015) to report these issues to your line manager. The researcher will inform you if this is going to happen.

The audio recordings will be downloaded onto a password protected computer owned by the University of Southampton, along with any other electronic data recorded. The audio recordings will be transcribed and the downloaded recordings archived in line with the University of Southampton policy. Data recorded in paper form will be stored in a locked filing cabinet during the study, and archived thereafter. You can request a copy of the interview transcript if you wish. The results of the study will be used in a PhD thesis and may be published in peer-reviewed and practice journals, however, no research participant will be identifiable from any publications.

For the purposes of monitoring research there is a possibility that the Hospital's Research and Development department will audit the data collected.

10. Are insurance provisions in place?

In the event that something does go wrong, and you are harmed during this research as a result of someone's negligence, then you may have grounds for a legal action for compensation against the University of Southampton, who are the sponsors for this study. You may however have to pay legal costs. The lead researcher, SN Michelle Baker-Moffatt, is a doctoral student at the University of

Southampton. The University of Southampton provides additional professional indemnity and clinical investigation insurance.

11. What will happen to the results of the research study?
The study results will contribute to the development of a PhD thesis and presented at academic and professional conferences and meetings. The findings may also be published in an academic or practice journal. It will not be possible to identify you from the results presented

12. Who is running the study?
The University of Southampton is acting as the sponsor for this study. This project is part of a PhD undertaken by SN Michelle Baker-Moffatt. The project is being supervised by Dr Sue Green and Dr Sandra Wilks at the University of Southampton

13. Who is organising and funding the research?
The University of Southampton is funding the research study through studentship arrangements. Portsmouth Hospitals Trust will not receive any funding if you participate in this research

14. Who has reviewed the study?
The research study has been reviewed and approved by the University of Southampton faculty ethics research and governance office, and by the Portsmouth Hospital NHS Trust Research Office

15. Where can I find out more about the study?
Should you require further information about this study, please contact SN Michelle Baker-Moffatt

Tel:

Email: Michelle.Baker-Moffatt@porthosp.nhs.uk

Appendix L: Participant consent form – Nurses

Consent Form

(*Version 4.0, 28.07.2015)*

Nurses' beliefs and practices concerning the maintenance of nasogastric tube patency: a qualitative study

Researcher name: Staff Nurse Michelle Baker-Moffatt
Ethics reference: PHT/2015/81
Participant ID:

*Please **initial** the box(es) if you agree with the statement(s):*

I have read and understood the information sheet *(version 4.0)* and have had the opportunity to ask questions about the study	Initial
I agree to take part in this research study and agree for my anonymised data, including that recorded on the Data Record Sheet, to be used for the purpose of this study	Initial
I understand my participation is voluntary and I may withdraw at any time prior to the publication of the study results without my legal rights being affected	Initial
I understand the interview will be audio recorded for accurate transcription by a professional transcriber	Initial
I agree that responsible individuals from regulatory authorities or from the NHS Trust where it is relevant to me taking part in this research may require access to the anonymised data for audit purposes	Initial
I understand that information collected about me during my participation in this study will be stored on a password protected computer and that this information will only be used for the purpose of this study. All files containing any personal data will be made anonymous	Initial

Name of participant (print name): Date: Signature:

……..

Name of Researcher: Date: Signature:

MICHELLE BAKER-MOFFATT…….....

Appendix M: Interview guide

Interview Guide

(*Version 2.0, 10.06.2015)*

Nurses' beliefs and practices concerning the maintenance of nasogastric tube patency: a qualitative study

Housekeeping:

Ensure the participant is comfortable

Briefly go through the Participant Information Sheet – answer questions

Complete Consent Form

Complete Data Record Sheet

Switch on recorder

Thank participant for taking the time to talk about their beliefs and practice concerning NG tubes

Begin questions:

First, could you tell me about your experience of caring for people with nasogastric tubes?

- Specific clinical examples
- What are the challenges?

In your experience how much of a problem do you think blocking of NG tubes is?

- Is it a challenge?

I am interested in what you think makes NG tubes block?

- Have you found increased blockages with any medication or particular patient groups?

What do you think is the most important way of keeping an NG tube working?

- Are NG tubes changed often?
- How often are they flushed?

Can you talk to me about how and when you might flush an NG tube?

- Talk me through when you might decide to flush an NG tube

What sort of training or education have you had on NG tube management?

Can you tell me what you know about biofilms?

Is there anything that you would like to tell me about NG tubes that we haven't covered already?

Closing - Many thanks for your time. I hope to have completed the study by January 2016. Would you like a copy of the study results?

Voucher given?

End

Appendix N: Data record form – Nurses

Data Record Form

Nurses' beliefs and practices concerning the maintenance of nasogastric tube patency

Project number	
Participant's name	
Contact details (Phone/email)	
Length of time qualified	
Years of experience with NGT	
Pay Band	

PIS given (Date)	
PIS discussed (Y/N)	
Consent signed (Date)	
Date of interview	
Recording transcribed (Date)	

Notes

Glossary of terms

Aliquot	a portion or sample taken for analysis
Autoclave	a pressure chamber used for sterilisation
BSC	biological safety cabinet
CFU	colony forming units
CE	cell elongation
DAPI	a fluorescent bacterial DNA stain
ddH_2O	sterile distilled water, purified water
DNA	deoxyribonucleic acid
DVC	direct viable count
EDIC	episcopic differential interference contrast
EF	epifluorescence
EPS	extracellular polymeric substance
FG	French gauge – external diameter measurement, 1 FG = ⅓ mm
In vitro	within the glass – testing in a laboratory setting using apparatus
In vivo	within the living – testing on whole, living organisms
ml	millilitre, unit of volume = a thousandth of a litre
µl	microlitre, unit of volume = one millionth of a cubic decimetre
µm	micrometre, unit of measurement = one millionth of a metre
MSDS	Material Safety Data Sheet
Petri dish	shallow cylindrical plastic lidded dish used to culture cells
pH	a measure of the acidity or basicity of an aqueous solution
Planktonic	suspended in a liquid
R2B	an appropriate low-nutrient broth
SYTO 9	a fluorescent bacterial DNA stain
TSA	tryptone soya agar
TSB	tryptone soya broth
Universal	30 ml clear container with a screw-top for laboratory use
VBNC	viable but non-culturable
Vortex	rapid stirring of a liquid (using a laboratory vortex machine)
V/V	volume/volume, used when mixing two liquids

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