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Microbial risk during the ward-pharmacy intravenous prescribing cycle

by

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

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MICROBIAL RISK DURING THE WARD-PHARMACY INTRAVENOUS PRESCRIBING CYCLE

by Peter David Austin

Parenteral nutrition (PN) can be a life-saving treatment in patients with intestinal failure but it can also cause morbidity and even death. One of the most important complications of PN is intravascular catheter infection (CI), the prevalence of which varies considerably between centres due to differences in indications for PN, patient characteristics, and procedures used to prescribe, compound, package and administer PN. The inter-relationships between several of these variables are poorly understood, including uncertainty about the mechanisms of transfer of microbes from one setting to another and the effect of infusate composition on microbial growth, which is complicated further by the use of different criteria used to define CI.

This thesis examined a series of specific hypotheses related to the above issues, through a combination of clinical and laboratory studies involving both observations and interventions. The overarching hypothesis was that: ‘simple measures in clinical and pharmaceutical environments can reduce potential exposure of intravenous lines and infusates to microbial contamination, limit microbial growth in intravenous feeds and reduce the rate of CI to an extent that depends on the definition used’.

It was found that infusate composition, prescription and associated packaging methods (freshly printed electronic < handwritten recycled paper), compounding environment (pharmaceutical < ward) and training and experience in aseptic techniques (greater < less) could significantly reduce microbial contamination. A series of laboratory studies and a systematic review with meta-analyses found the growth of microbes in PN infusates was species specific and affected by various nutrients and osmolarity. A clinical study involving a cohort of patients receiving PN found the CI rate to be strikingly affected by the definition of CI used (an actual difference > 10% or a relative difference > 400%).

The studies suggest that simple steps can be taken to reduce contamination rates using electronic prescriptions, optimising method of packaging, appropriate training and experience in aseptic techniques, all operating within a ward-pharmacy intravenous prescribing cycle, which needs to be tackled at multiple points. The variability of microbial species’ growth in PN makes it difficult to provide generic recommendations about the duration of PN infusion from a single bag (which affects microbial growth), and the variability of CI rates when various definition of CI are used is confusing and requires a consensus statement to be established using a combination of evidence based information and clinical wisdom.

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DECLARATION OF AUTHORSHIP

I, ... [please print name]

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.

[title of thesis] **.....**

.....

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as described in the section 'Publications and presentations arising from this thesis'

Signed: **.....**

Date: **.....**

MY ROLE IN THE WORK CONTAINED IN THIS THESIS

I conceived of the work contained within this thesis, and was responsible for the following:

- For sub-hypothesis one I designed the formulations of the PN infusates, set up the documentation for the preparation of the PN infusates, arranged for the required components to be available for the preparation of the PN infusates, arranged the preparation of the PN infusates, adjusted the pH of the PN infusates, inoculated all of the PN infusates, sampled all of the PN infusates, counted some of the plates for the numbers of cfu, undertook the literature search, populated all of the data into the statistics package, and carried out all of the statistics, including the meta-analyses.
- For sub-hypotheses two and four I took all of the swab samples, populated all of the data into the statistics package, and carried out all of the statistical analyses.
- For sub-hypothesis three I undertook the literature search, populated all of the data into the statistics package, and carried out all of the statistics, including the meta-analyses.
- For sub-hypothesis five I organised and supervised the preparation of all of the doses, took all of the environmental samples, populated all of the data into the statistics package, and carried out all of the statistics.
- For sub-hypothesis six I identified the relevant cases, established that no formal Ethical approval was required, collated all of the data from the medical records, assessed all of the cases against each of the definitions, populated all of the data into the statistics package, and carried out all of the statistics.

In addition, I completed the applications for funding to Pharmacy Research UK (formerly the Pharmacy Practice Research Trust) and to the Wessex Clinical Academic Careers Steering Group.

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS

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[b] Pharmacy department at University Hospital Southampton NHS Foundation Trust as part of a service development

[c] Based on a framework developed with the support of the 2010 Galen Award

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PAPERS

[a] Austin PD, Hand KS, Elia M. Factors that influence *Staphylococcus epidermidis* growth in parenteral nutrition with and without lipid emulsion: A study framework to inform maximum duration of infusion policy decisions, *Clinical Nutrition* 2012; 31(6): 974-980.

Austin PD, Elia M. Improved aseptic technique can reduce variable contamination rates of ward-prepared parenteral doses. *Journal of Hospital Infection* 2013; 83(2):160-163.

[a] Austin PD, Hand KS, Elia M. A Comparison of the Surface Contaminants of Handwritten Recycled and Printed Electronic Parenteral Nutrition Prescriptions and Their Transfer to Bag Surfaces During Delivery to Hospital Wards. *Journal of Parenteral and Enteral Nutrition* 2014 Feb;38(2):254-62.

[c] Austin PD, Hand KS, Elia M. Factors influencing *Candida albicans* growth in parenteral nutrition with and without lipid emulsion: Using an established framework to inform maximum duration of infusion policy decisions. *Clinical Nutrition* 2014 Jun;33(3):489-94.

[d] Austin PD, Hand KS, Elia M. Factors Influencing *Escherichia coli* and *Enterococcus durans* Growth in Parenteral Nutrition With and Without Lipid Emulsion to Inform Maximum Duration of Infusion Policy Decisions. *Journal of Parenteral and Enteral Nutrition* 2015 Nov;39(8):953-65.

[e] Austin PD, Hand KS, Elia M. Systematic review and meta-analysis of the risk of microbial contamination of parenteral doses prepared under aseptic techniques in clinical and pharmaceutical environments: an update. *Journal of Hospital Infection* 2015 Dec;91(4):306-318.

SUBMITTED PAPERS

[e] Austin PD, Hand KS, Elia M. The impact of definition and procedures used for absent blood culture data on the rate of intravascular catheter infection during parenteral nutrition.

[d] Austin PD, Hand KS, Elia M. A systematic review and meta-analyses of the effect of lipid emulsion on microbial growth in parenteral nutrition.

ABSTRACTS

Austin P, McKenzie S, Elia M. Improved technique can reduce variable contamination rates of ward-prepared parenteral doses. *Clinical Nutrition* 2011; 6(Suppl 1):184.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress held in Gothenburg, Sweden, from 3 – 6 September 2011.

[a] Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. Growth of *Staphylococcus epidermidis* differs in lipid alone compared to PN and is influenced by pH. *Clinical Nutrition* 2011; 6(Suppl 1):185.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress held in Gothenburg, Sweden, from 3 – 6 September 2011.

[a] Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. Glucose and lipid in parenteral nutrition independently influence growth of *Staphylococcus epidermidis*. *Clinical Nutrition* 2011; 6(Suppl 1):185.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress held in Gothenburg, Sweden, from 3 – 6 September 2011.

[a] Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. Total non-protein energy influences growth of Staphylococcus epidermidis in parenteral nutrition. *Proceedings of the Nutrition Society* 2011; 70 (OCE5), E337.

Presented at the British Society for Parenteral and Enteral Nutrition annual conference held in Harrogate, United Kingdom, from 29 – 30 November 2011.

[b] Austin P, McKenzie S, Ballard R, Elia M. 'Improved technique can reduce variable contamination rates of ward prepared parenteral doses'.

Presented at the University of Southampton Faculty of Medicine's inaugural Research Conference held in Southampton, United Kingdom, from 13 – 14 June 2012.

Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. The effects of lipid and glucose concentration in parenteral nutrition on the growth of *Candida albicans*. *Clinical Nutrition Supplements* volume 7, issue 1, September 2012: 204-205.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress held in Barcelona, Spain, from 8 – 11 September 2012.

The above abstract was judged to be an 'outstanding abstract' by the Congress.

Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. Greater growth of *Candida albicans* in lipid alone than in parenteral nutrition with and without a lipid component. *Clinical Nutrition Supplements* volume 7, issue 1, September 2012: 205.

Presented at the European Society for Parenteral and Enteral Nutrition annual congress held in Barcelona, Spain, from 8 – 11 September 2012.

[a] Austin P, Hand K, Longmaid K, Lamph D, Willis C, Elia M. Substitution of recycled prescriptions with printed electronic prescriptions for parenteral nutrition reduces microbial surface contamination. *Clinical Nutrition Supplements* volume 7, issue 1, September 2012: 205-206.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress held in Barcelona, Spain, from 8 – 11 September 2012.

[a] Austin P, Hand K, Longmaid K, Lamph D, Willis C, Elia M. Fewer contaminants on parenteral nutrition (PN) bags by packaging with printed electronic prescriptions compared to recycled prescriptions. *Clinical Nutrition Supplements* volume 7, issue 1, September 2012: 121.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress held in Barcelona, Spain, from 8 – 11 September 2012.

[a] Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. 'The effects of the presence or absence of lipid and glucose concentration in parenteral nutrition on the growth of *Staphylococcus epidermidis*'.

Presented at the 2013 University of Southampton Faculty of Medicine Research Conference held in Southampton, United Kingdom, on 12 June 2013.

[d] Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. The effects of glucose concentration and the inclusion of lipid in parenteral nutrition on the growth of *E. Coli* and *E. durans*. *Clinical Nutrition* volume 32, Supplement 1, September 2013: S94-S95.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress the Leipzig, Germany, from 31 August – 3 September 2013.

<u>The above abstract was judged to be an 'outstanding abstract' by the Congress.</u>

[d] Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. The effect of osmolarity on the growth of *E. coli* and *E. durans* in parenteral nutrition containing lipid. *Clinical Nutrition* volume 32, Supplement 1, September 2013: S96.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress the Leipzig, Germany, from 31 August – 3 September 2013.

[c] Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. 'The effects of lipid and glucose concentration in parenteral nutrition on the growth of *Candida albicans*'.

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Austin P, Hand K, Palmer J, Longmaid K, Lamph D, Willis C, Elia M. 'The effects of lipid and glucose concentration in parenteral nutrition on the growth of *Candida albicans*'.

Presented at the NIHR Southampton Biomedical Research Centre Symposium held in Southampton, United Kingdom on 26 March 2014.

[e] Austin P, Hand S, Elia M. A systematic review and meta-analysis of the frequency of microbial contamination of parenteral nutrition and other parenteral medicines prepared aseptically in clinical and pharmaceutical environments. Clinical Nutrition 33(supplement 1); 2014: S103 – S104.

Presented at the European Society for Clinical Nutrition and Metabolism annual congress held in Geneva, Switzerland, from 6 – 9 September 2014.

[e] Austin P, Hand S, Elia M. A systematic review and meta-analysis of the effect of additives to PN and other parenteral medicines made in clinical environments on the rate of microbial dose contamination. Clinical Nutrition 33(supplement 1); 2014: S106.

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ORAL PRESENTATIONS

[a] ‘Parenteral Nutrition Workshop’ included some data related to *S. epidermidis* from sub-hypothesis one during the New Zealand Hospital Pharmacists Association Annual Conference at the Sebel Trinity Wharf Hotel, Bay of Plenty, New Zealand, from 11 – 13 November 2011.

The above presentation was part of the Keynote Speaker role.

[a,b,c,d] ‘Microbial growth in Parenteral Nutrition’ as a presentation during the University of Southampton Nutrition and Metabolism PhD Student Meeting was about data from sub-hypothesis one at Southampton General Hospital, Hampshire, United Kingdom, on 12 February 2012.

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[a, c] ‘Microbial growth in PN’ as a presentation to the Pharmacy department at University Hospital Southampton NHS Foundation Trust was about data from sub-hypothesis one at Southampton General Hospital, Southampton, United Kingdom, on 28 November 2012.

[a, c, d] ‘Should the inclusion of lipid make a difference to the infusion duration of parenteral nutrition bags?’ as an interactive session was about data from sub-hypothesis one during the annual NHS Quality Assurance and Technical Services Symposium at the Crowne Plaza, Chester, United Kingdom, from 24 – 25 September 2013.

[e] ‘Pharmacy roles on a nutrition team’ as a presentation included some data from sub-hypothesis three during the Polish Society for Parenteral and Enteral Nutrition annual congress at the Hotel Warszawianka, Jachranka, Poland from 20 – 22 June 2014.

a, c, d, e ‘Microbial growth in parenteral nutrition with and without lipid’ as a presentation was about data from sub-hypothesis one and also included some data from sub-hypothesis three at Palmerston North Hospital, Palmerston North, New Zealand, on 6 November 2014.

a, c, d, e ‘Microbial growth in parenteral nutrition with and without lipid’ as the ‘Pharmatell session’ was about data from sub-hypotheses one and also included some data from sub-hypothesis three at the University of Auckland, Auckland, New Zealand, on 10 November 2014.

a, c, d, e ‘How long can a parenteral nutrition bag be infused for?’ as a presentation as part of the ‘Nutrition Support Teams Quarterly Regional Meeting’ was about data from sub-hypothesis one and also included some data from sub-hypothesis three at Auckland City Hospital, Auckland, New Zealand, on 10 November 2014.

a, c, d, e ‘Reducing catheter related infection risk: consensus and controversies’ as part of the symposium ‘How to make home parenteral nutrition Safer?’ included some data from sub-hypotheses one, three and five during the European Society for Clinical Nutrition and Metabolism annual congress at the International Congress Centre of Lisbon, Lisbon, Portugal, from 5 – 8 September 2015.

ABBREVIATIONS

A. calcoaceticus	*Acinetobacter calcoaceticus*
ANCOVA	analysis of covariance
ANOVA	analysis of variance
B. cepacia	*Burkholderia cepacia*
B. cereus	*Bacillus cereus*
BD	Becton, Dickinson and Company
BS EN ISO	British Standard European Norm International Organization for Standardization
B. subtilis	*Bacillus subtilis*
C. albicans	*Candida albicans*
CDC	Centers for Disease Control and Prevention
C. difficile	*Clostridium difficile*
cfu	colony forming units
CI	confidence interval(s)
cm	centimetres
CRD	United Kingdom National Health Service Centre for Reviews and Dissemination
CI	(intravascular) catheter infection (within an in situ intravenous catheter)
DNase	Deoxyribonuclease
E. aerogenes	*Enterobacter aerogenes*
EC	European Community
E. cloacae	*Enterobacter cloacae*
E. coli	*Escherichia coli*
E. durans	*Enterococcus durans*
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
ESPEN	European Society for Clinical Nutrition and Metabolism
EUR	Euro (currency)
g	gram
GBP	British pound (currency)
GMP	Good Manufacturing Practice

GRADE	Grading of Recommendations Assessment, Development and Evaluation
GR	microbial 'growth ratio' (log_{10} [cfu.mL^{-1} at 48 hours / cfu.mL^{-1} at time zero])
HELICS	Hospital in Europe Link for Infection Control through Surveillance
HEPA	high efficiency particulate air
HICPAC	Healthcare Infection Control Practices Advisory Committee
IBM	International Business Machines
IQR	interquartile range
K. aerogenes	*Klebsiella aerogenes*
kcal	kilocalorie(s)
kg	kilogram(s)
K. oxytoca	*Klebsiella oxytoca*
K. pneumonia	*Klebsiella pneumoniae*
KSH	Kieran Sean Hand
L	litre(s)
Ltd	Limited company
M	molar
Mcal	megacalorie(s)
M. furfur	*Malassezia furfur*
mL	millilitre(s)
MM	Matching Michigan
mmol	millimole(s)
mOsm	milliosmole(s)
MRSA	methicillin-resistant *Staphylococcus aureus*
n	number
NCPF	(Public Health England) National Collection of Pathogenic Fungi
NCTC	(Public Health England) National Collection of Type Cultures
NHS	(United Kingdom) National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NS	not significant
P	probability

PDA	Peter David Austin
P. aeruginosa	*Pseudomonas aeruginosa*
% v/v	percent volume in volume (i.e. mL per 100mL)
% w/v	percent weight in volume (i.e. grams per 100mL)
P. fluorescens	*Pseudomonas fluorescens*
PN	parenteral nutrition
ROC	receiver-operator characteristic
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RR	relative risk
S. aureus	*Staphylococcus aureus*
SD	standard deviation
S. epidermidis	*Staphylococcus epidermidis*
SEM	standard error of the mean
S. faecalis	*Streptococcus faecalis*
S. maltophilia	*Stenotrophomonas maltophilia*
S. marcescens	*Serratia marcescens*
S. pyogenes	*Streptococcus pyogenes*
SPSS	Statistical Package for the Social Sciences
S. saprophyticus	*Staphylococcus saprophyticus*
UK	United Kingdom
USA	United States of America
USD	United States dollar (currency)

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW

PN is the infusion of a feed directly into the vein using an administration set and an intravenous catheter (the 'plastics'). PN delivers essential and non-essential nutrients and aims to improve nutritional status and function or limit their deterioration in patients unable to assimilate adequate nutrients through their gastrointestinal tract. The indications for PN vary according to clinical factors, practices, care setting and country. For example, in the UK, one review published in 1999 indicated PN is used much less frequently for cancer (5% of patients[1]) than in other countries such as Sweden (80% of patients[1]). Furthermore, within the UK, the proportion of patients receiving PN in hospitals for cancer (3%[2]) is similar to that in the community (5%[1]), but several times lower than the proportion of patients receiving PN as hospital inpatients for post-operative ileus (over 16%[2]).

PN is comprised of a number of separate components: water, protein as amino acids, lipid (fat) as triglycerides, carbohydrate as glucose, electrolytes in various salts, vitamins and trace elements. Infusing each required component separately is undesirable since it would be impractical and time consuming. Such a practice could result in confusion and errors, incompatibilities such as precipitation within the 'plastics', and increased risk of CI (see 1.2.2 below) due to the high number of manipulations from touch transfer[3]. Therefore, instead of a series of separate infusions of each required component, compatible components are mixed together and administered as a single PN infusate. The formulation needs to meet the requirements of an individual patient but must also be stable. Single bags of a PN infusate may be called an 'all-in-one', 'big bag' or 'total nutrient admixture'. In some cases lipid-free PN rather than lipid PN is required (see 1.2.1 below).

A range of 'multichamber' PN bags can be sourced from commercial manufacturers, which have separate compartments of amino acids and glucose ('two chamber' bags; lipid-free PN) or amino acids, glucose and lipid ('three chamber' bags; lipid PN). Once the weak internal seals between the separate compartments are broken the

components within them can mix within a sealed system preventing contamination from the outside. These bags are not only simpler but can also be cost-effective since they are mass produced, can be stored unused at room temperature, and limit the compounding time required to combine individual components. However, the apparent simplicity of 'multichamber' PN bags can be misleading because the concentration and pattern of nutrient combinations is fixed and limited by the compatibility and stability of nutrient admixtures, and they always require additives such as vitamins[4]. Nevertheless, the appropriate use of 'multichamber' bags when suitable for individual patients has potential to improve practice and provision of PN services.

1.2 COMPLICATIONS OF PARENTERAL NUTRITION USE

PN administration can be associated with a variety of complications that include metabolic complications arising from inadequate or excessive provision of any PN component (or PN components in combination), or intravenous catheter complications such as occlusion or infection. It is therefore essential to ensure adequate clinical and laboratory monitoring of patients during PN administration to identify any indication of a potential complication (or complications) as early as possible. Two of the most frequently encountered complications of PN use are adverse effects on the liver that may lead to the exclusion of the lipid component, and infection.

1.2.1 INCLUSION OR EXCLUSION OF LIPID IN PARENTERAL NUTRITION INFUSATES

Over a prolonged period, lipid in PN can potentially cause adverse effects on the liver. Abnormal liver function markers are frequently seen in patients receiving long term PN[5] and once infection and/or the use of certain medicines are excluded as potential causes, the presence of lipid in PN is often considered to be a possibility. In such cases lipid may be administered only a few times a week and the energy lost from lipid replaced with glucose, even though replacement of lipid with glucose is also considered to have the potential to cause adverse effects on the liver. Indeed, total energy density may be more relevant than the specific quantity of lipid or glucose. Nevertheless, the exclusion of lipid from PN is not infrequently seen in

clinical practice, and furthermore some hospitals routinely exclude lipid from most PN infusates and instead administer it intermittently to avoid fatty acid deficiency even in the absence of abnormal liver function markers. Lipid may also be excluded from PN to ensure adequate stability, or to avoid hypersensitivity (allergy) in susceptible individuals. Therefore, some PN infusates include lipid (lipid PN), others exclude lipid (lipid-free PN), and sometimes lipid is infused alone (as lipid emulsion).

1.2.2 INTRAVASCULAR CATHETER INFECTION

CI refers to an infection linked to the presence of microbes within an intravenous catheter lumen. The catheter can be the primary infection source if the microbes were introduced through the hub of the catheter, but it may also act as a secondary infection source should the microbes 'seed' to the catheter lumen through the tip from another site. CI is a widespread problem that affects hospital inpatients and those patients managed on intravenous therapy in the community[6]. CI contributes to infective morbidity, line removal and death, and in those managed on intravenous therapy in the community CI can lead to distress and frequent hospitalisation.

It can be difficult to directly link the rate of contaminated doses administered through an intravenous catheter with the rate of CI (since other causes exist), but there is obvious concern that this might be the case. For example, the administration of contaminated PN has led to a number of reported deaths: 13 in Johannesburg (South Africa) in 1990[7] and another 8 in 1992[8]; 2 in Manchester (England) in 1994[9, 10]; 6 in Bloemfontein (South Africa) in 2004[11]; 3 in Mainz (Germany) in 2010[12, 13]; 9 in Alabama (USA) in 2011[14]; 3 in Chambery (France) in 2013[15]; and 1 in London (England) in 2014[16]. Nosocomial infections are a frequent major cause of morbidity, mortality and expense, making it necessary to introduce policies and practical clinical guidelines to reduce or eliminate them. Indeed, it has been estimated that over one year there were approximately 1.7 million cases of nosocomial infection in hospitals in the USA that were associated with almost 99,000 deaths, of which 31% were due to bloodstream infections[17]. Nosocomial infections also increase length of length of hospital stay, produce detrimental effects on well-being, with major costs to the healthcare system.

The financial burden of CI is considerable although it varies according to local tariffs and methods of management, and the severity and type of infection. The King's Fund estimated as early as 1992 that the cost was of between GBP 1650 and GBP 5,000 per episode[18]. The cost of an episode of CI in intensive care has been estimated at EUR 4,200 to EUR 13,030 in Europe in 2009[19], and as much as USD 34,508[20] and USD 56,167[21] in the USA in 1999 and 2001 respectively.

To limit clinical consequences and financial burden it is therefore essential to reduce potential exposure of intravenous lines and infusates to microbial contamination, limit growth of any microbial contaminants in intravenous feeds, and reduce the rate of CI in clinical practice.

1.3 STERILITY OF PARENTERAL NUTRITION INFUSATES AND THE USE OF ASEPTIC TECHNIQUES

Parenteral administration of any medicine bypasses important defence mechanisms of the body so there is a clear need to ensure sterility to limit the potential for infection. Strictly speaking a sterile medicine is one that contains no microbial contaminants but in practice there always remains a risk of contamination, because each of the five methods of terminal sterilisation (wet heat, dry heat, gas, irradiation and filtration) must be validated to ensure a reduction of the bioburden (microbial contaminants) to a sterility assurance limit of at least $1x10^{-6}$. Therefore, the probability of a dose being microbiologically contaminated cannot be greater than one in a million for it to be called 'sterile'.

Terminal sterilisation procedures can destabilise some medicines or their containers, or it may not be practical to supply terminally sterilised doses of a size to suit every patient. Medicines may therefore sometimes need to be terminally sterilised in a form that requires manipulation prior to administration to a patient. For example, solutions are often less stable than solid forms of drugs so that reconstitution of a terminally sterilised powder and/or dilution may be required prior to administration. There are a number of PN components that cannot be effectively terminally sterilised together because they may be destroyed or degraded, as is the case with certain vitamins. Therefore, PN infusates can consist of a number of terminally sterilised PN components which may be administered separately, or mixed together shortly before

use, for example in pharmaceutical environments or by mixing the contents of 'multichamber' PN bags in clinical environments.

The manipulation of terminally sterilised medicines prior to use (including PN components and infusates) is undertaken using a method called an aseptic technique where the operator manipulates the sterile starting components in such a way so as to minimise the risk of microbial contamination. Aseptic techniques vary and are dependent both on the operator and the circumstances. The most appropriate aseptic technique in one situation and environment may not be the most appropriate one in a different situation and environment. Therefore, careful training and validation in the use of aseptic techniques is essential to ensure correct techniques are utilised by operators.

However, adequate aseptic techniques alone cannot guarantee that a dose free from microbial contaminants will ultimately be prepared because random contamination of the dose from the environment could still occur during the manipulations. This is one reason it is often argued (and recommended, for example[22-24]) that aseptically prepared doses for parenteral administration should be made in a dedicated pharmacy unit offering a controlled environment, where the risk of environmental contamination can be reduced to very low levels, and significantly lower than in a clinical environment, such as a hospital ward or operating theatre.

Should an infusate for parenteral use become contaminated with microbes the extent to which the contaminants can survive and grow depends on the nature of the infusate. Indeed, the growth of different microbes varies in different intravenous infusion fluids[25, 26] and some can survive in some cytotoxic solutions[27]. Therefore, the type of infusate being administered may affect the risk of infection. It is intuitive that PN infusates would offer microbial contaminants a more desirable environment than some other infusates such as cytotoxics that are intended to cause cell death. The risk of microbial growth in PN infusates is increased by their potential storage prior to use and their administration by slow infusion, both of which provide more time for contaminant survival and subsequent multiplication.

In the UK, the high-risk nature of PN has led to the requirement[22] that all PN preparation and all additions directly into PN bags must only occur within dedicated

pharmaceutical environments that meet recognised standards[28, 29]. However, not all countries have a similar requirement, and even in the UK patients fed PN often still require ward-prepared flushes and medicines for parenteral use. These other doses may also pose a clinical risk to patients and a financial risk to the healthcare system.

1.4 CURRENT KNOWLEDGE GAPS

Pharmacists have been extremely successful in developing a niche in PN prescribing that capitalizes on their unique expertise in formulation and aseptic compounding, and often fluid and electrolyte balance, medicine logistics and financing. Furthermore, advances in legislation now allow suitable pharmacists to prescribe these complex medicines in some countries, including the UK. This makes pharmacists valuable in modern hospitals in nutrition support teams that care for PN patients. Their role could become even more valuable to patients and healthcare systems if they could demonstrate the value of evidence based, quality assured practice, particularly as it relates to their speciality, whilst identifying areas that could be improved by such practice. This challenge not only requires knowledge of the current evidence base but also identification of gaps in knowledge that need to be addressed in order to improve clinical practice.

This thesis will address a number of gaps in the current knowledge of PN use in order to identify practice that may help to reduce CI rates. These range from prescription methods, to types of infusions, including composition, to PN admixtures. In the section below these gaps are identified individually and a hypothesis is raised for testing. The gaps in knowledge and the sub-hypotheses raised to address them are then incorporated into an interrelated structured programme of work, with an overarching hypothesis. The next section identifies the specific gaps in knowledge, all of which were subsequently addressed by specific sub-hypotheses, and leads to the presentation of an overarching hypothesis.

1.4.1 PRESCRIPTION OF INFUSATE COMPOSITION

The duration over which a PN infusate is administered by slow intravenous infusion is dependent on the individual patient, the clinical circumstances, and local practice and policy. However, it would not be expected that a PN bag would be infused for

longer than 48 hours due to known physical or chemical instability of the infusate, or lack of data on stability. Most PN prescriptions indicate a bag is to be infused over a period of between 8 and 48 hours, but a number of guidelines may restrict this to a maximum of 24 hours for PN infusates of certain composition. In particular, there are a number of guidelines[6, 30-32] that recommend lipid emulsion or lipid-containing PN should not be infused beyond 24 hours, whereas it is acceptable to infuse lipid-free PN for periods up to 72 hours.

These guidelines restrict the versatility of prescription regimens, making the infusion procedures more complicated for nurses. Start-up regimens, such as those recommended by the NICE[33] and weaning regimens are easier to use when all the nutrients are administered together. However, restricting the infusion of lipid-containing PN to 24 hours (instead of 48 or even 72 hours) may increase the risk of infection due to the need to undertake more frequent manipulation of the central venous line. If lipid PN is avoided by replacing lipid with glucose, there is also the risk of increased infections due to hyperglycaemia, and potential problems with poor glycaemic control[34] and risk of developing essential fatty acid deficiency[35]. In addition, more frequent changes in regimens may increase work load and can create some confusion among nurses.

The above guidelines seem to be primarily based on the proposition that lipid is an independent risk factor for microbial growth, which in turn is based on a limited and inadequate evidence base. The European guidelines from ESPEN[32] are derived from clinical opinion and not on any cited research. The UK guidelines commissioned by the UK Department of Health (epic2)[31] again do not provide any supporting evidence from original research, but they refer to the 2002 American guidelines[30]. Recommendations from the latter guidelines stem from seven studies[36-42]. Of these, three are laboratory studies[40-42] that examined bacterial growth (up to 24 or 48 hours), but they are of little value since none compared lipid with non-lipid PN components. The remaining four studies[36-39] were also of little value since they were concerned with septicaemia and/or catheter colonisation: none set out to compare the effects of lipid-containing and lipid-free PN, and none to compare the effect of lipid infusions administered over less and more than 24 hours. Furthermore the proportion of patients receiving PN was unknown[36, 38, 39] and only 5.5% in one of them[37]. This hardly forms an adequate evidence-base for national and international guidelines.

Therefore, there is a need to gather the evidence that would allow objective examination of the proposition that microbes grow more rapidly in lipid PN than lipid-free PN. This would allow more robust recommendations to be made about the duration of infusion of feeds from the same PN bag, according to the presence of lipid.

The recently updated 2011 American 'Guidelines for the Prevention of Intravascular Catheter-Related Infections' from the National CDC[6] continues to recommend limiting the administration of lipid-containing intravenous feeds to 24 hours but that administration for longer periods of up to 72 hours continues to be acceptable for non-lipid feeds.

Despite the limited evidence of microbial growth in PN infusates, clinical practice has become widely entrenched throughout the world as a result of these influential guidelines. Therefore, there is a need to either confirm the recommendations of these guidelines or alternatively to provide an evidence base to support a change in established practice to limit the practical difficulties and clinical risks described above. It seems reasonable to approach the problem in two steps. First, to develop and apply an experimental framework that can examine whether lipid has a specific effect in stimulating the growth of microbes in PN infusates that dominates and overrides any other independent effects such as those produced by glucose concentration, energy density and pH of the feed. And second, to critically appraise the existing studies using standard protocols for systematic review, which may include met-analyses where appropriate.

In summary, the evidence base that underpins the national and international guidelines on the duration of infusion of lipid PN (on the assumption that microbes grow more quickly in lipid PN than in lipid-free PN) appears weak and incomplete[43-45]. This gap in knowledge can be addressed by examining the validity of the established guidelines[6, 30-32] through a rigorous and more complete analysis of the existing studies according to procedures demanded by systematic reviews with meta-analyses. Since several gaps in knowledge are identified this can be referred to as gap 1, which can be addressed by sub-hypothesis 1:

"Exclusion of lipid from PN infusates will limit bacterial growth compared to lipid-containing PN infusates, in line with current guidelines which have a weak evidence base."

1.4.2 METHOD OF PRESCRIPTION

Pharmacists have historically advised clinicians on the formulation of PN but recent changes in legislation now allow pharmacists to independently prescribe these complex medicines[46]. The responsibility for such prescribing would be expected to involve the mode of prescribing employed.

Several procedures are utilised to limit nosocomial infections such as hand washing, patient isolation until free of infection, choice of antibiotics and surveillance following outbreaks[47]. However, although 'contaminated objects' is a risk factor for microbial transmission[47], little attention has been placed on the possibility that PN prescriptions are a source of infection.

Prescriptions are often made on paper, which are recycled between the pharmacy, where aseptic services operate, and the clinical environment, where the patients are reviewed. These prescriptions may be a source of contamination entering the pharmacy aseptic suite, increasing not only the local bioburden but potentially also increasing the range of bacteria which may be pathogenic. This in turn increases the risk of contaminating a PN during compounding with microbes that may be pathogenic. Potentially difficult problems may arise if the prescription charts carry pathogenic multidrug resistant organisms, as has been reported in surgical patients[48]. However, no studies appear to have examined whether prescriptions for PN can be involved in such pathways of microbial transmission. This is surprising for at least two reasons. First, patients receiving PN often suffer from severe disease, are often immunocompromised and distributed in different wards where a wide variety of pathogenic organisms are found. Second, the World Health Organisation has recommended that physicians and nurses should follow practices that limit exposure to, and transfer of, infection, and that pharmacists in hospitals should distribute pharmaceuticals in a way that limits the potential transfer of infection[47].

One way in which the method of PN prescription may reduce this risk is to use electronic prescribing and to print daily prescriptions within the pharmaceutical environment for immediate use. However, widespread implementation of electronic prescription of PN would require significant investment that may be difficult to justify without an adequate evidence base.

In summary, there is a lack of evidence to determine whether freshly printed electronic prescriptions for PN offer an advantage over handwritten recycled printed paper prescriptions for PN to reduce delivery of microbial contaminants from the clinical to the pharmaceutical environment and *vice versa*. This gap in knowledge can be addressed by though microbiological assessment of the different ways of prescribing PN. This gap in knowledge can be referred to as gap 2, which can be addressed by sub-hypothesis 2:

"Changing the prescribing method for parenteral nutrition from recycling handwritten paper charts to prescribing electronically with fresh printing of prescriptions will reduce delivery of microbial contaminants from the clinical to the pharmaceutical environment."

1.4.3 COMPOUNDING ENVIRONMENT FOR INFUSATE

A number of recommendations have been made in the UK to reduce the risk of microbial contamination of aseptically prepared medicines for parenteral administration, including the following three. First, the British Standard BS EN ISO 14644-1: 1999[49] and EC GMP require dedicated units to operate to defined high standards when using aseptic techniques to prepare doses[28, 29]. Second, all aseptic dose preparation should be carried out in dedicated units with a minimum number of additions, according to the National UK guidelines[22-24]. Third, any doses that are prepared in a clinical environment should be used immediately or appropriately destroyed[28]. This last recommendation prevents batches from being prepared in clinical environments because whilst a batch generally involves fewer manipulations per final dose unit prepared, this could be offset by storage before use, which allows time for growth of any contaminating bacteria. For this reason only products prepared aseptically in a pharmaceutical environment may be stored before use, which restricts the opportunity for batch preparation to dedicated pharmacy units.

These recommendations have a theoretical basis and an intuitive appeal, but surprisingly no synthesis of experimental information is available to support them. For example, if experimental evidence can be found to show that there is no difference in microbial contamination rates between doses prepared in dedicated units and clinical wards, then there may be reluctance to undertake time consuming and costly work in dedicated pharmaceutical units.

In summary, there is a lack of evidence to determine whether the use of pharmaceutical rather than clinical environments offers a lower risk of contamination of doses for parenteral administration compounded under aseptic techniques.

This gap in knowledge (gap 3) can be addressed by undertaking a systematic review with meta-analyses to examine the rates of microbiological contamination of individual and batch doses prepared using aseptic techniques in different environments, and the effect of additions being made to sterile products under aseptic technique in clinical compared to pharmaceutical environments. It can be examined using the following hypothesis (sub-hypothesis 3):

“Undertaking aseptic manipulation of doses in a pharmaceutical environment in preference to a clinical environment (which has a higher microbial load) will reduce the dose contamination rate that can be achieved.”

1.4.4 DOSE PACKAGING AND TRANSFER

Once prescriptions have been used to prepare PN bags in pharmacy aseptic services, the prescription and the bag need to be transferred to the hospital ward or other location so that the PN can be administered to the patient.

Separate delivery of PN bags and PN prescriptions could be problematic for a variety of reasons including loss of prescriptions and administrative delays, both of which require human resource to resolve. Since both the prescription and feed are required simultaneously before administration can commence, it would be reasonable to expect both of them to be delivered to the ward at the same time. The simplest method of achieving this and preventing delays and confusion is to place the prescription next to the bag before sealing them together, often using plastic.

If PN bags are prepared in a hospital pharmaceutical environment that meets appropriate standards, such as those in use in the UK[28, 29], the feed itself should be effectively free of microbes. However, the sealed plastic encasing the bag and the trapped air or other materials placed between the surface of the bag and the surrounding plastic encasing of the bag are almost certainly not. Therefore, despite the practical attraction of sealing PN bags and prescriptions together for delivery to patients on the wards, microbes could be transferred from the prescription to the outer bag surface. This in turn could increase the risk of infection of the indwelling venous catheter through touch transfer, and this risk may be exacerbated further should staff mistakenly believe the entire content of the sealed plastic to be sterile rather than only the PN infusate itself. Indeed, this mistaken belief may even lead them to place the bag directly onto the 'sterile field' during aseptic manipulations (connection and disconnection) involving the patient's intravenous catheter.

In summary, there is a lack of evidence to determine whether packaging of compounded PN bags in direct contact with freshly printed electronic rather than handwritten recycled prescriptions during their delivery reduces the surface contamination of those PN bags when they arrive at the destination clinical environment (hospital ward).

This gap in knowledge (gap 4) can be addressed through microbiological assessment of the effect of packaging different types of prescription with compounded PN bags on the microbial surface contamination of those PN bags during transfer to the destination clinical environment. It can be specifically examined using the following hypothesis (sub-hypothesis 4):

"Packaging of compounded PN bags and prescriptions (with potential surface contaminants) separately rather than together for transfer to clinical environments will reduce microbial surface contaminants on arrival at the destination."

1.4.5 TRAINING AND EXPERIENCE IN ASEPTIC TECHNIQUES

Variable training and practice in the use of aseptic techniques is likely to differ between individual healthcare workers within a particular profession as well as between healthcare workers from different professions. This could result in variable

rates of dose contamination during preparation, and line contamination during administration (increased CI rates).

A recent systematic review[50] concluded that there was a lack of information on the impact of different aseptic techniques on the contamination rate of dose preparation within a consistent environment, identifying this as an important area for future research. Research in this area could support the intuitive rationale of ensuring appropriate education and training in the use of aseptic techniques is embedded into routine clinical practice.

In summary, there is a lack of evidence to determine whether improved aseptic technique can reduce variable contamination rates of doses prepared in a consistent environment. This gap in knowledge (gap 5) can be addressed through a comparison of the rate of contamination of doses prepared by operators more or less experienced and trained in the use of aseptic techniques. It can be tested using the following hypothesis (sub-hypothesis 5):

"Substituting staff with less training and experience in aseptic techniques in a clinical environment with more trained and experienced staff will reduce the rate of microbial contamination of doses prepared under aseptic technique."

1.4.6 DEFINITION OF INTRAVASCULAR CATHETER INFECTION

CI is a serious complication of intravenous fluid and drug administration, which is costly and associated with increased mortality[18-21]. Therefore, there has been considerable investment to reduce the number of CI and to use it as a benchmark for the quality of service provision. However, the different criteria used to determine CI could have major effects on the benchmark, and potentially also on the ranking order of institutions by CI rates. The variability in reported CI rates is surprisingly large, for example ranging from zero[51] to over 25%[52] in patients administered PN. Furthermore, the effects of interventions, such as the introduction of a nutrition team, has been reported to reduce CI in such patients by more than an order of magnitude[18], or by an average of 27 to 2.5 percent[53].

Such large variability may at least partly be due to choice of diagnostic criteria to assess CI and to other methodological differences, such as the extent to which confounding variables and missing data are taken into account. For example, ignoring missing blood cultures, which are required for some definitions of CI, could introduce bias, especially if the missing results did not occur randomly. Such factors could be responsible for creating invalid and misleading comparisons between centres and types of intervention.

In summary, there is a lack of evidence to determine the extent to which the number of CI is affected by different commonly used or recommended definitions, and by different methods for dealing with missing data. This gap in knowledge can be addressed through an assessment of the number of CI in a fixed patient cohort receiving PN (which is often considered to be at particular risk of CI, due in part to the potentially enhanced microbial growth in the nutrient rich PN infusates) according to different definitions for CI. This gap in knowledge can be referred to as gap 6, which can be tested using sub-hypothesis 6:

“Changes in CI rates depend on the definition of CI, which can vary widely.”

1.5 HYPOTHESIS

The gaps in knowledge identified in the previous section can be brought together and addressed collectively by the following overarching hypothesis:

“Simple measures in clinical and pharmaceutical environments can reduce potential exposure of intravenous lines and infusates to microbial contamination, limit microbial growth in PN and reduce the rate of CI to an extent that depends on the definition applied.”

This overarching hypothesis is made up of a series of interrelated sub-hypotheses which were based on the specific gaps in knowledge identified in the previous section. Brought together they can be used to address what can be described as the ward-pharmacy intravenous prescribing cycle (Figure 1.1.), whereby each step of the cycle is linked to the preceding step and also to a potential intervention that can minimise the risk of infection. Without such interventions there is not only a risk

that poor practice in one part of the cycle could influence that in another, but also that infection could be exacerbated further by the operation of a positive feedback loop. The individual interventions shown in Figure 1.1 are linked to the six sub-hypotheses, which are identified by a specific number (1 – 6 below), and which were referred to in section 1.4.

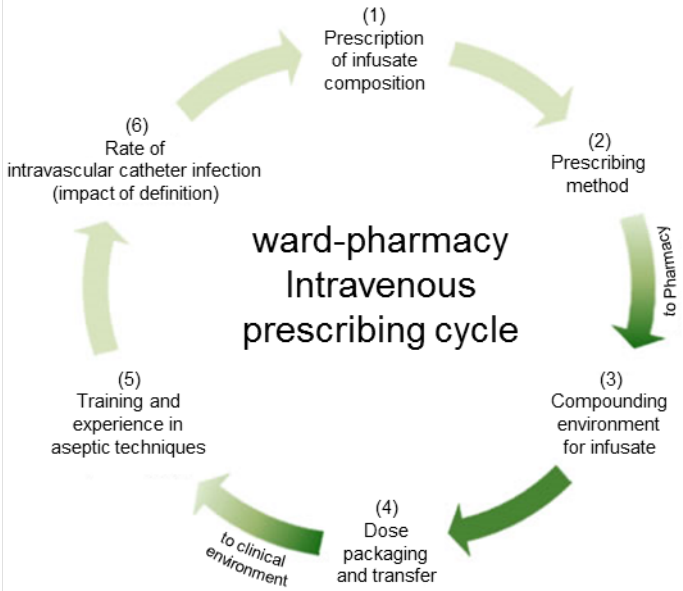

Figure 1.1 The ward-pharmacy intravenous prescribing cycle

Each of these hypotheses that may lead to a change in practice aim to examine whether or not a reduction in the contamination (or CI) rate and/or growth of bacteria can be achieved by:

(1) Exclusion of lipid from PN infusates in line with current guidelines, which have a weak evidence base.

(2) Changing the prescribing method from recycling handwritten paper prescription charts to prescribing electronically and using freshly printed prescriptions.

(3) Undertaking aseptic manipulation of doses in a pharmaceutical environment in preference to a clinical environment, which has a higher microbial load.

(4) Packaging of compounded PN bags and prescriptions (with potential surface contaminants) separately rather than together for transfer to clinical environments.

(5) Substituting staff with less training and experience in aseptic techniques in a clinical environment with more trained and experienced staff.

(6) Understanding the impact of definition when comparing CI rates between institutions or following interventions.

CHAPTER 2

METHODS

2.1 METHODS FOR SUB-HYPOTHESIS ONE

Sub-hypothesis one is that "exclusion of lipid from PN infusates will limit bacterial growth compared to lipid-containing PN infusates, in line with current guidelines which have a weak evidence base."

2.1.1 TESTING MICROBIAL GROWTH IN PARENTERAL NUTRITION INFUSATES

2.1.1.1 Preparation of parenteral nutrition infusates

The required physic-chemical stability was assessed for each PN infusate[54, 55] prior to compounding. All PN infusates were prepared within a validated EC GMP grade A environment in a NHS aseptic unit (Pharmacy Department of University Hospital Southampton NHS Foundation Trust; Southampton, UK) following standard aseptic procedures. Prior to removal of the prepared bags from the aseptic environment a dispensing pin (B. Braun; Allentown, USA) was inserted into the giving set port of each bag and used to remove a 25mL aliquot of the infusate into a 30mL luer-lock syringe (BD; Franklin Lakes, USA) prior to capping the dispensing pin with a universal plug (Vygon; Aachen, Germany).

2.1.1.2 Measurement and adjustment of pH of parenteral nutrition infusates

In a standard laboratory a glass electrode pH meter (Orion 3 Star pH Benchtop from Thermo Fisher Scientific; Singapore) calibrated with three standard buffers at pH 4.00±0.02, pH 7.00±0.02 and pH 9.22±0.02 (BDH Laboratory Supplies; Lutterworth, UK) was used to measure the pH of each 25mL PN aliquot. The pH of some PN infusates was modified by addition of hydrochloric acid and/or sodium hydroxide at concentrations of 2M, 0.2M and 0.02M through the bag additive ports through a 25mm 5-micrometer Versapor membrane syringe filter (Pall Corporation; Michigan, USA) under aseptic technique. Following each adjustment each bag was completely

inverted five times before a 10mL sample was taken from the dispensing pin after a 4mL discard (to account for dead space in the port). The pH of the 10mL sample was tested and the adjustment procedure repeated until the infusate pH was within 0.08 units of the target pH. The calibration of the pH meter was regularly checked (at least after checking initial PN infusate pH and after adjustment of PN infusate pH) with minor adjustments made to the calibration if necessary.

2.1.1.3 Microbes for inoculation

Three microbes were used to separately inoculate sets of PN infusates, all of which were sourced from the Health Protection Agency Culture Collections (Salisbury, UK): *S. epidermidis* NCTC 11047; *C. albicans* NCPF 3179; and *E. coli* NCTC 9001. The *E. coli* was inadvertently contaminated with a single strain of wild type *E. durans*, which was confirmed from multiple samples tested by Public Health England (Colindale, London, UK). This meant that both *E. coli* and *E. durans* were studied at the same time in the same infusates.

2.1.1.4 Inoculation of prepared parenteral nutrition infusates with microbes

Inoculations of prepared PN infusates were undertaken under aseptic technique within a validated positive pressure laminar flow hood (VLF K3 from Bassaire; Southampton, UK) in a category 2 microbiology laboratory (the Health Protection Agency Food, Water and Environmental Laboratory in Southampton, UK), operating at a controlled room temperature monitored using a calibrated recording device (Multiuse EVt2 from Comark; Dongguan, China). Once made up, each microbial stock solution was cultured to establish the cfu/mL of the primary inoculum in order to be able to calculate the appropriate volume of stock solution to inoculate each PN bag. Each prepared PN bag was gently warmed to room temperature for 2 hours prior to disinfection of the additive port using 70% v/v isopropyl alcohol and 2% w/v chlorhexidine gluconate wipes (Professional Disposables International Ltd; Flint, UK). A 3mL luer-lock syringe (BD; Franklin Lakes, USA) with a 19G 1.5 inch needle (Microlance 3 from BD; Drogheda, Ireland) was used to inject the stock culture through the additive port of each PN bag with the aim to yield 50cfu/mL, which is an initial count similar to that used in a number of other studies[56-59], and which would be expected to ensure detection on sampling and may reflect

contamination in clinical practice. For each inoculation the needle was inserted into the additive port to its maximum depth with the bag inclined down and away from the operator to limit any possibility of the inoculate pooling at the injection site. Immediately after inoculation, each bag was completely inverted twice and repeatedly compressed to ensure adequate mixing.

2.1.1.5 Sampling of microbial colony forming units from prepared and inoculated parenteral nutrition infusates

Sampling was undertaken under aseptic technique within a validated positive pressure laminar flow hood (VLF K3 from Bassaire; Southampton, UK) in the same category 2 microbiology laboratory (the Health Protection Agency Food, Water and Environmental Laboratory in Southampton, UK) used to inoculate the PN infusates. Each bag was sampled immediately after inoculation (at time zero) and after 24 and 48 hours. Between samplings each bag was hung on a stand to simulate conditions that operate in clinical practice. Immediately prior to sampling in the positive pressure laminar flow hood, each bag was gently squeezed three times whilst still suspended on the stand. Four mL discards taken from the dispensing pin using 5mL luer-lock syringes (BD; Franklin Lakes, USA) preceded the removal of aliquots for testing using new 1mL luer-lock syringes (BD; Franklin Lakes, USA). Immediately following sampling, the dispensing pin was resealed with a fresh universal plug (Vygon; Aachen, Germany) at each time point.

2.1.1.6 Measurement of microbial colony forming units in parenteral nutrition infusate samples

When required, ‘maximum recovery diluent’ (Health Protection Agency, Southampton, UK) was used for dilution of infusate samples prior to plating. Sample aliquots were evenly distributed over the surface of Columbia blood agar plates (Oxoid Ltd; Basingstoke, UK) using either spreaders (Prolab Diagnostics; Wirral, UK) for 0.2mL or 0.5mL volumes, or an automated spiral plater (Don Whitley Scientific Ltd, Shipley, UK) to dispense 0.05mL of sample from 5mL spiral cups (Don Whitley Scientific Ltd, Skipton, UK). Following plate incubation, a member of the Health Protection Agency Food, Water and Environmental Laboratory in Southampton independently assessed and reported plate growths. That operator was

blinded to the composition and pH of the samples that were being tested, although white or cream infusates indicated the presence of lipid.

2.1.1.7 Sample size

Sample size calculations were based on ANCOVA and undertaken using SPSS SamplePower version 2.0 (Chicago, Illinois, USA). A sample size of 16 bags for each of two groups (with and without lipid) and an assumed R^2 of 0.50 for three covariates in combination (cfu/mL at time zero, glucose concentration and pH) would detect an effect size according to Cohen's criteria of 0.38 (medium effect size, $f = 0.25$ and large effect size, $f = 0.40$) with 80% power and a p-value of 0.05. If R^2 was only 0.30 the same sample size would detect an effect size of 0.45 with the same power and p-value.

2.1.1.8 Statistics

If the distribution of cfu/mL for a test bacterium was not normally distributed, either at 24 or 48 hours, the results were log transformed prior to statistical analyses. Simple statistics such as mean changes in cfu/mL over time and associated measures of variations were obtained using ANOVA and repeated measures ANOVA. A general linear model was used to examine more detailed relationships between variables. ANCOVA was used in the absence of significant heterogeneity of regression (with type of infusate as fixed factor and other variables as covariates e.g. pH and glucose concentration). All the ANCOVA results can also be extracted from a single analysis of repeated measures ANOVA (24 and 48 hour cfu/mL) in which baseline cfu/mL is included as a covariate. Separate analyses (multiple linear regression) were used where there was significant heterogeneity of regression. Simple linear regression was used for comparisons with no covariates. The Student independent sample *t* test was also used. Unless otherwise stated, results are presented as mean ± SEM. The analyses were undertaken using SPSS versions 18 and 21 (Chicago, Illinois, USA).

2.1.2 SYSTEMATIC REVIEW OF MICROBIAL GROWTH IN PARENTERAL NUTRITION INFUSATES

2.1.2.1 Literature search

The literature search was undertaken on 22 February 2014 using all available years in three databases: Medline (OvidSP) from 1946; Embase (OvidSP) from 1947; and the complete Cochrane Library (John Wiley & Sons, Ltd).

The primary outcome was the extent of promotion or suppression of microbial growth over a 48-hour period in each of lipid emulsion, lipid PN and lipid-free PN, expressed as a microbial 'growth ratio' (GR; $\log_{10}$ [cfu.mL^{-1} at 48 hours / cfu.mL^{-1} at time zero]).

2.1.2.2 Search terms

Appendix A shows the search terms (including variations and pleural terms) used in combination with each other and the corresponding numbers of identified records (i.e. papers, publications or journal articles). Additional records were sought through cross-referencing and discussion with experts in the field.

2.1.2.3 Inclusion and exclusion criteria

The inclusion criteria were records in the English language that reported laboratory studies of microbial growth in lipid emulsion, lipid PN or lipid-free PN over a 48-hour period. Abstracts were excluded if they reported work that was subsequently published as full papers. Records were also excluded if they duplicated previous work or if the cfu/mL had not been reported at both time zero and at 48 hours (except for one publication[59] that reported the cfu/mL at time zero and at 40 hours). Furthermore, work that did not define infusate composition was not included.

2.1.2.4 Search procedure

A total of 14700 records were identified by the literature search: 7546 from Medline; 6145 from Embase; and 1009 from the Cochrane Library. The removal of duplicates

left 10623 records. Eight additional records[43-45, 60-64] were identified by cross-referencing, hand searching and discussion with experts in the field. This led to 10631 records, of which 69 remained after the exclusion of 10562 of the records during a review of the abstracts. The complete text of each of the remaining 69 records was printed for more detailed examination which resulted in exclusion of a further 45 records for the reasons indicated in Figure 2.1. This left 24 full text records[42-45, 56-75] for detailed review. The methodological stages of the literature search are summarised in Figure 2.1.

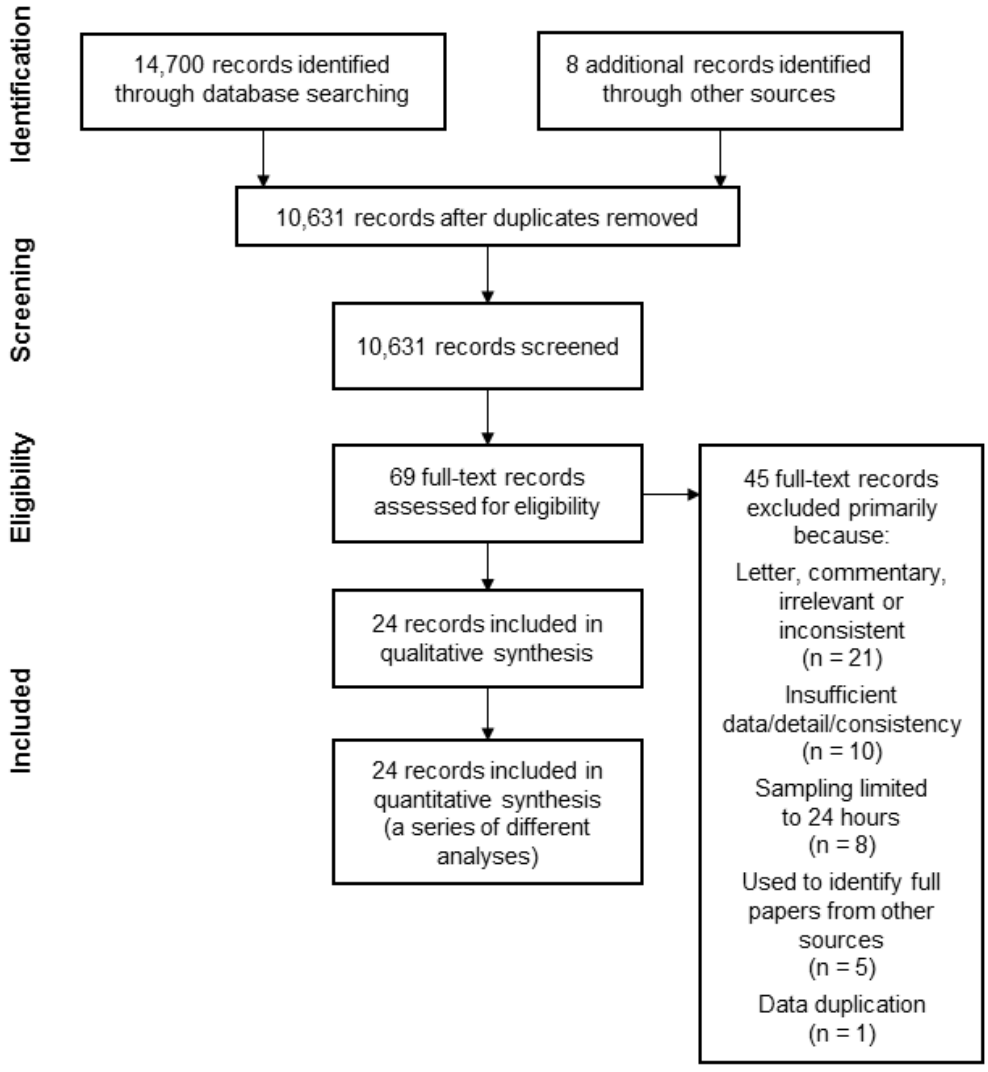

Figure 2.1 The methodological stages of the literature search used to identify studies that reported microbial 'growth' in lipid emulsion and parenteral nutrition with and without lipid over a 48 hour period

2.1.2.5 Sample size

The sample size was determined by the results of the literature search.

2.1.2.6 Statistics

Two main sets of analyses were undertaken: an unmatched analysis of all the identified data, except for *E. durans* in lipid emulsion (see above); and a matched analysis based on within study methodologies. The matched analysis involved estimation of the GR in PN of the same composition using the same methodology and the same (or almost the same) ambient conditions, except for the presence of lipid, which in most cases increased the energy density. Simple comparisons involved the use of unpaired and paired t tests and ANOVA according to requirements. General linear models, with GR as the dependent variable, were used to make adjustments for type of infusate (as a fixed factor), species of microbe (as a fixed factor) and glucose concentration (as covariate). Random effects meta-analyses were also carried out to compare matched datasets. Two-group random effects meta-analyses were used to examine the effect of isoenergetic (1 Mcal) exchange glucose for lipid whilst maintaining a fixed glucose concentration of 13.33% w/v and the addition of lipid (1 Mcal) to lipid-free PN while the glucose concentration remained constant at 13.33% w/v. A one-group random effects meta-analysis of B coefficients was used to examine the effect of percent non-protein energy as lipid on GR. The B coefficients were extracted from a general linear model with GR as the dependent variable and percent non-protein energy as lipid as the independent variable after adjustment for energy density (Mcal/2L of PN) (no fixed factor in model). The 95% confidence ellipses of GR-glucose concentration (unmatched datasets) were constructed using the central limit theorem[76]. Unless otherwise indicated, results are presented as GR mean ± SD**.** A two-tailed p-value < 0.05 was considered statistically significant. Analyses were undertaken using the software SPSS Statistics version 22 (Chicago, Illinois, USA) and Comprehensive Meta-Analysis version 2 (Biostat, Englewood, New Jersey, USA).

2.1.2.7 Quality of studies

Two of the authors (PDA and KSH) independently assessed the quality of each eligible publication for the purpose this review using the GRADE system[77, 78], with any disagreements resolved by discussion. The systematic review was undertaken using the guidelines recommended by the Cochrane Collaboration[79], the CRD[80], and the PRISMA guidelines[81].

2.2 METHODS FOR SUB-HYPOTHESES TWO AND FOUR

Sub-hypothesis two is that "changing the prescribing method for parenteral nutrition from recycling handwritten paper charts to prescribing electronically with fresh printing of prescriptions will reduce delivery of microbial contaminants from the clinical to the pharmaceutical environment", and sub-hypothesis four is that "packaging of compounded PN bags and prescriptions (with potential surface contaminants) separately rather than together for transfer to clinical environments will reduce microbial surface contaminants on arrival at the destination."

2.2.1 TYPES OF PRESCRIPTIONS AND BAGS

All of the PN prescriptions used were of the same size (A4 size; 21cm by 29.7cm), and consisted of a single-sided pre-printed proforma, which were either freshly printed electronic or recycled. Each freshly printed electronic prescription consisted of a single prescription on a single sheet printed within the pharmacy department immediately before use. These electronic prescriptions were each used only once, so that no prescription recycling from the ward back to pharmacy took place. Each handwritten recycled prescription sheet had space for up to six individual PN prescriptions that were completed using indelible ink by members of the Nutrition Team during their routine duties in clinical environments (hospital wards). When no more space remained on a handwritten recycled prescription sheet a new sheet was stapled to the top so that the base (lowermost) sheet was always the one that had been in circulation for the longest period. These handwritten recycled prescriptions were transferred between the clinical and pharmaceutical environments each day that a new PN bag was required, until the patient no longer required PN.

The choice of PN bag was determined by the Nutrition Team and not by the study methodology. The multichamber ('Oliclinomel' from Baxter; Lessines, Belgium) and gravity filled PN bags (using 'Freka Mix' bags from Fresenius Kabi; Bad Hersfeld, Germany and 'Ultrastab' bags from Baxter Healthcare SA; Zurich, Switzerland) included in the study were of an overall similar size. The median sampled surface areas and IQR of all the bags were identical to those that were destined to be packaged with either freshly printed electronic or handwritten recycled prescriptions (1027 (IQR 936, 1027) cm^2).

2.2.2 RANDOMISATION PROCEDURE

A binary number (0 or 1) was randomly established using computer software (Excel 2000 from Microsoft Corporation; Redmond, Washington, USA), using the formula '=RAND()' in the A1 spreadsheet cell and the formula 'IF(A1<0.5,0,1)' in the A2 spreadsheet cell. A result of less than 0.500000 (including zero) indicated the use of an electronic prescription, and a result of 0.500000 or greater indicated the use of a handwritten recycled prescription. All of the bacteriologists handling the samples were blinded to the randomisation.

2.2.3 TAKING SWAB SAMPLES

To assess the microbial load on prescriptions, the entire surface of the reverse and blank surface of the base sheet of freshly printed electronic and handwritten recycled prescriptions was sampled by a single operator (PDA), irrespective of how many sheets a handwritten recycled prescription consisted of.

To assess the microbial load on the surface of compounded bags following delivery packaged in direct contact with the prescription in heat sealed plastic, the entire back surface of PN bags was sampled (i.e. the bag surface without any paper label describing the bag content and with the instructions for administration, and the surface in contact with the prescription during delivery).

Non-sterile blue nitrile examination gloves (Premier Healthcare and Hygiene Ltd; Selangor, Malaysia) were worn when samples were taken using cellulose sponge stick swabs with neutralising buffer (3M; Juechen, USA). A fresh swab was used to

take each sample and was removed from the individual bag it was supplied in immediately prior to use. The first side of the swab was used in a horizontal direction over the surface to be sampled before the other side of the swab was used in a vertical direction over the same surface, avoiding the very edges each time. The sampled surface was then disinfected using 70% v/v isopropyl alcohol BP wipes (Azowipes from Synergy Health plc; Chorley, UK) in the pharmaceutical environment or 2% w/v chlorhexidine gluconate wipes (Professional Disposables International Ltd, Flint, UK) in a clinical environment, and allowed to dry. Immediately following sampling and surface disinfection, each swab was re-sealed in the individual bag from which it had been removed and promptly receipted into a category 2 microbiology laboratory within the Health Protection Agency Food, Water and Environmental Laboratory (in Southampton, UK), within their maximum recommended period of 24 hours.

2.2.4 MICROBIOLOGICAL INVESTIGATION OF SWAB SAMPLES

All of the microbiology was undertaken in a category 2 microbiology laboratory within the Health Protection Agency Food, Water and Environmental Laboratory (in Southampton, UK) using standard procedures. 10 mL of maximum recovery diluent (Health Protection Agency; Southampton, UK) was added into each of the bags containing a swab before they were mixed using an impactor (Stomacher from Don Whitley Scientific Ltd; Shipley, UK) for 60 seconds. Following impaction, the liquid from the bag was poured into a 30 mL ‘universal’ tube (Sterilin Ltd; Newport, UK) from which 0.5 mL aliquots were distributed onto a series of growth plates of different media (Plate Count Agar, Rose Bengal Chloramphenicol, Slanetz and Bartley and Baird Parker Medium; all from Oxoid Limited; Perth, Scotland) using spreaders (Prolab Diagnostics; Wirral, UK) to maximise the detection of specific potential contaminants (Table 2.1). Columbia Blood Agar and DNase agar plates (both from Oxoid Limited; Perth, Scotland) were used to investigate whether colonies growing on Baird Parker Medium were *S. aureus* (Figure 2.2).

Table 2.1 Media plates used to select particular potential surface contaminants

Media[a]	Purpose	Incubation
Plate Count Agar	To detect non-specific aerobic bacteria	30°C (range 29.1°C, 31.1°C) for 48 hours
Rose Bengal Chloramphenicol	To detect moulds and yeasts	25°C (range 23.6°C, 25.6°C) for 48 hours for yeasts and 5 days for moulds
Slanetz and Bartley	To detect faecal streptococci (Enterococci)	30°C (range 28.9°C, 30.9°C) for 4 hours followed by 37°C (range 35.6°C, 37.6°C) for 35 hours
Baird Parker Medium	To provide an initial indication of staphylococcal contaminants (subject to additional procedures below)	37°C (range 35.7°C, 37.7°C) for 48 hours
Columbia Blood Agar	For further investigation of colonies on Baird Parker Medium to test for non-specific staphylococci and *S. aureus* using colony morphology +/- latex agglutination test +/- tube coagulase test	37°C (range 35.7°C, 37.7°C) for 24 hours
DNase	A DNase test was used for further investigation of colonies on Baird Parker Medium to test for *S. aureus* if the colonies had a surrounding zone of clearing on Baird Parker Medium or if their morphology on Columbia Blood Agar was consistent with *S. aureus*	37°C (range 35.7°C, 37.7°C) for 24 hours
Brilliance MRSA 2	For further investigation of *S. aureus* colonies (blue colonies were tested for methicillin resistance against a battery of antibiotics)	37°C (range 35.7°C, 37.7°C) for 24 hours

[a] All media plates were sourced from Oxoid Limited; Perth, Scotland.

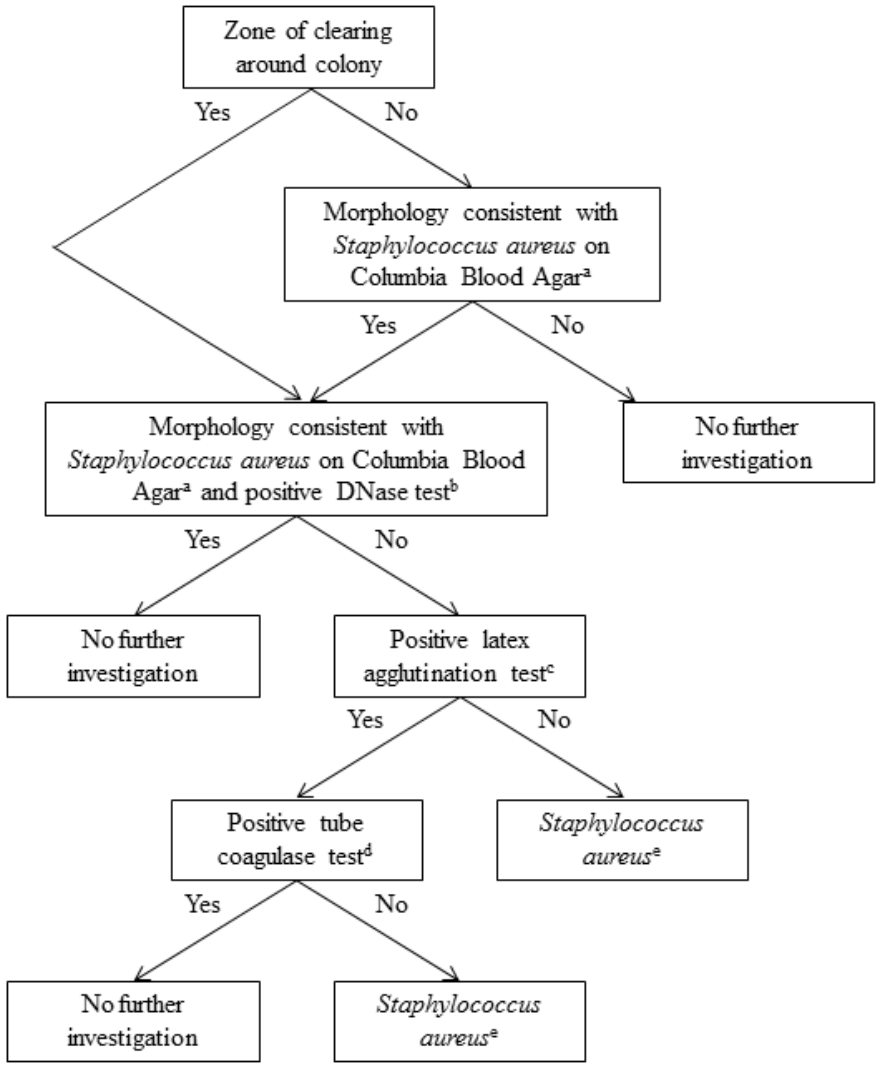

Figure 2.2 Procedure to establish whether colonies on Baird Parker Medium were *S. aureus*

[a] Large, circular, convex colonies with a white or golden colour
[b] Purple colour around colony on a blue background when toluene blue is added to the media surface or clear area on an opaque background when 1M hydrochloric acid is added to the media surface
[c] Using the test-kit Pastorex Staph-Plus (Bio-Rad Laboratories, Paris, France)
[d] Clotting after 4 or 24 hours when the sample bacteria are warmed at 37°C in Brain Heart e Infusion broth diluted 1 in 4 with reconstituted dehydrated lyophilised rabbit plasma
[e] Subsequently tested for methicillin resistance

Further investigations were carried out to determine whether colonies identified as *S. aureus* were a resistant form. The relevant colony was picked onto a 'brilliance MRSA 2' media plate (Oxoid; Perth, Scotland) using a sterile plastic inoculating loop (Medical Wire and Electronics; Corsham, UK). Any of these colonies that

indicated some antibiotic resistance (a blue colony colour following incubation) were taken to the clinical Health Protection Agency laboratory in Southampton for resistance testing against a battery of antibiotics where it was attempted to grow the colonies on growth plates with agar incorporation (by the clinical Health Protection Agency; Southampton, UK) of Cefoxitin, Erythromycin, Vancomycin, Teicoplanin, Gentamicin, Rifampicin, Fucidin, Tetracycline, Trimethoprim, Chloramphenicol, Mupirocin, Neomycin and Ciprofloxacin.

2.2.5 SAMPLE SIZE

Sample size calculations were undertaken using two levels of contamination to reflect the uncertainly and range of contaminations that are likely to apply in clinical practice. First, with a starting contamination rate of 99.9999% for the handwritten recycled prescriptions a sample size of 32 per group is sufficient to detect a reduced contamination rate of 79% in the electronic prescriptions group with 80% power and a significance of $P < 0.05$ (2-tailed). Second, with a starting contamination rate of 75% for the handwritten recycled prescriptions the same sample size is sufficient to detect a reduced contamination rate of 41% in the electronic prescriptions group with the same power and significance. The sample size calculations were based on Fisher's exact test and undertaken using SPSS SamplePower version 2.0 (IBM; Chicago, Illinois, USA).

2.2.6 STATISTICS

A comparison of the proportions of contaminated prescriptions between the recycled and electronic groups was made using Fisher's exact test, and RR was also calculated. Differences in the number of microorganisms (expressed as the number found on a prescription or bag surface by multiplying the result by a factor of 20) between the recycled and electronic groups were assessed non-parametrically using the Mann Whitney U test because of the skewed distribution of the number of contaminants, which did not normalize after logarithmic transformation.

Relationships between the number of contaminants and risk factors (for example, the total number of prescriptions written) were also examined non-parametrically using

the Spearman rank correlation coefficient. These analyses were undertaken using SPSS version 18 (IBM; Chicago, Illinois, USA).

2.3 METHODS FOR SUB-HYPOTHESIS THREE

Sub-hypothesis three is that "undertaking aseptic manipulation of doses in a pharmaceutical environment in preference to a clinical environment (which has a higher microbial load) will reduce the dose contamination rate that can be achieved."

2.3.1 LITERATURE SEARCH

The literature search was undertaken on 10 February 2014 with a wider protocol than that undertaken by the previous review[50].

The literature search included studies that involved microbial contamination with bacteria and/or fungi. The studies involved preparation of doses for parenteral administration to patients prepared under aseptic techniques, including simulation studies.

In cases where a single record reported more than one outcome, for example when using different preparation environments, each outcome was included as a separate study. Consistent data within the same record were combined only if whole groups of data could be combined.

The present literature search used an additional search term, truncated search terms, and combination of three search terms only if more than 5000 results were returned for any two search term combination. Three databases were used for all available years: Medline from 1946 onwards using OvidSP; Embase from 1947 onwards using OvidSP; and the Cochrane Library. Attempts were made to identify further records by hand searching.

2.3.2 SEARCH TERMS

Appendix B shows the search terms used: each of four search terms was combined with each of four further search terms, unless a combination returned more than 5000

results in which case a third search term group was added in an attempt to capture the most relevant results. Additional records were sought through cross-referencing and discussion with experts in the field.

2.3.3 INCLUSION AND EXCLUSION CRITERIA

Studies were excluded if they were not reported in the English language, if they only involved animals, or if they reported the rate of contamination of infusate stock (an infusate in a single container used to prepare multiple doses) rather than actual or simulated prepared doses (for example, contamination of multi-dose vials after repeated use). Studies were also excluded if they involved the use of blood or a blood component, if there was freezing/thawing of prepared doses, or if there was reuse of equipment during dose preparation (except when used in the preparation of a single batch). For an environment to qualify as a pharmaceutical environment the recognized standard of the cabinet in which the doses were prepared and the room in which that cabinet was situated had to be specified in the record (journal article).

2.3.4 SEARCH PROCEDURE

The literature search identified 42246 records (17662 from Medline, 20824 from Embase and 3760 from the Cochrane Library) and 28020 after duplicates had been removed. The title and abstract (if necessary and accessible) of each of the 28020 identified records was evaluated and excluded if it did not meet the above inclusion criteria. This left 137 records, which were individually subjected to a full text review to confirm relevance and compliance with the above criteria to yield a final total of 34 studies from 33 records[82-114]. Each of the final 19 studies from 17 records[83, 85-87, 89-93, 95, 104, 107, 114-118] identified in our 2009 search[50] were identified in the present search but 5 of those studies from 4 records[115-118] were excluded due to inadequate and/or inadequately described pharmaceutical environments. The methodological stages of the search are shown in Figure 2.3. As previously[50], the included studies were divided into groups according to whether doses were prepared in a clinical or pharmaceutical environment (hypothesis 1), whether doses had been prepared as individual lots or as part of a batch (hypothesis 2) and whether doses had been sampled without or before administration or during or after administration to a patient (due to a risk of contamination from manipulations after preparation and

potential differences in time between preparation and sampling which may have affected recovery of damaged microbial cells). Doses were considered to be either contaminated or not contaminated without any attempt to identify the density of any microbes present, and the types of microbe reported briefly summarised.

The methodological stages of the search are shown in Figure 2.3.

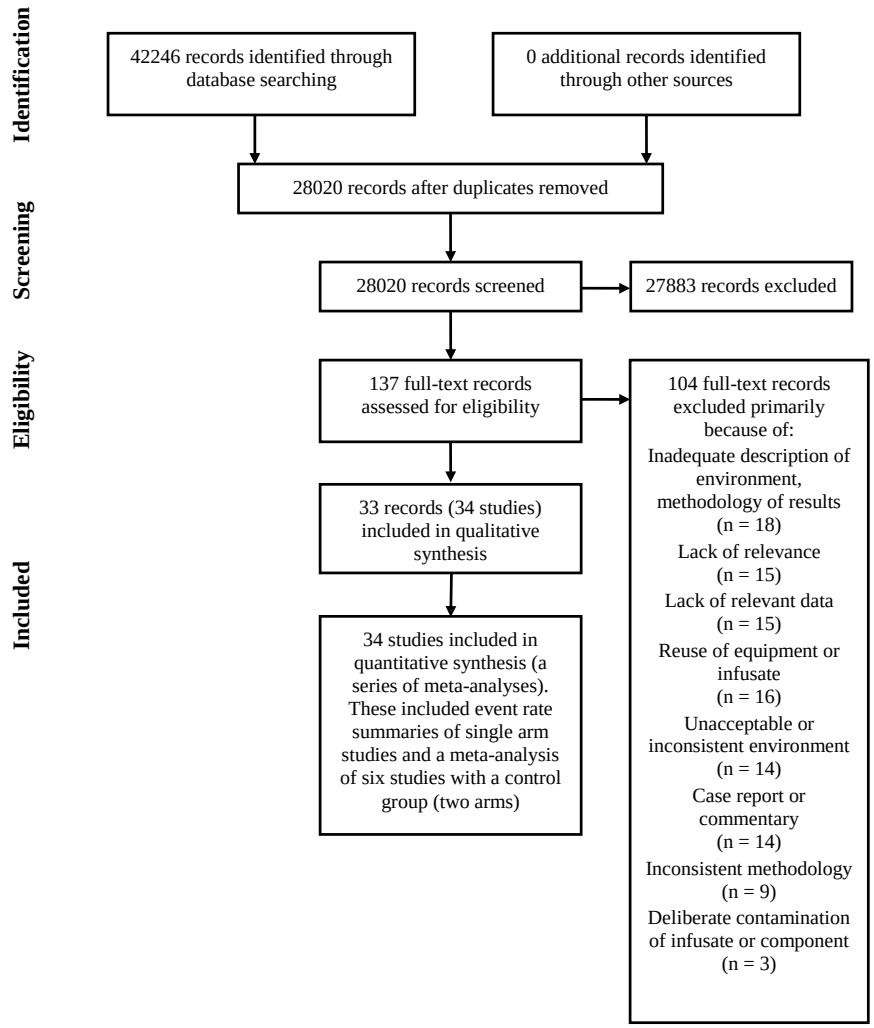

Figure 2.3 The methodological stages of the literature search used to identify studies that report the rate of contamination of doses prepared under aseptic techniques in clinical and pharmaceutical environments

2.3.5 SAMPLE SIZE

The sample size was determined by the results of the literature search.

2.3.6 STATISTICS

The point estimate, SEM and 95% CI for the contamination rate of each separate group was obtained by logarithmic (logit) transformation due to the skewed nature of the data. When there was zero contamination in a group a value of 0.5 contaminated doses was used to overcome the mathematical difficulties associated with logarithmic transformation (the log of zero is minus infinity). Data amalgamation and the meta-analyses were undertaken using a random effects model and the software Comprehensive Meta-Analysis version 2 (Biostat, Englewood, New Jersey, USA). One group meta-analyses were used for hypotheses 1 and 2 and a two group meta-analysis was used for hypothesis 3 due to the nature of the available studies. The random effects model was chosen because of the clinical heterogeneity of the studies, but the I^2 statistic is also presented. Comparisons between group means were undertaken using unpaired t-tests, with a two-tailed $P < 0.05$ considered statistically significant.

2.3.7 QUALITY OF STUDIES

The quality of the included studies was independently assessed by two people (PDA and KSH) using the GRADE system[77, 78] with subsequent discussion to resolve any disagreement. The recommendations of the CRD[80] and Cochrane[79] as well as the PRISMA guidelines[81] for reporting systematic reviews were considered at all stages during this review.

2.4 METHODS FOR SUB-HYPOTHESIS FIVE

Sub-hypothesis five is that “substituting staff with less training and experience in aseptic techniques in a clinical environment with more trained and experienced staff will reduce the rate of microbial contamination of doses prepared under aseptic technique.”

2.4.1 SYRINGE PREPARATION AND VALIDATION

Each syringe required 4 mL to be taken from a 10-mL glass ampoule of single strength (30 g/L) sterile tryptic soy broth aseptic test media (Torbay P.M.U., Torbay,

UK) into a 10-mL luer slip syringe (B. Braun, Melsungen, Germany) using a 19-gauge needle (BD microlance 3, BD, Drogheda, Ireland) to simulate the drawing up of an intravenous line flush, and the syringe sealed with a universal plug (Vygon, Aachen, Germany). Each operator was permitted to use any additional components necessary for their chosen aseptic techniques.

The prepared syringes were incubated at ambient room temperature (~22 °C) for seven days, and then at 32 ± 0.5 °C for a further seven days[28]. Each syringe was blindly assessed and reported as either contaminated (visual turbidity) or not contaminated by a single independent operator from the Quality Assurance Section of the Pharmacy Department. No species identification of contaminants was performed.

2.4.2 ENVIRONMENTAL CONTAMINATION (BIOBURDEN)

During each session, two settle plates (90-mm diameter tryptone soy agar plates; Cherwell Laboratories Ltd, Bicester, UK) were exposed to airborne contaminants falling on them during the aseptic manipulations. At the end of each session, a single pharmacist pressed two contact plates (45-mm diameter tryptone soy agar plates; Cherwell Laboratories Ltd) on to the working area. All of the monitoring plates were incubated at ambient room temperature (~22 °C) for three days, followed by incubation at 32 ± 0.5 °C for a further four days. Each plate was independently assessed and the number of cfu/plate was reported by the single independent pharmacy quality assurance operator. Growth on the settle plates was standardized to the recommended 4-hour period[28]. No species identification was undertaken.

2.4.3 WORKING SURFACE CLEANING

The pharmacy operator consistently elected to clean the working surface at the beginning of each session using 70% v/v isopropyl alcohol wipes (Sterets, Medlock Medical, Oldham, UK) as well as the neck of each ampoule, but none of the nurses chose to do so. In order to evaluate the effect of surface cleaning, further surface sampling was undertaken after completion of all syringe preparations. On two separate occasions, three contact plates were used to sample surface contamination at the front, middle and back of the bench and trolley surfaces used for syringe

preparation, both before and after cleaning by the pharmacy operator. The plates were prepared and reported using the same techniques as before.

2.4.4 SAMPLE SIZE

A large study of aseptic manipulations by pharmacy operators indicated a maximum contamination rate of 0.2%[119], and therefore a minimum of 500 pharmacy operator samples would be expected to be required to detect one with contamination. Using SamplePower 2 (Chicago, Illinois, USA), a sample size of 200 for the nurses is sufficiently large to detect a contamination rate of 3.2% (relative to 0.2% with 500 samples) with a p-value of 0.05 (2-tailed) and 80% power. A sample size of 300 would detect a contamination rate of 2.6% with the same criteria.

2.4.5 STATISTICS

Point estimates and 95% CI for contamination rates were obtained for the nurses and pharmacy operator using the Wilson method (using the statistical program Confidence Interval Analysis version 2[120]). All other analyses were undertaken using SPSS version 18 (Chicago, Illinois, USA) except for a meta-analysis, which was undertaken using Comprehensive Meta-Analysis version 2 (Biostat, Englewood, New Jersey, USA) to combine results of contamination rates obtained by different nurses. Fisher's exact test was used to examine differences in syringe contamination rates between the nurses and the pharmacy operator, and binary logistic regression was undertaken to further evaluate differences between nurses and wards. Student's unpaired (independent) t-test was used to examine differences in cfu/plate between operators and Student's paired t-test was used to examine contamination of the same surface before and after it was cleaned by the pharmacy operator. Unless otherwise stated, the results are presented as mean ± SEM.

2.5 METHODS FOR SUB-HYPOTHESIS SIX

Sub-hypothesis six is that "changes in CI rates depend on the definition of CI, which can vary widely."

2.5.1 STUDY COHORT

The study cohort comprised all adult hospital inpatients starting PN in a large UK teaching hospital (Southampton) between December 2009 and July 2010 inclusive.

2.5.2 ETHICS COMMITTEE APPROVAL

Prior to data collection the local Ethics committee confirmed no formal approval was required for this study in which data from medical records were to be obtained retrospectively by a single pharmacist (PDA).

2.5.3 ESTABLISHMENT OF INTRAVASCULAR CATHETER INFECTION

All new suspected CI episodes, including those which occurred more than once in the same patient during hospital stay, were identified from medical records. Suspected CI was defined as temperature ≥ 38ºC and/or documented clinical suspicion of CI for any reason. Infection and pyrexia due to other defined sources were excluded. From these suspected CI episodes, CI was established according to seven commonly used definitions. Two were simple pragmatic clinical markers: removal of the central venous catheter used for PN due to suspected CI; and stopping PN due to suspected CI. Another was a documented retrospective, multidisciplinary clinical diagnosis based on clear statements of confirmed CI following investigation of suspected CI. The remaining four definitions were published by various groups from the HELICS (2004)[121], the ESPEN (2009)[32], the MM project which was reported in 2010[122], and the CDC in 2011[6] (which refers to their 2008 definition[123]). In separate analyses, the 2011[6] and 2002[30] versions of the CDC definition were compared. All methods for diagnosing CI within each published definition were included (Appendix C). The CI rate was calculated as CI per PN episode. For the purposes of this sub-hypothesis the term ‘catheter infection’ is used to describe ‘intravascular catheter infection’ where ‘infection’ is used to indicate the process of infecting or the state of being infected (clinical or subclinical manifestations).

2.5.4 MICROBIOLOGICAL ASSESSMENT OF BLOOD CULTURES AND VENOUS CATHETER TIPS

Microbiological assessment of blood cultures and venous catheter tips was undertaken by the hospital pathology department. Each blood culture required 10mL blood, and a positive indication from a BacT/ALERT 3D microbial detection system (bioMérieux UK Limited) led to a Gram stain that was examined using immersion oil at x100 microscopy and culture on each of four agar plates (Oxoid Limited, UK) for at least 24 hours at 37°C: chocolate lysed blood agar and Columbia blood agar each in a 5% carbon dioxide environment; fastidious horse blood agar in an anaerobic environment; and cysteine lactose electrolyte deficient agar. Microbes growing were identified using relevant Analytical Profile Index (API) kits (bioMérieux UK Limited), and the British Society for Antimicrobial Chemotherapy guidelines were used to establish sensitivity breakpoints. Each tested venous catheter tip was vortexed in 1mL tryptone broth before 100microlitres was taken and incubated on a Columbia blood agar plate at 37°C for 48 hours. If any growth was detected within 48 hours, British Society for Antimicrobial Chemotherapy guidelines were used to establish microbial sensitivity breakpoints.

2.5.5 COMPARISON OF DIFFERENT DEFINITIONS FOR INTRAVASCULAR CATHETER INFECTION

In comparing CI rates established by two different definitions of CI two main types of analyses were undertaken: unpaired statistical comparisons, in which all the available data were used even if a diagnosis of CI in a particular subject could be established using one definition and not another; and pairwise comparisons, in which diagnosis had been made by both definitions. With the latter analysis any subject with missing data in either comparison arm was eliminated (pairwise deletions), e.g. due to missing blood cultures which prevented establishment of CI using certain definitions[6, 32, 121, 122]. Indeed, in some cases only one blood culture was required and in others more than one blood culture or simultaneous central and peripheral blood cultures[6, 32, 121-123]. A further type of analysis examined the risk of bias associated with the exclusion of subjects due to missing data (i.e. the possibility that excluded subjects systematically differed from included subjects). It also involved both sensitivity and ‘intention to categorise’ analyses. To examine whether the missing

data (absence of blood cultures) for the published definitions were not missing at random, the following two-step procedure was undertaken. First, the datasets for each of the three clinical definitions with complete datasets were split into two groups, one corresponding to episodes with complete data for a specific published definition, and the other corresponding to episodes with incomplete data for the same published definition. Second, the proportions of CI in these two newly formed groups were compared. In the sensitivity analysis all episodes associated with inadequate blood cultures were assigned to the CI group (model A) or to the no CI group (model B). In a third model (model C) the no CI group not only included all episodes with inadequate blood culture (as for model B), but also all episodes in which the same microbe responsible for a CI was identified at another site (for example from an infected intra-abdominal collection). Model C assumed that infections of the central venous catheter used for PN arose from another site (rather than *vice versa*). The second approach involved intention to categorise analysis using multiple imputation (see Statistics).

Two additional comparisons were undertaken: a historical comparison of two versions of the CDC criteria from 2002[30] and 2011[6]; and a comparison of CI episodes between two separate patient groups (i.e. patients involved in the first and second halves of the study).

2.5.6 SAMPLE SIZE

Sample size calculations assumed a CI rate of 15%, which is within the typically reported range for patients administered PN. A sample size of 150 episodes according to each of two definitions is sufficient to detect a difference between 15% and either 5% or 28% (for a lower and higher CI rate respectively) with 80% power and a p-value of <0.05 (two tailed). Since 25% values could be missing (due to inadequate blood sampling) it was aimed to achieve at least 188 episodes. Sample size calculations were undertaken using Power and Precision version 4 (Biostat, Englewood, New Jersey, USA).

2.5.7 STATISTICS

Analyses were based on 2×2 contingency tables for paired samples. For the intention to categorise analyses ten multiple imputations (see footnote to relevant table) assigned missing data (inadequate blood sampling) to CI or no CI. All variables had 'complete' datasets except for the five published definitions of CI (4.1–26.9% missing data). The agreement between definitions was assessed using the kappa statistic (Cohen's kappa) and Altman's 1991 classification for grades of agreement[124]. In subsequent comparisons the kappa for each of the 10 imputations were combined using random effects meta-analysis[125], and the pooled value for the number of CI rounded to the nearest integer was used for comparisons involving the McNemar and Fisher's exact tests. For the comparison between the 2002[30] and 2011[6] versions of the CDC definition, sensitivity and specificity was assessed with the new upgraded definition as the referent. In addition, the areas under the receiver-operator characteristic (ROC) curves were compared. The statistical analyses were undertaken using SPSS version 22 (IBM, Chicago, USA) and Comprehensive Meta-Analysis version 2.2.064 (Biostat, Englewood, USA). A p-value <0.05 (two tailed) was considered significant.

CHAPTER 3
PRESCRIPTION OF INFUSATE COMPOSITION

This chapter examines sub-hypothesis 1: “exclusion of lipid from PN infusates will limit bacterial growth compared to lipid-containing PN infusates, in line with current guidelines which have a weak evidence base.”

The aim of this section of the thesis was to develop and apply an experimental framework and to undertake a systematic review in order to examine whether lipid has a specific effect in stimulating microbial growth in PN infusates that dominates and overrides any other independent effects produced by other nutrients in the infusate.

3.1 TESTING MICROBIAL GROWTH IN PARENTERAL NUTRITION INFUSATES

3.1.1 METHODOLOGY

The detailed methods used are described in Chapter Two. Following pilot studies and for each microbe tested, a series of PN regimens (250 mL fills; the 2000 mL equivalents are shown in Table 3.1) were compounded in pharmacy aseptic services to provide a range of four clinically realistic lipid-free regimens each with a different osmolarity but with fixed electrolytes and micronutrients. A further four regimens were compounded that were identical except for the addition of a fixed quantity of lipid, altering the osmolarities. One of the lipid regimens consisted solely of the lipid component. In addition, three regimens were pH-adjusted. One lipid-free and one lipid-containing regimen were adjusted up to match the pH of the lipid alone and *vice versa*. Finally, for two microbes (*E. Coli* and *E. durans*) there were two additional lipid PN infusates to establish an increase in osmolarity due to sodium chloride at a fixed glucose concentration. Each infusate regimen was compounded in quadruplicate for each microbe examined. After checking the pH of each infusate, an inoculation took place with doses of specific strains of *S. epidermidis* (NCTC

11047), *C. albicans* (NCPF 3179) and *E. coli* (NCTC 9001) sourced through the local Health Protection Agency (HPA). The *E. coli* culture was inadvertently contaminated with *E. durans* (wild type; multiple samples confirmed as single strain by Public Health England Colindale, London, UK), which meant that *E. coli* and *E. durans* were studied at the same time in the same infusates. Each infusate was sequentially sampled for quantitative bacterial counting at time zero (immediately after inoculation) and at 24 and 48 hours. Storage of infusates during the test period was at a controlled and monitored room temperature (23.2°C (range 22.8°C – 23.7°C) for *S. epidermidis*, 21.5°C (range 20.0°C – 22.3°C) for *C. albicans*, and 21.1°C (20.4°C – 22.5°C) for *E. coli* and *E. durans*).

Table 3.1 The study parenteral nutrition (PN) regimens, all prepared in quadruplicate

PN regimen		A	B	C	D	E	F	G	H	I	J	K
Infusate type		Lipid-free PN	Lipid-free PN	Lipid-free PN	Lipid-free PN	Lipid PN	Lipid PN	Lipid PN	Lipid PN	Lipid alone	Lipid PN	Lipid PN
Volume	mL	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
Nitrogen[a]	g	9	9	9	9	9	9	9	9	0	9	9
Glucose	% w/v	8	11	14	17	8	11	14	17	0	8	8
Glucose	kcal	640	880	1120	1360	640	880	1120	1360	0	640	640
Lipid[b]	kcal	0	0	0	0	1000	1000	1000	1000	4000	1000	1000
Sodium	mmol	60	60	60	60	60	60	60	60	0	227	395
Potassium	mmol	60	60	60	60	60	60	60	60	0	60	60
Magnesium	mmol	10	10	10	10	10	10	10	10	0	10	10
Calcium	mmol	5	5	5	5	5	5	5	5	0	5	5
Phosphate	mmol	25	25	25	25	25	25	25	25	0	25	25
Vitamins[c]	mL	5	5	5	5	5	5	5	5	0	5	5
Trace elements[d]	mL	10	10	10	10	10	10	10	10	0	10	10
Calculated osmolarity	mOsm/l	871	1038	1204	1371	945	1110	1277	1443	270	1110	1277
pH adjusted	Y or N	N	Y[e]	N	N	N	Y[e]	N	N	Y[e]	N	N
Final pH of bag used unadjusted and (adjusted) for *S. epidermidis* inoculations	pH as mean ± SEM	6.24 ± 0.01	6.23 ± 0.02 (8.42 ± 0.02)	6.18 ± 0.02	6.22 ± 0.03	6.19 ± 0.03	6.16 ± 0.02 (8.45 ± 0.01)	6.12 ± 0.02	6.14 ± 0.03	8.47 ± 0.08 (6.19 ± 0.03)	Not tested	Not tested

Final pH of bag used unadjusted and (adjusted) for *C. albicans* inoculations	pH as mean ± SEM	6.19 ± 0.01	6.17 ± 0.01 (8.15 ± 0.01)	6.20 ± 0.03	6.17 ± 0.03	6.13 ± 0.01	6.11 ± 0.01 (8.16 ± 0.01)	6.08 ± 0.03	6.10 ± 0.02	8.35 ± 0.03 (6.10 ± 0.02)	Not tested	Not tested
Final pH of bag used unadjusted and (adjusted) for *E. coli* and *E. durans* inoculations	pH as mean ± SEM	6.208 ± 0.003	6.180 ± 0.000 (7.735 ± 0.031)	6.138 ± 0.018	6.145 ± 0.018	6.210 ± 0.012	6.123 ± 0.008 (7.763 ± 0.023)	6.065 ± 0.003	5.980 ± 0.011	7.758 ± 0.008 (6.198 ± 0.027)	6.045 ± 0.005	6.043 ± 0.008

[a] Synthamin 17EF (Baxter Healthcare Limited, Norfolk, UK)
[b] 20% Clinoleic (Baxter SA, Lessines, Belgium)
[c] As 1 vial of Cernevit (Baxter SA, Lessines, Belgium) in 5mL Water for Injection
[d] Additrace (Fresenius Kabi, Halden, Norway)
[e] Prepared in quadruplicate for both unadjusted and pH-adjusted (the pH of the lipid was adjusted to match that of both PN infusates, and *vice versa*)

3.1.2 RESULTS

The measured pH of each PN regimen is shown in Table 3.1, which illustrates that the lipid emulsions were alkaline (pH ~8.5) whereas all other regimens, including lipid-containing PN, were acidic (pH ~6.2).

In the PN bags that did not have their pH adjusted (n = 32 regimens A – H in Table 3.1) over a 48 hour period there was an increase in density of each microbe tested. There was only a slight progressive increase in the density of *S. epidermidis*, from a mean of 32.6 ± 1.4 cfu/mL at time zero to 43.4 ± 2.3 cfu/mL at 24 hours to 55.2 ± 2.9 cfu/mL at 48 hours ($P < 0.001$; repeated measures ANOVA), with significant increments from time zero to 24 or 48 hours ($P < 0.001$). The density of *C. albicans* progressively increased from a mean of 1.806 ± 0.015 $\log_{10}$ cfu/mL at time zero to 2.451 ± 0.044 $\log_{10}$ cfu/mL at 24 hours to 3.731 ± 0.059 $\log_{10}$ cfu/mL at 48 hours ($P < 0.001$; repeated measures ANOVA with no covariates), with significant increments from time zero to 24 or 48 hours ($P < 0.001$). There was a progressive increase in the density of *E. coli*, from a mean of 1.093 $\log_{10}$ cfu/mL at time zero to 1.846 ± 0.052 $\log_{10}$ cfu/mL at 24 hours to 2.241 ± 0.092 $\log_{10}$ cfu/mL at 48 hours ($P < 0.001$; repeated measures ANOVA adjusted for baseline $\log_{10}$ cfu/mL and pH), with significant increases from time zero to 24 hours ($P < .001$) and from time zero to 48 hours ($P < 0.001$). In the same PN infusates, there was also a progressive increase in the density of *E. durans*, from a mean of 0.843 $\log_{10}$ cfu/mL at time zero to 2.058 ± 0.038 $\log_{10}$ cfu/mL at 24 hours to 3.451 ± 0.059 $\log_{10}$ cfu/mL at 48 hours ($P < 0.001$; repeated measures ANOVA adjusted for baseline $\log_{10}$ cfu/mL and pH), with significant increases from time zero to 24 hours ($P < 0.001$) and from time zero to 48 hours ($P < 0.001$).

3.1.2.1 Growth of *S. epidermidis* in parenteral nutrition infusates

3.1.2.1.1 Effect of the glucose concentration in the presence and absence of lipid in parenteral nutrition

After adjustments for baseline cfu/mL (mean, 32.6 cfu/mL), pH, and glucose concentration there was a small but significant growth of *S. epidermidis* in PN with and without lipid (Table 3.2).

Table 3.2 Results of multiple regression analysis for *S. epidermidis* cfu/mL over time, after controlling for baseline cfu/mL and pH[a]

Variable (increment in model)	**B[b]**	**SEM**	***P***	**partial η squared[c]**
At 24 hours (n = 32)				
Presence of lipid (5% w/v compared to no lipid)[d]	9.67	5.25	0.076	0.112
Glucose concentration (per 10% w/v)	-4.95	6.62	0.461	0.020
pH (per 0.01 pH units)	-0.49	0.48	0.322	0.036
Baseline cfu/mL	0.32	0.29	0.290	0.041
At 48 hours (n = 32)				
Presence of lipid (5% w/v compared to no lipid)[d]	14.16	6.10	0.028	0.166
Glucose concentration (per 10% w/v)	25.55	7.69	0.003	0.290
pH (per 0.01 pH units)	0.54	0.56	0.349	0.033
Baseline cfu/mL	0.60	0.34	0.090	0.103

[a] In this model parenteral nutrition with or without lipid is a fixed factor whilst baseline cfu/mL, glucose concentration and pH are covariates.
[b] The B coefficient indicates the absolute change from baseline (32.56 cfu/mL) for the increment indicated of each variable in parentheses.
[c] The partial correlation coefficient squared (R^2) in this series is the same as partial η^2.
[d] Lipid; 1 = presence of lipid and 0 = absence of lipid in the statistical model.

At 24 hours, at a fixed glucose concentration the presence of 5% w/v lipid led to a mean increase of 9.7 ± 5.2 cfu/mL (P = 0.076), whereas for each 10% w/v increase in glucose there was a smaller, non-significant decrease of 4.95 ± 6.6 in cfu/mL (P = 0.461), with no significant effect of pH (P = 0.322).

At 48 hours, at a fixed glucose concentration the presence of 5% w/v lipid led to a mean increase of 14.2 ± 6.1 cfu/mL (P = 0.028), whereas for each 10% w/v increase in glucose there was an increase of 25.6 ± 7.7 in cfu/mL (P = 0.003), with no significant effect of pH (P = 0.349). Figure 3.1 shows that at a fixed lipid concentration (absence or presence of lipid at a final concentration of 5% w/v) an increase in glucose concentration stimulated growth by 48 hours. A simultaneous increase in the two energy sources stimulated growth to an even greater effect, with glucose energy producing a slightly greater effect than the same amount of lipid energy, although the differences were not significant.

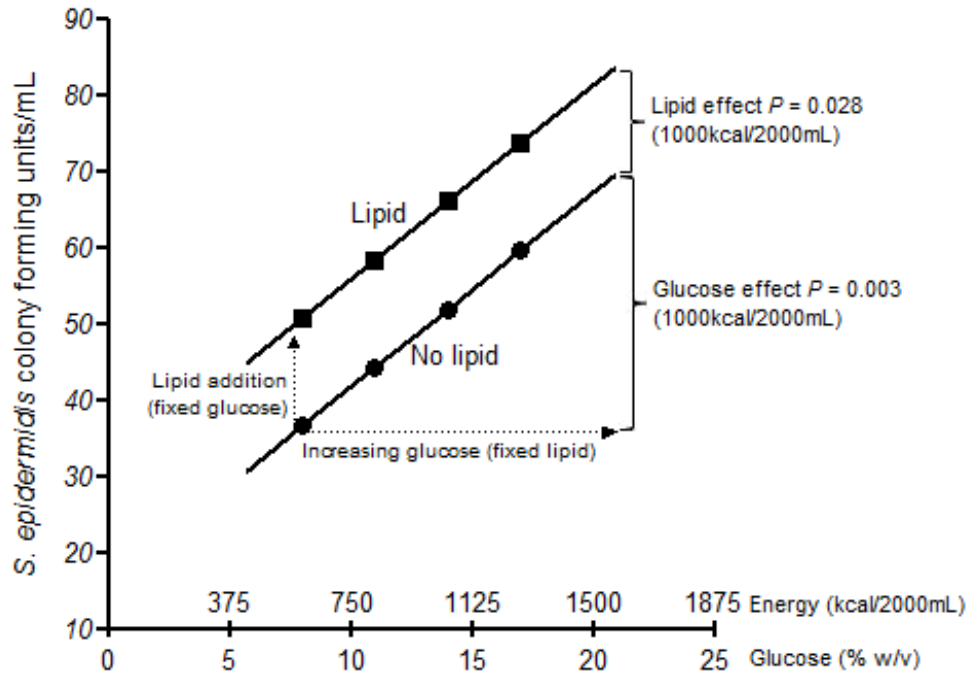

Figure 3.1 The independent effects of increasing glucose concentration and addition of lipid on *S. epidermidis* growth over 48 hours in complete PN regimens (based on an ANCOVA model with baseline cfu/mL and pH as covariates). The partial η^2 for the overall model (also R^2) at 48 hours was found to be 0.387, and that for the glucose concentration was 0.290

3.1.2.1.2 Effects of non-nitrogen energy density and the proportion of non-nitrogen energy derived from lipid

Table 3.3, which is based on multiple regression analysis, shows that the growth of *S. epidermidis* was stimulated by the energy density of the feed at 48 hours (reported as non-nitrogen energy per 2 L feed, since this volume is often used in clinical practice) stimulated growth of *S. epidermidis*, but the proportion of energy derived from lipid had no significant effect. The same pattern was observed at 48 hours, but at 24 hours there was little overall effect of either variable, although the growth tended to increase as the percent non-nitrogen energy from lipid increased (or as percent energy from glucose decreased; $P = 0.043$). pH had no significant effect at any time point.

Table 3.3 Results of multiple regression analysis examining the effect of energy density and proportion of non-nitrogen energy as lipid on *S. epidermidis* cfu/mL over time, after controlling for baseline cfu/mL and pH

Variable (increment in model)	**B**[a]	**SEM**	***P***	**partial η squared**[b]
At 24 hours (n = 32)				
Energy density (1Mcal non-nitrogen kcal per 2L)	-4.31	6.70	0.525	0.015
Percentage of non-nitrogen energy from lipid (per 10%)	2.82	1.33	0.043	0.143
pH (per 0.01 pH units)	-0.51	0.47	0.287	0.042
Baseline cfu/mL	0.34	0.29	0.254	0.048
At 48 hours (n = 32)				
Energy density (1Mcal non-nitrogen kcal per 2L)	27.66	8.01	0.002	0.306
Percentage of non-nitrogen energy from lipid (per 10%)	-2.56	1.59	0.119	0.087
pH (per 0.01 pH units)	0.56	0.57	0.329	0.035
Baseline cfu/mL	0.60	0.35	0.096	0.100

[a] The B coefficient indicates the absolute change from baseline (cfu/mL) for the increment of each variable indicated in parentheses.
[b] The partial correlation squared (R^2) in this series is the same as partial η^2.

Figure 3.2, based on the multiple regression model, is used to illustrate the qualitative and quantitative relationships at 48 hours. When non-protein (lipid and glucose) energy density is fixed (at 640 kcal, 1500 kcal and 2360 kcal per 2 L) an increase in the proportion of non-protein energy from lipid tends to decrease growth of *S. epidermidis*, although the effect is small and not significant; Table 3.3. In contrast, when the proportion of non-nitrogen energy from lipid is fixed, energy density has a much greater and significant effect on the growth of *S. epidermidis* at 48 hours.

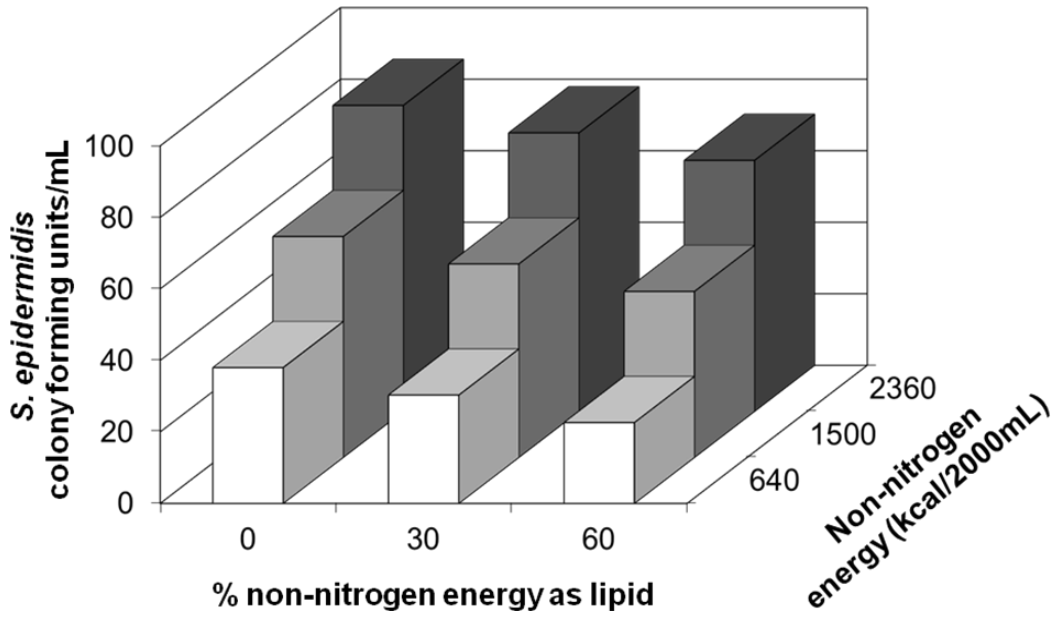

Figure 3.2 The independent effects of non-nitrogen energy and % non-nitrogen energy as lipid on growth of *S. epidermidis* at 48 hours, based on multiple regression analysis with baseline cfu/mL and pH as covariates. The % non-nitrogen energy as lipid was not significant ($P = 0.119$) and non-nitrogen energy in 2 L was significant ($P = 0.002$)

3.1.2.1.3 Effect of pH

Comparisons involving unadjusted pH

When lipid (pH 8.5 ± 0.08) was added to other components of the PN to prepare the lipid-containing PN, the pH of the final solution (6.2 ± 0.02) was essentially identical to that of lipid-free PN due to the low buffering capacity of lipid. The addition of lipid inherently affects the energy density of the PN admixture (Table 3.1). Growth of *S. epidermidis* was slower at the higher pH (lipid alone) for both the lower and higher energy density PN regimens, which was observed at both 24 hours and 48 hours (upper part of Table 3.4).

Table 3.4 Effect of pH and energy density on the number of *S. epidermidis* colony forming units per mL of infusate

Type of infusate	**Non-nitrogen kcal per 2L**	**pH ± SEM**	**Change in cfu/mL ± SEM at 24 hours**	***P*[a]**	**Change in cfu/mL ± SEM at 48 hours**	***P***	**Change in cfu/mL ± SEM, mean of values at 24 and 48 hours**	***P*[a]**
Regimens without pH adjustment (n = 4 per group)								
Lipid PN[b]	1880	6.2 ± 0.02	17.8 ± 4.3	0.008	19.2 ± 8.1	0.031	18.5 ± 5.0	0.008
Lipid alone[b]	4000	8.5 ± 0.08	-8.5 ± 4.3		-15.5 ± 8.1		-12.0 ± 5.0	
Lipid-free PN[c]	880	6.2 ± 0.02	-5.3 ± 4.0	0.556	10.5 ± 3.8	0.003	2.6 ± 2.0	0.002
Lipid alone[c]	4000	8.5 ± 0.08	-8.8 ± 4.0		-18.4 ± 3.8		-13.6 ± 2.0	
Lipid PN[d]	1880	6.2 ± 0.02	17.5 ± 5.8	0.067	27.8 ± 8.5	0.220	22.6 ± 5.3	0.062
Lipid-free PN[d]	880	6.2 ± 0.02	-4.2 ± 5.8		8.6 ± 8.5		2.2 ± 5.3	
Regimens without and with pH adjustment[e] (n = 4 per group)								
Lipid PN[f]	1880	6.2 ± 0.02	14.1 ± 5.1	0.425	27.5 ± 8.8	0.045	20.8 ± 6.5	0.086
LipidPN[f]	1880	8.4 ± 0.01	7.0 ± 5.1		-10.1 ± 8.8		-1.5 ± 6.5	
Lipid-free PN[g]	880	6.2 ± 0.02	-6.8 ± 4.2	0.070	12.7 ± 5.1	0.006	2.9 ± 2.5	0.001
Lipid-free PN[g]	880	8.4 ± 0.02	-21.0 ± 4.2		-21.8 ± 5.1		-21.4 ± 2.5	

Lipid alone[h]	4000	8.5 ± 0.08	-8.4 ± 3.6	0.077	-15.7 ± 5.6	0.068	-12.0 ± 2.7	0.011
Lipid alone[h]	4000	6.2 ± 0.03	3.3 ± 3.6		3.1 ± 5.6		3.2 ± 2.7	

[a] The p-value refers to the comparison of the infusate pairs (bb, cc, dd, ff, gg, and hh).
[b, c, d] Adjusted for baseline cfu/mL (b = 35.6 cfu/mL, c = 38.6 cfu/mL, d = 34.0 cfu/mL).
[e] The infusate with adjusted pH is the second of each pair, which is compared with the infusate with the unadjusted pH (the first of each pair).
[f, g, h] Adjusted for baseline cfu/mL (f = 34.8 cfu/mL, g = 38.6 cfu/mL, h = 36.0 cfu/mL).

Comparisons involving adjusted pH

When the lipid-containing PN (pH ~6.2) was alkalinised to produce a final pH that was comparable to that of the lipid alone, growth of *S. epidermidis* was suppressed (lower part of Table 3.4). When the pH of the lipid alone (pH ~8.5) was acidified to produce a final pH that was comparable to that of the lipid-containing PN (pH ~6.2) growth was enhanced.

3.1.2.1.4 Discussion

This study involving growth of *S. epidermidis* in various PN infusates identified factors that should be taken into account when making recommendations on the duration of infusion from PN bags. It found that the growth of *S. epidermidis* in PN is not solely affected by the presence of lipid, but also by the concentration of glucose and total non-nitrogen energy density of the feed. Although influential peer-reviewed guidelines[6, 31, 32] currently limit infusion of lipid-containing PN bags, but not lipid-free PN bags, to 24 hours, this study provides no evidence to support these guidelines, at least with respect to *S. epidermidis*, one of the commonest, if not the most common, organism isolated in adult CI[126-129].

The study found little overall growth in PN infusates with and without lipid and in lipid alone. In contrast to certain other organisms which can increase by up to five orders of magnitude (each order of magnitude representing a x10 increase in cfu/mL) in PN infusates over 48 hours[72, 73], this study with *S. epidermidis* showed an increase of less than a single order of magnitude, which did not even amount to a doubling, in keeping with a series of other observations[56-58]. This small overall effect in PN infusates with and without lipid and in lipid alone is probably of limited clinical significance and does not support a need for making recommendations that limit infusion from bags containing PN infusates to 48 hours.

Although the growth of *S. epidermidis* was slow, it was found to depend not only on the presence of lipid but also on the concentration of glucose, and the non-nitrogen energy density of the feed, both of which can be affected by withdrawal or lipid and/or replacement by glucose. Therefore, conclusions about the singular growth enhancing properties of organisms in lipid-containing PN infusates can be

misleading, especially if the effects of other associated variables are not taken into account. Indeed, when a lipid emulsion is incorporated into a PN admixture the final pH and the associated growth characteristics of *S. epidermidis* appear to resemble much more closely that of the PN without a lipid component (little/no growth or a decline in cfu/mL over time) than the lipid emulsion alone.

In this study growth of *S. epidermidis* was found to be suppressed in lipid alone compared to PN infusates with or without lipid. However, recommendations about duration of PN infusates do not apply to lipid alone because it is not generally infused over periods of more than 12 hours let alone 24 hours. The different growth rates in lipid alone and PN solutions may be due, at least in part, to the initially high pH of the lipid which was substantially lowered when it was added to lipid-free PN, the pH of which is dominated by the buffering capacity of the amino acids. The dependency of the growth of *S. epidermidis* on the pH of the PN infusates is confirmed by the upward adjustment of the PN infusates with and without lipid to the alkaline pH exhibited by lipid alone (pH ~ 8.4). The suppression of growth in this alkaline environment is of scientific interest because it elucidates factors influencing growth in different infusates, but it probably has little direct relevance to clinical practice. This is because such an upward adjustment of pH would destabilise the PN infusates, leading to calcium phosphate precipitation[130].

In order to put the novel findings of this study into context it is prudent to briefly review published studies on the growth potential of *S. epidermidis* in relevant infusates[40, 56-59, 131]. Whilst these other studies have provided some valuable information they are limited by lack of sample size calculations, lack of statistical modelling to take into account the effects of confounding variables, use of a single or uncertain number of samples per regimen, and experimental conditions that differ from each other or do not reflect those associated with clinical practice. For example, the ambient temperature in our study ranged from 22.8 °C to 23.7 °C compared to 25 °C[56, 58, 59, 131] or unspecified room temperature[40] or not specified at all[57]. However, despite these limitations, these other studies have provided some interesting information on the growth of *S. epidermidis* in PN admixtures with and without lipid emulsion[56-59], as well as in glucose[56, 57, 131] and lipid emulsions alone[40, 57]. In the studies examining growth of *S. epidermidis* in glucose alone there was a general reduction in cfu/mL at 24 and 48 hours in a concentration of 5% w/v (pH not

reported[56]), 12.5% w/v (pH 4.8) and 25% w/v (at pH 4.6)[57]. In a separate study involving a combination of 13 *Staphylococcus sp.* types including one *S. epidermidis* there was also a reduction in cfu/mL at 48 hours in 5% w/v glucose alone (pH between 4.8 and 4.9[131]). In the studies involving lipid alone, one of which involved 10% w/v Intralipid (Cutter Laboratories, Berkeley, California, USA) at pH 7.3[57] and another in which results of 5 different lipid emulsions of uncertain pH were reported together[40], there was substantial growth (an increase of one to four orders of magnitude) at 24 and 48 hours. In the studies involving PN admixtures with lipid (at pH 6.5 and 6.4[58], at pH 6.2 and pH 6.1[57] and at an uncertain pH[56]) and in those not containing lipid at pH 6.4 and pH 6.2[58] and at pH 6.1 and pH 6.2[57] there was either little growth of *S. epidermidis* (less than a doubling) or a decrease in cfu/mL at 24 and 48 hours. In contrast, one study[59] reported a substantial increase in the density of *S. epidermidis* in PN admixtures with a lipid emulsion at pH 5.64 and pH 6.14 and in a PN admixture without a lipid emulsion at pH 6.19, particularly when lipid was included in the PN. This study[59], which appeared to show more growth in PN containing lipid, did not adjust for the greater energy density of the lipid-containing PN, or for the presence of vitamins in the PN containing lipid.

A comparison of the present study with the above results raises at least three issues. First, the present study adds to the literature by demonstrating that the growth of *S. epidermidis* in a lipid emulsion alone and in PN admixtures is pH dependent. This is consistent with a pH study (not involving PN) showing substantially greater growth of *S. epidermidis* at pH 5.5 and pH 7.0 compared to pH 8.5[132]. Second, the present study found a suppression of *S. epidermidis* growth in lipid emulsion alone in contrast to other data suggesting a substantial increase in growth[40, 57]. This may be due to differences in the type of lipid emulsion used (this study used Clinoleic as the lipid emulsion, whereas the other studies[40, 57] did not), *S. epidermidis* strains, and ambient conditions, such as temperature and pH. For example, the temperature in our study ranged from 22.8°C to 23.7°C compared to an unspecified room temperature[40] or no indication of any temperature[57]. With respect to the pH of the lipid emulsion in our study it was 8.4 compared to 7.3 in another study[57] and not specified for the other study that combined the results from five lipids[40]. Some studies did not report the pH of the lipid emulsion they used in their study and this could be important, not least because marketed emulsions can range from pH 6 to pH 8.8, and specifications for individual marketed emulsions can vary by as much as 2.3 pH units (personal

communications from the UK Medical Information departments of Baxter, B. Braun and Fresenius Kabi on 19 January 2012). Third, the present study is consistent with the limited observations of *S. epidermidis* growth in PN admixtures in generally showing little or no growth.

None of the previously published studies[40, 56-59, 131] set out to examine the validity of the recommendations on the maximum duration of PN bag infusion or the independent effects of glucose, lipid emulsion and energy density of PN admixtures on the growth of *S. epidermidis*, whereas the current study did. In addition, the current study attempted to simulate PN typically used in practice by including trace elements and vitamins in multi-layered bags. In contrast, other studies with *S. epidermidis* have either not added trace elements and vitamins or added variable amounts of these nutrients[40, 57, 59, 131]. In addition, some studies undertook experiments using much smaller volumes of infusate in tubes that were kept open or intermittently opened for sampling[56], or in bottles[57, 131], or in unspecified types of container[40, 58, 59].

Overall, the studies of the growth of *S. epidermidis* (and other bacteria in these studies) in PN infusates[40, 56-59, 131], including the present study, do not support the recommendation that PN containing a lipid emulsion should be infused for shorter periods (maximum 24 hours) compared with PN without a lipid emulsion (up to 48 hours or longer).

3.1.2.2 Growth of *C. albicans* in parenteral nutrition infusates

The measured pH of each PN regimen is shown in Table 3.1, which illustrates that without any pH adjustment the lipid emulsion was alkaline (pH ~8.4) whereas all of the other regimens, including PN containing lipid, were acidic (pH ~6.1 to ~6.2). In the infusates without pH adjustment (n = 36) the mean baseline cfu/mL of *C. albicans* (1.810 ± 0.013 $\log_{10}$cfu/mL) did not differ significantly between lipid and lipid-free PN regimens and lipid alone (ANOVA, $P = 0.616$). In the PN infusates without pH adjustment (n = 32) the density of *C. albicans* progressively increased from a mean of 1.806 ± 0.015 $\log_{10}$cfu/mL at time zero to 2.451 ± 0.044 $\log_{10}$cfu/mL at 24 hours to 3.731 ± 0.059 $\log_{10}$cfu/mL at 48 hours ($P < 0.001$; repeated measures

ANOVA with no covariates), with significant increments from zero to 24 or 48 hours ($P < 0.001$).

3.1.2.2.1 Effect of the glucose concentration in the presence and absence of lipid in parenteral nutrition

Following adjustments for baseline log_{10}cfu/mL (mean, 1.806 log_{10}cfu/mL), pH, and glucose concentration, the *C. albicans* density increased over time in PN with and without lipid (Table 3.5). At 24 hours, at a fixed glucose concentration the presence of 5% w/v lipid led to a non-significant mean increase in log_{10}cfu/mL ($P = 0.449$), and for each 10% w/v increase in glucose there was a non-significant decrease in cfu/mL ($P = 0.074$), unaffected by pH ($P = 0.786$). At 48 hours, at a fixed glucose concentration the presence of 5% w/v lipid led to a non-significant decrease in log_{10}cfu/mL ($P = 0.127$), whereas each 10% w/v increase in glucose concentration led to a significant decrease in log_{10}cfu/mL ($P = 0.018$) (again without a significant effect of pH ($P = 0.240$)).

Table 3.5 Results of multiple regression analysis for *C. albicans* log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH[a]

Variable (increment in model)	B[b]	SEM	*P*	partial η squared[c]
At 24 hours (n = 32)				
Presence of lipid (5% w/v compared to no lipid)[d]	0.090	0.117	0.449	0.021
Glucose concentration (per 10% w/v)	-0.243	0.131	0.074	0.114
pH (per 0.01 pH units)	0.003	0.011	0.786	0.003
Baseline (per log_{10}cfu/mL)	0.763	0.547	0.174	0.067
At 48 hours (n = 32)				
Presence of lipid (5% w/v compared to no lipid)[d]	-0.246	0.156	0.127	0.084
Glucose concentration (per 10% w/v)	-0.438	0.174	0.018	0.190
pH (per 0.01 pH units)	-0.018	0.015	0.240	0.051
Baseline (per log_{10}cfu/mL)	-0.385	0.728	0.602	0.010

[a] In this model parenteral nutrition with or without lipid is a fixed factor whilst baseline log_{10}cfu/mL, glucose concentration and pH are covariates.
[b] The B coefficient indicates the absolute change (log_{10}cfu/mL) for the increment indicated of each variable in parentheses.
[c] The partial correlation coefficient squared (R^2) in this series is the same as partial η^2.
[d] Lipid; 1 = presence of lipid and 0 = absence of lipid in the statistical model.

Figure 3.3 not only shows these interrelationships but also the complex effect of isoenergetically exchanging lipid for glucose in the same volume of PN. Whilst the addition of lipid to a PN solution (1000kcal/2L) increases the energy density of PN and non-significantly reduces the growth of *C. albicans*, the removal of glucose (1000kcal/2L) to re-establish the same initial energy density significantly increases the growth of *C. albicans*. The overall effect is that substituting 1000kcal of lipid for 1000kcal of glucose in the same final volume of PN produces a non-significant decrease in the growth of *C. albicans* (3.825 v 3.521 log_{10}cfu/mL).

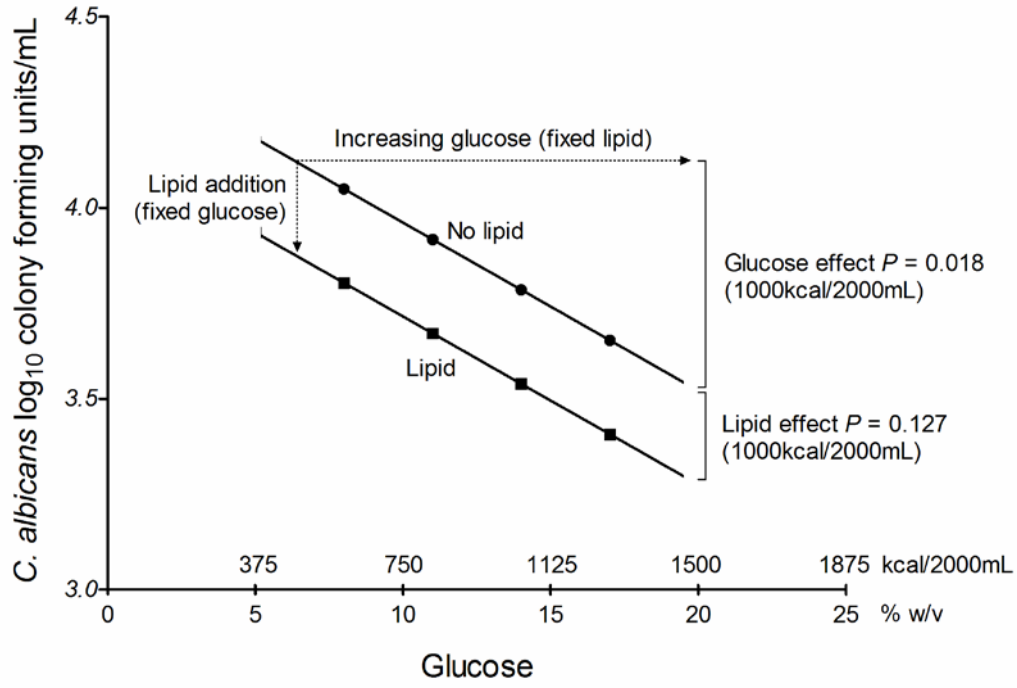

Figure 3.3 The independent effects of increasing glucose concentration and addition of lipid on *C. albicans* growth over 48 hours in complete PN regimens (based on ANCOVA with baseline $\log_{10}$cfu/mL and pH as covariates). The partial η^2 for the overall model (also R^2) at 48 hours was found to be 0.223, and that for the glucose concentration was 0.190

3.1.2.2.2 Effects of non-nitrogen energy density and the proportion of non-nitrogen energy derived from lipid

Table 3.6, which is based on multiple regression analysis, shows that *C. albicans* at 24 hours tended to be inhibited by increasing non-nitrogen energy density (*P* = 0.098) and was further inhibited at 48 hours, when the effect became both much larger and significant (*P* = 0.007). *C. albicans* density increased at 24 hours as the percent non-nitrogen energy from lipid increased (or as the percent energy from glucose decreased; *P* = 0.031), but this was not significant at 48 hours (*P* = 0.132). The pH had no significant effect on the growth of *C. albicans*, either at 24 or 48 hours (*P* = 0.839 and *P* = 0.228 respectively).

Table 3.6 Results of multiple regression analysis examining the effect of energy density and proportion of non-nitrogen energy as lipid on *C. albicans* log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH

Variable (increment in model)	**B**[a]	**SEM**	***P***	**partial η squared**[b]
At 24 hours (n = 32)				
Energy density (1Mcal non-nitrogen kcal per 2L)	-0.231	0.135	0.098	0.098
Percentage of non-nitrogen energy from lipid (per 10%)	0.062	0.027	0.031	0.161
pH (per 0.01 pH units)	0.002	0.011	0.839	0.002
Baseline (per log_{10}cfu/mL)	0.761	0.542	0.172	0.068
At 48 hours (n = 32)				
Energy density (1Mcal non-nitrogen kcal per 2L)	-0.520	0.179	0.007	0.239
Percentage of non-nitrogen energy from lipid (per 10%)	0.056	0.036	0.132	0.082
pH (per 0.01 pH units)	-0.018	0.014	0.228	0.053
Baseline (per log_{10}cfu/mL)	-0.398	0.717	0.583	0.011

[a] The B coefficient indicates the absolute change from baseline (log_{10}cfu/mL) for the increment of each variable indicated in parentheses.
[b] The partial correlation squared (R^2) in this series is the same as partial η^2.

Figure 3.4, constructed from the multiple regression model (Table 3.6), uses three fixed amounts of non-protein energy density (at 640kcal, 1500kcal and 2360kcal per 2L) to illustrate the relationships at 48 hours. While an increase in the energy density significantly suppressed the growth of *C. albicans*, the proportion of energy from fat (or glucose) had no significant effect.

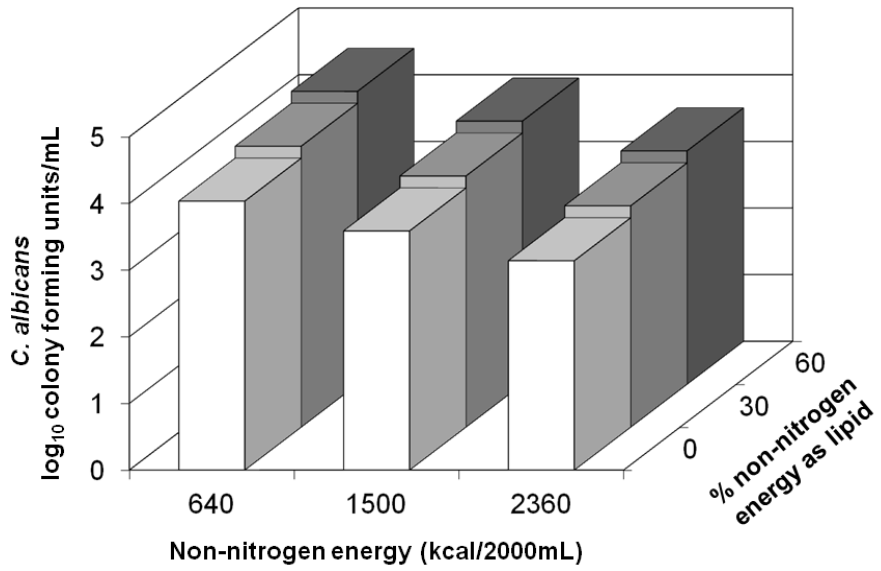

Figure 3.4 The independent effects of non-nitrogen energy in 2L (P = 0.007) and % non-nitrogen energy as lipid (per 10%, P = 0.132) in PN regimens on growth of *C. albicans* at 48 hours, based on multiple regression analysis with baseline $\log_{10}$cfu/mL and pH as covariates

3.1.2.2.3 Effect of pH

The effect of pH on the growth of *C. albicans* is reported separately below for lipid-free PN, lipid PN and lipid alone, using ANCOVA, with high and low pH as fixed factors.

Comparisons involving unadjusted pH

When lipid (pH 8.35 ± 0.03; PN regimen I in Table 3.1) was added to other components to prepare the lipid-containing PN, the final solution pH (6.11 ± 0.01; PN regimen F in Table 3.1) was virtually identical to that of the lipid-free PN (6.17 ± 0.01; PN regimen B in Table 3.1) due to the low buffering capacity of the lipid emulsion. The addition of lipid, whilst maintaining the same overall volume and glucose concentration, increases the energy density of the PN admixture to the extent shown in Table 3.1. Growth of *C. albicans* was greater in lipid alone (at a higher pH) than in PN with or without lipid (both at a lower pH) at both 24 and 48 hours. Growth was comparable between the PN with and without lipid at similar pH values

(upper part of Table 3.7). The regimens in each of these comparisons were neither isoenergetic nor equivalent in nutrient composition.

Table 3.7 Effect of pH and energy density on the number of *C. albicans* colony forming units per mL of infusate

Type of infusate (pH)	**Non-nitrogen kcal per 2L**	**pH mean pH units ± SEM**	**Change in log_{10} cfu/mL ± SEM at 24 hours**	***P*[a]**	**Change in log_{10} cfu/mL ± SEM at 48 hours**	***P*[a]**
Regimens without pH adjustment (n = 4 per group)						
Lipid PN[b]	1880	6.1 ± 0.01	0.793 ± 0.07	< 0.001	2.058 ± 0.13	0.002
Lipid alone[b]	4000	8.4 ± 0.03	1.776 ± 0.07		3.159 ± 0.13	
Lipid-free PN[c]	880	6.2 ± 0.01	0.697 ± 0.09	0.001	2.004 ± 0.12	0.001
Lipid alone[c]	4000	8.4 ± 0.03	1.774 ± 0.09		3.201 ± 0.12	
Lipid PN[d]	1880	6.1 ± 0.01	0.783 ± 0.09	0.586	1.989 ± 0.16	0.998
Lipid-free PN[d]	880	6.2 ± 0.01	0.706 ± 0.09		1.988 ± 0.16	
Regimens without and with pH adjustment[e] (n = 4 per group)						
Lipid PN[f]	1880	6.1 ± 0.01	0.719 ± 0.10	0.633	1.999 ± 0.15	0.869
Lipid PN[f]	1880	8.2 ± 0.01	0.793 ± 0.10		1.961 ± 0.15	
Lipid-free PN[g]	880	6.2 ± 0.01	0.679 ± 0.12	0.621	1.938 ± 0.14	0.487
Lipid-free PN[g]	880	8.2 ± 0.01	0.575 ± 0.12		1.774 ± 0.14	
Lipid alone[h]	4000	8.4 ± 0.03	1.781 ± 0.08	0.003	3.208 ± 0.10	0.121
Lipid alone[h]	4000	6.1 ± 0.02	2.400 ± 0.08		3.478 ± 0.10	

[a] The p-value refers to the comparison of the infusate pairs (bb, cc, dd, ff, gg, and hh).

[b, c, d] adjusted for baseline cfu/mL (b = 1.829 log_{10}cfu/mL, c = 1.823 log_{10}cfu/mL, d = 1.810 log_{10}cfu/mL).

[e] The infusate with adjusted pH is the second of each pair, which is compared with the infusate with the unadjusted pH (the first of each pair).

[f, g, h] adjusted for baseline cfu/mL (f = 1.837 log_{10}cfu/mL, g = 1.848 log_{10}cfu/mL, h = 1.831 log_{10}cfu/mL).

Comparisons involving adjusted pH

When the PN with and without lipid (pH ~6.1 and pH ~6.2 respectively; Table 3.7) were each alkalinised to produce a final pH that was comparable to that of the lipid alone (pH ~8.2; Table 3.7), there was no effect on the growth of *C. albicans* (lower part of Table 3.7). When the pH of the lipid alone (pH ~8.4; Table 3.7) was acidified to produce a final pH that was comparable to that of the lipid-containing PN (pH ~6.1; Table 3.7) growth was enhanced at 24 hours but not at 48 hours (lower part of Table 3.7).

3.1.2.2.4 Discussion

C. albicans density in PN infusates with and without lipid was found to increase over 48 hours by orders of magnitude, in agreement with a number of other studies[42, 56-59, 68, 71-73, 131]. Since this growth is substantially greater than a number of other potential PN contaminants such as *S. aureus*[56, 72, 73] or *S. epidermidis*[58], and since *C. albicans* is not an infrequent cause of CI, its behaviour in different PN infusates is expected to influence recommendations on maximum duration of bag infusion (24 hours or longer).

This study found an increase in energy density suppressed *C. albicans* in PN with and without lipid. A 1000kcal addition of lipid into PN at a fixed glucose concentration attenuated *C. albicans* growth (but not significantly, and paradoxically in an opposite way to that implied by guidelines[6, 31, 32]), but when 1000kcal of glucose was removed to re-establish the initial PN energy density, *C. albicans* growth increased (although not significantly differently from the initial value). This suggests that recommendations about the duration of PN bag infusion should consider not only the presence of lipid but also energy density.

This study raises two other concepts. First, it is unclear from this or other studies whether the suppressive effect of glucose concentration is direct or indirect. For example, since glucose concentration is the main determinant of PN osmolarity (and uniquely within the lipid and lipid-free PN groups in Figure 3.3), osmolarity may mediate at least some and perhaps most of the glucose effect, in which case recommendations about the maximum infusion duration of PN bags should consider

variations in osmolarity, including those due to nutrients such as sodium. Indeed, a study not involving PN found greater suppression of *C. albicans* as the sodium chloride concentration (and therefore osmolarity) increased over the range 0 to 200mmol/L (equivalent to 0 to 400mOsm/L)[133]. It is also possible that part of the decrease in *C. albicans* cfu/mL that occurs when lipid is added to lipid-free PN at a fixed concentration of glucose in a fixed volume (~70mOsm/L when 500mL lipid was included in 2L of PN) is due to osmolarity rather than being entirely a property of the lipid emulsion itself. Second, this study suggests caution is needed when extrapolating data from lipid emulsion alone to lipid PN, since *C. albicans* growth in the former is several orders of magnitude greater than the latter. This does not appear to be due to pH since upward adjustment of pH of the lipid PN to that of the lipid alone, and *vice versa*, had no significant effects on *C. albicans* growth at 48 hours (Table 3.7). However, in clinical practice adjustment of the pH of lipid emulsion is unlikely to occur without the addition of amino acid solution. However, it may be due to the much lower osmolarity of the lipid emulsion (270mOsmol/L; Table 3.1) than the PN admixtures (range 871 to 1443mOsmol/L; Table 3.1). This caution also applies to other individual PN components.

Previous studies[40, 42, 56-59, 68, 71, 131] are generally consistent with this study in showing *C. albicans* suppression in increasing glucose concentrations, but less consistent with the effect of lipid in PN. Some studies[40, 42, 56-59, 68, 71, 131] are conflicting and confusing due to differences in design, ambient conditions and other variables. For example, one study[131] of *C. albicans* growth in a 5% (w/v) glucose solution over a 48 hour period at 25°C found an increase in cfu/mL but another study[56] using the same infusate at the same temperature over the same period reported a decrease despite a higher starting cfu/mL. Five studies[40, 42, 57, 68, 71] reported wide variations in *C. albicans* growth in lipid alone at both lower and higher initial cfu/mL, and one study[71] found different lipid emulsions to variably affect *C. albicans* growth whilst another did not[40]. Out of four studies[57-59, 68] examining *C. albicans* growth in PN with and without a lipid component over 48 hours, three[57, 58, 68] reported an increase in growth and the other[59] a decrease. None of these studies controlled for variables such as the energy density, glucose concentration and pH of the PN, as in our study. However, three studies[57-59] did compare *C. albicans* growth in PN in the presence and absence of lipid at the same[57, 59] or similar[58] glucose concentrations (a difference of under 2% w/v glucose between the compared bags), although this was not

commented upon. One of these[57] reported that the presence of lipid enhanced the growth of *C. albicans*, another[59] reported a decrease, and the final one[58] reported high growth in PN with and without lipid emulsion. Similarly, two studies[58, 68] of *C. albicans* growth in PN of similar energy density (within 10kcal/100mL of each other) found the presence of lipid to enhanced growth, although interpretation is confounded by differences in glucose concentration and inoculation dose. To add to the variability, two studies[58, 59] reported an increase in *C. albicans* growth in PN when the proportion of energy derived from glucose increased but another study[57] reported the opposite.

Various reasons can explain such a wide array of results. These include the following: the use of different *C. albicans* strains (the preferred pH of some *C. albicans* strains can vary by as much as 3 pH units[134]); initial inoculation cfu/mL (potentially affecting the subsequent growth of *C. albicans*[135]); lipid emulsions of different pH (the same product from the same manufacturer can vary by up to 2.3 pH units[43]); use of different amino acid solutions (at the same pH, the amino acid composition can affect *C. albicans* growth[136]); PN of variable energy density; and different glucose concentrations (the presence of glucose supports growth[135, 137] but increasing glucose concentrations generally have a suppressive effect on *C. albicans* when utilisation of glucose is not enhanced[137], in agreement with the findings of this study). In addition, the published studies were undertaken at temperatures that were either not reported[40, 42, 57, 71] or over a wide range (~25°C[58, 59, 131], 25°C and 35°C[56], and 37°C[68]), some of which clearly do not represent the ambient temperature of wards where the infusions are administered. Furthermore, at least one of the studies[56] examined *C. albicans* growth in tubes exposed to oxygen in the air, which again does not reflect conditions associated with infusion of PN to patients. The situation becomes even more complex when there are interactions between different experimental variables. For example, since a change in temperature could affect *C. albicans* germination differently in various growth media (not in PN)[133] it could also differentially affect the viability of *C. albicans* in PN. Our study attempted to control for the variables discussed and to more closely reflect conditions used in clinical practice than some of these other studies. It did not specifically examine growth of *C. albicans* in PN during storage at 2-8°C, which has previously been shown to be inhibited[67].

The risks associated with growth of organisms in PN infusates need to be balanced with other clinical risks associated with administration of contaminant-free infusates. For example, whilst PN high in glucose concentration would reduce *C. albican*s growth, infusions of large amounts of glucose would increase the risk of hyperglycaemia which predisposes to infections, including CI. Furthermore, the risks associated with the growth of one microorganism in PN infusates should be balanced with the risks associated with the growth of others. For example, in our previous study[43] of *S. epidermidis* growth in the same PN formulations as in the present study, increasing glucose concentration induced growth (albeit to a small extent), in contrast to the decrease in *C. albicans* growth in the present study. This makes it difficult to make blanket recommendations about the maximum duration of different PN infusates.

Overall, the data from this and other studies of *C. albicans* growth in PN infusates do not seem to provide a body of evidence to limit the duration of PN bag infusion to a greater extent should they contain lipid. This conclusion is based on inadequate methodologies, difficulties in interpreting the results and lack of fundamental information about factors that control *C. albicans* proliferation in PN. A complexity is that the same cfu/mL may be associated with different proportions of filamentous to non-filamentous forms of *C. albicans*, which may affect pathogenicity[138]. A further difficulty in making blanket recommendations about the duration of PN bag infusion is that other potential contaminant types may behave differently to the fungus *C. albicans*, for example the Gram-positive bacterium *S. epidermidis* shows little growth[43], and Gram-negative bacteria such as *E. coli* may behave differently again. Until these points are investigated further, for example by the use of a clinical study or by undertaking a critical systematic review of the literature to examine the independent roles of each PN component along with regimen energy density and osmolarity for a number of potential PN contaminants, it is difficult to provide robust evidence based guidelines and to make policy decisions for implementation into routine clinical practice.

3.1.2.3 Growth of *E. coli* and *E. durans* in parenteral nutrition infusates

3.1.2.3.1 Growth of *E. coli* in parenteral nutrition infusates

Effect of the glucose concentration in the presence and absence of lipid in PN

In a general linear model, with glucose concentration, baseline $\log_{10}$cfu/mL and pH as covariates, the presence/absence of lipid emulsion a fixed factor, and glucose concentration*presence/absence of lipid as the interaction term, the following results were found: an increase in glucose concentration suppressed growth (the $\log_{10}$ cfu/mL of *E. coli*) at both 24 and 48 hours but only significantly at 48 hours (P = 0.170 at 24 hours, and P = 0.002 at 48 hours); the presence of lipid in PN increased growth of *E. coli* at both 24 and 48 hours (P = 0.011 at 24 hours, and $P < 0.001$ at 48 hours); and the way glucose affected growth of *E. coli* depended on whether the PN regimen was a lipid-containing or lipid-free PN (glucose concentration*presence/absence of lipid interaction, P = 0.368 at 24 hours and P = 0.026 at 48 hours). In view of the significant glucose concentration*presence/absence of lipid interaction at 48 hours the data were not examined by ANCOVA but by two separate regression analyses, one involving regression of glucose concentration on $\log_{10}$cfu/mL in the presence of the lipid emulsion and the other in the absence of a lipid emulsion (Table 3.8). At 24 hours the effect of increasing glucose concentration in reducing growth of *E. coli* was more pronounced in lipid PN than in lipid-free PN, but the effects were not significant (P = 0.202 in lipid-free PN, and P = 0.270 in lipid PN). At 48 hours (Figure 3.5 based on Table 3.8) the reverse pattern was observed: the effect of increasing glucose concentration in reducing growth of *E. coli* was less pronounced in lipid-PN and non-significant (P = 0.398), while in lipid-free PN it was larger and significant (P = 0.014).

Table 3.8 Results of multiple regression analysis to examine the effects of lipid and glucose on the growth of *E. coli* log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH[a]

Variable (increment in model)	**B[b]**	**SEM**	***P***	**partial η squared[c]**
At 24 hours for PN without lipid[d]				
Glucose concentration (per 10% w/v)	-0.295	0.219	0.202	0.132
pH (per 0.01 pH units)	0.000	0.019	0.992	0.000
Baseline (per log_{10}cfu/mL)	0.356	0.353	0.334	0.078
At 24 hours for PN with lipid[e]				
Glucose concentration (per 10% w/v)	-0.832	0.719	0.270	0.100
pH (per 0.01 pH units)	-0.016	0.028	0.582	0.026
Baseline (per log_{10}cfu/mL)	-0.130	0.359	0.723	0.011
At 48 hours for PN without lipid[f]				
Glucose concentration (per 10% w/v)	-0.903	0.313	0.014	0.410
pH (per 0.01 pH units)	-0.034	0.027	0.226	0.120
Baseline (per log_{10}cfu/mL)	-0.060	0.506	0.907	0.001
At 48 hours for PN with lipid[g]				
Glucose concentration (per 10% w/v)	-0.542	0.619	0.398	0.060
pH (per 0.01 pH units)	0.035	0.024	0.173	0.149
Baseline (per log_{10}cfu/mL)	0.076	0.309	0.809	0.005

[a] In the separate regression analyses involving lipid-PN and lipid-free PN adjustments were made for baseline log_{10}cfu/mL, glucose concentration and pH. For the lipid-free PN the mean of 1.571 log_{10}cfu/mL at 24 hours and the mean of 1.759 log_{10}cfu/mL at 48 hours was evaluated at the following covariate values: 1.226 log_{10}cfu/mL; pH = 6.168; and glucose concentration = 12.50%. For the lipid PN the mean of 2.120 log_{10}cfu/mL at 24 hours and the mean of 2.724 log_{10}cfu/mL at 48 hours was evaluated at the following covariate values: 0.963 log_{10}cfu/mL; pH = 6.094; and glucose concentration = 12.50%.

[b] The B coefficient indicates the absolute change (log_{10}cfu/mL) for the increment indicated in parentheses for each variable. For example, at 24 hours for PN without lipid for every 10% w/v increase in glucose concentration there is a decrease of 0.295 log_{10}cfu/mL.

[c] The partial correlation coefficient squared (R^2) in this series is the same as partial η^2.

[d] n = 16; regimens A to D in Table 3.1.

[e] n = 16; regimens E to H in Table 3.1.

[f] n = 16; regimens A to D in Table 3.1.

[g] n = 16; regimens E to H in Table 3.1.

The overall effect of including lipid in PN (assessed using the same general linear model described above (see Effect of glucose)) was to enhance the growth of *E. coli* ($P = 0.011$ at 24 hours, and $P < 0.001$ at 48 hours), which was confirmed by separate analyses involving PN with and without a lipid emulsion. At 24 hours, at a fixed glucose concentration, the $\log_{10}$cfu/mL of *E. coli* was greater in lipid PN than in lipid-free PN. The difference was significant at the three lower fixed glucose concentrations examined (0.757 $\log_{10}$cfu/mL at 8% w/v, 0.596 $\log_{10}$cfu/mL at 11% w/v, and 0.435 $\log_{10}$cfu/mL at 14% w/v; $P < 0.040$ at each point), but not at the highest glucose concentration examined (0.273 $\log_{10}$cfu/mL at 17% w/v; $P = 0.443$). At 48 hours, at a fixed glucose concentration, the $\log_{10}$cfu/mL of *E. coli* was greater in lipid PN than in lipid-free PN (Figure 3.5, based on Table 3.8). The difference was significant at the three highest glucose concentrations examined (0.918 $\log_{10}$cfu/mL at 11% w/v, 1.026 $\log_{10}$cfu/mL at 14% w/v, and 1.134 $\log_{10}$cfu/mL at 17% w/v; $P < 0.001$ at each point), and very close to significance at the lowest glucose concentration (0.810 $\log_{10}$cfu/mL at 8% w/v; $P = 0.059$).

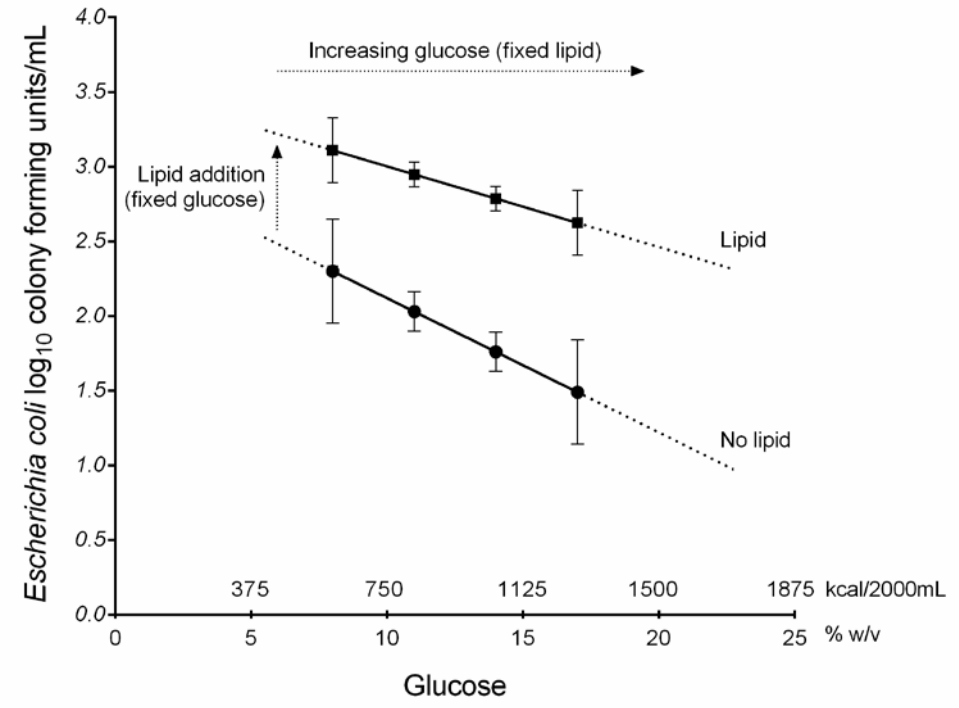

Figure 3.5 The independent effects of increasing glucose concentration and addition of lipid on *E. coli* growth over 48 hours in complete PN regimens (based on multiple regression analysis with baseline $\log_{10}$cfu/mL and pH as covariates). The bars represent ±1 SEM of the fitted modelled line at each glucose concentration indicated. The partial η^2 for the overall model (also R^2) at 48 hours was found to be 0.519 for lipid-free PN and 0.919 for lipid PN, and that for the glucose concentration in lipid-free PN was 0.410 compared to 0.060 in lipid PN

Effects of non-nitrogen energy density and the proportion of non-nitrogen energy derived from lipid

Table 3.9, which summarises the results of multiple linear regression with no fixed factor, shows that an increase in energy density by 1000kcal per 2L led to lower $\log_{10}$cfu/mL of *E. coli* at both 24 hours (0.373 ± 0.233 $\log_{10}$cfu/mL) and 48 hours (0.970 ± 0.290 $\log_{10}$cfu/mL) but the effect was significant only at 48 hours (P = 0.122 at 24 hours and P = 0.002 at 48 hours). Table 3.9 also shows that, at a given energy density, an increase in the proportion of lipid by 10% independently favoured growth of *E. coli* at both 24 hours (0.175 ± 0.039 $\log_{10}$cfu/mL; $P < 0.001$) and 48 hours (0.377 ± 0.049 $\log_{10}$cfu/mL; $P < 0.001$).

Table 3.9 Results of multiple regression analysis examining the effects of energy density and proportion of non-nitrogen energy as lipid on *E. coli* log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH[a]

Variable (increment in model)	**B[b]**	**SEM**	***P***	**partial η squared[c]**
At 24 hours[d]				
Energy density (1000kcal non-nitrogen energy per 2L)	-0.373	0.233	0.122	0.086
Percentage of non-nitrogen energy from lipid (per 10%)	0.175	0.039	< 0.001	0.426
pH (per 0.01 pH units)	-0.008	0.011	0.457	0.021
Baseline (per log_{10}cfu/mL)	0.119	0.230	0.610	0.010
At 48 hours[e]				
Energy density (1000kcal non-nitrogen energy per 2L)	-0.970	0.290	0.002	0.293
Percentage of non-nitrogen energy from lipid (per 10%)	0.377	0.049	< 0.001	0.689
pH (per 0.01 pH units)	-0.006	0.014	0.647	0.008
Baseline (per log_{10}cfu/mL)	0.105	0.286	0.715	0.005

[a] In this model baseline log_{10}cfu/mL, pH, energy density and percentage of non-nitrogen energy from lipid are covariates.
[b] The B coefficient indicates the absolute change from baseline (log_{10}cfu/mL) for the increment of each variable indicated in parentheses. For example, at 24 hours for every 1000kcal increase in non-nitrogen energy per 2L there is a decrease of 0.373 log_{10}cfu/mL.
[c] The partial correlation squared (R^2) in this series is the same as partial η^2.
[d] n = 32; regimens A to H in Table 1.
[e] n = 32; regimens A to H in Table 1.

Figure 3.6 (constructed from the multiple regression model at 48 hours; Table 3.9), shows that the effect of increasing energy density in significantly reducing log_{10}cfu/mL of *E. coli* ($P = 0.002$) was independent of the effect due to the proportion of non-nitrogen energy from lipid, which was found to significantly increase the log_{10}cfu/mL of *E. coli* ($P < 0.001$).

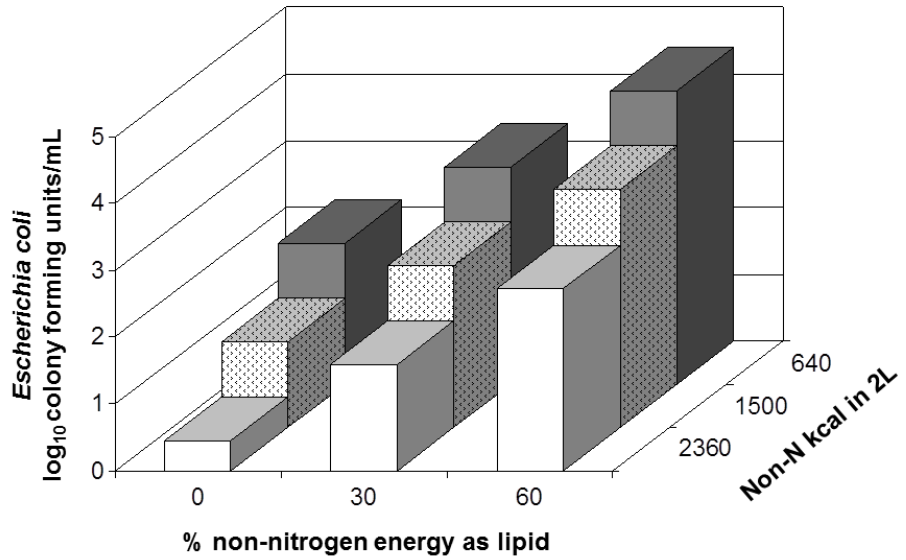

Figure 3.6 The independent effects of non-nitrogen energy in 2L (P = 0.002) and % non-nitrogen energy as lipid (per 10%, P < 0.001) in PN regimens on growth of *E. coli* at 48 hours, based on multiple regression analysis with adjustment for baseline $\log_{10}$cfu/mL and pH

Effect of pH

The effect of pH on the growth of *E. coli* is reported separately below for lipid-free PN, lipid PN and lipid alone, using ANCOVA, with high and low pH as fixed factors.

Comparisons involving unadjusted pH

After adjusting for baseline $\log_{10}$cfu/mL, *E. coli* was found to grow more in lipid alone, which had a higher pH (~7.6 in this study), than in PN with or without lipid, which had a lower pH (~6.2 in this study) (Table 3.10). There was also greater growth in lipid PN compared to lipid-free PN despite the higher energy density of the lipid PN.

Table 3.10 Results of linear regression analysis examining the effects of pH and energy density on *E. coli* log_{10}cfu/mL over time[a]

Type of infusate (pH)	Non-nitrogen kcal per 2L	Mean pH ± SEM	Change in log_{10} cfu/mL ± SEM at 24 hours	*P*[b]	Change in log_{10} cfu/mL ± SEM at 48 hours	P value[b]
Regimens without pH adjustment[c]						
Lipid PN[d]	1880	6.123 ± 0.008	1.020 ± 0.076	0.002	1.816 ± 0.158	0.002
Lipid alone[d]	4000	7.758 ± 0.008	1.6271 ± 0.076		3.106 ± 0.158	
Lipid-free PN[e]	880	6.180 ± 0.000	0.500 ± 0.073	< 0.001	0.944 ± 0.333	0.024
Lipid alone[e]	4000	7.758 ± 0.008	1.597 ± 0.073		2.925 ± 0.333	
Lipid PN[f]	1880	6.123 ± 0.008	1.023 ± 0.095	0.017	1.676 ± 0.159	0.034
Lipid-free PN[f]	880	6.180 ± 0.000	0.467 ± 0.095		0.910 ± 0.159	
Regimens without and with pH adjustment[g]						
Lipid PN[h]	1880	6.123 ± 0.008	1.073 ± 0.216	0.947	1.772 ± 0.093	< 0.001
Lipid PN[h]	1880	7.763 ± 0.023	1.095 ± 0.216		3.619 ± 0.093	
Lipid-free PN[i]	880	6.180 ± 0.000	0.396 ± 0.040	0.024	0.615 ± 0.126	0.001
Lipid-free PN[i]	880	7.735 ± 0.031	0.592 ± 0.040		1.999 ± 0.126	
Lipid alone[j]	4000	7.758 ± 0.008	1.640 ± 0.136	0.007	3.044 ± 0.171	0.011
Lipid alone[j]	4000	6.198 ± 0.027	2.604 ± 0.136		4.144 ± 0.171	

[a] In this model the regimen within each pair is a fixed factor and baseline log_{10}cfu/mL is a covariate.
[b] The p-value refers to the comparison of the infusate pairs (cc, dd, ee, gg, hh, and ii).
[c] n = 4 per group; using unadjusted regimens B, F and I from Table 1.
[d, e, f] controlled for baseline cfu/mL (d = 1.0539 log_{10}cfu/mL, e = 1.1618 log_{10}cfu/mL, f = 1.1591 log_{10}cfu/mL).
[g] n = 4 per group; using unadjusted and pH-adjusted regimens B, F and I from Table 1. The infusate with adjusted pH is the second of each pair, which is compared with the infusate with the unadjusted pH (the first of each pair).
[h, i, j] Adjusted for baseline cfu/mL (h = 1.0923 log_{10}cfu/mL, i = 1.3133 log_{10}cfu/mL, j = 1.1011 log_{10}cfu/mL).

Comparisons involving adjusted pH

When the pH of the PN infusate (with or without lipid) was adjusted upwards to match that of the lipid alone, growth of *E. coli* increased. When the pH of lipid alone was reduced to match that of PN, growth increased. The changes in log_{10}cfu/mL induced by altering the pH were smaller in PN, with or without lipid, than in lipid alone (t-test, $P < 0.001$).

Effect of osmolarity

In a general linear model with osmolarity, baseline log_{10}cfu/mL and pH as covariates, the presence/absence of lipid emulsion as a fixed factor, and osmolarity*glucose/sodium chloride as the interaction term, the following results were obtained: an increase in osmolarity significantly suppressed the log_{10}cfu/mL ($P = 0.003$ at 24 hours, and $P = 0.011$ at 48 hours); the presence of glucose in PN tended to suppress the growth of *E. coli* at 24 hours ($P = 0.508$) and to increase it at 48 hours ($P = 0.068$) but neither of the effects were significant; and the way increased osmolarity affected growth of *E. coli* in lipid PN was not significantly different when due to glucose compared to sodium chloride at 24 hours, but it was significantly different at 48 hours (osmolarity*glucose/sodium chloride interaction, $P = 0.629$ at 24 hours and $P = 0.018$ at 48 hours). The data were not examined by ANCOVA but by separate regression analyses to examine the effects of changing osmolarity using glucose or sodium chloride (Table 3.11, and Figure 3.7, which also shows the variation in log_{10}cfu/mL at each level of osmolarity).

Table 3.11 Results of multiple regression analysis examining the effects of osmolarity changes due to glucose and sodium chloride on *E.* coli log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH[a]

Variable (increment in model)	**B[b]**	**SEM**	***P***	**partial η squared[c]**
At 24 hours for lipid PN with osmolarity differences due to glucose with a fixed sodium chloride component[d]				
Osmolarity (per 100 mOsm/L)	-0.148	0.130	0.277	0.097
pH (per 0.01 pH units)	-0.015	0.028	0.595	0.024
Baseline (per log_{10}cfu/mL)	-0.133	0.359	0.717	0.011
At 24 hours for lipid PN with osmolarity differences due to sodium chloride with a fixed glucose component[e]				
Osmolarity (per 100 mOsm/L)	-0.224	0.061	0.006	0.631
pH (per 0.01 pH units)	-0.027	0.012	0.045	0.414
Baseline (per log_{10}cfu/mL)	0.027	0.286	0.927	0.001
At 48 hours for lipid PN with osmolarity differences due to glucose with a fixed sodium chloride component[f]				
Osmolarity (per 100 mOsm/L)	-0.097	0.111	0.402	0.059
pH (per 0.01 pH units)	0.036	0.024	0.168	0.152
Baseline (per log_{10}cfu/mL)	0.075	0.309	0.813	0.005
At 48 hours for lipid PN with osmolarity differences due to sodium chloride with a fixed glucose component[g]				
Osmolarity (per 100 mOsm/L)	-0.254	0.092	0.025	0.488
pH (per 0.01 pH units)	-0.025	0.018	0.189	0.205
Baseline (per log_{10}cfu/mL)	0.516	0.436	0.270	0.149

[a] In the separate regression analyses involving osmolarity changes using either sodium chloride or glucose adjustments were made for baseline log_{10}cfu/mL, glucose concentration and pH. At 24 hours, the glucose osmolarity mean of 2.120 log_{10}cfu/mL was evaluated at the following covariate values: baseline = 0.963 log_{10}cfu/mL; pH = 6.094; and osmolarity = 1193.750mOsm/L, and the sodium chloride mean of 2.361 log_{10}cfu/mL was evaluated at the following covariate values: baseline = 1.019 log_{10}cfu/mL; pH = 6.099; and osmolarity = 1110.667mOsm/L. At 48 hours, the glucose osmolarity mean of 2.724 log_{10}cfu/mL and the sodium chloride mean of 3.330 log_{10}cfu/mL were evaluated at the same covariate values as at 24 hours.

[b] The B coefficient indicates the absolute change (log_{10}cfu/mL) for the increment indicated in parentheses for each variable. For example, at 24 hours for lipid PN with osmolarity differences due to glucose with a fixed sodium chloride component for every 100 mOsm/L increase in osmolarity there is a decrease of 0.148 log_{10}cfu/mL.

[c] The partial correlation coefficient squared (R^2) in this series is the same as partial η^2.

[d] n = 16; regimens E to H from Table 1.
[e] n = 12; regimens E, J and K from Table 1.
[f] n = 16; regimens E to H from Table 1.
[g] n = 12; regimens E, J and K from Table 1.

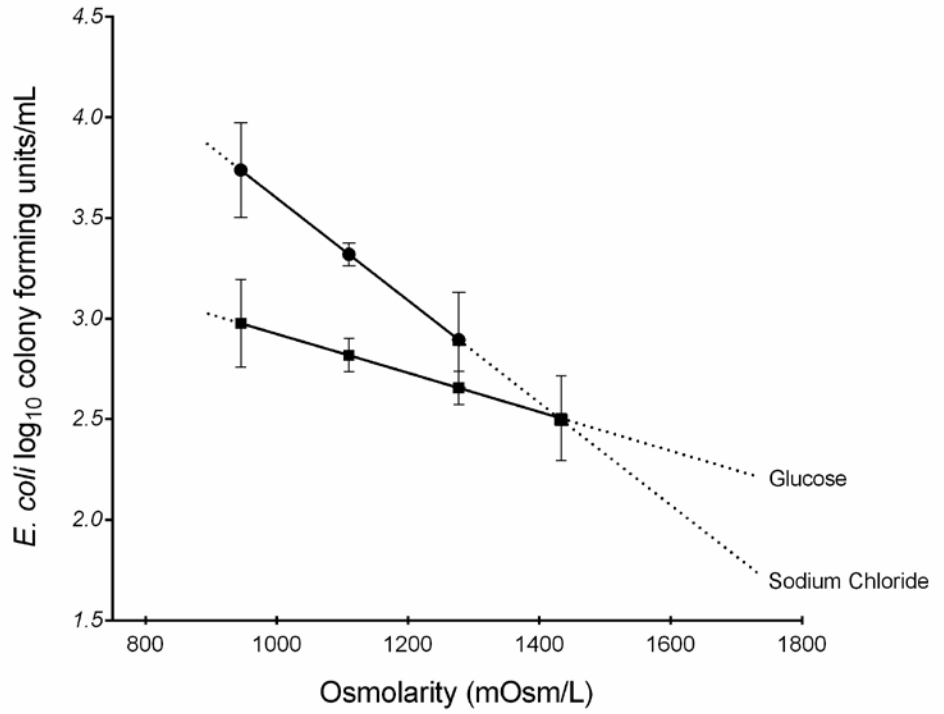

Figure 3.7 The independent effects of osmolarity due to glucose and sodium chloride in PN regimens containing lipid on growth of *E. coli* at 48 hours, based on multiple regression analysis with baseline log_{10}cfu/mL and pH as covariates. The bars represent ±1 SEM of the fitted modelled line at each glucose concentration indicated. The partial η^2 for the overall model (also R^2) at 48 hours was found to be 0.919 for the osmolarity of glucose and 0.681 for the osmolarity of sodium chloride

The results show that an increase in osmolarity due to glucose reduced the growth of *E. coli* in lipid PN (only investigated in lipid PN and not lipid-free PN) but the effect was not significant (P = 0.277 at 24 hours, and P = 0.402 at 48 hours). An increase in osmolarity due to sodium chloride was also associated with reduced growth in lipid PN (also only investigated in lipid PN and not lipid-free PN), which was greater than glucose and also significant (P = 0.006 at 24 hours, and P = 0.025 at 48 hours).

3.1.2.3.2 Growth of *E. durans* in parenteral nutrition infusates

Effect of the glucose concentration in the presence and absence of lipid in parenteral nutrition

The overall effect of glucose on log_{10}cfu/mL was assessed using a general linear model (see 3.1.2.3.1.1), which indicated that an increase in glucose concentration suppressed growth (the log_{10}cfu/mL of *E. durans*) at both 24 and 48 hours but only significantly at 48 hours ($P = 0.164$ at 24 hours, and $P = 0.029$ at 48 hours), and that the presence of lipid in PN increased growth of *E. durans* at both 24 and 48 hours but only significantly at 48 hours ($P = 0.055$ at 24 hours, and $P = 0.013$ at 48 hours). In addition, the way in which glucose affected growth of *E. durans* did not depend on whether the PN was lipid PN or lipid-free PN (glucose*presence/absence of lipid interaction, $P = 0.229$ at 24 hours and $P = 0.379$ at 48 hours). In view of the non-significant glucose*presence/absence of lipid interaction at both 24 and 48 hours the data were examined by ANCOVA (Table 3.12). At 24 hours the effect of an increase in glucose concentration in reducing growth of *E. durans* was not significant ($P = 0.470$) but at 48 hours (Figure 3.8 based on Table 3.12) it was significant ($P = 0.002$).

Table 3.12 Results of ANCOVA analysis to examine the effects of lipid and glucose on the growth of *E. durans* log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH[a]

Variable (increment in model)	**B[b]**	**SEM**	***P***	**partial η squared[c]**
At 24 hours[d]				
Presence of lipid (5% w/v compared to no lipid)[e]	0.282	0.103	0.011	0.218
Glucose concentration (per 10% w/v)	-0.136	0.186	0.470	0.020
pH (per 0.01 pH units)	0.002	0.010	0.822	0.002
Baseline (per log_{10}cfu/mL)	-0.116	0.107	0.287	0.042
At 48 hours[d]				
Presence of lipid (5% w/v compared to no lipid)[e]	0.586	0.097	< 0.001	0.577
Glucose concentration (per 10% w/v)	-0.593	0.174	0.002	0.300
pH (per 0.01 pH units)	-0.016	0.009	0.092	0.101
Baseline (per log_{10}cfu/mL)	0.022	0.101	0.831	0.002

[a] In the separate regression analyses involving lipid-PN and lipid-free PN adjustments were made for baseline log_{10}cfu/mL, glucose concentration and pH. The mean of 2.058 log_{10}cfu/mL at 24 hours and the mean of 3.451 log_{10}cfu/mL at 48 hours were evaluated at the following covariate values: baseline = 0.843 log_{10}cfu/mL; pH = 6.131; and glucose concentration = 12.50%.

[b] The B coefficient indicates the absolute change (log_{10}cfu/mL) for the increment indicated in parentheses for each variable. For example, at 24 hours the presence of 5% w/v lipid compared to no lipid leads to an increase of 0.282 log_{10}cfu/mL.

[c] The partial correlation coefficient squared (R^2) in this series is the same as partial η^2.

[d] n = 32; regimens A to H in Table 1.

[e] Lipid; 1 = presence of lipid and 0 = absence of lipid in the statistical model.

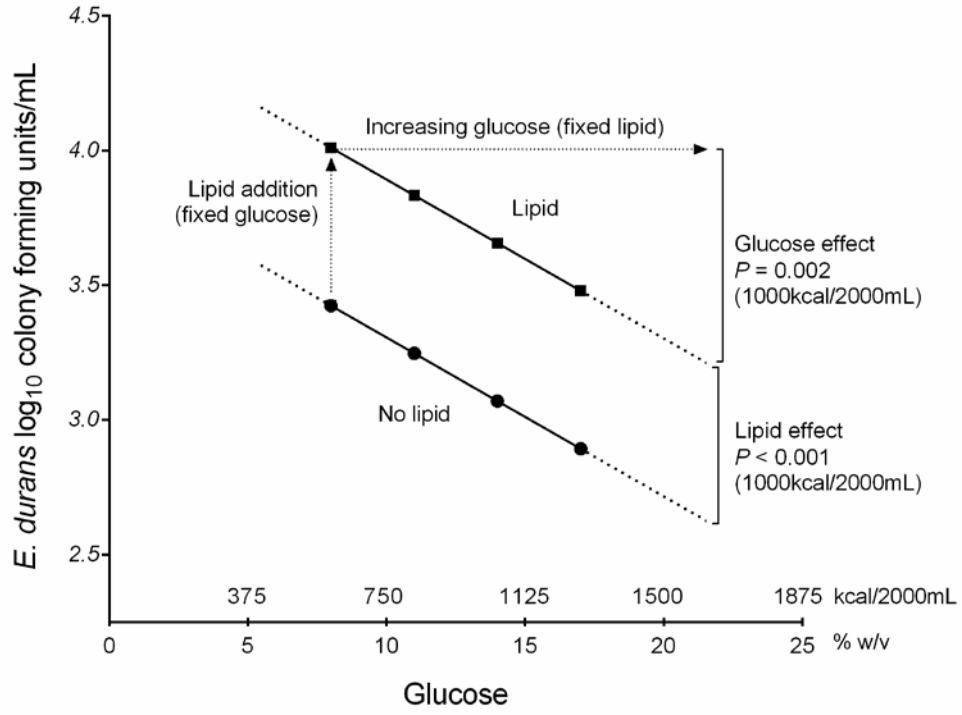

Figure 3.8 The independent effects of increasing glucose concentration and addition of lipid on *E. durans* growth over 48 hours in complete PN regimens (based on ANCOVA with adjustment for baseline log_{10}cfu/mL and pH). The partial η^2 for the overall model (also R^2) at 48 hours was found to be 0.840, and that for the glucose concentration was 0.300

The overall effect of including lipid in PN (assessed using the same general linear model described above (see 3.1.2.3.1.1)) was an enhancement in growth of *E. durans* (*P* = 0.055 at 24 hours, and *P* = 0.013 at 48 hours). Since there was no significant interaction between glucose concentration and the presence/absence of lipid emulsion (homogeneiety of regression; *P* = 0.229 at 24 hours, and *P* = 0.379 at 48 hours), the results were analysed using ANCOVA. The analysis (Table 3.12) showed that the inclusion of lipid in PN at a fixed glucose concentration significantly increased growth of *E. durans* both at 24 hours (*P* = 0.011) and at 48 hours (*P* < 0.001) (Figure 3.8).

Effects of non-nitrogen energy density and the proportion of non-nitrogen energy derived from lipid

The results in Table 3.13, obtained in the same manner as for *E. coli*, show that an increase in energy density by 1000kcal per 2L led to lower log_{10}cfu/mL of *E. durans* at both 24 hours (0.229 ± 0.229 log_{10}cfu/mL) and 48 hours (0.773 ± 0.215

log_{10}cfu/mL) but the effect was significant only at 48 hours (P = 0.327 at 24 hours and P = 0.001 at 48 hours).

Table 3.13 Results of multiple regression analysis examining the effects of energy density and proportion of non-nitrogen energy as lipid on *E. durans* log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH[a]

Variable (increment in model)	**B[b]**	**SEM**	***P***	**partial η squared[c]**
At 24 hours[d]				
Energy density (1000kcal non-nitrogen energy per 2L)	-0.229	0.229	0.327	0.036
Percentage of non-nitrogen energy from lipid (per 10%)	0.089	0.037	0.021	0.181
pH (per 0.01 pH units)	-0.006	0.012	0.640	0.008
Baseline (per log_{10}cfu/mL)	-0.120	0.101	0.247	0.049
At 48 hours[d]				
Energy density (1000kcal non-nitrogen energy per 2L)	-0.773	0.215	0.001	0.324
Percentage of non-nitrogen energy from lipid (per 10%)	0.237	0.034	< 0.001	0.640
pH (per 0.01 pH units)	-0.033	0.011	0.006	0.251
Baseline (per log_{10}cfu/mL)	-0.020	0.095	0.833	0.002

[a] In this model baseline log_{10}cfu/mL, pH, energy density and percentage of non-nitrogen energy from lipid are covariates.
[b] The B coefficient indicates the absolute change from baseline (log_{10}cfu/mL) for the increment of each variable indicated in parentheses. For example, at 24 hours an increase in energy density of 1000kcal of non-nitrogen energy per 2L leads to a decrease of 0.229 log_{10}cfu/mL.
[c] The partial correlation squared (R^2) in this series is the same as partial η^2.
[d] n = 32; regimens A to H in Table 1.

Table 3.13 also shows that at a given energy density an increase in the proportion of lipid by 10% independently favoured growth of *E. durans* at both 24 hours (0.089 ± 0.037 log_{10}cfu/mL; P = 0.021) and 48 hours (0.237 ± 0.034 log_{10}cfu/mL; P < 0.001). Figure 3.9 (constructed from the results of multiple regression analysis at 48 hours (Table 3.13)), shows that for *E. durans*, as for *E. coli*, the effect of increasing energy density in reducing the log_{10}cfu/mL at 48 hours (P = 0.001) was independent of that due to decreasing percentage of non-nitrogen energy from lipid (P < 0.001).

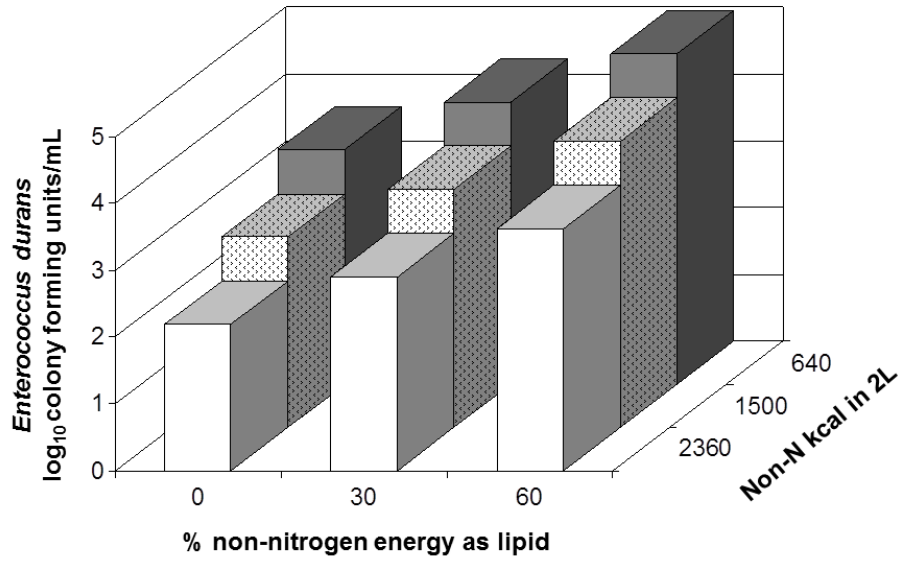

Figure 3.9 The independent effects of non-nitrogen energy in 2L (P = 0.001) and % non-nitrogen energy as lipid (per 10%, P < 0.001) in PN regimens on growth of *E. durans* at 48 hours, based on multiple regression analysis with baseline adjustment for log_{10}cfu/mL and pH

Effect of pH

The effect of pH on the growth of *E. durans* is reported separately below for lipid-free PN, lipid PN and lipid alone, using ANCOVA, with high and low pH as fixed factors.

Comparisons involving unadjusted pH

After adjusting for baseline log_{10}cfu/mL (as for *E. coli*), the behaviour of *E. durans* was qualitatively different from that of *E. coli* since the log_{10}cfu/mL of *E. durans* increased in PN with or without lipid (~pH 6.2 in this study) but was eliminated in lipid alone (~pH 7.6 in this study) by 48 hours (Table 3.14). The change in log_{10}cfu/mL between PN with or without lipid and the lipid alone was significant (t-test, P < 0.001).

Table 3.14 Results of linear regression analysis examining the effects of pH and energy density on *E. durans* log_{10}cfu/mL over time[a]

Type of infusate (pH)	Non-nitrogen kcal per 2L	Mean pH ± SEM	Change in log_{10} cfu/mL ± SEM at 24 hours	*P*[b]	Change in log_{10} cfu/mL ± SEM at 48 hours	*P* value[b]
Regimens without pH adjustment[c]						
Lipid PN[d] Lipid alone[d]	1880 4000	6.123 ± 0.008 7.758 ± 0.008	1.557 ± 0.220 1.075 ± 0.220	0.277	Growth[g] Zero cfu/mLat 48 hours[g]	Unable to compute[h]
Lipid-free PN[e] Lipid alone[e]	880 4000	6.180 ± 0.000 7.758 ± 0.008	1.282 ± 2.104 0.985 ± 2.104	0.946	Growth[g] Zero cfu/mL at 48 hours[g]	Unable to compute[i]
Lipid PN[f] Lipid-free PN[f]	1880 880	6.123 ± 0.008 6.180 ± 0.000	1.003 ± 0.042 0.909 ± 0.042	0.198	2.744 ± 0.077 2.155 ± 0.077	0.004
Regimens without and with pH adjustment[j]						
Lipid PN Lipid PN	1880 1880	6.123 ± 0.008 7.763 ± 0.023	Growth[g] Growth[g]	Unable to compute[l]	Growth[g] Zero cfu/mL[g]	Unable to compute[m]
Lipid-free PN[k] Lipid-free PN[k]	880 880	6.180 ± 0.000 7.735 ± 0.031	0.826 ± 0.043 0.754 ± 0.043	0.324	2.180 ± 0.060 1.329 ± 0.060	< 0.001
Lipid alone Lipid alone	4000 4000	7.758 ± 0.008 6.198 ± 0.027	Growth[f] Growth[f]	Unable to compute[n]	Zero cfu/mL[g] Zero cfu/mL[g]	Unable to compute[o]

[a] In this model the regimen within each pair is a fixed factor and baseline log_{10}cfu/mL is a covariate.
[b] The p-value refers to the comparison of the infusate pairs (cc, dd, ee, gg, hh, and ii).
[c] n = 4 per group; using unadjusted regimens B, F and I from Table 1.
[d, e, f] adjusted for baseline cfu/mL (d = 0.4756 log_{10}cfu/mL, e = 0.6296 log_{10}cfu/mL, f = 1.1052 log_{10}cfu/mL).
[g] Unable to compute since it is not possible to compute log_{10} of zero cfu/mL. There were zero cfu/mL at 24 hours for one sample of pH-adjusted lipid PN and one sample of pH-adjusted lipid alone, and for all samples at 48 hours for unadjusted lipid alone, pH-adjusted lipid alone and pH-adjusted lipid PN.
[h] The difference between the change in cfu/mL from baseline to 48 hours between the pair is significant (t-test, $P < 0.001$).

[i] The difference between the change in cfu/mL from baseline to 48 hours between the pair is significant ($P < 0.001$).
[j] n = 4 per group; using unadjusted and pH-adjusted regimens B, F and I from Table 1. The infusate with adjusted pH is the second of each pair, which is compared with the infusate with the unadjusted pH (the first of each pair).
[k] Adjusted for baseline cfu/mL (1.1811 $\log_{10}$cfu/mL).
[l] The difference between the change in cfu/mL from baseline to 48 hours between the pair is not significant (greater growth in the unadjusted group, independent t-test, $P = 0.903$).
[m] The difference between the change in cfu/mL from baseline to 48 hours between the pair is significant (independent t-test, $P < 0.001$).
[n] The difference between the change in cfu/mL from baseline to 48 hours between the pair is not significant (greater growth in the unadjusted group, independent t-test, $P = 0.103$).
[o] The difference between the change in cfu/mL from baseline to 48 hours between the pair is not significant although a t-test cannot be computed.

Comparisons involving adjusted pH

Adjustment of pH also elicited different responses in the log_{10}cfu/mL of *E. durans* than *E. coli*. When the pH of the PN infusate (with or without lipid) was adjusted upwards to that of the lipid alone the log_{10}cfu/mL of *E. durans* at 48 hours decreased in PN without lipid and was eliminated in PN with lipid. When the pH of lipid alone was reduced to match that of PN, *E. durans* was also eliminated. However, at the unadjusted (native) pH of PN, *E. durans*, like *E. coli*, grew more rapidly in lipid PN than lipid-free PN despite the greater energy density of the lipid PN.

Effect of osmolarity

The overall effect of osmolarity on log_{10}cfu/mL was assessed using a general linear model (see 3.1.2.3.1.4), which indicated that an increase in osmolarity had a significant overall effect in suppressing log_{10}cfu/mL (P = 0.025 at 24 hours, and P = 0.042 at 48 hours), and that the presence of glucose in PN increased growth of *E. durans* at 24 hours (P = 0.556) and at 48 hours (P = 0.078). In addition, the way in which an increase in osmolarity affected growth of *E. durans* was not significantly different in lipid-containing PN when due to glucose compared to sodium chloride at 24 hours, but it was significantly different at 48 hours (osmolarity*glucose/sodium chloride interaction, P = 0.320 at 24 hours and P = 0.018 at 48 hours). As for *E. coli*, the effects of glucose and sodium chloride concentrations were examined in separate regression analyses. The results (Table 3.15, and Figure 3.10) show that an increase in osmolarity due to glucose led to an increase in log_{10}cfu/mL of *E. durans* at 24 hours in lipid PN (only investigated in lipid PN and not lipid-free PN) (P = 0.846) but a reduction in log_{10}cfu/mL at 48 hours (P = 0.759). An increase in osmolarity due to sodium chloride was associated with reduced log_{10}cfu/mL in lipid PN (also not investigated in lipid-free PN), which did reach statistical significance (P = 0.021 at 24 hours, and P = 0.045 at 48 hours).

Table 3.15 Results of multiple regression analysis examining the effects of osmolarity changes due to glucose and sodium chloride on *E. durans* log_{10}cfu/mL over time, after controlling for baseline log_{10}cfu/mL and pH[a]

Variable (increment in model)	B[b]	SEM	*P*	partial η squared[c]
At 24 hours for lipid PN with osmolarity differences due to glucose with a fixed sodium chloride component[d]				
Osmolarity (per 100 mOsm/L)	0.023	0.118	0.846	0.003
pH (per 0.01 pH units)	0.017	0.025	0.507	0.037
Baseline (per log_{10}cfu/mL)	-0.185	0.116	0.137	0.175
At 24 hours for lipid PN with osmolarity differences due to sodium chloride with a fixed glucose component[e]				
Osmolarity (per 100 mOsm/L)	-0.237	0.083	0.021	0.504
pH (per 0.01 pH units)	-0.053	0.013	0.004	0.676
Baseline (per log_{10}cfu/mL)	-0.057	0.171	0.747	0.014
At 48 hours for lipid PN with osmolarity differences due to glucose with a fixed sodium chloride component[f]				
Osmolarity (per 100 mOsm/L)	-0.032	0.102	0.759	0.008
pH (per 0.01 pH units)	0.002	0.022	0.915	0.001
Baseline (per log_{10}cfu/mL)	0.011	0.100	0.916	0.001
At 48 hours for lipid PN with osmolarity differences due to sodium chloride with a fixed glucose component[g]				
Osmolarity (per 100 mOsm/L)	-0.170	0.072	0.045	0.414
pH (per 0.01 pH units)	-0.059	0.011	0.001	0.774
Baseline (per log_{10}cfu/mL)	0.274	0.148	0.100	0.301

[a] In the separate regression analyses involving osmolarity changes using either sodium chloride or glucose adjustments were made for baseline log_{10}cfu/mL, glucose concentration and pH. At 24 hours, the glucose osmolarity mean of 2.231 log_{10}cfu/mL was evaluated at the following covariate values: baseline = 0.506 log_{10}cfu/mL; pH = 6.094; and osmolarity = 1193.750mOsm/L, and the sodium chloride mean of 2.617 log_{10}cfu/mL was evaluated at the following covariate values: baseline = 0.5735 log_{10}cfu/mL; pH = 6.099; and osmolarity = 1110.667mOsm/L. At 48 hours, the glucose osmolarity mean of 3.797 log_{10}cfu/mL and the sodium chloride mean of 3.330 log_{10}cfu/mL were evaluated at the same covariate values as at 24 hours.

[b] The B coefficient indicates the absolute change (log_{10}cfu/mL) for the increment indicated in parentheses for each variable. For example, at 24 hours for lipid PN with osmolarity differences due to glucose with a fixed sodium chloride component an increase of 100mOsm/L leads to an increase of 0.023 log_{10}cfu/mL.

[c] The partial correlation coefficient squared (R^2) in this series is the same as partial η^2.

[d] n = 16; regimens E to H from Table 1.

[e] n = 12; regimens E, J and K from Table 1.

[f] n = 16; regimens E to H from Table 1.

[g] n = 12; regimens E, J and K from Table 1.

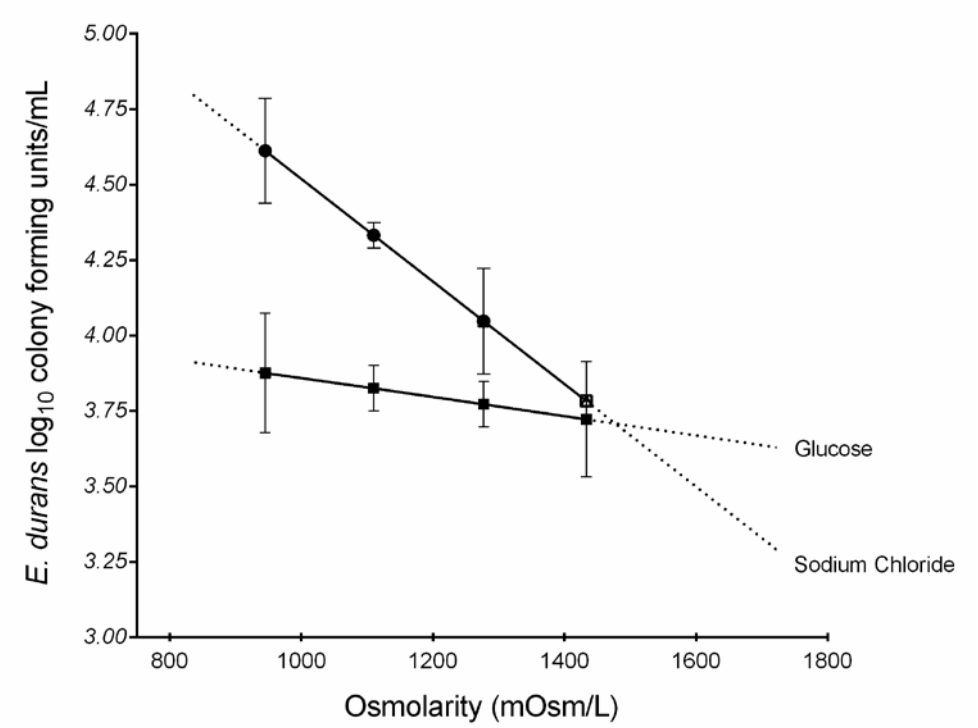

Figure 3.10 The independent effects of osmolarity due to glucose and sodium chloride in PN regimens containing lipid on growth of *E. durans* at 48 hours, based on multiple regression analysis with adjustment for baseline log_{10}cfu/mL and pH. The bars represent ±1 SEM of the fitted modelled line at each glucose concentration indicated. The partial η^2 for the overall model (also R^2) at 48 hours was found to be 0.284 for the osmolarity of glucose, and 0.881 for the osmolarity of sodium chloride

3.1.2.4 Discussion

This study found that the log_{10}cfu/mL of both *E. coli* and *E. durans* were suppressed by increasing glucose concentration in PN both with and without lipid, and that an increase in osmolarity suppressed growth, apparently according to the type of nutrient causing the osmolarity change. It also found that both the presence of lipid in PN (at fixed glucose concentration) and increasing the proportion of energy from lipid (with a corresponding reduction in the proportion of energy from glucose in a fixed volume of PN) enhanced the growth of these two organisms. The data contrast with the information gathered on other microorganisms (*S. epidermidis*[43] and *C. albicans*[44]) using the same methodology and same infusate formulations (with the exception of the osmolarity component) as in the present investigations. For example, unlike the present study in which increasing glucose concentrations suppressed growth of *E. coli* and *E. durans* (as well as in the study of *C. albicans*), glucose enhanced the growth of *S. epidermidis*. In addition, the significant

independent effect of the lipid emulsion in enhancing growth of *E. coli* and *E. durans* at a fixed glucose concentration was opposite to that found with *C. albicans*. Furthermore, increasing the proportion of non-nitrogen energy from lipid compared to glucose in the same volume of PN significantly increased the $\log_{10}$cfu/mL of *E. coli* and *E. durans*, but not *S. epidermidis* (non-significant decrease in $\log_{10}$cfu/mL at 48 hours) or *C. albicans* (non-significant increase in $\log_{10}$cfu/mL at 48 hours). Taken together these observations suggest that organisms behave differently in the same PN formulation, and given the wide range of PN formulations and differences in the composition of commercially available PN products, caution should be exercised in coming to overarching conclusions about the effects of lipid in PN on a wide array of Gram-positive, Gram-negative and fungal organisms. Fundamental research into the biology and nutritional biochemistry of different organisms in PN infusates may help establish sound principles to inform clinical practice. A systematic, critical review of the current evidence base may also help clarify the clinical issues and identify gaps in the current knowledge that need to be addressed.

The conclusion that the same medium affects the growth pattern of various microorganisms in different ways is not restricted to complete PN infusates. For example, the present study demonstrated the same lipid emulsion affected the growth of *E. coli* and *E. durans* in strikingly different ways. *E. coli* was found to grow in lipid alone but *E. durans* was killed when inoculated into lipid alone (in contrast its growth was enhanced in lipid PN compared to lipid-free PN). It is also evident from this study that it is inappropriate to extrapolate from observations based on the growth of organisms in lipid emulsions alone to lipid PN. Recommendations about the duration of infusion of lipid PN from a single bag should be based only on studies of PN.

Manipulation of the pH was also found to affect the growth of *E. coli* and *E. durans* in different ways. Generally, and depending on other conditions, a pH ~7 is considered ideal for the growth of *E. coli*[139] or enterococci (the genus to which the species *E. durans* belongs)[140], but both are reported to potentially grow in environments with a wide range of pH (~pH 4.5 to 9.0[139, 141], or even up to ~pH 9.6 for some strains of *E. durans*[142, 143]). In this study, the $\log_{10}$cfu/mL of *E. coli* was found to be enhanced in PN infusates at a higher rather than lower pH (~pH 7.6 v 6.2), whereas the reverse was observed with *E. durans*, which was suppressed in PN

without lipid and killed in PN with lipid at high pH (pH ~7.6) (but not at lower pH (pH ~6.2)). Since at 48 hours *E. durans* was also found to be killed in the lipid emulsion alone, which had a pH of ~7.6, it is possible that the eradication of *E. durans* in the lipid emulsion alone was due to the high pH rather than to a property of the nutrients within the lipid emulsion. Although large manipulations of pH can provide insights into the biology of the microorganisms they are not necessarily of direct relevance to clinical practice where the pH of the PN infusates falls well below those artificially induced in this study. Nevertheless, more detailed investigations would be informative because of the large variation in pH of commercially available components of PN mixtures, which may affect the final pH of the mixture in different ways. For example, the pH of different commercial lipid emulsions can vary from pH 6.0 to 8.8 and within batches may vary by up to 2.3 pH units[43] The pH of mixed adult multi-chamber PN infusates without additions (which is largely determined by the amino acid solutions that for adults can vary from pH 5.4 to 6.4 and within batches may vary by up to 0.8 pH units) can vary from pH 4.8 to 6.0 and within batches may vary by up to 1.2 pH units (personal communications from the UK Medical Information departments of B. Braun (on 10 October 2013) and Fresenius Kabi (on 18 October 2013)).

One of the major findings of this study concerns the effect of increasing osmolarity, which was found to suppress the $\log_{10}$cfu/mL of both *E. coli* and *E. durans* at 48 hours. In our previous studies with *S. epidermidis*[43] and *C. albicans*[44] it was not possible to assess whether changes in cfu/mL or $\log_{10}$cfu/mL respectively induced by altering the glucose concentration were due to the associated changes in osmolarity or to some other property of glucose because the only source of variation in osmolarity in the lipid and lipid-free PN infusates was entirely due to glucose (all other components were kept constant). For example, any antimicrobial effect of honey is not thought to be solely due to its high sugar content, which is largely responsible for the high osmolarity of honey[144]. In the present study an increase in osmolarity due to sodium chloride, which was varied independently of the glucose concentration, was found to suppress the growth of both *E. coli* and *E. durans*. This suppressive effect was also found to be more with sodium chloride than glucose, suggesting that properties of nutrients other than those due to osmolarity can influence growth of microorganisms. The present work with osmolarity, which appears to be the first of its kind in PN infusates, suggests the need to undertake

more detailed investigations in this area and to assess their clinical relevance. For example, an increase in volume (decrease in osmolarity) of PN could increase the growth of organisms such as *E. coli* and *E. durans*, whereas a decrease in volume (increase in osmolarity) could do the opposite. The addition or exclusion of nutrients that can substantially alter the osmolarity of PN infusates (for example the addition of large quantities of sodium chloride for patients with copious intestinal fluid effluents, or the removal of sodium chloride for salt overloaded patients) may influence microbial growth. The mechanisms by which osmolarity affects the growth of microorganisms and the mechanisms by which organisms adapt to hyperosmolarity are not entirely clear, although it is well recognised that the ability of microorganisms to withstand environments of increasing osmolarity varies considerably according to the species[145]. In the case of *E. coli* it is known that adaptive responses depend on the presence of potassium and glutamate[146], but it is unclear to what extent variations in the concentration of these nutrients in different PN formulations affect the growth of *E. coli*. It is also known that in a high osmolarity environments some compounds such as glycine betaine[147, 148] are osmoprotective to *E. coli*, allowing the organisms to withstand up to an approximate two-fold further increase in osmolarity[147].

This study has at least two limitations. First, each PN bag was inadvertently inoculated with both *E. coli* and *E. durans* (rather than *E. coli* alone) so there is a possibility of interactions between the two microbes. Although the growth patterns of *E. coli* in the PN infusates in our study were broadly consistent with those found in other studies (see below), this remains a possibility. *E. durans*, which is potentially pathogenic to humans[149, 150], was an unexpected contaminant of the reference *E. coli* used to inoculate the bags containing PN infusates. On the other hand, this contamination can be regarded as a fortunate since it allowed this study to illustrate that two different microorganisms can behave qualitatively and quantitatively differently in the same PN-related infusates at the same time under exactly the same conditions. Also, although the methodology ensured that the PN infusates had a fixed concentration of amino acids, electrolytes (except sodium chloride in the osmolarity comparisons), trace elements and vitamins it did not allow examination of the effects of these other nutrients on the growth of *E. coli* and *E. durans*.

There do not appear to be any previously published studies on the growth of *E. durans* in relevant infusates. Of the two most important enterococci in clinical practice, *E.* (*S.*) *faecalis* and *E. faecium*, neither seem to have been tested for growth in PN infusates and only *E. faecalis* in lipid emulsion alone[40, 65]. *E. faecalis* grew strongly in lipid emulsion alone by orders of magnitude, which is consistent with our findings with *E. durans*. However, there are a number of published studies on the growth of *E. coli* over 48 hours in relevant infusates. These include the following range of infusates: Lipid-free PN[69, 151-153]; lipid PN and lipid-free PN[57-59, 75]; and lipid emulsion alone[40, 65, 71, 154]. Several of the PN studies also involved the examination of glucose alone[57, 153] or lipid emulsion alone[57, 151], and one of the studies of lipid emulsion alone also involved the examination of glucose alone[65].

The results of these studies are generally consistent with the findings of the present study. *E. coli* was found to be suppressed by increasing concentrations of glucose alone[57, 65, 153] but to proliferate by orders of magnitude in lipid alone[40, 57, 65, 71, 151] (without a noticeable effect of the type of lipid[71, 154]). Growth in lipid PN was found to be greater than in lipid-free PN[57, 58, 75]. There was also greater growth in lipid emulsion alone compared to lipid PN[57] or lipid-free PN[57, 151]. A study also suggested possible differences in growth depending on the crystalline amino acid solution used, albeit at different concentrations[152]. The independent effects of energy density, osmolarity and glucose concentration on the growth of *E. coli* are unclear mainly because the studies were not designed to examine the independent effects of these variables. However, in PN with[57, 58] or without[57, 58, 153] lipid emulsion growth of *E. coli* was enhanced at lower glucose concentrations, typically associated with a lower osmolarity and energy density of the PN infusates. Finally, one study found no growth of *E. coli* in any of the PN formulations tested[59] and the reasons for this is unclear, although it may have been due to the use of a different strain of *E. coli* or different methodological procedures including use of different ambient conditions. In many studies it is difficult to be certain about the changes due to the presence of lipid or altered glucose concentrations, either because both changed simultaneously or because the formulations included other changes, such as alterations in the concentration of other nutrients, such as amino acids or the presence or absence of vitamins. In addition, in most studies with *E. coli* the ambient conditions did not reflect those used in clinical practice. For example, the studies were undertaken at different ambient temperatures (22°C[152], 24°C[69], 25°C[58, 59, 75], 37°C[153], both 25°C

and 37°C[65], or at an unspecified temperature[40, 57, 71, 151, 154]), utilised different containers to study microbial growth in PN infusates (bags[58] (without specifying if they were oxygen barrier bags), bottles[57, 71], plastic tubes or containers[69, 152, 154], or in unspecified containers[40, 59, 65, 75, 151, 153]), and four[57, 69, 152, 153] of the eight studies[57-59, 69, 75, 151-153] involving PN infusates did not utilise PN containing electrolytes, vitamins and trace elements. Furthermore, initial inoculations in the studies aimed to yield up to 100 cfu/mL[57-59, 71, 75], 1000 cfu/mL[152], 10 or 1000 cfu/mL[69], 2500 cfu/mL[154], 10,000 to 20,000 cfu/mL[40, 151], and 100,000 cfu/mL[65, 153], which are not all representative of the level of contamination that might be expected in clinical practice. None of the studies set out to examine whether changing osmolarity due to different components had an effect on growth independent of the presence of lipid or glucose.

In summary, growth of *E. coli* and *E. durans* in PN infusates was found to be greater in PN with than without a lipid component, although other variables such as glucose concentration and osmolarity were also identified as independent variables. There is a need to review guidelines about the types of PN infusate, including lipid emulsion, which may affect potential microbial contaminant growth in different PN formulations. This activity should take into account the different types of potential microorganisms, the frequency with which they are likely to cause PN related infections, the clinical risk they may present to a patient (including those with an altered immune function), and factors that affect the growth of each of these microorganisms, including osmolarity, and study conditions. A critical systematic review would help to inform practice.

3.2 SYSTEMATIC REVIEW: MICROBIAL GROWTH IN PARENTERAL NUTRITION INFUSATES

3.2.1 METHODOLOGY

The detailed methods are described in Chapter Two.

The literature search was undertaken on 22 February 2014 using all available years in three databases: Medline (OvidSP) from 1946; Embase (OvidSP) from 1947; and the

complete Cochrane Library (John Wiley & Sons, Ltd). The search terms and numbers of results are indicated in Appendix A.

The literature search identified 14700 records (7546 from Medline, 6145 from Embase and 1009 from the Cochrane Library) and 10623 after duplicates had been removed. The title and abstract of each of the 10623 identified records was evaluated and excluded if it did not meet the inclusion criteria. This left 69 records, which were individually subjected to a full text review to confirm relevance and compliance with the above criteria to yield a final total of 24 studies from 33 records[42-45, 56-75].

The infusates tested in the included studies were divided into lipid emulsion, lipid PN and lipid-free PN.

3.2.2 RESULTS

A total of 24 records[42-45, 56-75] with relevant data were identified. Of these 24 studies, 12 examined growth in lipid emulsion[42-45, 57, 62-65, 68, 70, 71], 14 in lipid PN[43-45, 56-59, 61, 67, 68, 70, 72, 74, 75], 15 in lipid-free PN[42-45, 57-60, 66, 68, 69, 72, 73, 75] and 10 in both types of PN[43-45, 57-59, 61, 68, 72, 75]. Growth in the 19 PN studies[42-45, 56-61, 66-70, 72-75] was assessed in the presence (n = 10 studies[42-45, 56, 58, 70, 73-75]), absence (n = 4 studies[57, 67-69]) and both the presence and absence (n = 5 studies[59-61, 66, 72]) of vitamins. The corresponding figures for trace elements were 12 studies[42-45, 56, 58, 59, 61, 67, 72, 74, 75], 6 studies[57, 60, 66, 68, 69, 73] and 1 study[70]. Some PN components used in some of the studies are currently unavailable in at least some countries.

The guideline groups recommending restriction of the duration of infusion of lipid-containing PN compared with lipid-free PN cited 9 records[40-42, 57, 59, 62, 68, 131, 155], of which only three[57, 59, 68] examined microbial growth in both PN with and without lipid. Furthermore, four of the records[40, 41, 131, 155] cited by the guideline groups did not meet the inclusion criteria of this review for a variety of reasons: lipid emulsion, lipid PN or lipid-free PN were not examined[131]; concern has been raised[156] over the method of expressing microbial growth[40]; no sampling was undertaken beyond 24 hours[41], which meant that the inclusion criterion for data at 48 hours could not be met; and the concentration of nutrients such as amino acids and lipid emulsion in the PN infusate had not been defined[155]. Some guideline groups cited even fewer

records: the CDC HICPAC group[6] cited 6 records[40-42, 57, 68, 131]; the Cochrane Collaboration[157] cited 4 studies[59, 62, 68, 155]; the epic project[158] cited no original studies but instead cited the CDC HICPAC group[6] and the Cochrane Collaboration[159] (from 2005[159] not 2013[157]); and the ESPEN group[32] came to a conclusion that was based on expert opinion rather than data in original records. The detailed summaries of the composition of individual test infusates (Appendix D) and the methodological procedures (Appendix E) are summarised below.

3.2.2.1 Composition of infusates

It is clear that the composition of the infusates varied widely within and between studies (Appendix D), and in only a minority of studies was the composition of the feeds containing lipid matched with those without lipid (see below). Confounding variables included: the use of 10% w/v or 20% w/v lipid (n = 4[42, 57, 58, 65] and n = 13[43-45, 56, 59, 61-63, 67, 70, 72, 74, 75] studies respectively) or both 10% w/v and 20% w/v lipid (n = 3 studies[64, 68, 71]) or no lipid (n = 4 studies[60, 66, 69, 73]); energy density of the infusates, which varied from 32 – 200 kcal/100mL; pH of the infusates, which varied from 7.30 – 8.47 for lipid emulsion and from 5.10 – 6.62 for PN; osmolarity of the PN infusates, which varied from 644 – 1986 mOsm/L; and final nutrient concentrations in the infusates, including those for amino acids (1.5 – 6.75% w/v), glucose (3.3 – 25.0% w/v), lipid (0 – 5% w/v) and percent non-nitrogen energy as lipid (0.0 – 71.0%). In some cases no information was available about characteristics of the infusate or its composition. For example, pH was not reported for any test infusate in n = 6 studies[56, 62, 64-66, 71] (25%). The within study differences included the use of different amino acid solutions of different composition[66, 67] and different concentrations of lipid (see above). The amino acid solutions used in the PN infusates were intended for administration to adult (not paediatric) patients.

3.2.2.2 Study conditions

As well as the composition of infusates, the study conditions and methodology also varied widely (Appendix E). For example, the ambient study temperature ranged from 8 – 37ºC, and various types of containers were used ranging from ‘tubes’[62] or ‘bottles’[71, 74] to sterile oxygen barrier PN bags[43-45]. In some cases the infusate storage temperature was unspecified[42, 57, 71] or nominal[60], and details of the container were

frequently not provided[42, 59, 60, 63-65, 67, 75]. Only a single sample was taken from test infusates in 8 studies[60-62, 65, 72-75], and no details of the number of samples taken were provided in 2 other studies[42, 70].

3.2.2.3 Species of microbe

The GR of 24 types of microbe were examined, which included a mixture of reference and clinical strains of 13 types of Gram-negative bacteria, 9 types of Gram-positive bacteria and 2 types of fungi (Appendix E and the individual microbes listed in the footnote to Table 3.16). Appendix E shows that the initial microbial inoculation into the infusates varied by up to 8 orders of magnitude, to yield approximately 0.01 – 1000000 cfu/mL of infusate at baseline.

Table 3.16 Unmatched and matched data analyses of microbial 'growth' in lipid emulsion and parenteral nutrition with and without lipid

		Unmatched data analysis[a]				**Matched data analysis[b]**
		Lipid emulsion v lipid PN v lipid-free PN	**Lipid emulsion v lipid PN**	**Lipid emulsion v lipid-free PN**	**Lipid PN v lipid-free PN**	**Lipid PN v lipid-free PN**
Descriptive statistics						
Records	n	24	24	24	24	7
Types of microbe	n	24[c]	23[d]	18[e]	21[f]	9[g]
Lipid emulsion	n	45	45	45		
	'Growth ratio' mean ± SD	2.636 ± 2.399	2.636 ± 2.399	2.636 ± 2.399		
Lipid PN	n	155	155		155	38
	'Growth ratio' mean ± SD	2.505 ± 2.939	2.505 ± 2.939		2.505 ± 2.939	1.231 ± 1.595
Lipid-free PN	n	151		151	151	38
	'Growth ratio' mean ± SD	1.552 ± 2.857		1.552 ± 2.857	1.552 ± 2.857	0.554 ± 1.576
Inferential statistics[h]						
Simple comparison[i]						
Type of infusate	*P*	0.006	0.785	0.022	0.004	0.067
	partial η^2	0.029	0.000	0.027	0.027	0.045
With adjustment for overall glucose concentration[j]						
Type of infusate (fixed factor)	*P*	0.008	0.001	0.625	0.037	0.069
	partial η^2	0.027	0.051	0.001	0.014	0.045
Glucose concentration (% w/v)	B coefficient[k]	- 0.132	- 0.195	- 0.098	- 0.132	0.000
	P	< 0.001	< 0.001	0.002	< 0.001	0.999
	partial η^2	0.074	0.100	0.051	0.081	0.000[l]

With adjustment for species of microbe[m]						
Type of infusate (fixed factor)	*P*	< 0.001	< 0.001	< 0.001	0.001	0.083
	partial η^2	0.086	0.079	0.077	0.043	0.051
Species of microbe[n]	*P*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	partial η^2	0.320	0.440	0.256	0.378	0.732
Infusate-microbe interaction	*P*	0.045	0.008	0.047	0.525	0.428
	partial η^2	0.096	0.123	0.079	0.036	0.124
With adjustment for overall glucose concentration and species of microbe[o]						
Type of infusate (fixed factor)	*P*	0.011	0.434	0.133	0.002	0.076
	partial η^2	0.029	0.004	0.013	0.033	0.054
Glucose concentration (% w/v)	B coefficient[k]	- 0.096	- 0.125	- 0.079	- 0.096	-0.037
	P	< 0.001	< 0.001	0.008	< 0.001	0.025
	partial η^2	0.055	0.073	0.041	0.058	0.085[l]
Species of microbe[n]	*P*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	partial η^2	0.306	0.431	0.250	0.353	0.755
Infusate-microbe interaction	*P*	0.020	0.007	0.038	0.326	0.382
	partial η^2	0.106	0.124	0.083	0.044	0.133

[a] Includes all the available data, except *E. durans* in lipid emulsion since it was completely eliminated over a 48-hour period (due to the mathematical difficulties of taking $\log_{10}$ of zero $cfu.mL^{-1}$ at 48 hours).

[b] A subset of all the available data, representing those data available for the same microbe in both lipid PN and lipid-free PN of the same composition in the same study, although in some cases the inclusion of lipid increased the energy density.

[c] *S. epidermidis*, *C. albicans*, *S. aureus*, *E. coli*, *P. aeruginosa*, *E. cloacae*, *K. pneumoniae*, *S. marcescens*, *E. aerogenes*, *S. faecalis*, *B. cereus*, *S. pyogenes*, *K. oxytoca*, *S. maltophilia*, *B. cepacia*, Flavobacterium, *A. calcoaceticus*, *S. saprophyticus*, *K. aerogenes*, *B. subtilis*, *E. durans*, *P. fluorescens*, Coryneform and *M. furfur*.

[d] *S. epidermidis*, *C. albicans*, *S. aureus*, *E. coli*, *P. aeruginosa*, *E. cloacae*, *K. pneumoniae*, *S. marcescens*, *E. aerogenes*, *S. faecalis*, *B. cereus*, *S. pyogenes*, *K. oxytoca*, *S. maltophilia*, *B. cepacia*, Flavobacterium, *A. calcoaceticus*, *S. saprophyticus*, *K. aerogenes*, *E. durans*, *P. fluorescens*, Coryneform and *M. furfur*.

[e] *S. epidermidis, C. albicans, S. aureus, E. coli, P. aeruginosa, E. cloacae, K. pneumoniae, S. marcescens, E. aerogenes, S. faecalis, B. cereus, S. pyogenes, B. cepacia, K. aerogenes, B. subtilis, E. durans, P. fluorescens* and *M. furfur*.
[f] *S. epidermidis, C. albicans, S. aureus, E. coli, P. aeruginosa, E. cloacae, K. pneumoniae, S. marcescens, E. aerogenes, B. cereus, K. oxytoca, S. maltophilia, B. cepacia,* Flavobacterium, *A. calcoaceticus, S. saprophyticus, K. aerogenes, B. subtilis, E. durans, P. fluorescens* and Coryneform.
[g] *S. epidermidis, C. albicans, S. aureus, E. coli, E. cloacae, K. pneumoniae, S. marcescens, K. aerogenes* and *E. durans*.
[h] The growth ratio was the dependent variable in the inferential statistics.
[i] ANOVA for comparisons between all three types of infusate and an independent samples t-test for comparisons between types of infusate pairs.
[j] General linear model with log ratio as the dependent variable, type of infusate as fixed factor and glucose concentration as covariate.
[k] The B coefficient indicates the absolute change in the log ratio from baseline for each increase of 1% w/v of glucose concentration.
[l] The matched analysis compared PN with and without lipid each at the same glucose concentration, which explains the partial eta squared of zero without species of microbe in the model, and in the model that includes species of microbe and infusate-microbe interaction the partial eta squared of 0.085 takes into account differences in glucose concentration between types of microbe.
[m] General linear model with log ratio as the dependent variable, type of infusate and species of microbe as fixed factors and an interaction term between type of infusate and species of microbe.
[n] Different strains grouped together.
[o] General linear model with log ratio as the dependent variable, type of infusate and species of microbe as fixed factors, glucose concentration as covariate and an interaction term between type of infusate and species of microbe.

3.2.2.4 Outcome: microbial 'growth ratio' (GR; $\log_{10}$ [cfu.mL^{-1} at 48 hours / cfu.mL^{-1} at time zero])

In both the unmatched and matched analyses that follow, GR is used to indicate microbial 'growth' or decline over a 48-hour period.

3.2.2.4.1 Unmatched data analysis

For all the data combined (n = 351 data points) the mean GR of 2.112 was associated with wide variability (SD = 2.874) which persisted when the results were restricted to lipid emulsion alone (GR of 2.636 ± 2.399; n = 45), lipid-containing PN (2.505 ± 2.939; n = 155) and lipid-free PN (GR of 1.552 ± 2.857; n = 151). Figures 3.11 and 3.12 show the striking variability in 'growth', with some studies showing a suppression in cfu (GR < 0), others little or no growth (GR ~0) and yet others 'growth' that varied by more than eight orders of magnitude (GR >8). Figures 3.11 and 3.12 and Table 3.16 allows an examination of the effect of glucose concentration

of PN infusates, type of infusate, species of microbe and the inclusion or addition of lipid emulsion.

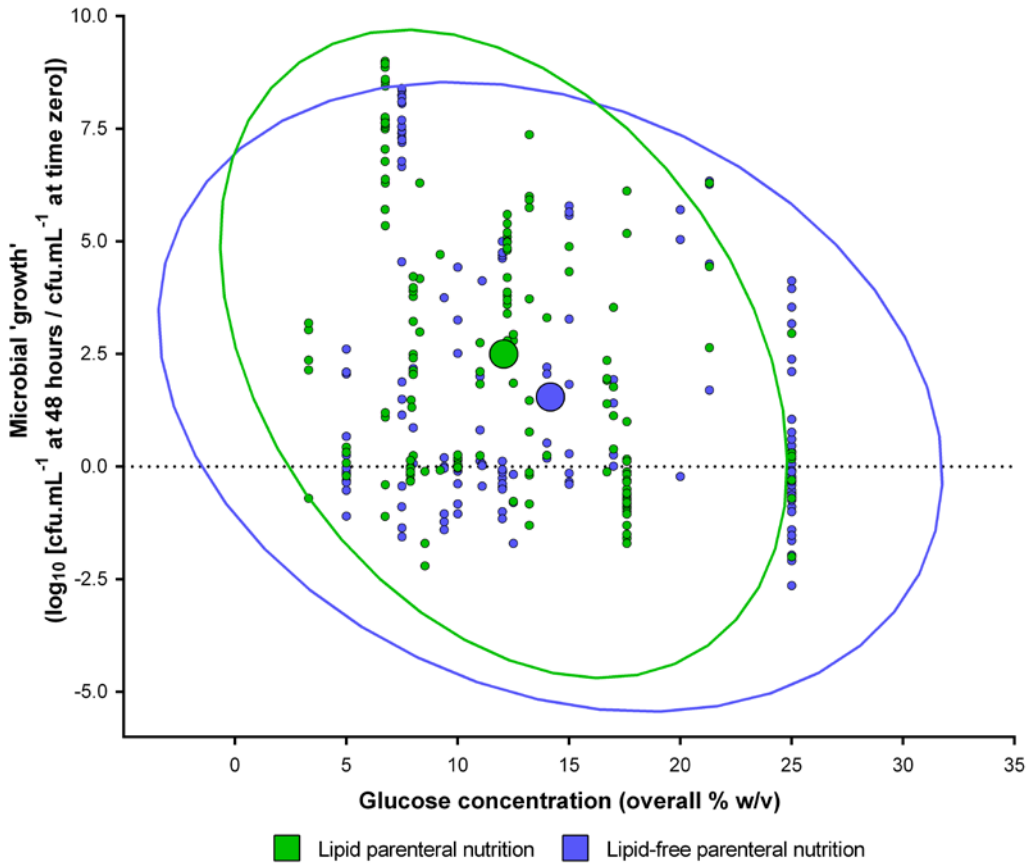

Figure 3.11 The effect of glucose concentration on microbial 'growth' in parenteral nutrition with and without lipid over a 48-hour period (unmatched data analysis)

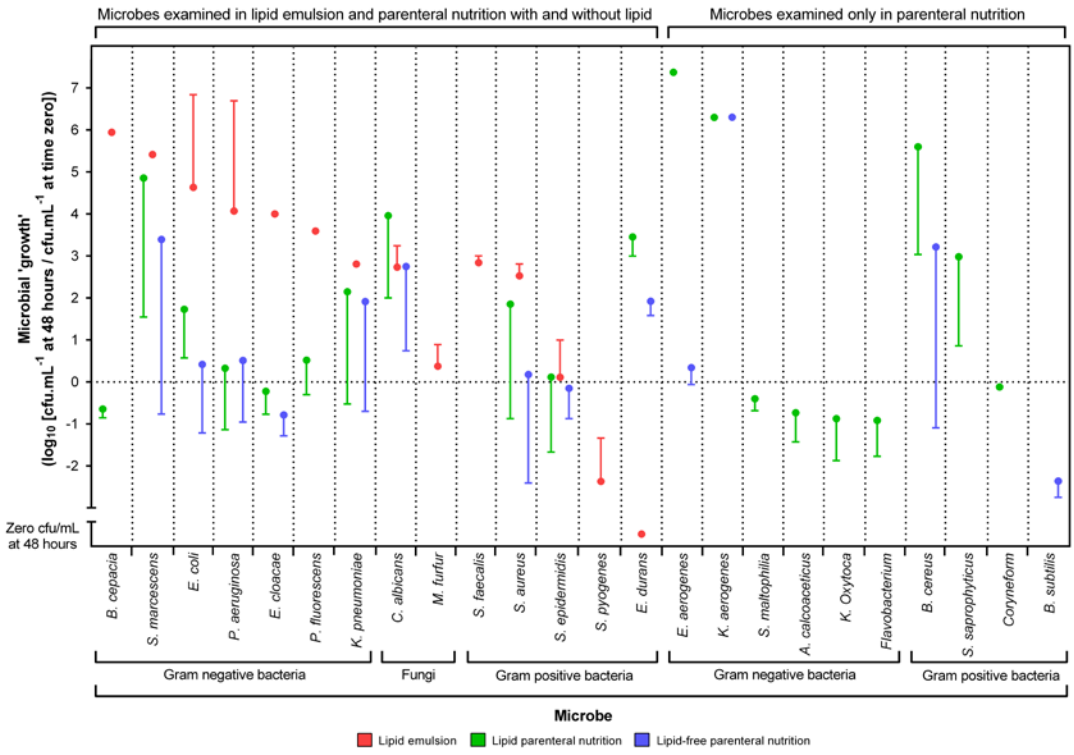

Figure 3.12 'Growth' of different species of microbe in lipid emulsion and in parenteral nutrition with and without lipid over a 48 hour period (unmatched data analysis)

Glucose concentration of PN infusates

Despite the enormous variability in growth between studies (Figure 3.11) the pooled results showed an inverse relationship between microbial growth in PN and the glucose concentration of PN (lipid-containing and lipid-free PN combined) (r = 0.305, $P < 0.001$). The relationship between glucose concentration and microbial growth did not differ significantly ($P = 0.356$) between lipid-containing PN (r = 0.344, $P < 0.001$) and lipid-free PN (r = 0.248, $P = 0.002$). Figure 3.11 not only shows that the values for the grand means for microbial growth in PN with and without lipid are close to each other, but also the visually striking overlap of the 95% confidence ellipses defining the variability of individual points. From Table 3.16, an increase in overall glucose concentration (as a covariate) was found to result in a significant microbial decline. This effect of increasing glucose concentration was not only observed with unmatched analyses involving combinations of lipid alone and PN with and without lipid, but also with matched or pair-wise analyses. However, in the unmatched analyses the glucose concentration was found to account for only 4.1

– 10.0% of the variability of the GR in the general linear models used (partial η^2 = 0.041 – 0.010).

Type of infusate

From Table 3.16, for all three types of infusate combined microbial growth was significantly affected by type of infusate (P = 0.006, with a tendency for the GR in lipid alone to be greater than in lipid PN, the latter to be greater than lipid-free PN), even after adjustment for glucose concentration (P = 0.008), species of microbe (P < 0.001) or both of these variables (P = 0.011). Eight of the twelve pair-wise comparisons were significant, but the type of infusate accounted for only 0.1 – 8.6% of the variability in GR (partial η^2 = 0.001 – 0.086).

Species of microbe

The four unmatched data analyses (shown in four separate columns in Table 3.16; lipid emulsion v lipid PN v lipid-free PN, lipid emulsion v lipid PN, lipid emulsion v lipid-free PN, and lipid PN v lipid-free PN) showed the species of microbe accounted for as much as 25.0 – 44.0% of the variability of the GR (partial η^2 = 0.250 – 0.440), which was statistically significant both before and after adjustment for glucose concentration (P < 0.001). The same analyses also showed a significant species of microbe-infusate interaction which accounted for a further 3.6 – 12.4% of the variability (partial η^2 = 0.036 – 0.124; significant for three of the four comparisons). Figure 3.12 shows that the raw data of GR varied substantially between microbes, when all three types of infusates were analysed either together or individually (P < 0.001 in each case). When the same analyses were repeated using microbe category (Gram-negative bacteria, Gram-positive bacteria and fungi) instead of species of microbe, microbe category and microbe category-infusate interaction were found to explain much less of the variability (3.5 – 9.1% and 0.9 – 10.5% respectively).

Inclusion or addition of lipid emulsion to parenteral nutrition

Figure 3.12 also reveals an overall GR for various types of microbe to be comparable in lipid PN and lipid-free PN, and higher in lipid emulsion alone. In comparisons involving all three types of infusate the mean GR was highest in lipid emulsion for 6

of the 9 microbes tested. The mean GR was also highest in lipid emulsion for 9 of the 11 microbes tested in lipid emulsion alone compared to lipid PN (significant for 5 microbes, or 3 after adjustment for glucose concentration) and for 8 of the 9 microbes tested in lipid emulsion alone and lipid-free PN (significant for 4 microbes, or 3 after adjustment for glucose concentration).

3.2.2.4.2 Matched data analysis

A total of 7 records[43-45, 57, 59, 68, 75] with relevant data on 9 types of microbe (listed in footnote of Table 3.16) were identified. Matched data analysis made use of data from the same study to examine the growth of the same microbe in lipid PN and lipid-free PN of the same composition. However, in some cases the inclusion of lipid increased the overall energy density of the PN admixture (Appendix D). One study[57] involved PN without micronutrients and another study[59] estimated rather than measured baseline cfu/mL.

For the lipid PN and lipid-free PN combined (n = 76) the mean GR of 0.893 was associated with wide variability (SD = 1.611), which was also observed when the results were examined in lipid PN alone (1.231 ± 1.595; n = 38), or lipid-free PN alone (0.554 ± 1.576; n = 38). Meta-analyses were restricted to data from only 5 records[43-45, 57, 75]. Two other records[59, 68] contained insufficient data for inclusion in the meta-analysis (n = 1 for each of type of infusate for each species of microbe tested).

<u>Glucose concentration of PN infusates</u>

In bivariate analysis, glucose concentration was found to be very weakly related to GR in lipid PN ($r^2 = 9 \times 10^{-6}$, $P = 0.984$) and lipid-free PN ($r^2 = 9 \times 10^{-6}$, $P = 0.987$). A general linear model that adjusted for the type of infusate also demonstrated a weak non-significant effect of glucose concentration (partial $\eta^2 < 0.0005$; Table 3.16). After further adjustment for the species of microbe and infusate-species of microbe interaction, an increasing glucose concentration produced a significant decline in GR (partial $\eta^2 = 0.085$; $P = 0.025$; Table 3.16).

Type of infusate

From Table 3.16, the type of infusate accounted for only 4.5 – 5.4% of the variability of the GR in the general linear models used (partial η^2 = 0.045 – 0.054). Furthermore, microbial growth was not significantly affected by type of infusate (P = 0.067), even after adjustment for glucose concentration (P = 0.069), species of microbe (P = 0.083) or both variables (P = 0.076).

Species of microbe

From Table 3.16, the species of microbe accounted for a substantial amount of the variability in GR (73.2 and 75.5% using the two general linear models that included species of microbe as a variable) (partial η^2 = 0.732 – 0.755). This was much higher than the variability accounted for by either glucose concentration of PN infusates (0.0 – 8.5%; partial η^2 = 0.000 – 0.085) or type of infusate (4.5 – 5.4%; partial η^2 = 0.045 – 0.054). In addition, microbial growth was significantly affected by the species of microbe inoculated into PN, irrespective of whether or not it was adjusted for glucose concentration (P < 0.001 in each case). When the data were split by species of microbe (Figure 3.13) the variability of growth between different types of microbe remained considerable, ranging from suppression (GR <1) to rapid growth (GR >6). The GR was found to vary significantly within each type of infusate or for both combined (P < 0.001 in each case).

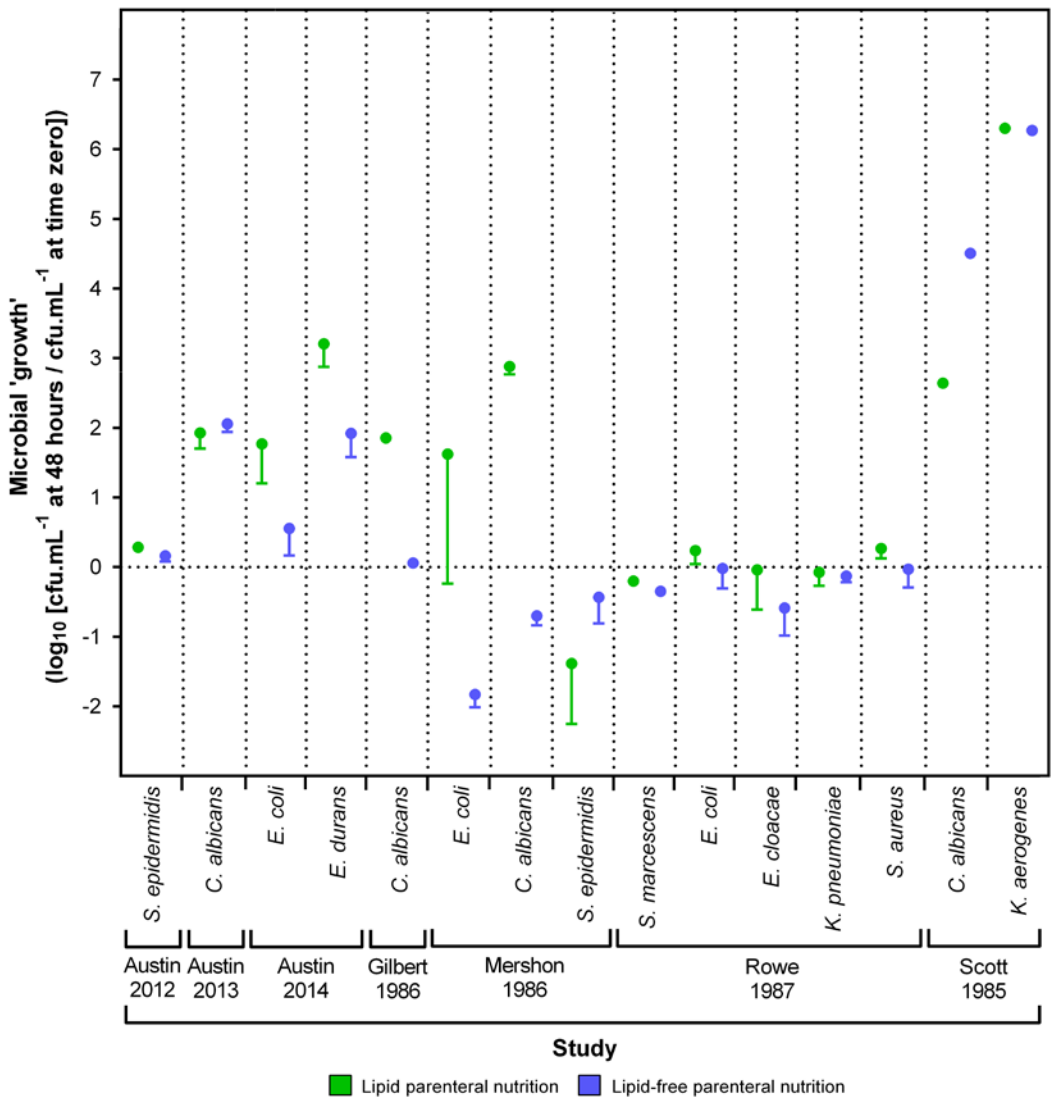

Figure 3.13 'Growth' of different species of microbe in lipid emulsion and in parenteral nutrition with and without lipid over a 48-hour period (matched data analysis)

Inclusion or addition of lipid emulsion to parenteral nutrition

Data for matched lipid PN and lipid-free PN were available from 5 records[43-45, 57, 75] for 7 types of microbe.

One study[68], which could not be included in the meta-analyses (because n = 1 for one species of microbe in each type of infusate; described above) reported the growth of the same species of microbe (*C. albicans*) in isoenergetically matched lipid PN and lipid-free PN. The GR was found to be greater in the lipid PN than lipid-free PN

(71.8 v 1.2 respectively). The methodology employed in the study was judged to be of poor quality (Appendix E).

The remaining studies were incorporated into three types of meta-analyses, with subgroup analyses by species of microbe. One of the meta-analyses examined the isoenergetic exchange of glucose for lipid (while maintaining the same energy density), another the addition of lipid to lipid-free PN (increasing the energy density of the infusate, while reducing the concentration of glucose and other nutrients), and the third the percent non-protein energy as lipid (using infusates of different energy density and maintaining the concentration of glucose). The types of microbe included in the meta-analyses were *S. epidermidis*, *C. albicans* and *E. coli*. The remaining types of microbe were not amenable to meta-analyses, either because information for each one of them was available from only one study (*S. aureus*[75], *E. cloacae*[75], *K. pneumoniae*[75], K. aerogenes[59] and *E. durans*[45]) and because sample size was only one for each type of infusate (*S. marcescens*[75]).

In each of the meta-analyses grouped by species of microbe (Figures 3.14 to 3.16) the visual variability of the data shown on the forest plots are confirmed by statistical tests of heterogeneity, which are shown in the figures. The first two meta-analyses were based on a glucose concentration of 13.33% w/v, which is equivalent to 1Mcal of glucose (1 gram glucose = 3.75kcal). The first meta-analysis (Figure 3.14) found the isocaloric (1Mcal) exchange of glucose for lipid at a fixed glucose concentration about13.33% w/v in PN led to an overall non-significant decrease in GR (difference in mean GR of -0.089 (95% CI -0.184, 0.006; $P = 0.067$)). The second meta-analysis (Figure 3.15) found that the addition of 1Mcal of lipid to lipid-free PN (to yield lipid PN with a greater energy density than the lipid-free PN) at a glucose concentration of 13.33% w/v led to an overall non-significant increase in the GR (difference in mean GR of 0.474 (95% CI -0.211, 1.160; $P = 0.175$)). The third meta-analysis (Figure 3.16) found an increase in the percent non-protein energy as lipid in PN led to an overall non-significant decrease in the GR (mean GR -0.001 (95% CI -0.005, 0.003; $P = 0.660$)).

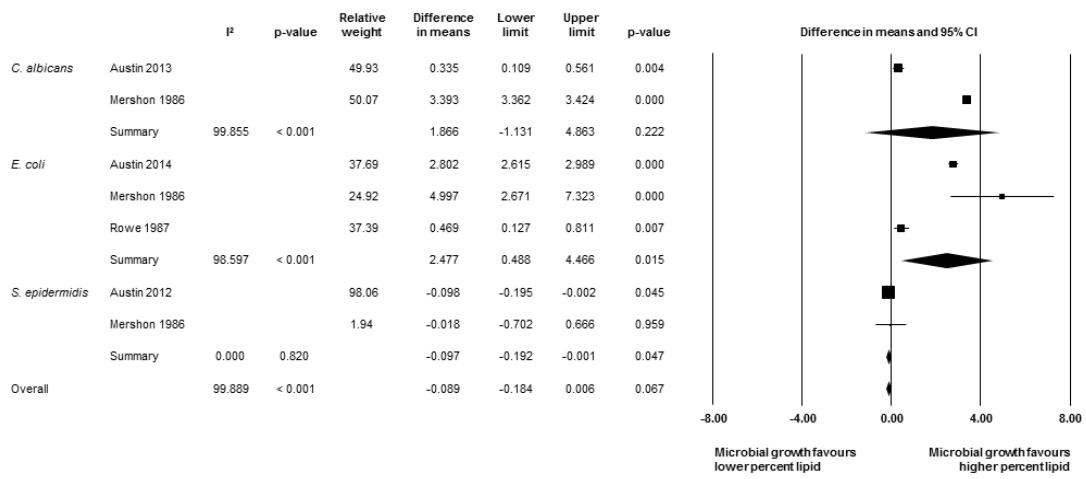

Figure 3.14 Meta-analysis of the effect of isoenergetic (1Mcal) exchange of glucose for lipid about a glucose concentration of 13.33% w/v in parenteral nutrition (matched data analysis). The p-value in the second column refers or the heterogeneity statistic I^2, and the p-value in the final column refers to the summary statistic of effect size

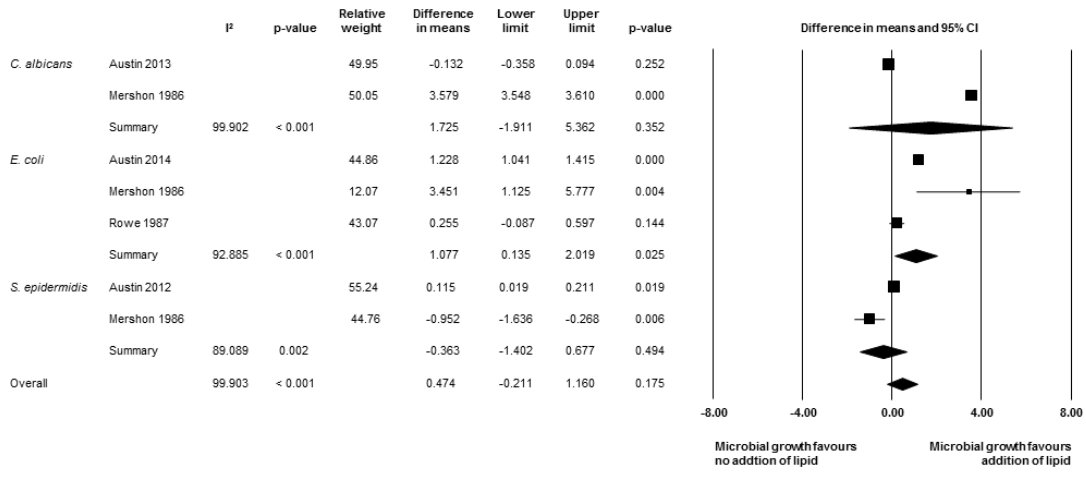

Figure 3.15 Meta-analysis of the effect of a 1Mcal addition of lipid to lipid-free PN to yield lipid PN (with a greater energy density) at a glucose concentration of 13.33% w/v in parenteral nutrition (matched data analysis). The p-value in the second column refers or the heterogeneity statistic I^2, and the p value in the final column refers to the summary statistic of effect size

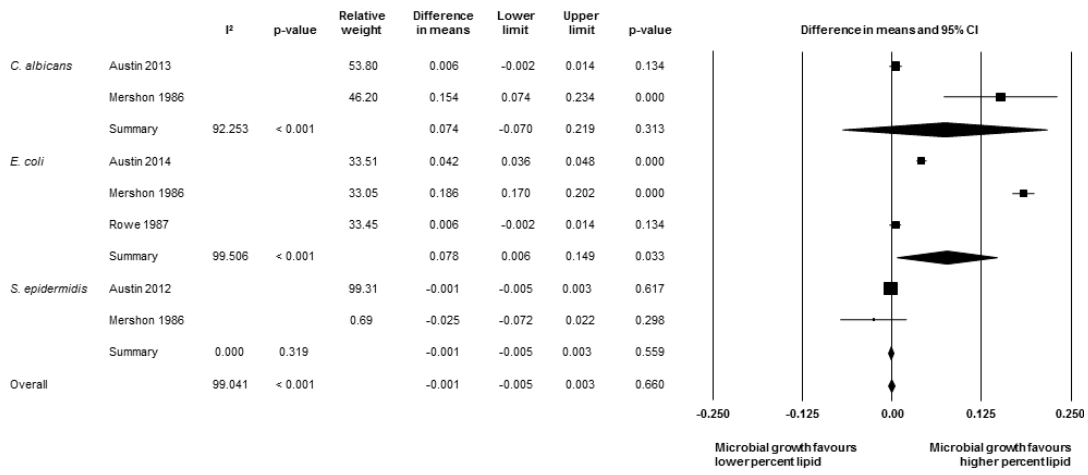

Figure 3.16 Meta-analysis of the effect of an increase in the percent non-protein energy as lipid in PN in parenteral nutrition (matched data analysis). The p-value in the second column refers or the heterogeneity statistic I2, and the p-value in the final column refers to the summary

3.2.2.4.3 Other confounding variables

Limited data within the studies examined indicate potential effects of a number of further confounding variables on microbial growth in PN infusates.

Electrolytes

None of the studies set out to examine the specific effects of the presence or quantity of different electrolytes or electrolyte salts in PN on microbial growth, apart from two. The first [45] found significant suppression of *E. coli* and *E. durans* as the concentration of sodium chloride increased between 30 and 198mmol/L in lipid PN, and the second[60] found little effect on a variety of microbes when both electrolytes and vitamins were incorporated into lipid-free PN.

Vitamins

Five studies examining the effect of vitamins on growth of microbes in PN infusates were reviewed.[59-61, 66, 72] Four of these[59, 61, 66, 72] were undertaken under the same ambient conditions using PN infusates of essentially identical composition except for the presence of vitamins which produced a very small increase in osmolarity,

(Appendices C and D). The other study examined growth in the presence and absence of both vitamins and electrolytes simultaneously[60]. Single samples were used in these studies except one that used 1 sample from three replicates[59] and another that used 3 samples from 2 replicates[66]. The results, which involved a wide range of organisms, were varied with some showing little or no difference in growth when vitamins were added to the infusates[59-61, 66, 72] and others showing apparent enhancement of growth[61]. One study showed a striking enhancement of growth of certain microbes[72] but this was not the case for other microbes tested in the same study, or the same microbe in other studies[61, 72] One of these studies showed a striking increase for two strains of *S. aureus* and not a third strain[61] in both lipid PN and lipid-free PN. The results were not amenable to meta-analysis.

Trace elements

Under essentially the same study conditions (Appendices C and D) one study[70] found that addition of trace elements to lipid-containing PN strikingly suppressed the growth of *P. fluorescens*. The effect was not observed in lipid emulsion, unless pH was adjusted downwards so that it was close to that of the PN infusate. The study was undertaken at 8ºC and the number of samples per test was not stated.

pH

Six studies examining the effect of pH on microbial growth were reviewed[43-45, 70, 72, 73]. Four of these[43-45, 70] examined whether differences in growth between lipid alone and lipid PN might be reproduced by a manipulating the pH of each type of infusate. In lipid alone and without manipulation of pH the cfu/mL of *S. epidermidis* decreased while in lipid-containing PN it increased[43]. Reducing the pH of the lipid alone from pH ~8.5 to pH ~6.2 (to reflect the pH of the lipid-containing PN) increased the cfu/mL but not to the same extent as in PN with no pH adjustment. In contrast, the growth of *C. albicans*[44] and *E. coli*[45] in lipid alone increased further when the pH was adjusted down, and in the case of *C. albicans* to a greater extent than in lipid PN. *E. durans* was eliminated in lipid alone at both higher and lower pH[45]. When the pH of PN with and without lipid was increased to be comparable to the pH of the lipid emulsion, the growth of *S. epidermidis*[43] and *E. durans*[45] was suppressed, that of *C. albicans*[44] showed little or no change, while that of *E. coli*[45]

was enhanced. Another study found that a reduction in pH from ~8.0 to approximately that found in PN (pH 4.7 or 4.3) suppressed the growth of *P. fluorescens*[70]. Two other studies[72, 73] examined the effect of increasing the pH of PN infusates. In a lipid-free PN infusate with a pH of 5.5 the growth of several microbes (*S. aureus, S. Marcescens* and *B. cereus*) was either unchanged or reduced over time, while in a lipid PN infusate with different amino acid composition and a pH of 6.0 there was in increase in growth of the same microbes over time[72]. When the pH of the first infusate (with and without a lipid component) was increased, microbial growth generally increased, and when the pH of the second infusate was reduced growth was also generally suppressed. The growth of *C. albicans* was unaffected by a change in pH. A furher study by the same group[73] involving lipid-free PN showed an increase in pH of an infusate with an initial pH of 5.5 was also found to increase the growth of several microbes (*S. aureus, S. marcescens* and *B. cereus*) except that of *C. albicans* which remained unaffected.

Osmolarity

One study[45] found that a sodium chloride or glucose induced increase in osmolarity, at a constant concentration of all other constituents, supressed the growth of *E. coli* and *E. durans*. The osmolarity effect on growth over a 48 hour period was significant for sodium chloride but not for glucose.

Container used to store the test infusates

Although the containers used for infusate storage varied between studies (Appendix E), none of them examined the effects of different containers on microbial growth.

Temperature

Four studies[56, 64, 65, 67] examined the effect of temperature on the growth of organisms in identical infusates, two studies involving lipid PN[56, 67] and the other two studies involving lipid alone[64, 65]. Three of these studies examined the effect of increasing the ambient temperature from 25ºC[56, 65] or unspecified room temperature[64] to 35 or 37ºC. One of the studies involving lipid PN examined the growth of some microbes at 22ºC, and at 4ºC or 6ºC, other microbes at 6 ºC and 10ºC and yet others at 4 and

22ºC. There were one to two samples from one to six replicates examining from one to twelve microbes per study. One study[67] involving lipid PN showed a general increase in growth with a rise in temperature, often to a striking extent. In contrast, another lipid PN study[56] showed a variable non-striking effect of temperature on growth, despite including several of the microbes examined in the first study. Of the two studies involving lipid alone[64, 65], one[65] found little effect of temperature on growth, and the other[64] a non-striking increase in growth by 48 hours.

Oxygen

Only three of the studies[43-45] used sterile oxygen barrier PN bags (Appendix E) that might be used in clinical practice, and none examined the effect of oxygen tension. In addition none has compared the effects of using oxygen permeable and impermeable bags, or compared growth in containers with samples and exposed and not exposed to oxygen in room air.

Crystalline amino acid solution in parenteral nutrition

Two studies examining the effect of different amino acid products were reviewed[66, 67]: one involving lipid-free PN without trace elements and vitamins but apparently with the same electrolyte and glucose concentrations[66]; and the other involving lipid PN in the presence of trace elements but not vitamins, with different electrolyte concentrations[67]. In the study involving lipid-free PN[66], which involved the use of three samples from two replicates, there was little or no microbial growth in one of the PN infusates containing the amino acid solution 'Synthamin', but a striking growth in most of the PN infusates containing different amino acid solutions. In the study involving lipid PN[67], involving two samples from three replicates, *K. pneumoniae* was found to be enhanced in PN containing the amino acid solution 'Vamin' and had little or no growth in PN containing 'Synthamin'. In contrast, in the same study *C. albicans* grew poorly in PN containing 'Vamin' and rapidly in PN containing 'Synthamin'.

3.2.3 QUALITY OF STUDIES

Out of a total of 24 studies there were 6 disagreements[57, 59, 61, 68, 72, 75] about the quality of studies that were resolved by discussion (1 study[75] was ultimately graded as being of moderate quality, 3 studies[57, 61, 72] low quality, and 2 studies[59, 68] very low quality). Overall, 3 studies[43-45] were graded as high quality, 1 study[75] as moderate quality, 4 studies[57, 58, 61, 72] were graded as low quality and 16 studies[42, 56, 59, 60, 62-71, 73, 74] very low quality. Principally, the very low quality scores were assigned because the studies either examined only one type of PN infusate or were associated with a generally poor methodology such as ambient temperature not likely to be encountered in routine clinical practice or inadequate sample size, for example n = 1 (Appendices C and D). The 5 studies[42, 57, 59, 62, 68] cited by the guideline groups that were included in the present work were all graded as either low[57] or very low[42, 59, 62, 68] quality. Of the total 24 studies, only 3 studies[43-45] were found to be primarily designed to examine the validity of the guidelines for duration of lipid PN and lipid-free PN administration under simulated conditions likely to exist in clinical practice.

3.2.4 DISCUSSION

This systematic review suggests that the evidence base to support a recommendation that infusion of PN from the same bag should be restricted to no more than a day (24 hours) if it contains lipid and up to two or more days if it does not contain lipid is weak. The review also indicates that other factors, including glucose concentration and the species of microbe, can have a substantial impact on microbial growth, and to a considerably greater extent than the inclusion of lipid in PN.

Microbial growth in PN was found to be widely variable and was predominantly sensitive to species of microbe. Some microbes were found to grow rapidly in both lipid and lipid-free PN (e.g. *C. albicans* and *S. marcescens*), others slowly (e.g. *S. epidermidis* and *P. aeruginosa*) and yet others were killed in both lipid and lipid-free PN while being able to grow in lipid alone (e.g. *E. cloacae*) (Figure 3.12). It is possible for an organism to be killed in lipid alone but to grow in both lipid and lipid-free PN (e.g. *E. durans*). Furthermore, individual studies have reported that the growth of microbes in certain PN infusates can be inhibited by formulations that include trace elements, e.g. *P. fluorescens*[70]. The finding that classification of

microbes as Gram-positive bacteria, Gram-negative bacteria or fungi captures only a small proportion of the variability in growth due to the microbe type not only indicates the difficulties in making simple general predictions about growth of microbial contaminants of lipid and lipid-free PN (Figure 3.12), but also in making recommendations about the duration of PN infusions according to whether they contain or do not contain lipid. In some PN infusates even the strain of a microbe, for example *S. aureus*, appears to affect its growth characteristics in both lipid PN and lipid-free PN[61] (see comments in the 'Species of microbe' sections in the Results of the unmatched and matched data analyses (3.2.2.4.1 and 3.2.2.4.2 respectively)). Since the changes in growth induced by the presence of lipid and other nutrients and by the physico-chemical characteristics of the infusates also appear to be strongly dependent upon the species of microbe (see below), those microbes likely to contaminate PN infusates should be taken into account, especially if general clinical recommendations are to be followed.

In unmatched data analysis involving a range of individually studied microbes it was difficult to observe any obvious difference in overall growth in lipid and lipid-free PN (Figure 3.13). Indeed, the presence of lipid in PN was found to account for only about 3% of the total variability in microbial growth both before and after adjustment for glucose concentration and species of microbe. In multivariate analysis an increasing glucose concentration suppressed growth and accounted for a greater proportion of the variability (5.8%) than the presence of fat in PN, while the species of microbe and infusate-microbe interaction in combination accounted for as much as ~40% of the variability. Growth in lipid emulsion alone was higher than in lipid PN (also lipid-free PN), which might be partly explained by physico-chemical differences, including pH, (lipid emulsion is alkaline and PN is acidic) and the presence of additional nutrients in PN which typically contains a range of macronutrients and micronutrients. It is therefore important not to extrapolate studies of lipid emulsion alone to lipid PN.

The matched data analysis involving a comparison of PN with and without the inclusion of lipid found no significant overall effect of the type of infusate on microbial growth, and a series of meta-analyses found no effect of isoenergetic exchange of glucose for lipid in the same PN volume, addition of lipid to lipid-free PN, or an increase in the percent non-protein energy as lipid, controlling for energy

density. However, subgroup analyses demonstrated important differences between microbes. The growth of *C. albicans* was not affected by the inclusion of lipid in PN no matter which of the three methods of analysis was used, whereas growth of *E. coli* was significantly increased by the inclusion of lipid in PN using all three methods of analysis. With *S. epidermidis* isocaloric exchange of glucose for lipid significantly reduced growth, but the reduction was not significant when lipid was added to lipid-free PN or when the analysis involved percent non-nitrogen energy as lipid as the independent variable. Microbes are diverse organisms and their responses to the ambient physico-chemical environment varies considerably, making it difficult to establish overarching patterns of microbial growth in PN infusates containing different concentrations and patterns of nutrients. Since the responses are microbe specific, recommendations about the infusion duration of PN infusates should take into account the growth characteristics of different microbial contaminants. They should also consider using greater weighting for microbes more frequently encountered, those that grow more rapidly in PN and those that have greater potential detrimental clinical consequences.

Another major finding of this review is that microbial growth can be substantially influenced by nutrients other than lipid and by the physico-chemical characteristics of the PN. The strongest indication for other nutrient effects comes from matched analyses, in which the growth of the same microbe is compared in PN formulation with and without specific groups of nutrients such as vitamins or trace elements, or in the case of amino acids, different formulations exchanged in the same final volume of PN. Despite the small number of studies and sample sizes there were strong indications that vitamins, trace elements and the amino acid profiles can have a striking effect on the growth of certain microbes in some PN formulations. Another potentially modifiable risk factor is the pH of the PN infusates, which was found to affect the growth of several microbes, including *S. aureus*, but not *C. albicans* in at least some PN infusates[72, 73]. Less work appears to have been carried out on the effect of osmolarity but at least one study[45] demonstrated a significant reduction in the growth of *E. coli* when the osmolarity of PN infusates was increased using sodium chloride. This raises the possibility that the effects of some macronutrients are at least partially mediated by changes in osmolarity. As with the presence of lipid in PN, the effects of these other modifiable risk factors appear to depend on the species of microbe, and the overall physico-chemical characteristics of the PN infusates. The

potential for interactions between the various risk factors is substantial, making it even more difficult to predict the growth patterns of different microbes in different nutrients, and to make overarching clinical recommendations about the duration of PN infusion from a single bag based on the overall growth characteristics of microbes.

Furthermore, clinical factors should also be taken into account. For example, since the currently recommended maximum duration of infusion of lipid PN is shorter than for lipid-free PN, more frequent changes of bags containing PN with lipid could potentially increase the risk of infection if more manipulations are required for more frequent PN bag changes. Furthermore, replacement of lipid for glucose may cause hyperglycaemia, which could also increase the risk of CI and other infections. A quantitative model which balances those risk factors that increase clinical infections with those that decrease clinical infections could help the guideline development process. Among such factors is the non-reuse of administration sets for PN[160] which may harbour microbes. Such a model would have greater validity if it was established using an evidence base that encompassed issues raised in this review, including those related to microbial biology in PN infusates, which are in need of further investigation.

3.2.5 LIMITATIONS

This systematic review is limited by the few data available, which became fewer as comparisons between types of infusate became matched or more specific. Indeed, several reports involved only limited test infusates and/or samples. Furthermore, much of the data were obtained from records that used different methodology, which was generally considered to be poor, involving conditions that often deviated from those operating under routine clinical care. For example, the ambient temperature used in some studies was outside the range normally used to infuse PN, the type of containers used were very different from those used in routine practice and potential exposure to oxygen, with unknown effects on microbial growth in PN. In addition, since this review included studies published over the last 40 years, some of the products used in the earlier studies are no longer available or not available in some countries. Since the studies involved formulations used in adults and not children (e.g. amino acid solutions) care should be taken in extrapolating the findings to

paediatric populations. Despite these limitations this study presents a summary of the current evidence base for microbial growth over a 48 hour period in PN infusates, and helps illustrate the difficulties in making robust recommendations about duration of infusates according to composition.

CHAPTER 4
PRESCRIBING METHOD AND DOSE PACKAGING AND TRANSFER

This chapter examines sub-hypotheses 2 and 4: “changing the prescribing method for parenteral nutrition from recycling handwritten paper charts to prescribing electronically with fresh printing of prescriptions will reduce delivery of microbial contaminants from the clinical to the pharmaceutical environment”; and “packaging of compounded PN bags and prescriptions (with potential surface contaminants) separately rather than together for transfer to clinical environments will reduce microbial surface contaminants on arrival at the destination.”

The aim of this section of the thesis was to develop and apply a study to examine whether freshly printed electronic prescription printouts could reduce viable surface contaminants compared to handwritten recycled paper PN prescriptions, and whether this could lead to lower surface contamination of PN bags if prescriptions and bags are packaged in direct contact with each other during delivery.

4.1 METHODOLOGY

The detailed methods are described in Chapter Two.

In comparing differences between freshly printed electronic and handwritten recycled paper prescriptions in the transfer of microbial contaminants in and out of pharmacy, three separate interrelated studies were undertaken, each of which was part of the ward-pharmacy cycle of microbial transfer (Figure 4.1).

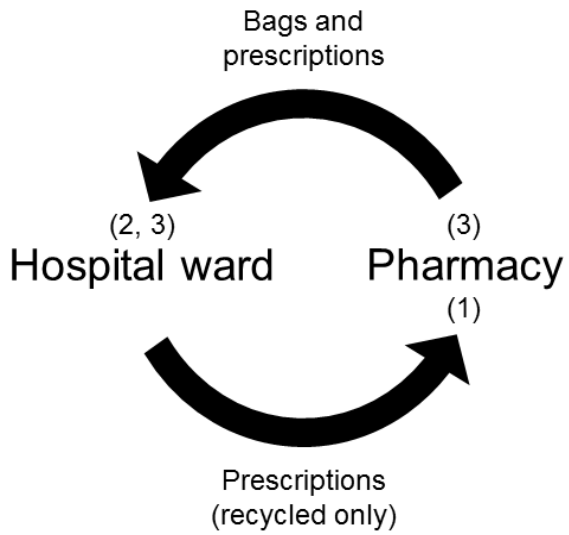

Figure 4.1 The ward-pharmacy cycle of microbial transfer

The first study involved measurements of the number and type of microorganisms contaminating prescriptions entering the pharmaceutical environment (Study 1), and the second study was of prescriptions arriving to the clinical environment after cycling through the pharmacy. The third study involved measurement of the number and species of microbes transferred to PN bag surfaces from contact with prescriptions during delivery to the clinical environment. The studies, which were undertaken based on adult wards of a busy NHS hospital with about 1200 beds, were randomised to be either electronic or recycled, and the operators who identified and counted the number of cfu were blinded to the randomisation procedure. A single pharmacist operator took all of the study samples and the same microbiological methods were used in each of the three studies.

For Study 1, a total of 64 prescriptions were sampled (n = 32 recycled and n = 32 electronic prescriptions) on their entry to the pharmaceutical environment. All of the swabs were receipted into a category 2 microbiology laboratory within their recommended timeframe (range of Study 1 samples from 6 - 383 minutes; maximum limit 24 hours), and stored under refrigeration at 2 ± 1°C if not tested the same day, for the microbiological procedures. A different set of prescriptions (n = 38 recycled and n = 34 electronic prescriptions) to that used in Study 1 was tested in Study 2. The duration from the samples being taken to their arrival at the category 2 laboratory in study 2 had a range of 4 - 149 minutes (maximum limit 24 hours). The prescriptions used in Study 3 were the same as those used in Study 2 (n = 38 packaged with

recycled prescriptions and n = 34 packaged with electronic prescriptions). Delivery of all of the Study 3 samples to the category 2 laboratory was within the maximum 24-hour limit (with ranges of 20 - 241 minutes and 6 - 150 minutes respectively).

4.2 RESULTS

No outbreaks of MRSA or *C. difficile* occurred during the study. However, of the 72 samples that had colonies growing on Baird Parker Medium (26 prescription samples and 46 bag samples), two (one prescription and one bag sample) were subsequently identified as including *S. aureus* (further details under Study 2 and Study 3 results below).

4.2.1 STUDY 1: PRESCRIPTIONS ENTERING THE PHARMACEUTICAL ENVIRONMENT

On arrival to the pharmaceutical environment (Table 4.1) a greater proportion of handwritten recycled prescriptions were contaminated with non-specific aerobes (94% v 44%, $P < 0.001$; RR 2.143 (95% CI 1.432, 3.206), $P < 0.001$). No significant differences were found with the other microorganisms which were infrequent contaminants (non-specific staphylococci, mould and yeast), and neither *S. aureus* nor enterococci were found.

Table 4.1 The presence or absence of contaminants on parenteral nutrition prescriptions and parenteral nutrition bags

	Recycled[a] proportion	**Electronic[b] proportion**	***P*[c]**
Prescriptions on arrival to pharmaceutical environment			
Non-specific aerobes	0.94 (30/32)	0.44 (14/32)	< 0.001
Non-specific Staphylococci	0.09 (3/32)	0.00 (0/32)	0.238
S. aureus	0.00 (0/32)	0.00 (0/32)	NS[d]
Moulds	0.00 (0/32)	0.06 (2/32)	0.492
Yeasts	0.03 (1/32)	0.00 (0/32)	1.000
Enterococci	0.00 (0/32)	0.00 (0/32)	NS[d]
Prescriptions on arrival to clinical environment			
Non-specific aerobes	0.76 (29/38)	0.50 (17/34)	0.028
Non-specific Staphylococci	0.11 (4/38)	0.03 (1/34)	0.361
S. aureus	0.03 (1/38)	0.00 (0/34)	1.000
Moulds	0.08 (3/38)	0.12 (4/34)	0.700
Yeasts	0.00 (0/38)	0.00 (0/34)	NS[d]
Enterococci	0.00 (0/38)	0.00 (0/34)	NS[d]
Bags compounded in pharmaceutical environment before delivery			
Non-specific aerobes	0.42 (16/38)	0.56 (19/34)	0.345
Non-specific Staphylococci	0.08 (3/38)	0.03 (1/34)	0.617
S. aureus	0.00 (0/38)	0.00 (0/34)	NS[d]
Moulds	0.08 (3/38)	0.12 (4/34)	0.700
Yeasts	0.00 (0/38)	0.03 (1/34)	0.472
Enterococci	0.00 (0/38)	0.00 (0/34)	NS[d]
Bags on arrival to clinical environment			
Non-specific aerobes	0.63 (24/38)	0.41 (14/34)	0.097
Non-specific Staphylococci	0.18 (7/38)	0.00 (0/34)	0.012
S. aureus	0.03 (1/38)	0.00 (0/34)	1.000
Moulds	0.11 (4/38)	0.12 (4/34)	1.000
Yeasts	0.00 (0/38)	0.00 (0/34)	NS[d]
Enterococci	0.00 (0/38)	0.00 (0/34)	NS[d]

[a] Handwritten recycled prescriptions or parenteral nutrition bags randomised to be packaged in direct contact with handwritten recycled prescriptions during delivery to the clinical environment (hospital ward).

[b] Freshly printed Electronic prescriptions or parenteral nutrition bags randomised to be packaged in direct contact with freshly printed electronic prescriptions during delivery to the clinical environment (hospital ward).

[c] Using Fisher's exact test.

[d] NS, not significant (result not computable).

When the results were analysed quantitatively (Table 4.2) a similar general pattern emerged. A greater number of non-specific aerobic organisms were isolated from handwritten recycled than freshly printed electronic prescriptions (recycled prescriptions median 130 organisms (IQR = 65, 260) v electronic prescriptions median 0 organisms (IQR 0, 75); $P < 0.001$), but no significant differences were found for non-specific staphylococci, mould or yeast.

Table 4.2 The number and type of contaminants on parenteral nutrition prescriptions and parenteral nutrition bags

	Recycled[a] median organisms (IQR)	**Electronic[b] median organisms (IQR)**	***P*[c]**
Prescriptions on arrival to pharmaceutical environment	**n = 32**	**n = 32**	
Non-specific aerobes	130 (65, 260)	0 (0, 75)	< 0.001
Non-specific Staphylococci	0 (0, 0)	0 (0, 0)	0.078
S. aureus	0 (0, 0)	0 (0, 0)	1.000
Moulds	0 (0, 0)	0 (0, 0)	0.154
Yeasts	0 (0, 0)	0 (0, 0)	0.317
Enterococci	0 (0, 0)	0 (0, 0)	1.000
Prescriptions on arrival to clinical environment	**n = 38**	**n = 34**	
Non-specific aerobes	120 (15, 320)	10 (0, 40)	0.001
Non-specific Staphylococci	0 (0, 0)	0 (0, 0)	0.219
S. aureus	0 (0, 0)	0 (0, 0)	0.344
Moulds	0 (0, 0)	0 (0, 0)	0.583
Yeasts	0 (0, 0)	0 (0, 0)	1.000
Enterococci	0 (0, 0)	0 (0, 0)	1.000
Unused bags compounded in pharmaceutical environment	**n = 38**	**n = 34**	
Non-specific aerobes	0 (0, 80)	20 (0, 80)	0.368
Non-specific Staphylococci	0 (0, 0)	0 (0, 0)	0.386
S. aureus	0 (0, 0)	0 (0, 0)	1.000
Moulds	0 (0, 0)	0 (0, 0)	0.583
Yeasts	0 (0, 0)	0 (0, 0)	0.290
Enterococci	0 (0, 0)	0 (0, 0)	1.000
Bags on arrival to clinical environment	**n = 38**	**n = 34**	
Non-specific aerobes	40 (0, 80)	0 (0, 40)	0.036
Non-specific Staphylococci	0 (0, 0)	0 (0, 0)	0.009
S. aureus	0 (0, 0)	0 (0, 0)	0.344
Moulds	0 (0, 0)	0 (0, 0)	0.836
Yeasts	0 (0, 0)	0 (0, 0)	1.000
Enterococci	0 (0, 0)	0 (0, 0)	1.000

[a] Handwritten recycled prescriptions or parenteral nutrition bags randomised to be packaged in direct contact with handwritten recycled prescriptions during delivery to the clinical environment (hospital ward).

[b] Freshly printed electronic prescriptions or parenteral nutrition bags randomised to be packaged in direct contact with freshly printed electronic prescriptions during delivery to the clinical environment (hospital ward).

[c] Using the Mann Whitney U test.

Considering only the handwritten recycled prescriptions (Table 4.3), significant relationships were found between the total number of non-specific aerobes on the one hand, and the total number of prescriptions, the number of prescriptions used by pharmacy, the number of prescription sheets, and the number of times the prescription had been recycled, on the other (r values ranging from 0.586 to 0.634, $P < 0.001$). Similar relationships were found for non-specific staphylococci (r values ranging from 0.417 to 0.480, $P < 0.020$ in each case), but no significant relationships were found with the other organisms.

Table 4.3 Spearman rank correlation coefficients between the number of surface contaminants found on handwritten recycled parenteral nutrition prescriptions and risk factors

	Prescriptions on arrival to pharmaceutical environment (n = 32)		**Prescriptions on arrival to clinical environment (n = 38)**	
	Correlation coefficient	***P***	**Correlation coefficient**	***P***
Non-specific aerobic contaminants				
Total number of prescriptions[a]	0.615	< 0.001	0.406	0.011
Prescriptions used by pharmacy[b]	0.614	< 0.001	0.402	0.012
Number of prescription sheets[c]	0.634	< 0.001	0.416	0.009
Number of times prescription had been recycled[d]	0.586	< 0.001	0.375	0.020
Non-specific Staphylococci				
Total number of prescriptions[a]	0.425	0.015	-0.027	0.873
Prescriptions used by pharmacy[b]	0.417	0.018	-0.057	0.735
Number of prescription sheets[c]	0.480	0.005	-0.159	0.341
Number of times prescription had been recycled[d]	0.430	0.014	-0.028	0.870

[a] The total number of parenteral nutrition prescriptions that were separate from any other medicine prescriptions.
[b] The number of prescriptions that had been used to compound a parenteral nutrition bag in pharmacy. Since some patients had their parenteral nutrition stopped after prescriptions had been written and before they had been made the number of prescriptions used by pharmacy was not necessarily the same as the total number of prescriptions written.
[c] The number of prescription sheets stapled together with each sheet consisting of up to six individual prescriptions.
[d] The number of times the prescriptions for the same patient went through the pharmacy department.

4.2.2 STUDY 2: PRESCRIPTIONS ARRIVING TO THE CLINICAL ENVIRONMENT

On arrival to the clinical environment (Table 4.1) a greater proportion of handwritten recycled prescriptions were contaminated with non-specific aerobes than freshly printed electronic prescriptions (76% v 50%, *P* = 0.028; RR 1.526 (95% CI 1.044,

2.232), $P = 0.029$), and although the trend was in the same direction with non-specific staphylococci, which were present less frequently, the differences were not statistically significant (11% v 3%, $P = 0.361$; RR 3.579 (95% CI 0.420, 30.477), $P = 0.243$). No significant differences were found with the other microorganisms that were infrequent contaminants (*S. aureus* and mould), and neither yeast nor enterococci were found.

When the results were analysed quantitatively (Table 4.2) the non-specific aerobic organism contamination of handwritten recycled prescriptions was significantly greater than that associated with the freshly printed electronic prescriptions (recycled prescriptions median 120 organisms (IQR 15, 320) v electronic prescriptions median 10 organisms (IQR 0, 40); $P = 0.001$). No significant differences were found for non-specific staphylococci, *S. aureus* or mould.

Considering only the handwritten recycled prescriptions (Table 4.3), significant inter-relationships were found between the total number of non-specific aerobes and the total number of prescriptions, the number of prescriptions used by pharmacy, the number of prescription sheets, and the number of times the prescription had been recycled (r values from 0.375 to 0.416, $P \leq 0.020$). No significant relationships were found for non-specific staphylococci (r values from -0.159 to -0.027, $P > 0.050$), and no significant relationships were found with the other organisms.

One *S. aureus* colony was found on a single handwritten recycled prescription arriving on the clinical environment. The methicillin resistance initially indicated (by growth on a media plate that incorporated antibiotic (brilliance MRSA 2 plate from Oxoid; Perth, Scotland)) was not confirmed on testing against a battery of antibiotics.

4.2.3 STUDY 3: BAGS BEFORE PACKAGING AND FOLLOWING DELIVERY

4.2.3.1 Surfaces of bags prior to packaging

There were no significant differences in contamination of the external surface of unused bags randomly allocated to the recycled and electronic prescription groups (see Tables 4.1 and 4.2 for the results).

4.2.3.2 Surfaces of bags following delivery

On arrival to the clinical environment (Table 4.1) a greater proportion of the bags packaged with handwritten recycled rather than freshly printed electronic prescriptions were contaminated with non-specific aerobes, but this was not significant (63% v 41%, $P = 0.097$; RR 1.534 (95% CI 0.959, 2.453), $P = 0.074$). A greater proportion of the bags randomly allocated to the handwritten recycled prescription group were contaminated with non-specific staphylococci compared to the freshly printed electronic prescriptions (18% v 0%, $P = 0.012$). No significant differences were found with the other microorganisms (*S. aureus* and mould), and neither yeast nor enterococci were found.

When the results were analysed quantitatively (Table 4.2), both a greater number of non-specific aerobic organisms and a greater number of non-specific staphylococci were isolated from the bags randomised to be packaged with handwritten recycled rather than freshly printed electronic prescriptions, and in each case the difference was significant (non-specific aerobes on handwritten recycled prescriptions median 40 organisms (IQR 0, 80) v freshly printed electronic prescriptions median 0 organisms (IQR 0, 40); $P = 0.036$; non-specific staphylococci on handwritten recycled prescriptions median 0 organisms (IQR 0, 0) v freshly printed electronic prescriptions median 0 organisms (IQR 0, 0); $P = 0.009$). No significant differences were found for *S. aureus* or mould.

Considering only the handwritten recycled prescriptions (Table 4.3), no significant relationships were found between any of the organisms and the total number of prescriptions, the number of prescriptions used by pharmacy, the number of prescription sheets, or the number of times the prescription had been recycled.

Two *S. aureus* colonies were found on the surface of a single bag arriving on the clinical environment that had been packaged in contact with a handwritten recycled prescription during delivery. As in Study 2, methicillin resistance was initially indicated but was not confirmed on subsequent more specific testing.

4.3 DISCUSSION

This study shows that both PN prescriptions and PN bags are a source of contaminants and that substituting freshly printed electronic for handwritten recycled prescriptions reduces this contamination. The study also suggests that contamination is likely to be greater when it involves handwritten recycled prescriptions that have been in circulation for longer. This can be explained by the relationships that were found between the numbers of contaminants on the one hand and the total number of prescriptions, the number of prescriptions used by pharmacy, the number of prescription sheets and the number of times prescription had been recycled on the other (Table 4.3).

The key issue is whether the greater contamination of handwritten recycled prescriptions is of clinical significance. Whilst this study did not set out to address this issue it raises the possibility that this may be so, because of the greater microbial burden on handwritten recycled prescriptions that may include pathogenic organisms such as *S. aureus*. In this study, *S. aureus* was found on two occasions, once on a handwritten recycled prescription, and once on a bag that had been in contact with a handwritten recycled prescription. This may initially appear infrequent, affecting less than five percent of prescriptions delivered to hospital wards but given that handwritten recycled PN prescriptions are recycled each day for the duration of PN (a recent mean of ~12 days and median of 8 days in the UK[2]), the likelihood of transfer of potentially pathogenic organisms increases accordingly. Non-specific aerobes were found much more frequently than *S. aureus* on both the prescriptions and bags and these colonies are expected to include *S. epidermidis*, a common skin contaminant, and frequent cause of catheter-related infection[127, 129]. Non-specific staphylococci were also found more frequently than *S. aureus*, and these colonies are expected to primarily consist of contaminants originating from the skin of hospital workers. Furthermore, patients receiving PN for more prolonged periods are often sick, immunosuppressed and more susceptible to infection. The presence of catheters that bypass the body's natural defences against pathogens increases the risk further. In addition, handwritten recycled prescriptions could increase the spread of MRSA or *C. difficile* during local outbreaks of these organisms, but no such outbreaks were encountered during this study. Whilst there were no outbreaks of *C. difficile* during this study it is possible that spore forming microbes could be of particular clinical

relevance. For example, the recent death caused by contaminated PN in London (England) was as a result of *B. cereus*[16]. However, routine testing for spore forming microbes was not undertaken due to the high cost and because no *C. difficile* was detected during pilot studies.

Whilst this study provides proof of concept, much larger studies are needed to determine whether cross contamination of microbial organisms using PN prescriptions and PN bags are a definitive clinical risk to patients, and whether any risk can be further reduced, for example by using a different type of paper. Larger studies with more power are also needed to establish significant differences in contamination rates of prescriptions harbouring infrequent types of bacteria, which are potentially pathogenic. In the absence of definitive information about the clinical significance of surface contamination of PN prescriptions and PN bags, a value judgement is required when establishing policies on prescription methods but if a reduction can be readily achieved by changing the type of prescription then it seems reasonable, and possibly safer, to adopt electronic prescription of PN. However, the resources required to implement electronic prescription of PN can be substantial, and are not limited to the purchase, maintenance and upgrading of computer hardware and software. For example, local software validation, the development of a contingency plan in the event of computer or software failure and necessary operator training can make the introduction of electronic PN prescribing daunting. Nevertheless, if implementation is practical and affordable then our study would support the use of an electronic rather than a recycled method of parenteral nutrition prescription.

CHAPTER 5
COMPOUNDING ENVIRONMENT FOR INFUSATE

This chapter examines sub-hypothesis 3: “undertaking aseptic manipulation of doses in a pharmaceutical environment in preference to a clinical environment (which has a higher microbial load) will reduce the dose contamination rate that can be achieved.”

The aim of this section of the thesis was to undertake a systematic review to establish and compare the reported contamination rates of doses made under aseptic techniques in pharmaceutical and clinical environments, and to consider the effect of making additions to sterile doses under aseptic techniques.

5.1 METHODOLOGY

The detailed methods used are described in Chapter Two.

The literature search was undertaken on 10 February 2014 using the search software of Medline, Embase and the Cochrane Library. The search terms and numbers of results are indicated in Appendix B. It can be seen from Appendix B that a third search term was required on five occasions.

The literature search identified 42246 records (17662 from Medline, 20824 from Embase and 3760 from the Cochrane Library) and 28020 after duplicates had been removed. The title and abstract of each of the 28020 identified records was evaluated and excluded if it did not meet the inclusion criteria. This left 137 records, which were individually subjected to a full text review to confirm relevance and compliance with the above criteria to yield a final total of 34 studies from 33 records[82-114].

The included studies were divided into groups according to whether doses had been prepared in a clinical or pharmaceutical environment (Study 1), whether doses had been prepared as individual lots or as part of a batch (Study 2), and whether doses had been sampled without or before administration or during or after administration

to a patient (due to a risk of contamination from manipulations after preparation and potential differences in time between preparation and sampling which may have affected recovery of damaged microbial cells).

5.2 RESULTS

A grand total of 16552 doses eligible for inclusion were identified from 34 studies taken from 33 records[82-114], which are summarised in Appendix F. Of the total 33 records, only 7 records involved head-to-head comparisons. One record[92] compared batch doses and individual lots in a clinical environment and 6 records[86, 89, 92, 99, 100, 106] compared additives and no additives to sterile doses in a clinical environment. Figure 4.1 shows the forest plot obtained when all the study data were combined in a meta-analysis grouped according to environment (pharmaceutical or clinical) and method of dose preparation (individual or batch). The majority (94%) of the doses that had been prepared as individual lots in clinical environments (n = 4141) had been sampled during or after administration, and all of the other doses had been sampled without or prior to administration. When only the 4141 doses prepared as individual lots in clinical environments were included there was a non-significantly higher frequency of contamination of doses sampled without or prior to administration than during or after administration (5.3% (95% CI 2.7%, 10.0%; n = 3889 doses) (I^2 = 93.07%; $P < 0.001$) v 2.3% (95% CI 0.5%, 10.1%; n = 252 doses) (I^2 = 56.45%; $P = 0.101$); $P = 0.314$).

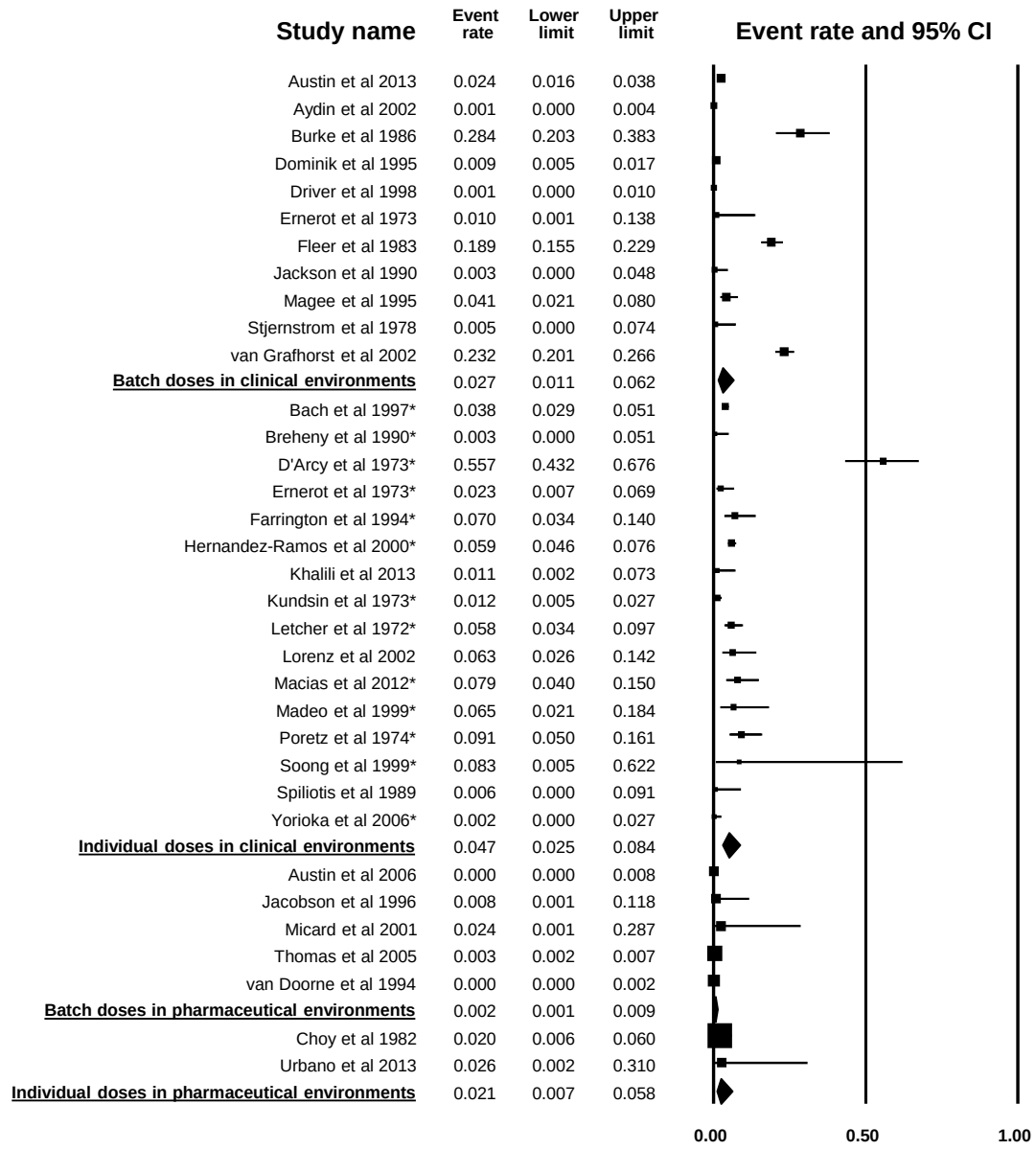

Figure 5.1 Forest plot and summary statistics of the frequency of the contamination rates of parenteral doses prepared aseptically in clinical and pharmaceutical environments

Nineteen[84, 85, 87, 88, 90, 92-95, 98-104, 106, 110, 113] of the 22 studies[82, 84, 85, 87-90, 92-95, 98-104, 106, 110, 112, 113] with contamination reported the species of microbe. In pharmaceutical environments this was limited to coagulase negative staphylococci (including *S. epidermidis*)[88, 110], *Bacillus sp.*[88, 110], and *Propionibacterium sp.*[88]. The same microbes were identified in clinical environments[84, 85, 87, 90, 93-95, 98, 100, 101, 104, 106, 113], as well as typically more pathogenic microbes such as *S. aureus*[85, 113], *S. marcescens*[85], *Klebsiella sp.*[95], *Enterobacter sp.* [95, 102] and fungi (including *Candida sp.*)[92, 95, 100].

5.2.1 STUDY 1: DOSE PREPARATION IN A CLINICAL COMPARED TO A PHARMACEUTICAL ENVIRONMENT

5.2.1.1 Individual and batch doses combined

5.2.1.1.1 All identified doses

The analysis involved 16552 doses from 34 studies (33 records[82-114]). Of these, 10272 doses from 27 studies (26 records[82, 84-87, 89-96, 98-104, 106-109, 113, 114]) had been prepared in clinical environments and 6280 doses from 7 studies (7 records[83, 88, 97, 105, 110-112]) had been prepared in pharmaceutical environments. When all the data were combined there was a significantly higher frequency of contamination of doses prepared in clinical than pharmaceutical environments (3.7% (95% CI 2.2%, 6.2%; n = 10272 doses) (I^2 = 95.35%; P < 0.001) v 0.5% (95% CI 0.1%, 1.6%; n = 6280 doses) (I^2 = 69.18%; P = 0.003); P = 0.007). The between study contamination was more variable in the clinical than pharmaceutical environment (range 0.1% to 55.7% v 0.0% to 2.6% respectively).

5.2.1.1.2 Doses sampled without or prior to administration

There were 12663 doses from 21 studies (21 records[82-84, 87, 88, 90, 91, 94, 96-98, 101, 104, 105, 108-113]) that had been sampled without administration or prior to administration, of which 6383 doses from 14 studies (14 records[82, 84, 87, 90-92, 94, 96, 98, 101, 104, 108, 109, 113]) had been prepared in clinical environments and 6280 doses from 7 studies (7 records[83, 88, 97, 105, 110-112]) had been prepared in pharmaceutical environments. When all the data were combined there was a significantly higher frequency of contamination of doses prepared in clinical than pharmaceutical environments (2.5% (95% CI 1.2%, 5.5%; n = 6383 doses) (I^2 = 95.69%; P < 0.001) v 0.5% (95% CI 0.1%, 1.6%; n = 6280 doses) (I^2 = 69.18%; P = 0.003); P = 0.044). The between study contamination was more variable in the clinical than pharmaceutical environment (range 0.1% to 28.4% v 0.0% to 2.6% respectively).

5.2.1.1.3 Doses sampled during or after administration

It was not possible to compare doses prepared in clinical and pharmaceutical environments that had been sampled during or after administration due to lack of data in the pharmaceutical environment.

5.2.1.2 Individual doses

5.2.1.2.1 All identified doses

The analysis involved 4309 doses from 18 studies (18 records[85, 86, 88, 89, 92, 93, 95, 98-103, 106-108, 111, 114]). Of these, 4141 doses from 16 studies (16 records[85, 86, 89, 92, 93, 95, 98-103, 106-108, 114]) had been prepared in clinical environments and 168 doses from 2 studies (2 records[88, 111]) had been prepared in pharmaceutical environments. When all the data were combined there was a non-significantly higher frequency of contamination of doses prepared in clinical than pharmaceutical environments (4.7% (95% CI 2.5%, 8.4%; n = 4141 doses) (I^2 = 91.64%; P < 0.001) v 2.1% (95% CI 0.7%, 5.8%; n = 168 doses) (I^2 = 00.00%; P = 0.856); P = 0.190). The between study contamination was more variable in the clinical than pharmaceutical environment (range 0.2% to 55.7% v 2.0% to 2.6% respectively).

5.2.1.2.2 Doses sampled without administration or prior to administration

There were 420 doses from 5 studies (5 records[88, 98, 101, 108, 111]) that had been sampled without administration or prior to administration, of which 252 doses from 3 studies (3 records[98, 101, 108]) had been prepared in clinical environments and 168 doses from 2 studies (2 records[88, 111]) had been prepared in pharmaceutical environments. When all the data were combined there was a non-significantly higher frequency of contamination of doses prepared in clinical than pharmaceutical environments (2.3% (95% CI 0.5%, 10.1%; n = 252 doses) (I^2 = 56.45%; P = 0.101) v 2.1% (95% CI 0.7%, 5.8%; n = 168 doses) (I^2 = 00.00%; P = 0.856); P = 0.923). The between study contamination was more variable in the clinical than pharmaceutical environment (range 0.6% to 6.3% v 2.0% to 2.6% respectively).

5.2.1.2.3 Doses sampled during or after administration

It was not possible to compare doses prepared in clinical and pharmaceutical environments that had been sampled during or after administration due to lack of data in the pharmaceutical environment.

5.2.1.3 Batch doses

5.2.1.3.1 All identified doses

The analysis involved 12243 doses from 16 studies (16 records[82-84, 87, 90-92, 94, 96, 97, 104, 105, 109, 110, 112, 113]). Of these, 6131 doses from 11 studies (11 records[82, 84, 87, 90-92, 94, 96, 104, 109, 113]) had been prepared in clinical environments and 6112 doses from 5 studies (5 records[83, 97, 105, 110, 112]) had been prepared in pharmaceutical environments. When all the data were combined there was a significantly higher frequency of contamination of doses prepared in clinical than pharmaceutical environments (point estimate 2.7% (95% CI 1.1%, 6.2%; n = 6131 doses) (I^2 = 96.48%; P < 0.001) v 0.2% (95% CI 0.1%, 0.9%; n = 6112 doses) (I^2 = 56.49%; P = 0.056); P < 0.001). The between study contamination was more variable in the clinical than pharmaceutical environment (range 0.1% to 28.4% v 0.0% to 2.4% respectively).

5.2.1.3.2 Doses sampled without or prior to administration

Since all of the identified doses had been sampled without or prior to administration, a comparison of doses prepared in clinical and pharmaceutical environments that had been sampled without or prior to administration yields the same results as all of the combined data (above).

5.2.1.3.3 Doses sampled during or after administration

It was not possible to compare doses prepared in clinical and pharmaceutical environments that had been sampled during or after administration due to lack of data in either the clinical or pharmaceutical environment.

5.2.2 STUDY 2: DOSE PREPARATION AS INDIVIDUAL LOTS OR AS PART OF A BATCH

5.2.2.1 Clinical and pharmaceutical environments combined

5.2.2.1.1 All identified doses

The analysis involved 16552 doses from 34 studies (33 records[82-114]). Of these, 4309 doses from 18 studies (18 records[85, 86, 88, 89, 92, 93, 95, 98-103, 106-108, 111, 114]) had been prepared as individual lots and 12243 doses from 16 studies (16 records[82-84, 87, 90-92, 94, 96, 97, 104, 105, 109, 110, 112, 113]) had been prepared as part of a batch. When all the data were combined there was a significantly higher frequency of contamination of doses prepared as individual lots than as part of a batch (4.4% (95% CI 2.5%, 7.6%; n = 4309 doses) (I^2 = 90.77%; P < 0.001) v 1.3% (95% CI 0.5%, 3.0%; n = 12243 doses) (I^2 = 96.68; P < 0.001); P = 0.022). The between study contamination was more variable for doses prepared as individual lots than as part of a batch (range 0.2% to 55.7% v 0.0% to 28.4% respectively).

5.2.2.1.2 Doses sampled without administration or prior to administration

There were 12663 doses from 21 studies (21 records[82-84, 87, 88, 90-92, 94, 96-98, 101, 104, 105, 108-113]) that had been sampled without administration or prior to administration, of which 420 doses from 5 studies (5 records[88, 98, 101, 108, 111]) had been prepared as individual lots and 12243 doses from 16 studies (16 records[82-84, 87, 90-92, 94, 96, 97, 104, 105, 109, 110, 112, 113]) had been prepared as part of a batch. When all the data were combined there was a non-significantly higher frequency of contamination of doses prepared as individual lots than as part of a batch (point estimate 2.7% (95% CI 1.2%, 6.0%; n = 420 doses) (I^2 = 28.53%; P = 0.231) v 1.3% (95% CI 0.5%, 3.0%; n = 12243 doses) (I^2 = 96.68%; P < 0.001); P = 0.231). The between study contamination was more variable for doses prepared as part of a batch than as individual lots (range 0.0% to 28.4% v 0.6% to 6.3% respectively).

5.2.2.1.3 Doses sampled during or after administration

It was not possible to compare doses prepared as individual lots and as part of a batch that had been sampled during or after administration due to lack of data for doses prepared as part of a batch.

5.2.2.2 Clinical environments

5.2.2.2.1 All identified doses

The analysis involved 10272 doses from 27 studies (26 records[82, 84-87, 89-96, 98-104, 106-109, 113, 114]). Of these, 4141 doses from 16 studies (16 records[85, 86, 89, 92, 93, 95, 98-103, 106-108, 114]) had been prepared as individual lots and 6131 doses from 11 studies (11 records[82, 84, 87, 90-92, 94, 96, 104, 109, 113]) had been prepared as part of a batch. When all the data were combined there was a non-significantly higher frequency of contamination of doses prepared as individual lots than as part of a batch (4.7% (95% CI 2.5%, 8.4%; n = 4141 doses) (I^2 = 91.64%; P < 0.001) v 2.7% (95% CI 1.1%, 6.2%; n = 6131 doses) (I^2 = 96.48%; P < 0.001); P = 0.299). The between study contamination was more variable for doses prepared as individual lots than as part of a batch (range 0.2% to 55.7% v 0.1% to 28.4% respectively).

5.2.2.2.2 Doses sampled without or prior to administration

There were 6383 doses from 14 studies (14 records[82, 84, 87, 90-92, 94, 96, 98, 101, 104, 108, 109, 113]) that had been sampled without or prior to administration, of which 252 doses from 3 studies (3 records[98, 101, 108]) had been prepared as individual lots and 6131 doses from 11 studies (11 records[82, 84, 87, 90-92, 94, 96, 104, 109, 113]) had been prepared as part of a batch. When all the data were combined there was a non-significantly higher frequency of contamination of doses prepared as part of a batch than as individual lots (point estimate 2.7% (95% CI 1.1%, 6.2%; n = 6131 doses) (I^2 = 96.48%; P < 0.001) v 2.3% (95% CI 0.5%, 10.1%; n = 252 doses) (I^2 = 56.48%; P = 0.101); P = 0.856). The between study contamination was more variable for doses prepared as part of a batch than as individual lots (range 0.1% to 28.4% v 0.6% to 6.3% to 28.4% respectively).

5.2.2.2.3 Doses sampled during or after administration

It was not possible to compare doses prepared as individual lots and as part of a batch that had been sampled during or after administration due to lack of data for doses prepared as part of a batch.

5.2.2.3 Pharmaceutical environments

5.2.2.3.1 All identified doses

The analysis involved 6280 doses from 7 studies (7 records[83, 88, 97, 105, 110-112]). Of these, 168 doses from 2 studies (2 records[88, 111]) had been prepared as individual lots and 6112 doses from 5 studies (5 records[83, 97, 105, 110, 112]) had been prepared as part of a batch. When all the data were combined there was a significantly higher frequency of contamination of doses prepared as individual lots than as part of a batch (2.1% (95% CI 0.7%, 5.8%; n = 168 doses) (I^2 = 00.00%; *P* = 0.856) v 0.2% (95% CI 0.1%, 0.9%; n = 6112 doses) (I^2 = 56.49%; *P* = 0.056); *P* = 0.002). The between study contamination was more variable for doses prepared as part of a batch than as individual lots (range 0.0% to 2.4% v 2.0% to 2.6% respectively).

5.2.2.3.2 Doses sampled without or prior to administration

Since all of the identified doses had been sampled without or prior to administration, a comparison of doses prepared as individual lots and as part of a batch that had been sampled without or prior to administration yields the same results as all of the combined data (above).

5.2.2.3.3 Doses sampled during or after administration

It was not possible to compare doses prepared as individual lots and as part of a batch that had been sampled during or after administration, since no relevant doses that had been prepared as either individual lots or as part of a batch had been identified.

5.2.3 STUDY 3: UNDERTAKING ADDITIONS TO TERMINALLY STERILISED DOSES

The maximum expected contamination rate of doses terminally sterilised according to appropriate and validated procedures is 1 per million[161].

5.2.3.1 Clinical and pharmaceutical environments combined

It was not possible to combine doses from clinical and pharmaceutical environments, since no studies that reported the contamination rate of sterile doses with and without additives undertaken in pharmaceutical environments had been identified.

5.2.3.2 Clinical environments

The analysis involved 1723 doses from 6 studies (6 records[86, 89, 92, 99, 100, 106]). Of these, additions had been made to 1108 doses and no additions had been made to 615 doses. All of the doses had been prepared as individual lots and sampled during or after administration. Figure 5.2 shows the forest plot when all of the study data were combined in a meta-analysis. There was a significantly higher frequency of contamination of doses with additives than without additives (risk ratio 2.121 (95% CI 1.093, 4.114); $P = 0.026$), with a low statistical heterogeneity ($I^2 = 22.50\%$; $P = 0.265$).

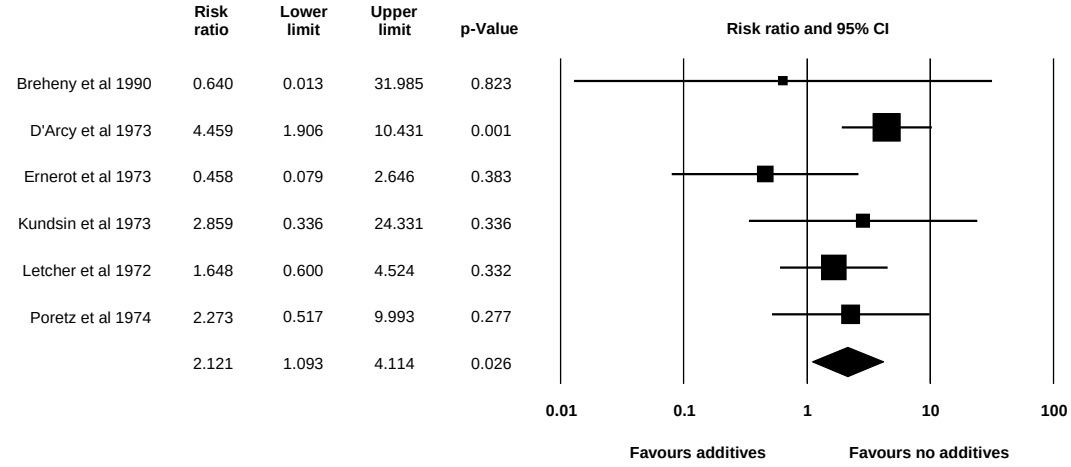

Figure 5.2 Forest plot of random effects meta-analysis comparing contamination rates of sterile parenteral doses with and without additives in clinical environments

5.2.3.3 Pharmaceutical environments

It was not possible to compare sterile doses with and without additives in pharmaceutical environments, since no relevant studies had been identified.

5.2.4 QUALITY OF STUDIES

For the purpose of this review two raters (PDA and KSH) graded all of the included studies as low to very low quality, with 3 disagreements within these categories. After discussion, the majority of the studies were graded as low quality primarily because they were nonrandomised, and 4 studies[89, 105, 107, 111] were graded as very low quality primarily due to low sample size[105, 107, 111] and limited procedural detail and high contamination rate[89].

5.3 DISCUSSION

This update identified more than double the number of doses than the 2009 review[50], which we have attempted to summarise to help inform judgements when establishing policy and clinical practice that ultimately aim to reduce patient infection rates. Overall, the contamination frequency was lower when doses had been prepared in pharmaceutical than clinical environments, but reported rates were often unacceptably high in both settings. For example, the mean reported study frequency

of microbial contamination of doses prepared under aseptic techniques in pharmaceutical environments could be more than 100 times higher than that expected from following the procedures recommended in Europe (over 2.0% compared to 0.02%[162]), and more than 2,750 times higher in clinical environments than that expected in a pharmaceutical environment (over 55.0% compared to 0.02%). The greater number of studies identified in this update meant that a previously non-significant but intuitive finding of the previous review[50] achieved statistical significance in the present review (Hypothesis 3).

When reported, the species of microbe found in the pharmaceutical environments had generally less pathogenic potential than that found in clinical environments, which also had a broader range of microbes, including various types of Gram-negative bacteria and fungi. This could in part reflect the use of strict governance procedures in pharmaceutical environments such as the strict use of special operator clothing to minimise shedding of microbes (and particles) and the filtration of air through 0.2 micron HEPA filters to largely limit the types of microbes to common commensals such as coagulase negative staphylococci. Not only are such procedures not followed in clinical environments, the closer proximity to patients acts as an additional source of a potentially broader range of more pathogenic microbes.

5.3.1 STUDY 1: DOSE PREPARATION IN A CLINICAL COMPARED TO A PHARMACEUTICAL ENVIRONMENT

There was a consistently lower frequency of contamination of doses prepared in pharmaceutical environments compared to clinical environments. However, this finding was not found to be statistically significant for doses prepared as individual lots, despite up to more than a two-fold difference in the overall frequency of dose contamination (4.7% v 2.1% for all individual doses combined, and 2.3% v 2.1% for only those individual doses sampled without administration or prior to administration). This lack of statistical significance could at least in part be explained by the limited data identified for doses prepared as individual lots in pharmaceutical environments (n = 168) (a potential type 2 error due to inadequate statistical power), which could have been compounded by necessary mathematical corrections during the analyses (see limitations below). A consistently narrower range of between study frequencies of dose contamination was found for doses prepared in pharmaceutical

than clinical environments. The lower frequency and variability of contamination of doses prepared in pharmaceutical than clinical environments is intuitive since pharmaceutical facilities are constructed and operated to restrict the number of environmental microbes, incorporate specialised equipment operated by staff who have more consistent and extensive training in the validation in the use of aseptic techniques based on standard protocols, which can reduce the rate of dose contamination[82]. Indeed, the perceived benefits of using pharmaceutical rather than clinical environments for aseptic preparation of parenteral doses have been noted in national documents, such as in the UK[23, 24], and particularly for high-risk products such as PN[22].

In addition to the potential clinical benefits it is also necessary to consider the economic consequences. None of the reviewed studies undertook a cost-effectiveness analysis but the start-up costs for building a new facility to create an appropriate pharmaceutical environment are expected to be high (e.g. several million national currency units in Europe or the United States). A cost-effectiveness analysis dealing only with ongoing costs (e.g. operator training and monitoring for environmental contaminants) needs to take into account factors such as the availability of expert advice and the provision of parenteral doses to hospital staff in a ready to use form, which reduces expenses in other areas.

5.3.2 STUDY 2: DOSE PREPARATION AS INDIVIDUAL LOTS OR AS PART OF A BATCH

For all the doses in both clinical and pharmaceutical environments combined, contamination was found to be higher in doses prepared as individual lots rather than as part of a batch. This difference was found to be significant in pharmaceutical environments but not in clinical environments. It is intuitive that individual doses would be a higher risk than batch doses in pharmaceutical environments since the risks of batch preparation are offset by fewer environmental contaminants, less variable techniques and the availability of specialised equipment. It is also intuitive that potential benefits of batch preparation would be lost in an uncontrolled environment with greater contaminants where more variable techniques are employed and no specialised equipment for batch production is available. These findings support recommendations to limit the expiry of parenteral doses prepared

under aseptic techniques in clinical environments, for example to 24 hours in the UK[22], which effectively preclude batch preparation, and which do not apply to pharmaceutical environments (although different additional requirements do apply).

5.3.3 STUDY 3: THE EFFECT OF UNDERTAKING ADDITIONS TO TERMINALLY STERILISED DOSES

It is reasonable to suggest that aseptic manipulations of a sterile dose can only increase the risk of microbial contamination, but there is limited evidence for such an effect. Unlike the 2009 review[50], which reported no significant effect of making additions to sterilised doses, this updated review found a significantly higher contamination rate of making additions to sterile doses than not making additions (a risk ratio of 1.459 (P = 0.682) and 2.121 (P = 0.026) respectively). This difference can be explained by use of a meta-analysis based on only 3 studies[86, 89, 92] with high statistical heterogeneity (I^2 = 66.45%, P = 0.055) in the 2009 review[50], and a meta-analysis based on 6 studies[86, 89, 92, 99, 100, 106] with lower heterogeneity (I^2 = 22.50%, P = 0.265) in the present review. This finding is consistent with the intuitive idea that aseptic manipulations should be minimised in uncontrolled environments such as hospital wards whenever possible. Nevertheless, adequate protocols and training are still required when it is necessary to prepare doses in clinical environments under aseptic technique. The updated conclusion that additions to sterile doses in clinical environments increase the contamination rate is in line with the findings for the previous two hypotheses.

5.4 LIMITATIONS

The evidence base was limited and generally dependent on poor quality studies which weaken the conclusions of this paper. One of the main limitations is that the studies did not primarily set out to examine the hypotheses raised in this review and so did not use the most appropriate methodologies to address these hypotheses. Furthermore, although there are substantially more studies in the current review than in the 2009 review[50], there is still the possibility that a type 2 error may have arisen when testing specific hypotheses. For example, there were only 168 individual doses prepared in pharmaceutical environments identified. The risk of type 2 error may have also been increased by the need to add 0.5 contaminated doses to a group when

it in reality there were no contaminated doses (for mathematical reasons that allow statistical procedures to be carried out). For example, the rate of contamination of doses prepared as individual lots in a pharmaceutical environment in one study[111] was reported as 0.0% (zero contaminated doses from a total of 18 doses) but in the analysis it was assumed to be 2.4% (0.5 contaminated doses from a total of 18 doses). The effect of this mathematical complication is attenuated as the sample size increases. Another potential limitation is that the studies spanned over a period of over 40 years (1972 to 2013), most of which were more than 10 years old (79% (27 from 34 studies)), which raises the possibility that the overall results do not exactly reflect current practice with currently used products. Finally, the general lack of head-to-head trials (7 from a total of 33 records) has meant that in some cases less robust analyses had to be used. In standard meta-analyses involving head-to-head trials, the differences between two groups of individual studies are established and amalgamated (two group meta-analysis). In contrast, in the present work for hypotheses 1 and 2 the average results from studies involving each group were amalgamated separately (one group meta-analysis) and then compared with each other. This increases the risk of bias since the products tested and conditions in the two comparator groups are less well matched. For example, 38% (3889 from 10272) of the doses prepared in clinical environments had been sampled during or after administration compared to none (from 6280) of the doses prepared in pharmaceutical environments. Furthermore, 40% (4141 from 10272) of doses prepared in clinical environments had been prepared as individual lots compared to 3% (168 from 6280) in pharmaceutical environments.

CHAPTER 6

TRAINING AND EXPERIENCE IN ASEPTIC TECHNIQUES

This chapter examines sub-hypothesis 5: “substituting staff with less training and experience in aseptic techniques in a clinical environment with more trained and experienced staff will reduce the rate of microbial contamination of doses prepared under aseptic technique.”

The aim of this section of the thesis was to develop and undertake a study to test whether improved aseptic technique can reduce variable contamination rates of doses prepared on wards (in clinical environments).

6.1 METHODOLODY

The detailed methods used are described in Chapter Two. In brief, one pharmacy operator and five nurses, each trained in aseptic techniques according to the requirements of their respective professions, participated in the study. The pharmacy operator was experienced in the techniques whereas the nurses were not, since they were newly trained. The infusate drawn up to simulate the preparation of intravenous line flushes was a growth medium (certified sterile) in order to enhance the detection of any microbial contaminants introduced into the doses during preparation. Each operator was requested to use the aseptic techniques they had been formally trained to implement. The syringes were prepared in the treatment room of one of two wards in a single hospital. No individual session was shared between the pharmacy operator and any nurse. The airborne and surface environmental contamination (bioburden) was monitored during each session.

One pharmacy operator and five nurse operators prepared simulated flushes on hospital wards over an eight-month period. Each simulated flush was required the operator to draw up a 4mL fill of a liquid growth media taken from a glass ampoule into a 10mL luer slip syringe using the aseptic technique they had been trained to use. The pharmacy operator had undergone more detailed training in aseptic

techniques than the nurses. In addition, the pharmacy operator underwent further validation of their training over a period of many years to ensure sustained competency in keeping with GMP[28, 29], which was not the case for the nurses, who only had a single training session and little practical experience.

The environment used to prepare the imitation flushes was monitored for airborne and surface microbial contaminants that could have affected the contamination rate achieved by each operator. An assessment as to whether each flush became contaminated during preparation was carried out and reported by a single blinded independent operator (from the Quality Assurance section of the Pharmacy department at University Hospital Southampton NHS Foundation Trust).

6.2 RESULTS

A total of 778 doses were prepared over the eight-month period during 18 sessions lasting a total of 1130 minutes (Table 6.1).

Table 6.1 Operator dose preparation time and contamination rates

Operator	Ward (ward 1 or ward 2)	Sessions (n)	Total syringes prepared (n)	Syringes with contaminant growth (n)	Total time (minutes)
Nurse 1	1	1	50	1	120
Nurse 2	1	2	56	1	115
Nurse 3	1	2	30	5	70
Nurse 4	2	2	98	5	183
Nurse 5	2	1	42	7	95
5 nurses summary	1 and 2	8	276	19	583
Pharmacy operator 1	2	10	502	0	547

6.2.1 DOSE CONTAMINATION

The pharmacy operator achieved a significantly lower dose contamination rate than the nurses ((0.0% (95% CI 0.0%, 0.8%)) vs (6.9% (95% CI 4.5%, 10.5%)); Fisher's exact test; $P < 0.001$). Contamination differed significantly between nurses (2 - 17% of doses; binary logistic regression, $P = 0.018$). Within the nurse-prepared doses, the mean time for syringe preparation varied from 1.87 to 2.40 minutes. Using the nurse

with the median dose preparation speed (2.26 minutes per syringe) as a referent, three of the other four nurses had significantly different dose contamination. Separate binary logistic regression analysis found no significant effect of the ward ($P = 0.266$) and duration of dose preparation ($P = 0.205$) on the contamination of the doses prepared by the nurses.

Binary logistic regression showed significant variation in contamination rates between individual nurses, and this was supported by the presence of significant heterogeneity ($I^2 = 66.46\%$; $P = 0.018$) when the contamination rates obtained by different nurses were amalgamated using a fixed effect meta-analysis. Therefore, it was deemed more appropriate to combine the results achieved by the different nurses using a random effects meta-analysis. This revealed an overall contamination rate of 7.4% (95% CI 3.1%, 16.4%), which was significantly higher ($P = 0.0032$) than that obtained by the pharmacist operator (0.1% (95% CI 0.0%, 1.6%)), established using the logit transformation. (Since the actual contamination rate was zero for the pharmacy operator (a value that causes mathematical difficulties with logarithmic transformations, a value of 0.5 infections was used with the meta-analysis package.)

6.2.2 DOSE PREPARATION TIME

The pharmacy operator prepared the doses more quickly than the nurse operators (mean 2.11 ± 0.013 minutes vs 1.09 ± 0.003 minutes; t-test; $P < 0.001$).

6.2.3 ENVIRONMENTAL CONTAMINATION

All of the ‘settle plate’ results were standardised to a 4-hour exposure from an actual range of 25 to 131 minutes. No significant difference in environmental airborne contamination was found between the pharmacy operator and nurse sessions (mean 4-hour ‘settle plate’ growths 94.3 ± 14.839 nurse operators vs 91.0 ± 8.278 pharmacy operator; t-test; $P = 0.843$).

Working surface contamination immediately following dose preparation was found to be significantly lower for the pharmacy operator compared to the nurses (mean ‘contact plate’ growths 31.9 ± 6.110 nurse operators vs 9.5 ± 2.907 pharmacy operator; t-test; $P = 0.003$).

6.2.4 WORKING SURFACE CLEANING

The pharmacy operator was found to significantly reduce surface contaminants by cleaning the working surface (mean 'contact plate' growths 16.9 ± 4.157 before cleaning vs 0.0 ± 0.000 after cleaning on two separate dates; paired samples t-test; *P* = 0.002). No effect difference was found for the use of trolley or bench, the location on the trolley or bench, or the two different sampling dates.

No additional working surface sampling was undertaken for any of the nurses because none chose to clean the surface before dose preparation.

6.3 DISCUSSION

The preparation of intravenous flushes is a very simple but nevertheless common and important procedure in clinical practice. The results indicate that the pharmacy operator was able to prepare more doses, more quickly, and with significantly less contamination than the nurses, who achieved rates consistent with other reports[50] (and Chapter five).

The most likely reason for the differences in contamination rates between the pharmacy operator and the nurses concerns their respective training and experience, which focused on different operational procedures. For example, the pharmacy operator cleaned the working surface prior to use, whereas the nurses did not. This explains the lower working surface contamination at the end of the pharmacy operator sessions, which could have reduced the flush contamination rate by decreasing touch-transfer of environmental contaminants. Another difference between procedures is that these were carried out more quickly by the pharmacy operator than the nurses. In the absence of any differences in airborne contaminants during the pharmacy and nurses' sessions the slower dose preparation by the nurses led to a greater duration of unsealed component exposure to these environmental contaminants. The slower manipulations undertaken by the nurses may reflect less practical experience in practiced aseptic techniques. The nurses in this study were trained but inexperienced in the use of aseptic techniques. In general, aseptic pharmacy operators are comparatively experienced in the use of aseptic techniques since they form more a focus of their routine work. In contrast typical nurses have to

integrate aseptic techniques with a wide range of other patient care duties and they may not practice their aseptic techniques as frequently as a specialised aseptic pharmacy operator.

Training of UK aseptic pharmacy operators is comprehensive and typically takes a minimum of several months in order to be able to incorporate both practical skills and relevant theory, including the principles of GMP[28, 29]. Following their training, the pharmacy operators are regularly validated to ensure maintenance of adequate strict aseptic techniques[163]. In contrast, the training in aseptic techniques that nurses receive is often much shorter in duration, less comprehensive and not followed by regular validation in the same way. Had the pharmacy operator's and nurses' training and experience been equivalent then it is possible that no significant differences would have been detected. However, it is often the case that different professions may approach the same issue in different ways and this can lead to inevitable divergences in practice. Therefore, it is of importance when designing and implementing training and validation to ensure this reflects best practice from all relevant professional groups and that the necessary reinforcement is put in place to ensure that best practice is sustained over time.

A further finding of this study is that there were significant differences in contamination rates between nurses. The extent to which this is due to differences in training, experience and/or a failure to embed what has been taught into routine practice is uncertain.

The study has at least two limitations. First, the operators were not chosen randomly and therefore are not representative of the general professional populations that may have differences in operational procedures, which may be related to contamination rates. Second, the number of nurses (n = 5) was small and there was only one pharmacy operator. Nevertheless, the number of nurses was sufficiently large to show large and significant between subject differences in contamination rates achieved. The better performance of the pharmacy operator could be interpreted as indicating what can be achieved by putting appropriate training into practice.

This study found differences in contamination rates of aseptically prepared doses between a pharmacy operator and nurses, and between nurses. These findings, which

may be magnified for more complex procedures, have implication for embedding techniques learned from appropriate education and training into routine clinical practice, which may reduce clinical infection rates and increase productivity. There may be an extended role for pharmacy aseptic services in supporting the development of education and validation of nurse operators in the use of appropriate aseptic techniques.

CHAPTER 7
RATE OF INTRAVASCULAR CATHETER INFECTION (IMPACT OF DEFINITION)

This chapter examines sub-hypothesis 6: “Changes in CI rates depend on the definition of CI, which can vary widely.”

The aim of this section of the thesis was to establish and compare the CI rate in a fixed patient cohort using different definitions, and to assess the effects, if any, of missing blood culture data.

7.1 METHODOLOGY

The detailed methods used are described in Chapter Two. During the study period PN was started 163 times and associated with 193 PN episodes with no CI, suspected CI or CI over 2259 PN-days (range 1-115 days per episode). The cohort comprised surgical (n = 154), medical (n = 25) and haemato-oncology patients (n = 14) (54% male/46% female; age of 58.04 ± 1.25 (SEM) years, and range 17 - 89 years). PN was administered through 223 central intravenous catheters (no peripheral administration).Table 7.1 provides descriptive statistics for CI using all available data for each definition. In all cases of suspected CI at least one blood culture was typically taken and reported: 6 episodes of suspected CI did not have blood culture but all of those episodes had catheter tip culture. However, this was often not sufficient to satisfy the requirement of the published definitions, in which cases the data were effectively absent (missing).

Table 7.1 Intravascular catheter infection rates during parenteral nutrition

Definition of intravascular catheter infection[a]	**Episodes with available data (n)**[b]	**Intravascular catheter infection (%)**	
		n	**%**
Intravenous catheter removal due to suspected intravascular catheter infection	193	43	22.3
Parenteral nutrition stopped due to suspected intravascular catheter infection	193	71	36.8
Retrospective clinical diagnosis	193	25	13.0
HELICS	144	13	9.0
ESPEN	141	3	2.1
MM	185	23	12.4
CDC	185	19	10.3
CDC 2002[c]	185	22	11.9

[a] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).
[b] Episodes with complete blood culture data, when required for a particular definition.
[c] The 2002 version of the Centres for Disease Control and Prevention for comparison with the 2011 version of the same definition.

7.2 RESULTS

7.2.1 AVAILABLE DATA ANALYSIS

Table 7.2 shows both unpaired and paired comparisons of CI rates, some involving complete case analysis and others incomplete case analysis.

Table 7.2 A matrix of 21 unpaired and 21 paired comparisons of intravascular catheter infection rates based on the available data analysis using different definitions of intravascular catheter infection

		Available case analysis (unpaired analysis)			**Available case analysis (paired analysis)**			
First definition[a]	**Second definition[a]**	**Episodes (n for first definition, second definition)**	**Intravascul-ar catheter infection (% for first definition, second definition)**	**Fisher exact test (*P*)**	**Episodes (n for first definition, second definition)**	**Intravascul-ar catheter infection (% for first definition, second definition)**	**Kappa ± SEM[b]**	**McNemar test (*P*)**
Intravenous catheter removal due to suspected intravascular catheter infection	Parenteral nutrition stopped due to suspected intravascular catheter infection	193, 193	22.3, 36.8	0.003	193, 193	22.3, 36.8	0.636 ± 0.057	< 0.001
Intravenous catheter removal due to suspected intravascular catheter infection	Retrospective clinical diagnosis	193, 193	22.3, 13.0	0.023	193, 193	22.3, 13.0	0.508 ± 0.078	0.001

Intravenous catheter removal due to suspected intravascular catheter infection	HELICS	193, 144	22.3, 9.0	0.001	144, 144	16.7, 9.0	0.541 ± 0.102	0.007
Intravenous catheter removal due to suspected intravascular catheter infection	ESPEN	193, 141	22.3, 2.1	< 0.001	141, 141	15.6, 2.1	0.127 ± 0.091	< 0.001
Intravenous catheter removal due to suspected intravascular catheter infection	MM	193, 185	22.3, 12.4	0.014	185, 185	21.1, 12.4	0.541 ± 0.080	0.002
Intravenous catheter removal due to suspected intravascular catheter infection	CDC[c]	193, 185	22.3, 10.3	0.002	185, 185	21.1, 10.3	0.440 ± 0.085	< 0.001

Parenteral nutrition stopped due to suspected intravascular catheter infection	Retrospective clinical diagnosis	193, 193	36.8, 13.0	< 0.001	193, 193	36.8, 13.0	0.356 ± 0.062	< 0.001
Parenteral nutrition stopped due to suspected intravascular catheter infection	HELICS	193, 144	36.8, 9.0	< 0.001	144, 144	25.0, 9.0	0.412 ± 0.088	< 0.001
Parenteral nutrition stopped due to suspected intravascular catheter infection	ESPEN	193, 141	36.8, 2.1	< 0.001	141, 141	23.4, 2.1	0.133 ± 0.070	< 0.001
Parenteral nutrition stopped due to suspected intravascular catheter infection	MM	193, 185	36.8, 12.4	< 0.001	185, 185	35.7, 12.4	0.325 ± 0.065	< 0.001

Parenteral nutrition stopped due to suspected intravascular catheter infection	CDC[c]	193, 185	36.8, 10.3	< 0.001	185, 185	35.7, 10.3	0.286 ± 0.063	< 0.001
Retrospective clinical diagnosis	HELICS	193, 144	13.0, 9.0	0.299	144, 144	13.2, 9.0	0.650 ± 0.102	0.109
Retrospective clinical diagnosis	ESPEN	193, 141	13.0, 2.1	< 0.001	141, 141	12.1, 2.1	0.274 ± 0.127	< 0.001
Retrospective clinical diagnosis	MM	193, 185	13.0, 12.4	1.000	185, 185	12.4, 12.4	0.851 ± 0.059	1.000
Retrospective clinical diagnosis	CDC[c]	193, 185	13.0, 10.3	0.428	185, 185	12.4, 10.3	0.839 ± 0.064	0.219
HELICS	ESPEN	144, 141	9.0, 2.1	0.018	141, 141	7.8, 2.1	0.409 ± 0.164	0.008
HELICS	MM	144, 185	9.0, 12.4	0.376	144, 144	9.0, 13.2	0.790 ± 0.082	0.031
HELICS	CDC[c]	144, 185	9.0, 10.3	0.852	144, 144	9.0, 11.1	0.809 ± 0.083	0.375
ESPEN	MM	141, 185	2.1, 12.4	< 0.001	141, 141	2.1, 12.1	0.274 ± 0.127	< 0.001
ESPEN	CDC[c]	141, 185	2.1, 10.3	0.003	141, 141	2.1, 9.9	0.329 ± 0.144	0.001
MM	CDC[c]	185, 185	12.4, 10.3	0.623	185, 185	12.4, 10.3	0.893 ± 0.053	0.125

[a] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).

[b] The summary kappa ± SEM was found to be: 0.503 ± 0.057 for all comparisons combined (n = 21); 0.454 ± 0.078 for clinical-published comparisons (n = 12); 0.613 ± 0.104 for published-published comparisons (n = 6); and 0.501 ± 0.088 for clinical-clinical comparisons (n = 3).

[c] The comparison between the 2002 and 2011 versions of the CDC definition yields a kappa ± SEM of 0.918 ± 0.047 and a McNemar p-value of 0.250 based on 185 episodes for comparison in an available case analysis with n = 22 (11.89%).

7.2.1.1 Unpaired comparisons

The CI rates varied from 2.1-12.4% for the published definitions, and up to 36.8% when the clinical definitions were included. Significant differences were found in 15 of the 21 comparisons (Fisher's exact test). In all cases, the frequency was lower using the published definitions, all of which required blood cultures.

7.2.1.2 Paired comparisons

As with the unpaired comparisons of CI, the paired comparisons (same subjects for each comparison) varied widely between definitions, ranging from 2.1 - 14.6% for the published definitions, and up to 36.8% when the clinical definitions were included. There was systematic over or underestimation in 16 out of the 21 pairwise comparisons (76.2%) (McNemar test). Figure 7.1 illustrates these results and the wide variability in agreement (kappa statistic) between 21 sets of comparisons, with a range of kappa values of 0.127 - 0.893 (Table 7.2). The strength of agreement beyond chance was more frequently 'poor' or 'fair' (42.9%; 9/21 comparisons) than 'good' or 'very good' (33.3%; 7/21 comparisons). A random effects meta-analysis involving amalgamation of the 21 kappa statistics yielded a summary statistic of 0.503 ± 0.057 (SEM), corresponding to only 'moderate' agreement. A 'moderate' agreement was also found for two subgroup comparisons (amalgamated kappa ± SEM: 0.454 ± 0.078 for clinical-published comparisons; and 0.501 ± 0.088 for clinical-clinical comparisons), and a 'good' agreement found for published-published comparisons (amalgamated kappa ± SEM: 0.613 ± 0.104).

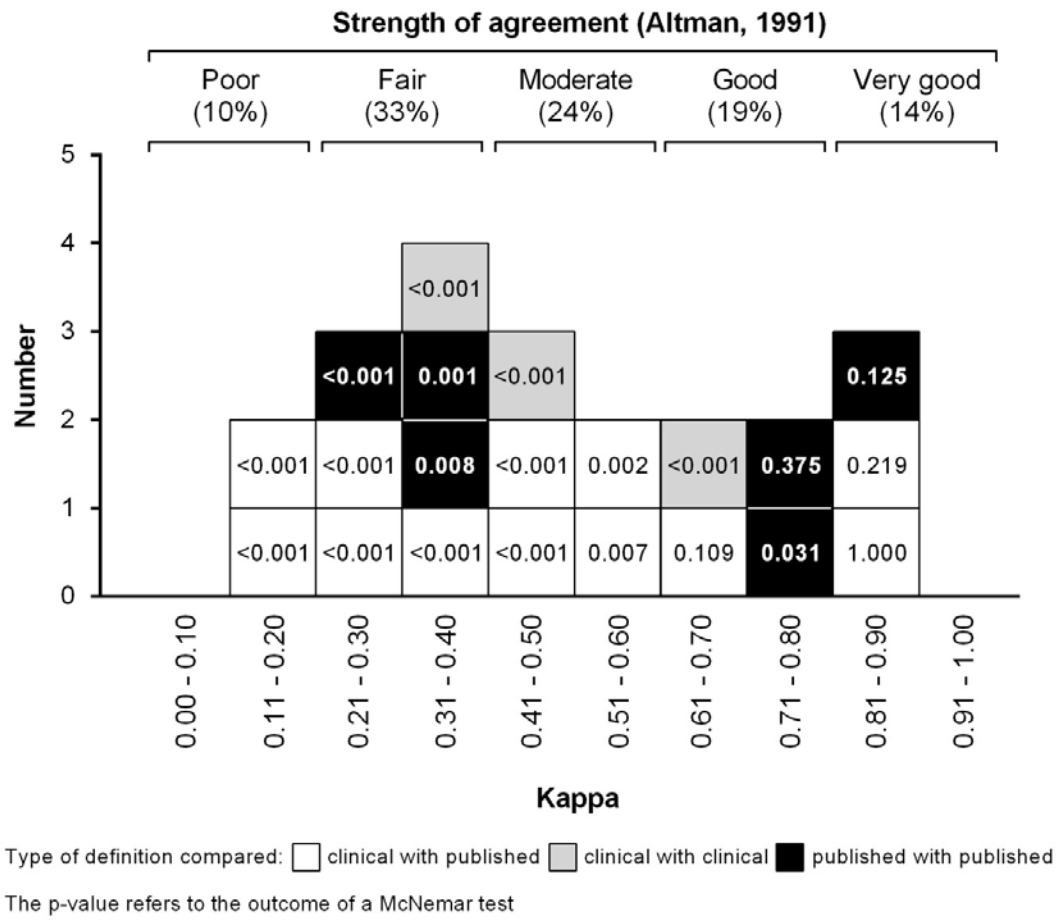

Figure 7.1 The distribution of kappa values (strength of agreement beyond chance) for intravascular catheter infection episodes according to different definitions (n = 141 to 193 for different definitions) using available case analyses. The p-value within each box indicates the McNemar test for paired comparisons

7.2.2 ANALYSES DEALING WITH MISSING DATA

7.2.2.1 Potential bias of missing data

Table 7.3 reports on the existence of potential selection bias when only the known results are used (i.e. whether missing data for the published definitions were randomly missing). When each complete clinical definition dataset was subdivided according to the availability (group 1) or unavailability (group 2) of data elements from alternative (published) definitions, group 2 had a higher CI frequency than group 1 in 14 of the 15 comparisons, being at least 1.5 times higher for 13 comparisons (4 significant). Furthermore, within each published definition (5 comparisons) imputed data showed significantly higher CI frequencies in group 2 for

each comparison. Except for one result which was 21.3-fold higher in group 2 than group 1, the remaining results were 3.5 to 4.5-fold higher in group 2.

Table 7.3 Intravascular catheter infection rates by type of analysis and published definition of intravascular catheter infection

	Available case analysis			Sensitivity analysis			Intention to categorise analysis[b]	
Intravascular catheter infection definition[a]	**Sample size (n)**	**Intravascular catheter infection (%)**	**Sample size (n)**	**Intravascular catheter infection (%)**			**Sample size (n)**	**Intravascular catheter infection (%)**
				Model A[c]	**Model B[d]**	**Model C[e]**		
HELICS	144	9.0	193	32.1	6.7	4.7	193	16.9
ESPEN	141	2.1	193	28.5	1.6	1.6	193	13.8
MM	185	12.4	193	16.1	11.9	8.3	193	13.9
CDC	185	10.3	193	14.0	9.8	6.7	193	11.4
CDC 2002[f]	185	11.9	193	15.5	11.4	8.3	193	13.2

[a] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).

[b] Intention to categorise analysis was carried out after multiple imputation. Ten imputations for each case were undertaken using a Mersenne Twister random number generator with a fixed starting point of 2000000 with the following predictor variables: patient age (range 17 to 89 years); gender (male or female); days of inpatient parenteral nutrition (range 1 to 115 days); patient type (surgical, medical or haemato-oncology); and each of the five published definitions of intravascular catheter infection.

[c] In model A all episodes with inadequate blood data are assigned to intravascular catheter infection.

[d] In model B all episodes with inadequate blood data case are assigned to no intravascular catheter infection.

[e] In model C all episodes with inadequate blood data are assigned to no intravascular catheter infection and all episodes of catheter intravascular catheter infection with another source that grew the same microbe were assigned to no intravascular catheter infection.

[f] The 2002 version of the Centres for Disease Control and Prevention for comparison with the 2011 version of the same definition.

7.2.2.2 Sensitivity analysis

The sensitivity results varied widely (Table 7.4), irrespective of whether the results of the same CI definitions were expressed according to the type of model (14.0 - 32.1% using model A, 1.6 - 11.9% using model B and 1.6 - 8.3% using model C) or the results of the same model were expressed according to the type of CI definition (Figure 7.2).

Table 7.4 Potential bias in intravascular catheter infection rates when the clinical definitions with complete datasets (n = 193 episodes) were divided into two groups, one corresponding to episodes with complete data for the published definitions (group 1) and the other corresponding to episodes with missing data for the published definitions (group 2)

Intravascular catheter infection definition[a]	**Group 1[b] sample size (n)**	**Group 1[b] intravascular catheter infection (complete dataset) (%)**	**Group 2[b] sample size (n)**	**Group 2[b] intravascular catheter infection (missing data) (%)**	**Difference in intravascular catheter infection between groups 1 and 2 (%)**	**Fisher's exact test, *P*[c]**
Intravenous catheter removal due to suspected intravascular catheter infection						
HELICS	144	16.6	49	38.8	22.2	0.002
ESPEN	141	15.6	52	40.4	24.8	0.001
MM	185	21.1	8	50.0	28.9	0.075
CDC	185	21.1	8	50.0	28.9	0.075
CDC 2002[d]	185	21.1	8	50.0	28.9	0.075
Parenteral nutrition stopped due to suspected intravascular catheter infection						
HELICS	144	25.0	49	71.4	46.4	< 0.001
ESPEN	141	23.4	52	73.1	49.7	< 0.001
MM	185	35.7	8	62.5	26.8	0.146
CDC	185	35.7	8	62.5	26.8	0.146
CDC 2002[d]	185	35.7	8	62.5	26.8	0.146

Retrospective clinical diagnosis						
HELICS	144	13.2	49	12.2	1.0[e]	1.000
ESPEN	141	12.1	52	15.4	3.3	0.629
MM	185	12.4	8	25.0	12.6	0.277
CDC	185	12.4	8	25.0	12.6	0.277
CDC 2002[d]	185	12.4	8	25.0	12.6	0.277

[a] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).

[b] Group 1 represents all cases of no suspected intravascular catheter infection plus cases of suspected intravascular catheter infection if adequate blood culture data for the relevant definition were available and group 2 represents cases of suspected intravascular catheter infection if adequate blood culture data for the relevant definition were not available.

[c] Two-tailed comparison between group 1 and group 2.

[d] The 2002 version of the Centres for Disease Control and Prevention for comparison with the 2011 version of the same definition.

[e] The only case in this table where the intravascular catheter infection rate was higher in group 1 than group 2.

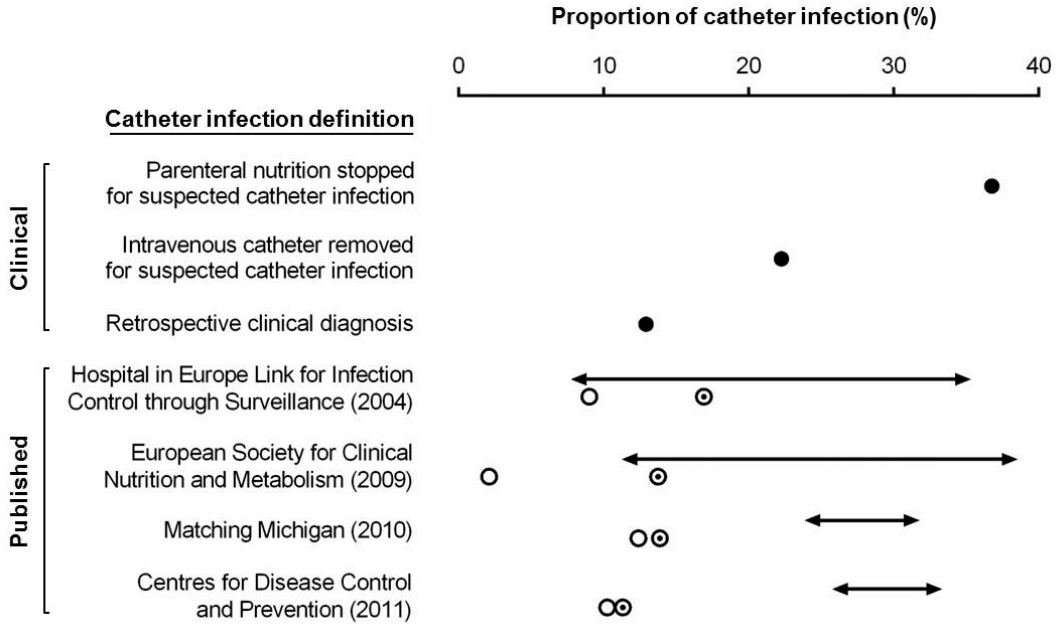

Figure 7.2 Intravascular catheter infection rates by definition and type of analysis. Solid circles = available case analyses (clinical definitions with complete datasets), horizontal arrows = sensitivity analyses using models A, B and C, open circles = available case analyses (published definitions with incomplete datasets), and open circle with dot = intention to categorise analyses. All episodes associated with inadequate blood cultures were assigned to the intravascular catheter infection group (model A) or to the no intravascular catheter infection group (model B). In a third model (model C) the no intravascular catheter infection group not only included all episodes with inadequate blood culture (as for model B), but also all episodes in which the same microbe responsible for an intravascular catheter infection was identified at another site (for example from an infected intra-abdominal collection). Model C assumed that infections of the central venous catheter used for PN arose from another site (rather than *vice versa*).

The wide variation in pairwise comparison (kappa values 0.076 - 1.00; 'poor' to 'very good' agreement) were mostly associated with significant under or overestimation between models (McNemar test) (Table 7.5). When the extreme sensitivity analyses involving models A and C were compared (Table 7.5) even larger differences in the CI proportion were observed in association with more significant results ($P < 0.001$ for each comparison). The individual kappa values remained variable at 0.076 - 0.642 ('poor' to 'good' agreement).

Table 7.5 Agreement beyond chance (kappa statistics) and systematic bias (McNemar test) associated with pairwise comparisons of intravascular catheter infection rates obtained using models A, B and C of the sensitivity analyses (n = 193)[a]

Definition of intravascular catheter infection[b]	**Model[a]**	**Kappa**	**McNemar test, *P***
Comparisons of models (using the same intravascular catheter infection definition)			
HELICS	A v B	0.265 ± 0.061	< 0.001
HELICS	A v C	0.187 ± 0.055	< 0.001
HELICS	B v C	0.808 ± 0.093	0.125
ESPEN	A v B	0.076 ± 0.042	< 0.001
ESPEN	A v C	0.076 ± 0.042	< 0.001
ESPEN	B v C	1.000 ± 0.000	1.000
MM	A v B	0.828 ± 0.059	0.008
MM	A v C	0.642 ± 0.083	< 0.001
MM	B v C	0.801 ± 0.072	0.016
CDC[c]	A v B	0.803 ± 0.067	0.008
CDC[c]	A v C	0.615 ± 0.091	< 0.001
CDC[c]	B v C	0.796 ± 0.080	0.031
Comparisons of definitions (using the same intravascular catheter infection model)			
HELICS v ESPEN	A	0.890 ± 0.036	0.039
HELICS v MM	A	0.412 ± 0.069	< 0.001
HELICS v CDC[d]	A	0.400 ± 0.069	< 0.001
ESPEN V MM	A	0.239 ± 0.075	0.001
ESPEN V CDC[d]	A	0.249 ± 0.074	< 0.001
MM v CDC[d]	A	0.919 ± 0.040	0.125
HELICS v ESPEN	B	0.359 ± 0.152	0.002
HELICS v MM	B	0.696 ± 0.089	0.002
HELICS v CDC[d]	B	0.728 ± 0.091	0.070
ESPEN v MM	B	0.209 ± 0.102	< 0.001
ESPEN v CDC[d]	B	0.253 ± 0.119	< 0.001
MM v CDC[d]	B	0.893 ± 0.053	0.125
HELICS v ESPEN	C	0.488 ± 0.177	0.031
HELICS v MM	C	0.702 ± 0.105	0.016
HELICS v CDC[d]	C	0.808 ± 0.093	0.125
ESPEN v MM	C	0.297 ± 0.134	< 0.001
ESPEN v CDC[d]	C	0.359 ± 0.152	0.002
MM v CDC[d]	C	0.888 ± 0.064	0.250

[a] Model A = all episodes with inadequate blood culture data are assigned to intravascular catheter infection in model; model B = all episodes with inadequate blood culture data are assigned to no intravascular catheter infection; and model C = all episodes with

inadequate blood culture data are assigned to no intravascular catheter infection and all episodes in which the same microbe responsible for a CI was identified at another site (for example from an infected intra-abdominal collection) were also assigned to no intravascular catheter infection (analyses based on data in Table 7.3).
[b] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).
[c] For the 2002 version of the CDC definition the comparisons are as follows: A v B, kappa = 0.823 ± 0.060 and McNemar $P = 0.008$; A v C, kappa = 0.659 ± 0.083 and McNemar $P < 0.001$; and B v C, kappa = 0.825 ± 0.069 and McNemar $P = 0.031$.
[d] The comparisons between the 2011 and 2002 versions of the CDC definition are as follows: model A, kappa = 0.938 ± 0.035 and McNemar $P = 0.250$; model B, kappa = 0.918 ± 0.047 and McNemar $P = 0.250$; and model C, kappa = 0.888 ± 0.064 and McNemar $P = 0.250$.

7.2.2.3 Intention to categorise analysis

The different CI rates (% of total PN episodes) between the intention to categorise and available case analyses ranged from 1.1 - 11.7% (based on Table 7.4). The intention to categorise analysis (modelled estimates of missing data) yielded less variable results than the sensitivity analysis (extreme assumptions about missing data). The kappa values varied from 0.181 - 0.506 ('poor' to 'moderate' agreement), which were mostly associated with non-significant under or overestimation between models (McNemar test) (Table 7.6). Figure 7.2 shows CI rates were consistently higher using intention to categorise than available case analyses for the published definitions. Both were consistently lower than those obtained by the clinical definitions, which had complete data.

Table 7.6 Agreement beyond chance (kappa) and systematic bias (McNemar test) associated with pairwise comparisons of intravascular catheter infection rates obtained using intention to categorise analysis (n = 193)[a]

Definitions compared[b]	**Episodes of intravascular catheter infection (%; first v second definition)[c]**	**Kappa ± SEM[d]**	**McNemar test, *P*[e]**
HELICS v ESPEN	16.9 v 13.8	0.181 ± 0.069	0.430
HELICS v MM	16.9 v 13.9	0.501 ± 0.055	0.327
HELICS v CDC[f]	16.9 v 11.4	0.506 ± 0.057	0.043
ESPEN v MM	13.8 v 13.9	0.194 ± 0.059	1.000
ESPEN v CDC[f]	13.8 v 11.4	0.210 ± 0.062	0.500
MM v CDC[f]	13.9 v 11.4	0.194 ± 0.059	0.125

[a] The intention to categorise analysis was based on multiple imputation and data presented in Table 7.3. Ten imputations for each case were undertaken using a Mersenne Twister random number generator with a fixed starting point of 2000000 with the following predictor variables: patient age (range 17 to 89 years); gender (male or female); days of inpatient parenteral nutrition (range 1 to 115 days); patient type (surgical, medical or haemato-oncology); and each of the five published definitions of intravascular catheter infection.
[b] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).
[c] The range for the 10 multiple imputations is presented.
[d] The summary result for the 10 multiple imputations is presented.
[e] The single McNemar test was undertaken for each comparison using the pooled summary data for the 10 imputations.
[f] For the 2002 version of the CDC definition the intravascular catheter infection was 13.2%. The comparisons between the 2011 and 2002 versions of the CDC definition are: kappa ± SEM = 0.865 ± 0.018; and McNemar range *P* = 0.219.

7.2.3 SPECIFIC COMPARISONS

7.2.3.1 Historical comparison of a single definition

A historical comparison was made between two versions of the CDC criteria (2002[30] and 2011[6]), both of which lacked adequate blood culture results for the same 8 episodes. The results using available case analysis were comparable with respect to CI (11.9% for the 2002 version v 10.3% for the 2011 version; Table 7.4), and associated with 'very good' agreement (kappa = 0.918 ± 0.047; Table 7.2) and a non-significant McNemar test result (*P* = 0.250). With the newer version as the referent, the earlier version had a sensitivity of 100.0% and a specificity of 98.2%, with an area under the ROC curve of 0.991 ± 0.006 (SEM). Very similar results were obtained using intention to categorise analysis (12.3% CI for the 2002 version v

10.7% for the 2011 version, with 90.5 – 100.0% sensitivity and 95.9 – 98.3% specificity).

7.2.3.2 Comparison between two chronological periods

Another specific comparison involved splitting all the data into two chronological periods to compare the CI proportion during the first part of the study with the second part so that differences between periods could be examined by CI definition. Both the available case and intention to categorise analyses (Table 7.7) showed an overall reduction in CI between the first and second period of 2.9 – 11.3% depending on the definition used.

Table 7.7 A comparison of intravascular catheter infection rates when the complete datasets (n = 93) were divided into two chronological groups, one corresponding to the first 97 episodes (chronological group 1) and the other corresponding to the second 96 episodes (chronological group 2)

Definition of intravascular catheter infection [a]	**Total episodes in comparison (n)**	**Chronological group 1**[b] **sample size (n)**	**Chronological group 1**[b] **intravascular catheter infection (%)**	**Chronological group 2**[b] **sample size (n)**	**Chronological group 2**[b] **intravascular catheter infection (%)**	**Difference in intravascular catheter infection between chronological groups 1 and 2 (%)**	**Fisher's exact test, *P*[c]**
Available case analysis							
Intravenous catheter removal due to suspected intravascular catheter infection	193	97	23.7	96	20.8	2.9	0.730
Parenteral nutrition stopped due to suspected intravascular catheter infection	193	97	39.2	96	34.4	4.8	0.551
Retrospective clinical diagnosis	193	97	18.6	96	7.3	11.3	0.031

HELICS	144	70	14.3	74	4.1	10.2	0.042
ESPEN	141	69	4.4	72	0.0	4.4	0.115
MM	185	90	15.5	95	9.5	6.0	0.266
CDC	185	90	14.4	95	6.3	8.1	0.090
CDC 2002[d]	185	90	15.5	95	8.4	7.1	0.174
Intention to categorise analysis[e]							
HELICS	193	97	22.0[f]	96	11.9[f]	10.1	0.081[g]
ESPEN	193	97	15.2[f]	96	12.4[f]	2.8	0.139[g]
MM	193	97	18.5[f]	96	9.8[f]	8.7	0.679[g]
CDC	193	97	16.1[f]	96	6.6[f]	9.5	0.040[g]
CDC 2002[d]	193	97	17.7[f]	96	8.7[f]	9.0	0.085[g]

[a] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).
[b] Chronological group 1 is the chronological first half of the episodes (n = 97) and chronological group 2 is the chronological second half of the episodes (n = 96).
[c] Two-tailed comparison between chronological group 1 and chronological group 2.
[d] The 2002 version of the Centres for Disease Control and Prevention for comparison with the 2011 version of the same definition.
[e] The intention to categorise analysis was based on multiple imputation (see Methods).
[f] The pooled result for the 10 multiple imputations is presented.
[g] The single Fisher's exact test was undertaken for each comparison using the pooled summary data for the 10 imputations.

The available case analysis showed significant reductions in CI for two of the definitions (retrospective clinical diagnosis and HELICS[121]) but not for the other five. With the intention to categorise analysis there was a significant reduction in CI for one definition[6]. For the published definitions there was no consistent increase or decrease of the CI proportion between the results of the available case and intention to categorise analyses.

7.3 DISCUSSION

This cohort study found that the CI frequency was strongly affected by definition of CI (2.1 - 36.8%) and by procedures used to deal with missing data. The study also quantified the discrepancies in suspected CI rates due to the use of different criteria, which were considered large enough to be frequently problematic in clinical practice. The findings have important implications about using CI as a benchmark for quality of clinical practice[2].

Since CI frequency can be reduced by more than an order of magnitude by changing the definition of CI, care should be taken not to just ascribe such a reduction to improved clinical practice. Furthermore, even the same definition can produce misleading results if used inconsistently (see below). Similarly the use of different procedures (including no procedure) to analyse missing data can also be problematic, as can the use of different versions of the same definition. In a comparison between two versions of the CDC definition (2011[6] and 2002[30]), CI was more prevalent (although not statistically significant) when using the earlier version, which identified 3 more CI episodes. The results (Table 7.3) indicate missing data (absence of sufficient blood cultures) for published definitions were not randomly missing, since the 'missing' datasets were associated with a 1.5 - 3 fold higher CI prevalence when examined using a procedure involving clinical definitions, and even higher when using intention to categorise procedure. Assuming that a missing blood culture is negative can be risky, with potential distortion of results. Therefore, any reports of CI incidence should specify whether such assumptions have been made. Furthermore, allocating suspected CI to no CI or CI requires careful consideration since different methods yield different results. The sensitivity and intention to categorise analyses confirmed that the potential errors can be large (a difference > 25% depending on definition), especially when the proportions of missing data is

large. While intention to categorise analysis of missing data may reduce selection bias, as suggested by the findings of this study, there is no substitute for a complete dataset analysis, which means that every effort should be made to minimise missing data. Retrospective clinical diagnosis can be valuable, especially when a multidisciplinary team is involved, but it has little or no value for prospective management. Furthermore, since retrospective analysis is generally not blinded there may be a risk of reporting bias, especially when there are pressures to reduce CI rates. This study also indicates that even when individual methods are used consistently, changes over time may depend on definition used. For example, in the before-and-after analysis involving the first and second part of the study significant 'improvements' were demonstrated using some definitions but not others.

Since this study did not aim to examine accuracy of different CI definitions, the kappa statistic was used to assess strength of agreement between definitions. Nevertheless, this study raises clinical concerns about the methods that are used to establish CI rates. First, it is somewhat disturbing that published definitions produced the observed variability (actual difference 10.30% or relative difference over 400%) (and even more pronounced differences when clinical definitions were used). Second, most pairwise comparisons demonstrated only 'poor' to 'moderate' agreement beyond chance, and the overall amalgamated summary for all pairwise comparisons was only 50.3% agreement beyond chance. Third, most pairwise comparisons in the sensitivity and intention to categorise analyses showed significant bias (systematic over or underestimation relative to the other method). For example, a 'traditional' paired peripheral and central blood culture definition (included in one definition[32]) was associated with a significantly lower CI rate than each of the other definitions. Given the potentially large effects observed in this study, it seems important that local and national initiatives should consider such issues in more detail. Although there are guidelines recommending hospital audits of CI rates without methodological recommendations[33] and reports describing suspected CI or CI may be based on expert opinion rather than any one definition[2], consideration should also be given to clinical governance issues and the use of consistent and reproducible reference methods that would allow more meaningful comparisons to be made within and between centres over time.

The published definitions provide various criteria or groups of criteria to establish CI, including quantitative or semi-quantitative blood cultures and time to blood culture positivity. Since all of the information even within the same definition of CI may not be routinely available, CI cannot be excluded. Despite this limitation the present study indicates widely different CI rates using the same clinical and laboratory information. It also suggests the need to establish a more practical way for measuring and comparing rates and the scientific and clinical communities need to agree on the most appropriate workable CI definition. The following could be considered: criteria for a suspected CI; reason for choosing a particular definition; simultaneous infection with the same species of microbe at a different site; inadequate blood cultures; changes in data management; and updates to existing CI definitions. Furthermore, care should be taken not to be confused by commonly used terminology (e.g. 'catheter associated (or suspected or related) bloodstream infection'), which may aim to indicate different aspects of CI such as likelihood of the intravenous catheter being the primary septic site (despite the frequent difficulties of establishing the primary site with certainty). This can be confusing and lead to misinterpretation and inaccurate reporting so some authorities avoid using such terminology altogether. There is a clear need to establish consistent terminology, as well as consistent criteria and methodology for assessing 'catheter infection', so that meaningful comparisons can be made within and between centres whilst facilitating methods to investigate the mechanisms by which such infections develop.

Future research could improve clarity by addressing the above issues and allow more meaningful comparisons to be carried out, especially when timing of patient transfers and characteristics of unmatched cohorts are involved. In this study no adjustment was made for the paired comparisons since they involved the same subjects, but for different groups of subjects appropriate adjustments could become important. In the meantime practical and theoretical work needs to guide and justify the considerable investment in services and research aiming to reduce CI rates. As a minimum, national guidance should address the need for consistent CI definition(s), consistent procedures to identify suspected CI and a consistent approach for dealing with inadequate blood culture and simultaneous infection at another site. Such clarity may be particularly important when there are incentives for reporting a lower CI rate or penalties for reporting a higher CI rate.

7.4 LIMITATIONS

This study considered several frequently used or reported CI definitions but did not systematically identify and examine all available definitions. It also involved a typical cohort of patients from a single centre for which not all the data for each definition were available. Nevertheless, the findings raised pragmatic, valid and relevant issues for routine clinical practice, including those relating to inadequate blood sampling for microbial cultures. Another limitation is that the study was undertaken retrospectively following detailed examination of medical records. Prospective or combined prospective and retrospective studies could offer additional insights.

CHAPTER 8

SUMMARY AND CONCLUSION

8.1 MAIN FINDINGS

This work has developed and strengthened the concept of the ward-pharmacy intravenous prescribing cycle, which can be appreciated by reference to Figure 8.1.

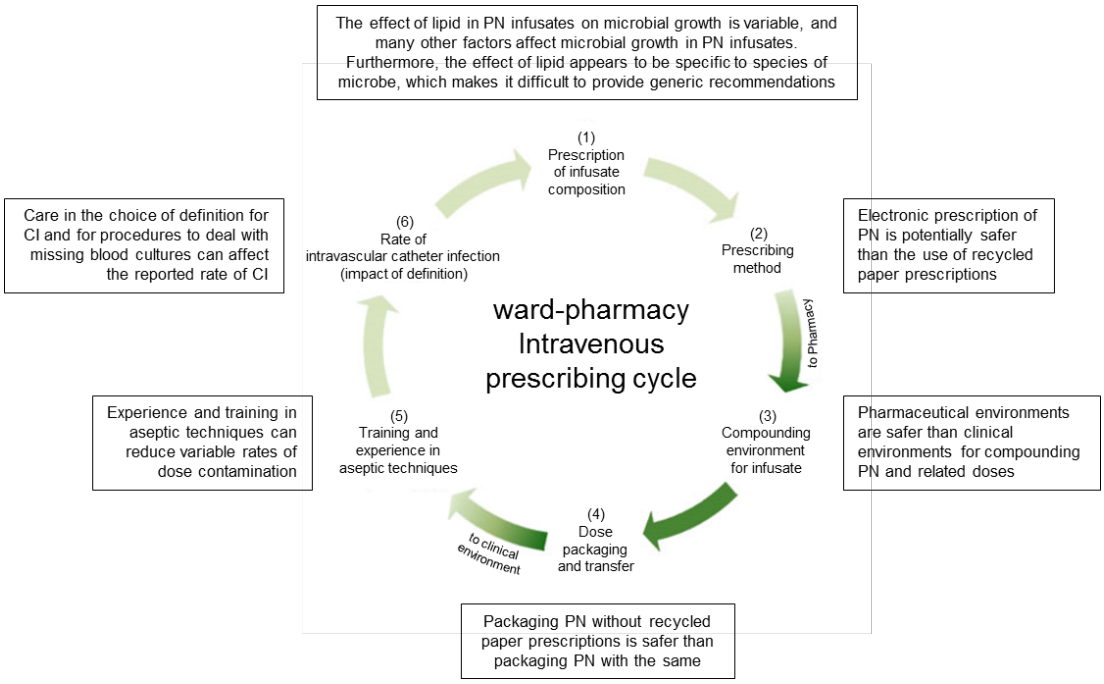

Figure 8.1 Evidence found for steps of the ward-pharmacy intravenous prescribing cycle

8.2 INTERVENTIONS AND INTER-RELATIONSHIPS

The idea that multiple evidence-based interventions can affect microbial risk within the ward-pharmacy intravenous prescribing cycle is founded on the sequential inter-dependence of the various steps within the cycle. Below are examples of how each of the six steps within the cycle can affect the next step and potentially other steps directly, ultimately influencing the reported CI rate:

(1) The prescription of PN infusates of particular composition can affect the choice of prescription method. for example, if considered a lower microbial risk, lipid-free PN may require a more complex prescription proforma.

(2) The prescription method can affect the compounding environment. For example, the environmental contaminants in the clinical environment where handwritten recycled paper prescriptions are written may be transferred to the compounding environment.

(3) The compounding environment for PN infusates can affect dose packaging and transfer. For example, standard operating procedures in pharmaceutical environments are expected to include the method of packaging and transfer of compounded products.

(4) The packaging and transfer of compounded doses can affect choice and use of aseptic techniques in clinical environments. For example, administration of PN bags with contaminated surfaces may require the use of different aseptic techniques to administration of PN bags without contaminated surfaces.

(5) The effect of training and experience in aseptic techniques can independently affect the reported CI rate. For example, the use of inadequate aseptic technique when administering PN may infect the intravenous catheter resulting in an episode of CI.

(6) The rate of CI according to different definitions can affect the prescription of PN infusates of particular composition. For example, a high rate may change the prescription of PN to infusates considered to have a lower risk of microbial growth.

In addition, each of the six steps within the cycle may directly affect other steps within the cycle. For example, training and experience in aseptic techniques apply to pharmaceutical environments as well as to hospital wards. Further examples include the potential effect of microbial risk of handwritten recycled paper prescriptions on choice of aseptic technique.

8.3 IMPLICATIONS OF INTERVENTIONS AND INTER-RELATIONSHIPS

The ward-pharmacy intravenous nutrition prescribing cycle involves a number of steps, and each of those steps appears amenable to intervention that can affect the overall microbial risk to patients administered PN. Since each step within the ward-pharmacy intravenous prescribing cycle can influence microbial risk, action is required at each of the steps, especially since one step can have a direct influence on more than one of the other steps potentially further increasing the risk to patients.

The extent to which these interventions, alone or in combination, affect the CI rate in practice depends on the definition of CI used and requires further research. In addition, the strength of evidence found for each step in the cycle varies and there are some weaknesses that need to be addressed in future research that could further strengthen the success of pharmacists in developing a niche in PN prescribing. For example, whilst judicious PN prescription may require a consideration of the potential for microbial growth in different PN formulations, it is not solely dependent on the presence or absence of lipid and furthermore the relative effects of a number of PN components are yet to be determined. In addition, whilst a synthesis of the current evidence base for compounding PN and related doses in pharmaceutical rather than clinical environments favoured the use of the pharmaceutical environments, all of the studies were rated as either ‘low’ or ‘very low’ quality (using the GRADE system[77, 78]) and higher quality evidence is desirable. This work found a benefit of an improvement in training and greater experience in manipulating doses for parenteral administration under aseptic technique, although further studies would again improve the evidence base. The best way of implementing this training in practice in a pragmatic manner remains to be explored. The evidence for the use of freshly printed electronic rather than handwritten recycled paper prescriptions for PN to reduce microbial risk transferring between the clinical and pharmaceutical environments is more convincing, but there is a need to extend the studies to different care settings that may utilise different procedures and harbour different types of microbe. In addition, there is a clear indication that different CI definitions, and different procedures used to handle missing blood culture, can affect the reported rate of CI. This means a consensus is required that would allow meaningful

evaluations of clinical practice, audits, quality improvement interventions and comparisons both within and between centres.

8.4 CONCLUSION

The work contained within this thesis found the overarching hypothesis “simple measures in clinical and pharmaceutical environments can reduce potential exposure of intravenous lines and infusates to microbial contamination, limit microbial growth in PN and reduce the rate of CI to an extent that depends on the definition applied” to be true. To achieve this effectively there needs to be an appropriate operational infrastructure to ensure that the ward-pharmacy intravenous prescribing cycle operates with minimal microbial risk utilising quality assured practices. At the same time, the same infrastructure could be used to encourage and undertake further research to strengthen the evidence base, which can in turn be used to improve practice further. A pharmacist would be eminently suitable to oversee this task.

APPENDIX A

The terms and number of results for the literature search undertaken on 22nd February 2014 to identify microbial growth in parenteral nutrition infusates over a 48-hour period

Database	Search row	Search terms	Results (n)
MEDLINE (OvidSP) from 1946	1	parenteral nutrition.mp. or parenteral nutrition*.tw. or intravenous nutrition.mp. or intravenous nutrition*.tw. or total nutrient.mp. or total nutrient*.tw. or total parenteral.mp. or total parenteral*.tw. or glucose.mp. or glucose*.tw. or lipid.mp. or lipids.mp. or lipid*.tw. or fat.mp. or fats.mp. or fat*.tw. or amino acid.mp. or amino acids.mp. or amino acid*.tw.	1881979
	2	(bacterium or bacteria).mp. or bact*.tw. or (fungus or fungi).mp. or fung*.tw. or microbe.mp. or micro*.tw.	2425980
	3	(grow or growth).mp. or grow*.tw. or (inoculate or inoculation or inoculated).mp. or inoculat*.tw. or (inhibit or inhibition or inhibited).mp. or inhibit*.tw.	2576179
	4	(solution or solutions).mp. or solut*.tw. or (emulsion or emulsions).mp. or emul*.tw. or (infusion or infusions).mp. or (infusate or infusates).mp. or infus*.tw. or (admixture or admixtures).mp. or admix*.tw.	729406
	5	1 and 2 and 3 and 4	7796
	6	limit 5 to english language	7546
EMBASE Classic and EMBASE (OvidSP) from 1947	1	parenteral nutrition.mp. or parenteral nutrition*.tw. or intravenous nutrition.mp. or intravenous nutrition*.tw. or total nutrient.mp. or total nutrient*.tw. or total parenteral.mp. or total parenteral*.tw. or glucose.mp. or glucose*.tw. or lipid.mp. or lipids.mp. or lipid*.tw. or fat.mp. or fats.mp. or fat*.tw. or amino acid.mp. or amino acids.mp. or amino acid*.tw.	2450002
	2	(bacterium or bacteria).mp. or bact*.tw. or (fungus or fungi).mp. or fung*.tw. or microbe.mp. or micro*.tw.	3059443

	3	(grow or growth).mp. or grow*.tw. or (inoculate or inoculation or inoculated).mp. or inoculat*.tw. or (inhibit or inhibition or inhibited).mp. or inhibit*.tw.	3849577
	4	(solution or solutions).mp. or solut*.tw. or (emulsion or emulsions).mp. or emul*.tw. or (infusion or infusions).mp. or (infusate or infusates).mp. or infus*.tw. or (admixture or admixtures).mp. or admix*.tw.	1073181
	5	1 and 2 and 3 and 4	6577
	6	limit 5 to english language	6145
The Cochrane Library (John Wiley & Sons, Ltd) (all text search)	#1	"parenteral nutrition" or parenteral nutrition* or "intravenous nutrition" or intravenous nutrition* or "total nutrient" or total nutrient* or "total parenteral" or total parenteral* or "glucose" or glucose* or "lipid" or "lipids" or lipid* or "fat" or "fats" or fat* or "amino acid" or "amino acids" or amino acid*	75460
	#2	"bacterium" or "bacteria" or bact* or "fungus" or "fungi" or fung* or "microbe" or micro*	81951
	#3	"grow" or "growth" or grow* or "inoculate" or "inoculation" or "inoculated" or inoculat* or "inhibit" or "inhibition" or "inhibited" or inhibit*	84343
	#4	"solution" or "solutions" or solut* or "emulsion" or "emulsions" or emul* or "infusion" or "infusions" or "infusate" or "infusates" or infus* or "admixture" or "admixtures" or admix*	56541
	#5	#1 and #2 and #3 and #4	1009

APPENDIX B

The terms and number of results for the literature search undertaken on 10th February 2014 to identify parenteral doses prepared under aseptic techniques in clinical and pharmaceutical environments

	Search row	Search terms	Results (n)
MEDLINE (OvidSP)	1	(syringe or syringes).mp. or syring*.tw.	21041
	2	(bag or bags).mp. or bag*.tw.	18909
	3	(infusion or infusions).mp. or infus*.tw.	258728
	4	(vial or vials).mp. or vial*.tw.	6066
	5	(microbial or microbiological).mp. or micro*.tw.	2017452
	6	(bacterium or bacteria).mp. or bact*.tw.	598873
	7	(fungus or fungi).mp. or fung*.tw.	134157
	8	(contaminated or contamination).mp. or contam*.tw.	166465
	9	prepared.mp. or prep*.tw. or manufactured.mp. or manuf*.tw. or compounded.mp. or compound*.tw.	1110191
	10	(1 and 5) or (1 and 6) or (1 and 7) or (1 and 8) or (2 and 5) or (2 and 6) or (2 and 7) or (2 and 8) or (3 and 5 and 9[b]) or (3 and 6 and 9[c]) or (3 and 7) or (3 and 8) or (4 and 5) or (4 and 6) or (4 and 7) or (4 and 8)	19123
	11	limit 10 to english language	17662
EMBASE Classic and EMBASE (OvidSP)	1	(syringe or syringes).mp. or syring*.tw.	32435
	2	(bag or bags).mp. or bag*.tw.	30560
	3	(infusion or infusions).mp. or infus*.tw.	352153
	4	(vial or vials).mp. or vial*.tw.	10079
	5	(microbial or microbiological).mp. or micro*.tw.	2221231
	6	(bacterium or bacteria).mp. or bact*.tw.	952057
	7	(fungus or fungi).mp. or fung*.tw.	252530
	8	(contaminated or contamination).mp. or contam*.tw.	253438
	9	prepared.mp. or prep*.tw. or manufactured.mp. or manuf*.tw. or compounded.mp. or compound*.tw.	1697262

	10	(1 and 5) or (1 and 6) or (1 and 7) or (1 and 8) or (2 and 5) or (2 and 6) or (2 and 7) or (2 and 8) or (3 and 5 and 9[b]) or (3 and 6 and 9[c]) or (3 and 7) or (3 and 8) or (4 and 5) or (4 and 6) or (4 and 7) or (4 and 8)	23099
	11	limit 10 to english language	20824
The Cochrane Library (Wiley Online Library)[a]	#1	"syringe" or "syringes" or syring*	1364
	#2	"bag" or "bags" or bag*	4467
	#3	"infusion" or "infusions" or infus*	36233
	#4	"vial" or "vials" or vial*	1325
	#5	"microbial" or "microbiological" or micro*	66334
	#6	"bacterium" or "bacteria" or bact*	25856
	#7	"fungus" or "fungi" or fung*	2759
	#8	"contaminated" or "contamination" or contam*	3453
	#9	"prepared" or prep* or "manufactured" or manuf* or "compounded" or compound*	64682
	#10	(#1 and #5) or (#1 and #6) or (#1 and #7) or (#1 and #8) or (#2 and #5) or (#2 and #6) or (#2 and #7) or (#2 and #8) or (#3 and #5 and #9[b]) or (#3 and #6[c]) or (#3 and #7) or (#3 and #8) or (#4 and #5) or (#4 and #6) or (#4 and #7) or (#4 and #8)	3760

[a] All document search.

[b] The combination of search terms 3 and 5 yielded 57265 results in Medline, 33340 results in Embase and 7464 results in the Cochrane Library, and 4848, 671 and 876 results respectively when search term 9 was also included in the combination.

[c] The combination of search terms 3 and 6 returned 6363 results in Medline and 9036 results in Embase, and 689 and 784 results respectively when search term 9 was included in the combination. The third search term was not required for the combination of search terms 3 and 6 in the Cochrane Library since 1565 results were returned.

APPENDIX C

The criteria of the published intravascular catheter infection definitions

Intravascular catheter infection definition [a]	Overall term used to describe intravascular catheter infection [b]	First sub-term used for intravascular catheter infection definition	Criteria for first sub-term used for intravascular catheter infection	Second sub-term used for intravascular catheter infection definition	Criteria for second sub-term used for intravascular catheter infection	Third sub-term used for intravascular catheter infection definition	Criteria for third sub-term used for intravascular catheter infection
HELICS	Central venous catheter-related infection	Local central venous catheter-related infection (no positive blood culture)	Quantitative or semi-quantitative central venous catheter culture[c] and pus/inflammation at the insertion site or tunnel	General central venous catheter-related infection (no positive blood culture)	Quantitative or semi-quantitative central venous catheter culture[c] and clinical signs improve within 48 hours after catheter removal	Central venous catheter-related bloodstream infection	Bloodstream infection[d] 48 hours before or after catheter removal and positive culture with the same microbe from central venous catheter[c] or greater growth in central than peripheral culture[e] or

							differential delay to positivity of blood cultures[f] or same microbe from pus at the catheter insertion site
ESPEN	Catheter related sepsis	Catheter-related bloodstream infection	Quantitative or semi-quantitative culture of the catheter when catheter removed or exchanged over a guide wire	Catheter-related bloodstream infection	When catheter is left in place, paired quantitative blood cultures or paired qualitative blood cultures from a peripheral vein and from the catheter, with a differential time to positivity		
MM	Catheter -linked infection	Catheter-associated bloodstream infection	Bloodstream infection[g] and one or more central venous catheters at time of blood culture or up to 48 hours following catheter removal, and signs and	Catheter-related blood stream infection	Bloodstream infection[g] and one or more central venous catheters at time of blood culture or up to 48 hours following catheter removal, and positive	Catheter-suspected blood stream infection	Negative blood cultures in the presence of parenteral antimicrobials, and clinical evidence of a systemic response to infection, and clinical condition

			symptoms and positive laboratory results not primarily related to an infection at another site		quantitative or semiquantitative culture[h] of same microbe[i] from central venous catheter blood or catheter tip and peripheral blood, or a differential delay to positivity of blood cultures[j]		improves after central venous catheter removal, in the absence of another likely source of infection
CDC	Intravascular catheter-related infection	Central line associated bloodstream infection	Primary bloodstream infection[k] when a central venous catheter was in use during the previous 48-hour period, which was not bloodstream related to an infection at another site[l]	Catheter-related bloodstream infection	Specific laboratory testing to identify the catheter as the source of a bloodstream infection[k,m]		

CDC 2002[n]	Catheter-related infection	Catheter-associated bloodstream infection	Bloodstream infection[o] when a central venous catheter was in use during the previous 48-hour period[p]	Catheter-related bloodstream infection	Bacteraemia and/or fungaemia in a patient with an intravascular catheter		

[a] HELICS = Hospital in Europe Link for Infection Control through Surveillance (2004), ESPEN = European Society for Clinical Nutrition and Metabolism (2009), MM = Matching Michigan (2010), CDC = Centres for Disease Control and Prevention (2011).

[b] Overall term used to describe intravascular catheter infection in the catheter infection definition incorporates up to three sub-terms, depending on the definition.

[c] Quantitative central venous catheter culture ≥ 1000 colony forming units per mL or semi-quantitative central venous catheter culture > 15 colony forming units.

[d] For this definition, bloodstream infection is defined in one of two ways: one positive blood culture from a recognised pathogen or the patient has one or more of fever > 38°C, chills or hypotension, and two positive blood cultures for a common skin contaminant from two separate blood samples drawn within 48 hours of each other; or the patient has one or more of fever > 38°C, chills or hypotension, and either one positive blood culture with a skin contaminant when an intravascular line is in place and antibiotics are started or a positive blood antigen test. Within these definitions, a skin contaminant is coagulase-negative staphylococci, *Micrococcus sp.*, *Propionibacterium acnes*, *Bacillus sp.* or *Corynebacterium sp.*

[e] Quantitative blood cultures with a ratio > 5 for central venous catheter/peripheral.

[f] When blood cultures drawn simultaneously, central venous catheter blood culture positive two hours or less before peripheral blood culture.

[g] For this definition, laboratory-confirmed bloodstream infection is defined in one of two ways: one or more recognized pathogens from one blood culture; or if the microbe cultured is a common skin organism *then i*t must have been cultured from two or more blood cultures drawn on separate occasions, or from one blood culture in a patient in whom antimicrobial therapy has been started in the presence of fever >38°C, chills, or hypotension. Within these definitions, a common skin organism is diphtheroids (*Corynebacterium spp.*), *Bacillus spp.* (not *Bacillus anthracis*), *Propionibacterium spp.*, coagulase-negative staphylococci (excludes sensitive *Staphylococcus aureus*), viridans group streptococci, *Aerococcus spp.* and *Micrococcus spp.*

[h] Quantitative catheter culture >1000 colony forming units per mL or >1000 colony forming units per catheter segment or semiquantitative catheter culture >15 colony forming units per catheter segment.

[i] Same species and antibiogram.

[j] Simultaneous quantitative blood cultures with a ratio > 5 for central venous catheter/peripheral.

[k] For this definition, laboratory-confirmed bloodstream infection is defined in one of two ways: a recognized pathogen cultured from one or more blood cultures is not related to an infection at another site; or the patient has one or more of fever > 38°C, chills, or hypotension, and signs and symptoms and positive laboratory results are not related to an infection at another site, and a common skin contaminant is cultured from two or more blood cultures (from one or more bottles from each blood draw) drawn on separate occasions within two days of each other. Within these definitions, a common skin contaminant is diphtheroids

(*Corynebacterium spp.*), *Bacillus spp.* (not *Bacillus anthracis*), *Propionibacterium spp.*, coagulase-negative staphylococci (including *Staphylococcus epidermidis*), viridans group streptococci, *Aerococcus spp.* and *Micrococcus spp.* Within these definitions, recognised pathogens include *Staphylococcus aureus, Enterococcus spp., Escherichia coli, Pseudomonas spp., Klebsiella spp., Candida spp.,* and "others".

[l] This definition recognises this may overestimate the true incidence since some bloodstream infections may not be caused by the central venous catheter.

[m] Unable to be included in this study since no criteria provided.

[n] The 2002 version of the Centres for Disease Control and Prevention for comparison with the 2011 version of the same definition.

[o] For this definition, laboratory-confirmed bloodstream infection is defined in one of two ways: a recognized pathogen cultured from one or more blood cultures is not related to an infection at another site; or the patient has one or more of fever > 38ºC, chills, or hypotension, and a common skin contaminant cultured from two or more blood cultures drawn on separate occasions and/or a common skin contaminant cultured from at least one blood culture from a patient with an intravenous catheter and the physician institutes appropriate antimicrobial therapy and/or a positive antigen test on blood, and in each case signs and symptoms with positive laboratory results are not related to an infection at another site. Within these definitions, provided examples of common skin contaminants are diphtheroids, *Bacillus spp., Propionibacterium spp.*, coagulase-negative staphylococci, or micrococci.

[p] If the time interval between onset of infection and device use is > 48 hours, there should be compelling evidence that the infection is related to the central line.

APPENDIX D

Summary of the composition of individual test infusates used within each study included in the data analyses

Record	Type of infusate[a]	Concentration of lipid emulsion used in infusate (% w/v)	Inclusion of electrolytes in the infusate	Inclusion of vitamins in the infusate	Inclusion of trace elements in the infusate	Final pH of infusate	Final osmolarity of infusate (mOsm/L)	Final concentration of amino acids in infusate (% w/v)	Final concentration of glucose in infusate (% w/v)	Final concentration of lipid in infusate (% w/v)	Final energy density in infusate (kcal/100mL)	Final proportion non-nitrogen energy as lipid in infusate (%)
Austin 2012	Lipid emulsion	20	No	No	No	8.47	270	0.00	0.00	20.00	200	100
	Lipid PN	20	Yes	Yes	Yes	6.19	945	2.81	8.00	5.00	93	61
	Lipid PN	20	Yes	Yes	Yes	6.16	1110	2.81	11.00	5.00	105	53
	Lipid PN	20	Yes	Yes	Yes	6.12	1277	2.81	14.00	5.00	117	47
	Lipid PN	20	Yes	Yes	Yes	6.14	1443	2.81	17.00	5.00	129	42
	Lipid-free PN	None	Yes	Yes	Yes	6.24	871	2.81	8.00	0.00	43	0

	Lipid-free PN	None	Yes	Yes	Yes	6.23	1038	2.81	11.00	0.00	55	0
	Lipid-free PN	None	Yes	Yes	Yes	6.18	1204	2.81	14.00	0.00	67	0
	Lipid-free PN	None	Yes	Yes	Yes	6.22	1371	2.81	17.00	0.00	79	0
Austin 2013	Lipid emulsion	20	No	No	No	8.35	270	0.00	0.00	20.00	200	100
	Lipid PN	20	Yes	Yes	Yes	6.13	945	2.81	8.00	5.00	93	61
	Lipid PN	20	Yes	Yes	Yes	6.11	1110	2.81	11.00	5.00	105	53
	Lipid PN	20	Yes	Yes	Yes	6.08	1277	2.81	14.00	5.00	117	47
	Lipid PN	20	Yes	Yes	Yes	6.10	1443	2.81	17.00	5.00	129	42
	Lipid-free PN	None	Yes	Yes	Yes	6.19	871	2.81	8.00	0.00	43	0
	Lipid-free PN	None	Yes	Yes	Yes	6.17	1038	2.81	11.00	0.00	55	0
	Lipid-free PN	None	Yes	Yes	Yes	6.20	1204	2.81	14.00	0.00	67	0
	Lipid-free PN	None	Yes	Yes	Yes	6.17	1371	2.81	17.00	0.00	79	0
Austin 2014	Lipid emulsion	20	No	No	No	7.76	270	0.00	0.00	100.00	200	100
	Lipid PN[b]	20	Yes	Yes	Yes	6.21	945	2.81	8.00	5.00	93	61
	Lipid PN[b]	20	Yes	Yes	Yes	6.05	1110	2.81	8.00	5.00	93	61
	Lipid PN[b]	20	Yes	Yes	Yes	6.04	1277	2.81	8.00	5.00	93	61
	Lipid PN	20	Yes	Yes	Yes	6.12	1110	2.81	11.00	5.00	105	53
	Lipid PN	20	Yes	Yes	Yes	6.07	1277	2.81	14.00	5.00	117	47
	Lipid PN	20	Yes	Yes	Yes	5.98	1443	2.81	17.00	5.00	129	42

	Lipid-free PN	None	Yes	Yes	Yes	6.21	871	2.81	8.00	0.00	43	0
	Lipid-free PN	None	Yes	Yes	Yes	6.18	1038	2.81	11.00	0.00	55	0
	Lipid-free PN	None	Yes	Yes	Yes	6.14	1204	2.81	14.00	0.00	67	0
	Lipid-free PN	None	Yes	Yes	Yes	6.15	1371	2.81	17.00	0.00	79	0
D'Angio 1987	Lipid PN	10	Yes	Yes	Yes	6.50	644	2.83	3.30	3.30	61	58
	Lipid PN	10	Yes	Yes	Yes	6.40	1391	2.83	16.70	3.30	114	21
	Lipid-free PN	None	Yes	Yes	Yes	6.40	767	4.25	5.00	0.00	37	0
	Lipid-free PN	None	Yes	Yes	Yes	6.20	1698	4.25	25.00	0.00	117	0
Deitel 1975	Lipid emulsion	10	No	No	No	Not reported	Not reported	0.00	0.00	10.00	110	100
Didier 1998	Lipid PN	20	Yes	Yes	Yes	Not reported	Not reported	5.00	17.60	4.00	130	36
Duffett-Smith 1979	Lipid-free PN	None	Yes	No	No	Not reported	Not reported	1.58	10.00	0.00	46	0
	Lipid-free PN	None	Yes	No	No	Not reported	Not reported	1.96	10.00	0.00	48	0
	Lipid-free PN	None	Yes	No	No	Not reported	Not reported	2.79	10.00	0.00	51	0
	Lipid-free PN	None	Yes	No	No	Not reported	Not reported	2.98	10.00	0.00	52	0

	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	2.98	10.00	0.00	52	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	1.58	15.00	0.00	66	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	1.96	15.00	0.00	68	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	2.79	15.00	0.00	71	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	2.98	15.00	0.00	72	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	1.58	20.00	0.00	86	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	2.79	20.00	0.00	91	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	2.98	20.00	0.00	92	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	1.58	25.00	0.00	106	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	1.96	25.00	0.00	108	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	2.79	25.00	0.00	111	0
	Lipid-free PN	None	Yes	Yes	No	Not reported	Not reported	2.98	25.00	0.00	112	0
Fossum 1988	Lipid PN[c]	20	Yes	No	Yes	5.10	900[f]	2.33	13.22	3.97	102	43
	Lipid PN[c]	20	Yes	No	Yes	Not reported	Not reported	2.26	9.21	3.97	86	52

Gilbert 1986	Lipid emulsion	10	No	No	No	7.70	260	0.00	0.00	10.00	110	100
	Lipid PN	20	Yes	No	No	5.70	1277	4.25	12.50	5.00	117	50
	Lipid-free PN	None	Yes	No	No	5.60	1840	4.25	25.00	0.00	117	0
Holmes 1979	Lipid-free PN	None	NR	No	No	5.79	830	4.25	5.00	0.00	37	0
	Lipid-free PN	None	NR	No	No	5.75	1330	4.25	15.00	0.00	77	0
	Lipid-free PN	None	NR	No	No	5.67	1830	4.25	25.00	0.00	117	0
Jeppsson 1987	Lipid emulsion	20	No	No	No	8.10	370[g]	0.00	0.00	20.00	200	100
	Lipid PN	20	Yes	Yes	Yes	5.40	923[g]	2.31	7.87	3.94	80	56
	Lipid PN[d]	20	Yes	Yes	No	5.40	910[g]	2.32	7.91	3.95	80	56
	Lipid PN[d]	20	Yes	Yes	No	5.40	901[g]	2.33	7.94	3.97	81	56
Keammerer 1983	Lipid emulsion	10[e]	No	No	No	Not reported	Not reported	0.00	0.00	10.00	110	100
	Lipid emulsion	10[e]	No	No	No	Not reported	Not reported	0.00	0.00	10.00	110	100
	Lipid emulsion	20	No	No	No	Not reported	Not reported	0.00	0.00	20.00	200	100
Kuwahara 2010a[j]	Lipid PN	20	Yes	No	Yes	6.00	4[h]	3.33	12.22	1.73	80	26
	Lipid PN	20	Yes	Yes	Yes	6.00	4[h]	3.33	12.22	1.73	80	26
	Lipid PN	20	Yes	Yes	Yes	5.50	4[h]	2.67	8.53	1.82	63	35
	Lipid-free PN	None	Yes	Yes	Yes	5.50	5[h]	2.94	9.39	0.00	49	0

Kuwahara 2010b[j]	Lipid-free PN	None	Yes	Yes	No	5.60	4[h]	3.00	12.00	0.00	60	0
	Lipid-free {N	None	Yes	Yes	No	5.50	5[h]	2.94	9.39	0.00	49	0
	Lipid-free PN	None	Yes	Yes	No	5.50	6[h]	3.33	11.10	0.00	58	0
Kuwahara 2013	Lipid PN	20	Yes	No	Yes	Not reported	Not reported	2.70	6.75	2.00	58	43
	Lipid PN	20	Yes	Yes	Yes	Not reported	Not reported	2.69	6.74	2.00	58	43
	Lipid-free PN	None	Yes	No	Yes	6.60	856[g]	3.00	7.50	0.00	42	0
	Lipid-free PN	None	Yes	Yes	Yes	Not reported	Not reported	2.99	7.49	0.00	42	0
Llop 1993	Lipid PN	20	Yes	Yes	Yes	6.50	1041	3.40	8.00	4.00	86	56
	Lipid PN	20	Yes	Yes	Yes	6.62	1964	6.75	15.00	5.00	137	45
Melly 1975	Lipid emulsion	10	No	No	No	7.80	280	0.00	0.00	10.00	110	100
	Lipid-free PN	None	Yes	Yes	Yes	5.90	1910	2.61	25.00	0.00	110	0
Mershon 1986	Lipid emulsion	10	No	No	No	7.30	300	0.00	0.00	10.00	110	100
	Lipid PN	10	No	No	No	6.20	1000	1.50	12.50	1.50	73	14
	Lipid PN	10	No	No	No	6.10	1950	1.50	25.00	1.50	123	8
	Lipid-free PN	None	No	No	No	6.20	920	1.50	12.50	0.00	56	0
	Lipid-free PN	None	No	No	No	6.10	1900	1.50	25.00	0.00	106	0

Obayashi 2003	Lipid emulsion	20	No	No	No	7.50	1[i]	0.00	0.00	20.00	200	100
Powell 1986	Lipid emulsion	10	No	No	No	Not reported	Not reported	0.00	0.00	10.00	110	100
	Lipid emulsion	20	No	No	No	Not reported	Not reported	0.00	0.00	20.00	200	100
Rowe 1987	Lipid PN	20	Yes	Yes	Yes	5.75	790	3.00	5.00	5.00	82	71
	Lipid PN	20	Yes	Yes	Yes	5.74	1067	3.00	10.00	5.00	102	56
	Lipid PN	20	Yes	Yes	Yes	5.65	1091	3.00	25.00	5.00	162	33
	Lipid-free PN	None	Yes	Yes	Yes	5.74	740	3.00	5.00	0.00	32	0
	Lipid-free PN	None	Yes	Yes	Yes	5.71	1018	3.00	10.00	0.00	52	0
	Lipid-free PN	None	Yes	Yes	Yes	5.62	1851	3.00	25.00	0.00	112	0
Scott 1985	Lipid PN	20	Yes	Yes	Yes	6.14	1746	2.60	21.30	3.30	129	28
	Lipid PN	20	Yes	Yes	Yes	5.64	992	2.90	8.30	3.30	78	50
	Lipid-free PN	None	Yes	No	Yes	6.19	1913	2.60	21.30	0.00	96	0
	Lipid-free PN	None	Yes	Yes	Yes	6.19	1986	2.60	21.30	0.00	96	0
Shiro 1995	Lipid emulsion	20	No	No	No	Not reported	Not reported	0.00	0.00	20.00	200	100
Wilkinson 1973	Lipid-free PN	None	No	No	No	6.30	1670	4.25	25.00	0.00	117	0
	Lipid-free PN	None	Yes	Yes	No	6.30	1750	4.25	25.00	0.00	117	0

[a] Some records use the same infusate formulation for more than one trial.
[b] Sodium chloride content difference affects the overall osmolarity.

[c] Contain different amino acid solutions.
[d] Differ in phosphate content since the only phosphate in one is from the lipid emulsion.
[e] Different lipid emulsions.
[f] Approximate osmolality (mOsm/kg).
[g] Osmolality (mOsm/kg).
[h] Approximate osmotic pressure ratio to physiological saline.
[i] Osmolality (mOsm/kg) compared to “saline solution”.
[j] Kuwahara 2010a and Kuwahara 2010b are two records from the same year, a = “Growth of Microorganisms in Total Parenteral Nutrition Solutions Containing Lipid” and b = “Growth of Microorganisms in Total Parenteral Nutrition Solutions Without Lipid”.

APPENDIX E

Summary of the methodological procedures used within each study included in the data analyses

	Lipid emulsion data points (n)[a]	**Lipid PN data points (n)[a]**	**Lipid-free PN data points (n)[a]**	**Samples per data point (n)**	**Infusate test volume (mL)**	**Container used for infusates during the study period**	**Ambient study temperature (°C)**	**Species of microbe tested (n)[b]**	**Approximate baseline inoculation (cfu/mL)**	**Measured baseline cfu/mL[c]**	**Type of microbial strain used**
Austin 2012	1	4	4	1 sample from each of 4 replicates	250	Sterile oxygen barrier PN bags	23.2	1	10 – 100	Yes	Reference
Austin 2013	1	4	4	1 sample from each of 4 replicates	250	Sterile Oxygen barrier PN bags	21.5	1	10 – 100	Yes	Reference
Austin 2014	2	8	8	1 sample from each of 4 replicates	250	Sterile Oxygen barrier PN bags	21.1	2	10 – 100	Yes	Reference and clinical

D'Angio 1987	0	10	10	1 sample from each of 3 replicates	100	Sterile empty evacuated containers	25	5	10 – 100	Yes	Reference
Deitel 1975	13	0	0	Variable[d]	4.9	Not specified	25 and 37	7	10,000 – 1,000,000	Yes	Clinical
Didier 1998	0	24	0	2 samples from 1 replicate	3	Sterile test tubes	25 and 35	12h	10 - 100	Yes	Clinical
Duffett-Smith 1979	0	0	19	Total 3 samples from 2 replicates[e]	39	Universal containers	22[m]	3	100 – 10,000	Yes	Reference and clinical
Fossum 1988	0	13	0	2 samples from 3 replicates	49	Not specified	22	9	1 – 100	Yes	Reference
Gilbert 1986	1	1	1	1 sample from 8 replicates	9	Borosilicate tubes closed with venting steel caps	37	1	10,000	No	Clinical

Holmes 1979	0	0	15	Total 3 samples from 2 replicates	49	Aseptic plastic container with plastic screw cap	24	5	100 – 10,000	Yes	Reference and clinical
Jeppsson 1987	1	9	0	Not specified	50	Sterile test tubes	8	4	10 – 1,000	Yes	Reference and clinical
Keammerer 1983	12	0	0	2 samples from 1 replicate[f]	500	Bottles	Unspecified room temperature	4	10 – 1,000	Yes	Reference and clinical
Kuwahara 2010a[o]	0	25	3	1 sample from 1 replicate, plated onto 2 plates	10	Sterile plastic tubes	23 – 26	4	10 – 1,000	Yes	Reference and clinical
Kuwahara 2010b[o]	0	0	23	1 sample from 1 replicate, plated onto 2 plates	10	Sterile plastic tubes	24 – 27	4	1 – 1,000	Yes	Reference and clinical

Kuwahara 2013	0	24	24	1 sample from 1 replicate, plated onto 2 plates	100	Sterile plastic flasks	23 -26	4	0.01 – 10	Yes	Reference and clinical
Llop 1993	0	4	0	1 sample from 1 replicate	100	Bottles	25 and 37	1	10 – 1,000	Yes	Clinical
Melly 1975	5	0	3	Not specified	Not specified	Not specified	Unspecified room temperature	5	1,000 – 100,000	Yes	Reference and clinical
Mershon 1986	3	6	6	1 sample from 4 replicates	Not specified[p]	Bottles	Unspecified room temperature	3	10 – 1,000	Yes	Clinical
Obayashi 2003	2	0	0	1 sample from 2 replicates	0.95	Not specified	30	2	100 – 1,000	Yes[n]	Reference and clinical
Powell 1986	4	0	0	1 sample from 6 replicates[g]	3	Not specified	35 and unspecified room temperature	1g	100 – 1,000	Yes[n]	Clinical

Rowe 1987	0	13	14	1 sample from 1 replicate, plated onto 3 plates	19	Not specified	25	5	10 – 1,000	Yes	Clinical
Scott 1985	0	6	5	1 sample from 3 replicates	19	Not specified	25	3	50	No	Reference
Shiro 1995	1	0	0	1 sample from 1 replicate	Not specified	Tubes	37	1	100 – 1,000	Yes	Reference
Wilkinson 1973	0	0	12	1 sample from 1 replicate plated on 2 plates at each of 2 dilutions	1000	Not specified	Nominal 25	6	10 – 10,000	Yes	Clinical

N/A = not applicable.

[a] each data point represents a ratio (the $\log_{10}$ of the cfu/mL at 48 hours divided by the cfu/mL at time zero).

[b] different strains counted as the same species of microbe.

[c] If not measured the baseline cfu/mL had been estimated.

[d] one sample from one replicate at baseline and two samples from one replicate at 48 hours.

[e] information extracted from the method cited in a separate (Holmes 1979).

[f] unclear if duplicate plates from one sample or two plates from two samples at each time point.

[g] six clinical isolates of same microbe combined.

[h] multiple strains per microbe tested.

[i] no vitamins in some of the PN formulations.

[j] vitamins and trace elements present in some, but not all, of the PN formulations.
[k] vitamins present when electrolytes present in the PN formulations.
[l] no trace elements in some of the PN formulations.
[m] article reports storage at 4°C “when appropriate”.
[n] article text does not indicate baseline samples were taken but results presented indicate that samples were taken at baseline.
[o] Kuwahara 2010a and Kuwahara 2010b are two records from the same year, a = “Growth of Microorganisms in Total Parenteral Nutrition Solutions Containing Lipid” and b = “Growth of Microorganisms in Total Parenteral Nutrition Solutions Without Lipid”.
[p] the fill volume of the 250mL bottles used was not stated.

APPENDIX F

Summary of studies that reported the frequency of microbial contamination of parenteral doses prepared under aseptic techniques in clinical and pharmaceutical environments

Study	Country	Dose	Individual or batch preparation (or treated as one or the other)	Preparation environment[a]	Administration to patients[b]	Additives/repackaging group		Control group (no additives)	
						Total doses (n)	Contaminated doses (n)	Total doses (n)	Contaminated doses (n)
Austin et al 2006	England	Growth medium	Batch	Pharmaceutical	No	1002	0	-	-
Austin at al 2013	England	Growth medium	Batch	Clinical	No	778[c]	19[c]	-	-
Aydin et al 2002	Turkey	Propofol with and without lidocaine	Batch	Clinical	No	1920[d]	1[d]	-	-
Bach et al 1997	Germany	Anaesthetic agents	Individual	Clinical	Yes	1228*	47	-	-
Breheny et al 1990[86]	Australia	Parenteral nutrition	Individual	Clinical	Yes	150*	0	96	0
Burke et al 1986	United States	5% w/v Glucose	Batch	Clinical	No	95	27	-	-

Choy et al 1982	United States	Various, including parenteral nutrition	Individual	Pharmaceutical	No	150[e]	3[e]	-	-
D'Arcy et al 1973	Ireland	Various	Individual	Clinical	Yes	61*	34	40	5
Dominik et al 1995	Germany	Contrast media	Batch	Clinical	No	1000	9	-	-
Driver et al 1998	United States	Various for obstetric theatre use	Batch	Clinical	No	756	0	-	-
Ernerot et al 1973a	Sweden	Various	Batch	Clinical	No	50	0	-	-
Ernerot et al 1973b	Sweden	Various	Individual	Clinical	Yes	131*	3	40	2
Farrington et al 1994	England	Midazolam or propofol	Individual	Clinical	Yes	100*	7	-	-
Fleer et al 1983	The Netherlands	Parenteral nutrition	Batch	Clinical	No[f]	428	81	-	-
Hernandez-Ramos et al 2000	Mexico	Various	Individual	Clinical	Yes	1011*	60	-	-
Jackson et al 1990	United States	Insulin	Batch	Clinical	No	159[g]	0		
Jacobson et al 1996	United States	Filgrastim (G-CSF)	Batch	Pharmaceutical	No	60[h]	0[h]	-	-
Khalili et al 2013	Iran	Crystalloid fluids	Individual	Clinical[i]	No	92[j]	1[j]	-	-

Kundsin et al 1973	United States	Not stated	Individual	Clinical	Yes[k]	432*	5	247	1
Letcher et al 1972	United States	'Medications'	Individual	Clinical	Yes	224[l*]	13[l]	142[l]	5[l]
Lorenz et al 2002	Austria	Propofol	Individual	Clinical	No[f]	80[m]	5[m]	-	-
Macias et al 2012	Mexico	Various[n]	Individual	Clinical	Yes	101*	8	-	-
Madeo et al 1999	England	Epidural analgesia	Individual	Clinical	Yes	46[o*]	3[o]	-	-
Magee et al 1995	England	Anaesthetics, 0.9% w/v Sodium Chloride and growth medium	Batch	Clinical	No	195	8	-	-
Micard et al 2001	France	Meglumine gadoterate	Batch	Pharmaceutical	No	20	0	-	-
Poretz et al 1974	United States	0.9% w/v Sodium Chloride in 5% w/v Glucose in Ringer's lactate	Individual	Clinical[i]	Yes[j]	110*	10	50	2
Soong et al 1999	Australia	Propofol	Individual	Clinical	Yes	5*	0	-	-
Spiliotis et al 1989	Greece	Parenteral nutrition	Individual	Clinical	No	80[p]	0[p]	-	-
Stjernstrom et al 1978	Sweden	'Saline' and growth medium	Batch	Clinical	No	100	0	-	-

Thomas et al 2005	United States	Growth medium	Batch	Pharmaceutical	No	2030[q]	7[q]	-	-
Urbano et al 2013	Japan	Growth medium	Individual	Pharmaceutical	No	18	0	-	-
van Doorne et al 1994	The Netherlands	Growth medium	Batch	Pharmaceutical[r]	No	3000	1	-	-
van Grafhorst et al 2002	The Nethelands	Growth medium	Batch	Clinical[i]	No	650	151	-	-
Yorioka et al 2006	Japan	Electrolytes and dobutamine	Individual	Clinical	Yes	290*	0	-	-

Asterisks (*) indicate doses that were sampled during or after administration, and the absence of an asterisk indicates doses that were sampled without or prior to administration.

Ernerot et al 1973a and Ernerot et al 1973b: different aspects examined within the same record.

[a] A clinical environment includes hospital wards or operating theatres and to be classified as a pharmaceutical environment the record must state compliance with a recognised standard for both the preparation cabinet and immediate room surrounding that cabinet environment must be specified.

[b] 'Yes' if the doses were sampled during or after administration and 'no' if the doses were sampled without or prior to administration.

[c] In this study 19 of 276 doses prepared by nurses were contaminated 0 of 502 doses prepared by a pharmacy operator were contaminated.

[d] The data from part 2 of this study has been excluded since it involved unacceptable methodology (a delay in drawing up the dose).

[e] Of the 150 prepared doses, 52 were parenteral nutrition and all of the 3 contaminated doses were parenteral nutrition.

[f] These doses were administered to patients after they had been sampled.

[g] This records reports that one additional prepared dose was misplaced and not tested.

[h] This includes only those doses prepared in a standardised pharmaceutical environment.

[i] Only the data from a clinical environment is included since the nature of the pharmaceutical environment used is unacceptable/unclear.

[j] Excludes data from vial residues.

[k] Simulated patient administration.

[l] This is the data reported from the containers rather than the associated giving sets.

[m] The data from sample 2 of group I has been excluded since it represented the same doses, and the data from group II has been excluded due to unacceptable conditions.

[n] The methodology excluded patients receiving electrolytes, antibiotics or cancer chemotherapy.
[o] This is the data reported from cases without reuse of administration sets.
[p] This is the data from sampling immediately after dose preparation and not those same doses sampled after infusion (when 3 contaminated samples were identified).
[q] This is the data from standardised pharmaceutical conditions since the environment used for the negative control doses is unclear.
[r] This excludes the doses prepared in uncontrolled pharmaceutical environments.

REFERENCES

1. Van Gossum A, Bakker H, Bozzetti F, et al. Home parenteral nutrition in adults: a european multicentre survey in 1997. ESPEN-Home Artificial Nutrition Working Group. *Clin Nutr* 1999; **18**(3): 135-40.
2. Stewart JAD, Mason DG, Smith N, Protopapa K, Mason M. A Mixed Bag: An enquiry into the care of hospital patients receiving parenteral nutrition. London: NCEPOD, 2010.
3. Clark-Christoff N, Watters VA, Sparks W, Snyder P, Grant JP. Use of triple-lumen subclavian catheters for administration of total parenteral nutrition. *Journal of parenteral and enteral nutrition* 1992; **16**(5): 403-7.
4. Position Statement on the use of multi-chamber parenteral nutrition bags for use in adult patients: British Pharmaceutical Nutrition Group.
5. Lloyd DA, Gabe SM. Managing liver dysfunction in parenteral nutrition. *The Proceedings of the Nutrition Society* 2007; **66**(4): 530-8.
6. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. *American journal of infection control* 2011; **39**(4 Suppl 1): S1-34.
7. Rothberg AD. Lessons from the investigation into intravenous fluid-related neonatal deaths. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* 1992; **81**(8): 397-8.
8. Frean JA, Arntzen L, Rosekilly I, Isaacson M. Investigation of contaminated parenteral nutrition fluids associated with an outbreak of Serratia odorifera septicaemia. *The Journal of hospital infection* 1994; **27**(4): 263-73.
9. Editorial. Two children die after receiving infected TPN solutions. *Pharm J Pharmaceutical Journal* 1994; **252**: 596.
10. Infected drip-feeds killed children. The Independent newspaper. 1995 23 February.
11. Green J, Rondganger L. Polluted hospital drip kills six babies: South Africa Health Systems Trust, 11 March 2004.
12. Third baby dies after receiving contaminated hospital drip. Deutsche Welle. 2010 24 August.
13. Walker T. Investigation exonerates staff of babies' deaths in German clinic. Deutsche Welle. 2010 27 August.

14. Sack K, Williams T. Deaths of 9 Alabama Patients Tied to Intravenous Supplement. The New York Times newspaper (page A20 of the New York edition). 2011 31 March.
15. Institut Pasteur. Press release: Paris, March 17, 2015. Chambéry babies – Official recognition of the new bacterium identified by the Institut Pasteur, Rouxiella chamberiensis.
16. Editorial. Parenteral nutrition product recalled following potential contamination. *The Pharmaceutical Journal* 2014; **292**(7814): 595.
17. Klevens RM, Edwards JR, Richards CL, Jr., et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Health Rep* 2007; **122**(2): 160-6.
18. Lennard-Jones JE (working party chair). A Positive Approach to Nutrition as Treatment. Appendix F: Potential Cost Saving to a Hospital by a Nutrition Team from a Reduction in Catheter Infection Rate in Patients Receiving Parenteral Nutrition: King's Fund Centre, 1992.
19. Tacconelli E, Smith G, Hieke K, Lafuma A, Bastide P. Epidemiology, medical outcomes and costs of catheter-related bloodstream infections in intensive care units of four European countries: literature- and registry-based estimates. *The Journal of hospital infection* 2009; **72**(2): 97-103.
20. Digiovine B, Chenoweth C, Watts C, Higgins M. The attributable mortality and costs of primary nosocomial bloodstream infections in the intensive care unit. *American journal of respiratory and critical care medicine* 1999; **160**(3): 976-81.
21. Dimick JB, Pelz RK, Consunji R, Swoboda SM, Hendrix CW, Lipsett PA. Increased resource use associated with catheter-related bloodstream infection in the surgical intensive care unit. *Archives of surgery* 2001; **136**(2): 229-34.
22. National Patient Safety Agency. Patient safety alert 20 Promoting safer use of Injectable medicines. 2007.
23. Chapter 8 and Chapter 10 of Health Circular HC(76)9: Report of the working party on the addition of drugs to intravenous infusion fluids (the 'Breckenridge Report'). Department of Health and Social Security Health Services Division. London; 1976.
24. Paragraph 46 in A Spoonful of Sugar (Medicines Management in NHS Hospitals). London: Audit Commission for Local Authorities and the National Health Service in England and Wales; 2001.

25. Crichton EP. Infusion fluids as culture media. *American journal of clinical pathology* 1973; **59**(2): 199-202.
26. Guynn JB, Jr., Poretz DM, Duma RJ. Growth of various bacteria in a variety of intravenous fluids. *American journal of hospital pharmacy* 1973; **30**(4): 321-5.
27. Karstens A, Kramer I. Viability of micro-organisms in novel anticancer drug solutions. *The European Journal of Hospital Pharmacy Science* 2007; **13**(2): 27-32.
28. Beaney AM. Quality assurance of aseptic preparation services. 4th ed. London ; Chicago: Pharmaceutical Press; 2006.
29. Great Britain Medicines and Healthcare Products Regulatory Agency. Rules and Guidance for Pharmaceutical Manufacturers and Distributors (The Orange Guide). London: Pharmaceutical Press; 2014.
30. O'Grady NP, Alexander M, Dellinger EP, et al. Guidelines for the prevention of intravascular catheter-related infections. Centers for Disease Control and Prevention. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control* 2002; **51**(RR-10): 1-29.
31. Pratt RJ, Pellowe CM, Wilson JA, et al. epic2: National evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. *The Journal of hospital infection* 2007; **65 Suppl 1**: S1-64.
32. Pittiruti M, Hamilton H, Biffi R, MacFie J, Pertkiewicz M. ESPEN Guidelines on Parenteral Nutrition: central venous catheters (access, care, diagnosis and therapy of complications). *Clin Nutr* 2009; **28**(4): 365-77.
33. Nutrition Support for Adults: Oral Nutrition Support, Enteral Tube Feeding and Parenteral Nutrition. National Institute for Clinical Excellence. London; 2006.
34. van den Berghe G, Wouters P, Weekers F, et al. Intensive insulin therapy in critically ill patients. *The New England journal of medicine* 2001; **345**(19): 1359-67.
35. Austin P, Stroud M. Prescribing Adult Intravenous Nutrition. London: Pharmaceutical Press; 2007.
36. Hanna HA, Raad I. Blood products: a significant risk factor for long-term catheter-related bloodstream infections in cancer patients. *Infection control*

and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America 2001; **22**(3): 165-6.

37. Raad I, Hanna HA, Awad A, et al. Optimal frequency of changing intravenous administration sets: is it safe to prolong use beyond 72 hours? *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* 2001; **22**(3): 136-9.
38. Saiman L, Ludington E, Dawson JD, et al. Risk factors for Candida species colonization of neonatal intensive care unit patients. *The Pediatric infectious disease journal* 2001; **20**(12): 1119-24.
39. Avila-Figueroa C, Goldmann DA, Richardson DK, Gray JE, Ferrari A, Freeman J. Intravenous lipid emulsions are the major determinant of coagulase-negative staphylococcal bacteremia in very low birth weight newborns. *The Pediatric infectious disease journal* 1998; **17**(1): 10-7.
40. Crocker KS, Noga R, Filibeck DJ, Krey SH, Markovic M, Steffee WP. Microbial growth comparisons of five commercial parenteral lipid emulsions. *Journal of parenteral and enteral nutrition* 1984; **8**(4): 391-5.
41. Jarvis WR, Highsmith AK. Bacterial growth and endotoxin production in lipid emulsion. *Journal of clinical microbiology* 1984; **19**(1): 17-20.
42. Melly MA, Meng HC, Schaffner W. Microbial growth in lipid emulsions used in parenteral nutrition. *Archives of surgery* 1975; **110**(12): 1479-81.
43. Austin PD, Hand KS, Elia M. Factors that influence Staphylococcus epidermidis growth in parenteral nutrition with and without lipid emulsion: A study framework to inform maximum duration of infusion policy decisions. *Clin Nutr* 2012; **31**(6): 974-80.
44. Austin PD, Hand KS, Elia M. Factors influencing Candida albicans growth in parenteral nutrition with and without lipid emulsion: Using an established framework to inform maximum duration of infusion policy decisions. *Clin Nutr* 2013; **33**(3): 489-94.
45. Austin PD, Hand KS, Elia M. Factors Influencing Escherichia coli and Enterococcus durans Growth in Parenteral Nutrition With and Without Lipid Emulsion to Inform Maximum Duration of Infusion Policy Decisions. *Journal of parenteral and enteral nutrition* 2014; **39**(8): 953-65.
46. United Kingdom Health and Social Care Act. 2001.
47. Ducel G, Fabry J, Nicolle L. Prevention of hospital-acquired infections: a practical guide. Malta: World Health Organisation, 2002.

48. Teng SO, Lee WS, Ou TY, Hsieh YC, Lee WC, Lin YC. Bacterial contamination of patients' medical charts in a surgical ward and the intensive care unit: impact on nosocomial infections. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2009; **42**(1): 86-91.
49. BS EN ISO 14-644-1: Cleanrooms and associated controlled environments - part 1: classification of air cleanliness: British Standards Institution, 1999.
50. Austin PD, Elia M. A systematic review and meta-analysis of the risk of microbial contamination of aseptically prepared doses in different environments. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques* 2009; **12**(2): 233-42.
51. Seisdedos Elcuaz R, Conde Garcia MC, Castellanos Monedero JJ, Garcia-Manzanares Vazquez-de Agredos A, Valenzuela Gamez JC, Fraga Fuentes MD. [Central venous catheters-related infections in patients with parenteral nutrition]. *Nutricion hospitalaria* 2012; **27**(3): 775-80.
52. Wischmeyer PE, Weitzel L, Mercaldi K, et al. Characteristics and current practice of parenteral nutrition in hospitalized patients. *Journal of parenteral and enteral nutrition* 2013; **37**(1): 56-67.
53. Appendix 3: Potential cost saving to a hospital by a nutrition team from a reduction in catheter infection rate in patients receiving parenteral nutrition: The British Association for Parenteral and Enteral Nutrition, 1994.
54. Surecall. Stability Data - Adult 2-in-1 Regimens Containing Synthamin (reference 09V4R2). Newbury, UK: Baxter Healthcare Ltd; 2009.
55. Surecall. Stability Data - Adult 3-in-1 Regimens (ClinOleic) (reference 09V4R4). Newbury, UK: Baxter Healthcare Ltd; 2009.
56. Didier ME, Fischer S, Maki DG. Total nutrient admixtures appear safer than lipid emulsion alone as regards microbial contamination: growth properties of microbial pathogens at room temperature. *Journal of parenteral and enteral nutrition* 1998; **22**(5): 291-6.
57. Mershon J, Nogami W, Williams JM, Yoder C, Eitzen HE, Lemons JA. Bacterial/fungal growth in a combined parenteral nutrition solution. *Journal of parenteral and enteral nutrition* 1986; **10**(5): 498-502.
58. D'Angio R, Quercia RA, Treiber NK, McLaughlin JC, Klimek JJ. The growth of microorganisms in total parenteral nutrition admixtures. *Journal of parenteral and enteral nutrition* 1987; **11**(4): 394-7.

59. Scott EM, Gorman SP, Wyatt TD, Magill EA. Growth of microorganisms in total parenteral nutrition mixtures and related clinical observations. *Journal of clinical and hospital pharmacy* 1985; **10**(1): 79-88.
60. Wilkinson WR, L.L. F, J.N. P. Growth of Microorganisms in Parenteral Nutrition Fluids. *Drug intelligence & clinical pharmacy* 1973; **7**: 226-32.
61. Kuwahara T, Kaneda S, Shimono K, Inoue Y. Effects of lipid emulsion and multivitamins on the growth of microorganisms in peripheral parenteral nutrition solutions. *Int J Med Sci* 2013; **10**(9): 1079-84.
62. Shiro H, Muller E, Takeda S, Tosteson TD, Goldmann DA, Pier GB. Potentiation of Staphylococcus epidermidis catheter-related bacteremia by lipid infusions. *The Journal of infectious diseases* 1995; **171**(1): 220-4.
63. Obayashi A, Oie S, Kamiya A. Microbial viability in preparations packaged for single use. *Biological & pharmaceutical bulletin* 2003; **26**(5): 667-70.
64. Powell DA, Durrell DE, Marcon MJ. Growth of Malassezia furfur in parenteral fat emulsions. *The Journal of infectious diseases* 1986; **153**(3): 640-1.
65. Deitel M, Kaminsky VM, Fuksa M. Growth of common bacteria and Candida albicans in 10% soybean oil emulsion. *Canadian journal of surgery Journal canadien de chirurgie* 1975; **18**(6): 531-5.
66. Duffett-Smith T, Allwood MC. Further studies on the growth of micro-organisms in total parenteral nutrition solutions. *Journal of Clinical Pharmacy* 1979; **4**(4): 219-25.
67. Fossum K, Kure R, Nygaard K. Growth of micro-organisms in all-in-one TPN-admixtures containing lipids. *Clin Nutr* 1988; **7**(2): 73-9.
68. Gilbert M, Gallagher SC, Eads M, Elmore MF. Microbial growth patterns in a total parenteral nutrition formulation containing lipid emulsion. *Journal of parenteral and enteral nutrition* 1986; **10**(5): 494-7.
69. Holmes CJ, Allwood MC. The growth of micro-organisms in parenteral nutrition solutions containing amino acids and sugars. *International Journal of Pharmaceutics* 1979; **2**(5): 325-35.
70. Jeppson R, Johansson M, Tengborn J. Bacterial growth properties in refrigerated all-in-one TPN mixtures. *Clinical Nutrition* 1987; **6**(1): 25-9.
71. Keammerer D, Mayhall CG, Hall GO, Pesko LJ, Thomas RB. Microbial growth patterns in intravenous fat emulsions. *American journal of hospital pharmacy* 1983; **40**(10): 1650-3.

72. Kuwahara T, Shimono K, Kaneda S, Tamura T, Ichihara M, Y. N. Growth of Microorganisms in Total Parenteral Nutrition Solutions Containing Lipid. *Int J Med Sci* 2010; **7**(3): 101-9.
73. Kuwahara T, Kaneda S, Shimono K, Inoue Y. Growth of Microorganisms in Total Parenteral Nutrition Solutions Without Lipid. *Int J Med Sci* 2010; **7**(1): 43-7.
74. Llop JM, Mangues I, Perez JL, Lopez P, Tubau M. Staphylococcus saprophyticus sepsis related to total parenteral nutrition admixtures contamination. *Journal of parenteral and enteral nutrition* 1993; **17**(6): 575-7.
75. Rowe CE, Fukuyama TT, Martinoff JT. Growth of microorganisms in total nutrient admixtures. *Drug intelligence & clinical pharmacy* 1987; **21**(7-8): 633-8.
76. Nixon RM, Wonderling D, Grieve RD. Non-parametric methods for cost-effectiveness analysis: the central limit theorem and the bootstrap compared. *Health economics* 2010; **19**(3): 316-33.
77. Guyatt GH, Oxman AD, Kunz R, et al. What is "quality of evidence" and why is it important to clinicians? *BMJ* 2008; **336**(7651): 995-8.
78. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008; **336**(7650): 924-6.
79. Higgins J, Green S. (editors) Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
80. Systematic reviews : CRD's guidance for undertaking reviews in health care. [3rd ed.]. ed. York: Centre for Reviews and Dissemination; 2009.
81. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine* 2009; **151**(4): 264-9, W64.
82. Austin P, Elia M. Improved aseptic technique can reduce variable contamination rates of ward-prepared parenteral doses. *The Journal of hospital infection* 2013; **83**(2): 160-3.
83. Austin P, Dixson S. Hub fluid does not increase microbiological contamination of prepared and stored syringes. *Pharmaceutical Journal* 2006; **276**(7383): 47-9.

84. Aydin N, Aydin N, Gultekin B, Ozgun S, Gurel A. Bacterial contamination of propofol: the effects of temperature and lidocaine. *Eur J Anaesthesiol* 2002; **19**(6): 455-8.
85. Bach A, Motsch J, Schmidt H, et al. In-use contamination of propofol. A clinical study. *Eur J Anaesthesiol* 1997; **14**(2): 178-83.
86. Breheny F, McGechie D, Toohey M. Contamination of parenteral nutrition solutions not a hazard with additions made at ward level. *Anaesth Intensive Care* 1990; **18**(4): 547-9.
87. Burke KG, Larson E, Maciorowski L, Adler DC. Evaluation of the sterility of thermodilution room-temperature injectate preparations. *Crit Care Med* 1986; **14**(5): 503-4.
88. Choy FN, Lamy PP, Burkhart VD, Tenney JH. Sterility-testing program for antibiotics and other intravenous admixtures. *American journal of hospital pharmacy* 1982; **39**(3): 452-6.
89. D'Arcy PF, Woodside W. Drug additives: a potential source of bacterial contamination of infusion fluids. *Lancet* 1973; **2**(7820): 96.
90. Dominik RH, Segebade IE, Taenzer V. Risk of microbial contamination of iodinated contrast media on multiple use of large-volume bottles. *Eur J Radiol* 1995; **19**(3): 198-205.
91. Driver RP, Jr., Snyder IS, North FP, Fife TJ. Sterility of anesthetic and resuscitative drug syringes used in the obstetric operating room. *Anesth Analg* 1998; **86**(5): 994-7.
92. Ernerot L, Thoren S, Sandell E. Studies on microbial contamination of infusion fluids arising from drug additions and administration. *Acta Pharm Suec* 1973; **10**(2): 141-6.
93. Farrington M, McGinnes J, Matthews I, Park GR. Do infusions of midazolam and propofol pose an infection risk to critically ill patients? *Br J Anaesth* 1994; **72**(4): 415-7.
94. Fleer A, Senders RC, Visser MR, et al. Septicemia due to coagulase-negative staphylococci in a neonatal intensive care unit: clinical and bacteriological features and contaminated parenteral fluids as a source of sepsis. *Pediatr Infect Dis* 1983; **2**(6): 426-31.
95. Hernandez-Ramos I, Gaitan-Meza J, Garcia-Gaitan E, Leon-Ramirez AR, Justiniani-Cedeno N, Avila-Figueroa C. Extrinsic contamination of

intravenous infusates administered to hospitalized children in Mexico. *Pediatric Infectious Disease Journal* 2000; **19**(9): 888-90.

96. Jackson EA, Gallo BM. Sterility of insulin in prefilled disposable syringes. *American journal of hospital pharmacy* 1990; **47**(11): 2508-10.
97. Jacobson PA, West NJ, Spadoni V, Maksym CJ, Pierson C. Sterility of filgrastim (G-CSF) in syringes. *Ann Pharmacother* 1996; **30**(11): 1238-42.
98. Khalili H, Sheikhbabayi M, Jamalifar H, Dalili D, Samadi N. Bacterial contamination of single-and multiple-dose vials after multiple use and intravenous admixtures in three different hospitals in Iran. *Iranian Journal of Pharmaceutical Research* 2013; **12**(1): 205-9.
99. Kundsin RB, Walter CW, Scott JA. In-use testing of sterility of intravenous solutions in plastic containers. *Surgery* 1973; **73**(5): 778-81.
100. Letcher KI, Thrupp LD, Schapiro DJ, Boersma JE. In-use contamination of intravenous solutions in flexible plastic containers. *American journal of hospital pharmacy* 1972; **29**(8): 673-7.
101. Lorenz IH, Kolbitsch C, Lass-Florl C, et al. Routine handling of propofol prevents contamination as effectively as does strict adherence to the manufacturer's recommendations. *Canadian Journal of Anaesthesia* 2002; **49**(4): 347-52.
102. Macias A, De La Torre A, Huertas-Jimenez MA, Silva Y, Gonzalez L, Polanco C. Endemic IV fluid contamination in hospitalized children in Mexico. A problem of serious public health consequences. *American journal of infection control* 2012; **40 (5)**: e180-e1.
103. Madeo M, Samaan AK, Allison W, Wilson JA, Martin CR. Contamination of bags for continuous epidural infusion. *Acute Pain* 1999; **2**(3): 125-8.
104. Magee L, Godsiff L, Matthews I, Farrington M, Park GR. Anaesthetic drugs and bacterial contamination. *Eur J Anaesthesiol Suppl* 1995; **12**: 41-3.
105. Micard S, Rieutord A, Prognon P, Hassan M, Brion F. Stability and sterility of meglumine gadoterate injection repackaged in plastic syringes. *International Journal of Pharmaceutics* 2001; **212**(1): 93-9.
106. Poretz DM, Guynn JB, Jr., Duma RJ, Dalton HP. Microbial contamination of glass bottle (open-vented) and plastic bag (closed-nonvented) intravenous fluid delivery systems. *American journal of hospital pharmacy* 1974; **31**(8): 726-32.

107. Soong WA. Bacterial contamination of propofol in the operating theatre. *Anaesth Intensive Care* 1999; **27**(5): 493-6.
108. Spiliotis J, Arvaniti A, Tsirigotis M, Angelopoulou H, Kalfarentzos F. Contamination rates of total parenteral nutrition bags prepared under aseptic conditions. *Intensive Therapy and Clinical Monitoring* 1989; **10**(10): 302-3.
109. Stjernstrom G, Gunnarsson B, Wikner H. Studies on microbiological contamination of in-use IV-fluids. *Acta Pharm Suec* 1978; **15**(3): 169-74.
110. Thomas M, Sanborn MD, Couldry R. I.V. admixture contamination rates: traditional practice site versus a class 1000 cleanroom. *Am J Health-Syst Pharm* 2005; **62**(22): 2386-92.
111. Urbano N, Modoni S, Schillaci O. Media Fill Test for validation of autologous leukocytes separation and labelling by (99m)Tc-HmPAO. *Nucl Med Biol* 2013; **40**(1): 104-8.
112. van Doorne H, Bakker JH, Meevis RF, Marskamp A. Influence of background air on microbial contamination during simulated i.v.-admixture preparation. *Journal of Clinical Pharmacy & Therapeutics* 1994; **19**(3): 181-7.
113. van Grafhorst JP, Foudraine NA, Nooteboom F, Crombach WH, Oldenhof NJ, van Doorne H. Unexpected high risk of contamination with staphylococci species attributable to standard preparation of syringes for continuous intravenous drug administration in a simulation model in intensive care units. *Crit Care Med* 2002; **30**(4): 833-6.
114. Yorioka K, Oie S, Oomaki M, Imamura A, Kamiya A. Particulate and microbial contamination in in-use admixed intravenous infusions. *Biological & pharmaceutical bulletin* 2006; **29**(11): 2321-3.
115. Lawrence J, Turner M, Gilbert P. Microbial contamination and growth in total parenteral nutrition solutions. *Journal of clinical pharmacy and therapeutics* 1988; **13**(2): 151-7.
116. Reiter PD. Sterility of intravenous fat emulsion in plastic syringes. *Am J Health-Syst Pharm* 2002; **59**(19): 1857-9.
117. Takagi J, Khalidi N, Wolk RA, Tjolsen E, de Leon R, Wesley JR. Sterility of total parenteral nutrient solutions stored at room temperature for seven days. *American journal of hospital pharmacy* 1989; **46**(5): 973-7.
118. Weil DC, Arnow PM. Safety of refrigerated storage of admixed parenteral fluids. *Journal of clinical microbiology* 1988; **26**(9): 1787-90.

119. Needle R. Clinical governance in aseptic compounding. *Hospital Pharmacist* 2000; **1**(7): 192.
120. Altman DG, Gardner MJSwc. Statistics with confidence : confidence intervals and statistical guidelines. 2nd ed. / edited by Douglas G. Altman ... [et al.] ed. [Great Britain]: BMJ Books; 2000.
121. Surveillance of Nosocomial Infections in Intensive Care Units: Hospital in Europe Link for Infection Control through Surveillance (HELICS) protocol 6.1, 2004.
122. Matching Michigan Adult data collection template. Reference number 0952. 27 January 2010: National Patient Safety Agency.
123. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *American journal of infection control* 2008; **36**(5): 309-32.
124. Altman DG. Practical statistics for medical research: Chapman and Hall; 1991.
125. Sun S. Meta-analysis of Cohen's kappa. *Health Services and Outcomes Research Methodology* 2011; **11**: 145-63.
126. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2004; **39**(3): 309-17.
127. Collins CJ, Fraher MH, Bourke J, Phelan D, Lynch M. Epidemiology of catheter-related bloodstream infections in patients receiving total parenteral nutrition. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009; **49**(11): 1769-70; author reply 71-2.
128. Marra AR, Camargo LF, Pignatari AC, et al. Nosocomial bloodstream infections in Brazilian hospitals: analysis of 2,563 cases from a prospective nationwide surveillance study. *Journal of clinical microbiology* 2011; **49**(5): 1866-71.
129. Opilla M. Epidemiology of bloodstream infection associated with parenteral nutrition. *American journal of infection control* 2008; **36**(10): S173 e5-8.

130. Allwood MC, Kearney MC. Compatibility and stability of additives in parenteral nutrition admixtures. *Nutrition* 1998; **14**(9): 697-706.
131. Maki DG, Martin WT. Nationwide epidemic of septicemia caused by contaminated infusion products. IV. Growth of microbial pathogens in fluids for intravenous infusion. *Journal of Infectious Diseases* 1975; **131**(3): 267-72.
132. Korting HC, Lukacs A, Vogt N, Urban J, Ehret W, Ruckdeschel G. Influence of the pH-value on the growth of Staphylococcus epidermidis, Staphylococcus aureus and Propionibacterium acnes in continuous culture. *Zentralblatt fur Hygiene und Umweltmedizin = International journal of hygiene and environmental medicine* 1992; **193**(1): 78-90.
133. Northrop FD, Ljubojevic S, Davies JM. Influence of Na+ and anions on the dimorphic transition of Candida albicans. *Microbiology* 1997; **143 (Pt 12)**: 3757-65.
134. Kabli S. Morphogenesis of Two *Candida albicans* Strains as Influenced by Growth Media, pH Value and Incubation Temperature. American-Eurasian J Agric & Environ Sci; 2006. p. 127-32.
135. Cuenca-Estrella M, Diaz-Guerra TM, Mellado E, Rodriguez-Tudela JL. Influence of glucose supplementation and inoculum size on growth kinetics and antifungal susceptibility testing of Candida spp. *Journal of clinical microbiology* 2001; **39**(2): 525-32.
136. Brennan MF, O'Connell RC, Rosol JA, Kundsin R. The growth of Candida albicans in nutritive solutions given parenterally. *Archives of surgery* 1971; **103**(6): 705-8.
137. Vidotto V, Accattatis G, Zhang Q, Campanini G, Aoki S. Glucose influence on germ tube production in Candida albicans. *Mycopathologia* 1996; **133**(3): 143-7.
138. Si H, Hernday AD, Hirakawa MP, Johnson AD, Bennett RJ. Candida albicans white and opaque cells undergo distinct programs of filamentous growth. *PLoS pathogens* 2013; **9**(3): e1003210.
139. Escherichia coli 0157: H7. New Zealand Ministry for Primary Industries Web site. http://www.foodsafety.govt.nz/elibrary/industry/Escherichia_Coli-Organism_Invades.pdf Published May 2001, Accessed October 20, 2013.
140. Slanetz LW, Bent DF, Bartley CH. Use of the membrane filter technique to enumerate enterococci in water. *Public Health Rep* 1955; **70**(1): 67-72.

141. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. *Microbiology* 2009; **155**(Pt 6): 1749-57.
142. Sherman J, Wing H. Streptococcus Durans N. Sp. *J Dairy Sci* 1937; **20**(3): 165-7.
143. Clarke PH. Growth of streptococci in a glucose phenolphthalein broth. *Journal of general microbiology* 1953; **9**(2): 350-2.
144. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: a review. *Iranian journal of basic medical sciences* 2013; **16**(6): 731-42.
145. Kunin CM, Chambers ST. Osmoprotective Properties for Bacteria of the Renal Papilla and Urine: Role of Betaines as Osmoprotectant Molecules. In: Kass EH, Svanborg C, ed. *Host-parasite interactions in urinary tract infections: 4th International symposium on pyelonephritis: Papers.* Chicago; 1989: 327-332. 1989.
146. Lucht JM, Bremer E. Adaptation of Escherichia coli to high osmolarity environments: osmoregulation of the high-affinity glycine betaine transport system proU. *FEMS microbiology reviews* 1994; **14**(1): 3-20.
147. Chambers S, Kunin CM. The osmoprotective properties of urine for bacteria: the protective effect of betaine and human urine against low pH and high concentrations of electrolytes, sugars, and urea. *The Journal of infectious diseases* 1985; **152**(6): 1308-16.
148. Chambers ST, Kunin CM, Miller D, Hamada A. Dimethylthetin can substitute for glycine betaine as an osmoprotectant molecule for Escherichia coli. *Journal of bacteriology* 1987; **169**(10): 4845-7.
149. Stepanovic S, Jovanovic M, Lavadinovic L, Stosovic B, Pelemis M. Enterococcus durans endocarditis in a patient with transposition of the great vessels. *Journal of medical microbiology* 2004; **53**(Pt 3): 259-61.
150. Vijayakrishnan R, Rapose A. Fatal Enterococcus durans aortic valve endocarditis: a case report and review of the literature. *BMJ case reports* 2012; **2012**.
151. Melly MA, Meng HC, Schaffner W. Microbiol growth in lipid emulsions used in parenteral nutrition. *Archives of surgery* 1975; **110**(12): 1479-81.
152. Duffett-Smith T, Allwood M. Further studies on the growth of micro-organisms in total parenteral nutrition solutions. *Journal of clinical pharmacy and therapeutics* 1979; **4**(4): 219-25.

153. Gelbart SM, Reinhardt GF, Greenlee HB. Multiplication of nosocomial pathogens in intravenous feeding solutions. *Applied microbiology* 1973; **26**(6): 874-9.
154. Kim CH, Lewis DE, Kumar A. Bacterial and fungal growth in intravenous fat emulsions. *American journal of hospital pharmacy* 1983; **40**(12): 2159-61.
155. Sherertz RJ, Gledhill KS, Hampton KD, et al. Outbreak of Candida bloodstream infections associated with retrograde medication administration in a neonatal intensive care unit. *The Journal of pediatrics* 1992; **120**(3): 455-61.
156. Holmes CJ. Microbial growth comparisons of five commercial parenteral lipid emulsions. *Journal of Parenteral & Enteral Nutrition* 1985; **9**(6): 765-6.
157. Ullman AJ, Cooke ML, Gillies D, et al. Optimal timing for intravascular administration set replacement. *The Cochrane database of systematic reviews* 2013; **9**: CD003588.
158. Loveday HP, Wilson JA, Pratt RJ, et al. epic3: national evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. *The Journal of hospital infection* 2014; **86 Suppl 1**: S1-70.
159. Gillies D, O'Riordan L, Wallen M, Morrison A, Rankin K, Nagy S. Optimal timing for intravenous administration set replacement. *The Cochrane database of systematic reviews* 2005; (4): CD003588.
160. Ayers P, Adams S, Boullata J, et al. A.s.p.e.N. Parenteral nutrition safety consensus recommendations. *Journal of parenteral and enteral nutrition* 2014; **38**(3): 296-333.
161. Sharp J. Quality in the manufacture of medicines and other healthcare products. London: Pharmaceutical Press; 2000: 331-60.
162. Pharmaceutical inspection convention co-operation scheme (PIC/S). Recommendation on the validation of aseptic processes; 2011: document reference PI 007-6, Interpretation of data (section 6).
163. Sizer T, Duncombe R, Needle R, Sewell G. Universal operator broth transfer validation (version 9). *UK Pharmaceutical Aseptic Services Committee* 2005.