# Contamination of hospital tap water: the survival and persistence of *Pseudomonas aeruginosa* on conventional and 'antimicrobial' outlet fittings


## SUMMARY

***Background***: *Pseudomonas aeruginosa* infections have been linked to contaminated hospital taps, highlighting the potential for tap outlet fittings (OF) to harbour biofilm. *P. aeruginosa* may be transferred to OFs via contaminated cleaning-cloths. Suggested interventions include flushing regimens and alternative OF designs.

***Aim***: To investigate the transfer of *P. aeruginosa* from a contaminated cleaning-cloth to conventional and 'antimicrobial/anti-biofilm' OFs and to determine whether this contamination persists and/or leads to contamination of tap water.

***Methods***: Microfibre cloths contaminated with *P. aeruginosa* ($10^8$CFU/mL) were used to wipe four different types of OF (one of conventional design (OF-A) and three marketed as 'antimicrobial' and/or 'anti-biofilm' (OF- B, -C and –D)). OFs were inserted into an experimental water distribution system for up to 24-hours. Survival was assessed by culture. Single and multiple water samples were collected and cultured for *P. aeruginosa*.

***Findings***: The median number of *P. aeruginosa* transferred from cloth to OF was $5.7x10^5$CFU (OF-A), $1.9x10^6$CFU (OF-B), $1.4x10^5$CFU (OF-C) and $2.9x10^6$CFU (OF-D). Numbers declined on all OFs during the 24-hour period with log reductions ranging from 3.5 (OF-C) to 5.2 (OF-B; $p>0.05$). All water samples delivered immediately after OF contamination contained *P. aeruginosa* at ≥10CFU/100mL. Contamination of water delivered from OF-A persisted despite continued flushing. Water delivered from OF-B did not contain *P. aeruginosa* beyond the first flush.

***Conclusion***: Contaminated cleaning-cloths can transfer *P. aeruginosa* to OFs, leading to contamination of tap water. Whilst not removing the potential for contamination, 'antimicrobial/anti-biofilm' OFs may prevent *P. aeruginosa* from continually contaminating water delivered from the outlet.


## INTRODUCTION

Water outlets, particularly taps and associated pipework, are a recognised reservoir for microorganisms, providing a large surface area for biofilms to develop and to harbour potential pathogens, such as *Pseudomonas aeruginosa*[1]. In the UK, *P. aeruginosa* is responsible for 3% of all reported monospecies bacteraemias[2]. There are also reports of non-bacteraemic infections[3,4]. The infection of immunocompromised individuals by *P. aeruginosa* carries a risk of fatality[5] but, as reporting is voluntary, the exact incidence and mortality of *P. aeruginosa* infections is unknown. The association between hospital taps and *P. aeruginosa* infections is well documented[6,7], however, it was not until 2013, that the Department of Health (DoH) issued guidance relating to *P. aeruginosa* control[8].

*P. aeruginosa* readily forms biofilms[9], and the design and/or materials associated with different tap components, such as thermostatic mixing valves (TMVs)[10], solenoid valves[11] and outlet fittings (OFs)[12], have been shown to facilitate biofilm formation. *P. aeruginosa* is able to persist on wet and dry surfaces for extended periods of time[11,13].

Between November 2011 and January 2012, 25 babies admitted to neonatal intensive care units in Northern Ireland acquired *P. aeruginosa.* These incidents were attributed to contaminated OFs [12]. However , in a laboratory-based investigation, colonisation of OFs was minimal[11]. An acknowledged limitation of this study was that the tap assemblies were inoculated systemically, simulating contamination of the water supply. Hospital taps, however, may also be exposed to contamination originating from the clinical environment, i.e. retrograde contamination.

Retrograde contamination could occur in a number of different ways, many resulting from human behaviour. OFs can become contaminated via splashback from a contaminated waste trap[14] or surface[15] (e.g. while rinsing contaminated equipment) or through inappropriate disposal and/or splashing of contaminated patient fluids[16]. The potential spread of contamination during cleaning has also been demonstrated[17]. Contamination of environmental surfaces via microfibre cloths is well documented[18,19], and can occur even when using the previously published fold-and-refold method[20]. Data on the potential for microfibre cloths to contaminate OFs has not been published. However, in response to reports of OF contamination and DoH guidance, several manufacturers have engineered a variety of 'antimicrobial' or 'anti-biofilm' solutions, designed to prevent the survival of microorganisms introduced to the OF, and/or to minimise the adhesion of microorganisms and detritus.

The aim of this study was to investigate retrograde contamination, specifically whether conventional and 'antimicrobial/anti-biofilm' OFs can become contaminated with *P. aeruginosa* via cleaning cloths and if this contamination can persist and/or lead to contamination of tap water.

## METHODS

### Preparation of test suspension

Cryopreserved *Pseudomonas aeruginosa*, previously isolated from an experimental water distribution system (EWDS)[11], was resuscitated from beads (Technical Service Consultants Ltd., Lancashire, UK) onto Tryptone Soya agar (Oxoid, Basingstoke, UK) and subcultured onto Cetrimide (CN) agar (Oxoid). Liquid cultures were prepared by suspending a colony in 100mL of nutrient broth (Oxoid) and incubating at 37°C with shaking at 120 rpm for 16-hours.

### Selection of outlet fittings (OFs)

Four different OFs were selected for this study: one of conventional design (OF-A) and three (currently on the market or in development) designed to reduce the risk of microbial colonisation. In comparison to OF-A, a multi-layered plastic rosette[11], OF-B is simpler in design and comprises a single-bore with a copper interior lining. OF-C has a similar simplified design but is made from a plastic that the manufacturer claims reduces impurity (and bacterial) adherence. OF-D has a conventional-style flow-straightening device (similar to OF-A) but made from a silver-impregnated plastic. A copper alloy sheath (>76% Cu) projects beyond this internal device.

### Contamination of outlet fittings via microfibre cloth

Microfibre cleaning cloths (80% polyester and 20% polyamide; Arco, Hull, UK) were cut into 10cm$^2$ swatches, weighed and inoculated with 10mL of the bacterial test suspension (~$10^8$colony forming units (CFU)/mL). Each swatch was wrung and reweighed, before being used to wipe the accessible

surfaces of between 1 and 9 OFs for 10 seconds. Cloth-contaminated OFs were inserted into taps on the EWDS and left *in situ* for 15 minutes, one-, four-, eight-, 12- or 24-hours.

**Survival of *P. aeruginosa* on outlet fittings**

At the appropriate time point, OFs (n=3) were removed from the taps and transferred to 5mL sterile thiosulphate Ringers solution (Oxoid) or, to (when it was required) neutralise potential antimicrobial ions, 5mL Dey-Engley neutralising broth (Sigma-Aldrich, Dorset, UK). Each OF was vortexed with sterile glass beads for two minutes and the resulting suspension serially diluted (ten-fold). Aliquots (100 µL) of each dilution were plated onto CN agar and incubated at 37°C for 24-48 hours. 3mL of the remaining suspension was filtered through a 0.2µm membrane (Pall Life Sciences, Portsmouth, UK) which was transferred to CN agar and incubated at 37°C for 24-48 hours. Each experiment was repeated on four separate occasions.

**Detection of *P. aeruginosa* in water dispensed from contaminated outlet fittings**

OFs (contaminated as previously described) were inserted into the EWDS. Each tap was flushed (30-seconds, 4.5 L/min) either once over a 24-hour period (OF-A only), or five times over a 20-minute period to simulate an infrequently- or frequently-used tap respectively (all OF types). A TMV associated with each tap ensured the temperature of the water delivered from each outlet was ~43°C. Chlorine levels were approximately 0.03-0.22mg/L. During each flush, water samples (500mL) were collected in sample bottles containing 20mg/L sodium thiosulphate (Scientific Laboratory Supplies, Nottingham, UK). The presence of *P. aeruginosa* was determined by filtering 100mL of each water sample through a 0.2µm membrane. Membranes were transferred to CN agar and incubated at 37°C for 24-48 hours. Colonies were enumerated and results recorded as above (≥10CFU/100mL) or below (1-9CFU/100mL) the hospital alert limit for augmented care[8], or below the detection limit of the assay (<1CFU/100mL). Experiments were repeated on four separate occasions.

**Data analysis**

Data analysis was performed using GraphPad Prism (version 7.00 for Windows). Parametric data were compared using unpaired T-tests or one-way ANOVA followed by Šídák's multiple comparisons test. Non-parametric data were compared using Kruskal-Wallis one-way ANOVA in combination with Dunn's multiple comparisons test. Statistical significance was set at $p<0.05$. Chi-square ($X^2$) tests were performed to test for independence between contamination levels of flushed water and the number of flushes.

**RESULTS**

**Contamination of outlet fittings via contaminated microfibre cloths**

*P. aeruginosa* was transferred to all OFs via contaminated microfibre cloths. The median number (n=12) of *P. aeruginosa* transferred was $5.7 \times 10^5$CFU (OF-A), $1.9 \times 10^6$CFU (OF-B), $1.4 \times 10^5$CFU (OF-C) and $2.9 \times 10^6$CFU (OF-D). Significantly fewer bacteria were transferred to OF-C than to any other OF ($p<0.05$). No other differences were significant.

**Survival of *P. aeruginosa* over time**

Figure 1 illustrates the reduction in viable *P. aeruginosa* recovered from the different OFs up to 24-hours after contamination. For all OF types, significant $\log_{(10)}$ reductions were observed by 15-minutes ($p<0.05$). There were also significant $\log_{(10)}$ reductions between one- and four-hours for all OF types, with reductions of 2.7 (OF-A), 3.7 (OF-B), 2.2 (OF-C) and 2.6 (OF-D). The rate of *P. aeruginosa* reduction decreased after the first four-hours, with significant $\log_{(10)}$ reductions of 1.8 (OF-A), 1.6 (OF-B), 1.3 (OF-C) and 2.6 (OF-D) over the following 20-hour period. No significant reduction was seen between four and eight-hours ($p>0.05$) and the subsequent four-hour period saw variable trends across the OF designs, with only OF-B demonstrating a significant $\log_{(10)}$ reduction of 1.1.

No significant difference in *P. aeruginosa* viability was observed after 24-hours between the conventional and 'antimicrobial/anti-biofilm' OFs. OF-B showed greater $\log_{(10)}$ reduction than OF-A at 12-hours ($p<0.05$), however this did not carry through to the 24-hour time point.

**Detection of *P. aeruginosa* in water dispensed from contaminated outlet fittings**

Taps fitted with OF-A were flushed once to simulate an infrequently-used tap. All water samples (n=6) taken immediately after contamination of the OF were positive for *P. aeruginosa* at ≥10CFU/100mL (above the augmented care alert limit). The level of *P. aeruginosa* in all water collected at 15-minutes, one-, four- and 12-hours after contamination also exceeded the alert limit. *P. aeruginosa* persisted on the surface of the OF and 24-hours after contamination, 3/6 (50%) of water samples delivered through OF-A contained *P. aeruginosa* at levels ≥10CFU/100mL.

Regardless of OF design, increased usage of the tap correlated with reduced water contamination (Table I). In all cases, water delivered immediately after contamination of the OFs (flush 1) was positive for *P. aeruginosa* at ≥10CFU/100mL. Contamination of water delivered from OF-A persisted despite continued flushing (or usage) and 6/12 (50%) in the fifth flush contained *P. aeruginosa* at levels of ≥10CFU/100mL (Table I). In contrast, by the second flush, the level of *P. aeruginosa* in water delivered through OF-B was always below the detection limit of the assay. Whilst two (17%) and one (8%) of the 12 water samples collected during the second and third use of taps fitted with OF-C respectively contained *P. aeruginosa* at ≥10CFU/100mL, the majority of samples (11/12, 92%) were below detection by the fourth flush. *P. aeruginosa* was recovered from water delivered from OF-D at ≥10CFU/100mL until the fourth flush, by which point no *P. aeruginosa* was detected in 8/12 (67%) samples. In contrast to OF-B and –C, water dispensed by contaminated OF-D contained *P. aeruginosa* at detectable levels in 2/12 (17%) samples by the fifth flush. $X^2$ tests indicated that the level of contamination was not independent from the number of flushes that had occurred for all four OF types (i.e. there is an association between flushing and the observed reduction in *P. aeruginosa* counts) (n=12. OF-A: $X^2$ (4) = 10.7 $p<0.05$; OF-B $X^2$ (4) =60.0 $p<0.0001$; OF-C: $X^2$ (4) = 46.2, $p<0.0001$; OF-D $X^2$ (4) = 49.3, $p<0.0001$).

**DISCUSSION**

Although the colonisation of OFs with *P. aeruginosa* has been reported since the 1960s[21], very few studies have investigated how such contamination can occur. Of those that have, the focus has been on post-incident investigation[12] or systemic contamination[11]. This is the first study to investigate the

transfer of *P. aeruginosa* from a cloth to an OF (i.e. cross-contamination during cleaning). The high load of *P. aeruginosa* used to inoculate the microfibre cloths was comparable to contamination levels found in cleaning-cloths in previous studies[22] and resulted in OF contamination at levels comparable to that recovered from the OFs implicated in the Northern Ireland incidents ($1.8x10^5$CFU)[12]. *P. aeruginosa* can survive on dry surfaces[13] and in the absence of flushing, *P. aeruginosa* survived on the surface of all OFs for at least 24 hours.

The antimicrobial effect of copper and silver ions is well documented and such studies have led to the design and manufacture of OFs that incorporate 'antimicrobial' materials[23,24]. However, results from laboratory-based assays do not necessarily translate when applied[25]. Copper surfaces can, in laboratory studies, achieve a 6-$\log_{(10)}$ reduction in bacterial viability[26]. In contrast, within the healthcare setting, copper surfaces have been shown to reduce the level of environmental contamination by just ~2-$\log_{(10)}$[27,28], and despite microbial reductions being significantly greater than on control surfaces, copper surfaces are still contaminated at levels above the accepted limit for healthcare(2.5CFU/$cm^2$)[29]. The results of the current study demonstrate that OFs can become contaminated when wiped with a contaminated cloth, and this contamination can persist. In comparison to the conventional OF (OF-A), the OFs incorporating antimicrobial materials including copper (i.e. OF-B and –D) did not significantly reduce microbial contamination over a 24-hour period. The clinical tap industry is increasingly incorporating copper and brass components within new designs whilst also attempting to reduce surface roughness to minimise niches for bacterial attachment. However, a study by Zeiger *et al* highlighted the importance of surface roughness in the efficiency of copper contact killing, demonstrating a superior killing effect by electroporated copper compared to polished or deposited copper surfaces[30].

The transfer of *P. aeruginosa* to OFs led to contamination of the first sample of water delivered from all outlets at ≥10CFU/100mL. Should the tap be used less frequently (i.e. once within a 24-hour period) then the ability of *P. aeruginosa* to persist on the surface of conventional OFs could result in 'high-risk' water (containing ≥10CFU/100mL[8]) being delivered 24-hours after the contamination event. Regardless of OF design, *P. aeruginosa* levels in water dispensed from affected taps reduced with increased tap usage. The 'antimicrobial/anti-biofilm' OFs reduced contamination more efficiently than conventional OFs over a series of flushes. The contamination of water delivered by OF-B and OF-C was reduced to a level below the detection limit of the assay (<1CFU/100mL) after one 30-second flush. However, unlike OF-B, OF-C did not consistently clear residual contamination, meaning that water samples delivered during the second (2/12) and third flush (1/12) were contaminated at ≥10CFU/100mL. OF-B and –C are both single bore OFs. OF-D is more complex and was less efficient than OF-B and –C at clearing contamination. This could be due to the additional flow control provided by its internal device, which is similar to OF-A. It is possible that antimicrobial agents from OF-D acted upon *P. aeruginosa* in the water sample, hence its superior performance to OF-A.

When wiped with a contaminated microfibre cloth, significantly fewer bacteria were transferred to OF-C than to any of the other OFs, which, when considered alongside the rapid removal of contamination through tap usage, implies that *P. aeruginosa* is unable to attach as easily or as strongly to this OF design. OF-C is marketed as being 'anti-biofilm'. However, it is unclear whether clearance of *P. aeruginosa* was an artefact of being retrofitted into another manufacturer's tap, or due to the materials used. Not all OFs are able to maintain flow regulation performance across all

spout designs. It is feasible that turbidity of flow and the resulting shear forces may enhance sloughing of attached bacteria. Nonetheless, as was observed during the current investigation, poor flow regulation can lead to splashing (data not presented). Implications of water (and its microbial content) spreading to the surrounding environment must be taken into consideration, both in droplet and aerosol form[31].

This study has demonstrated that OFs could become contaminated with *P. aeruginosa* in a retrograde manner, for example, during cleaning. If not immediately removed, this contamination can persist and lead to contamination of tap water. Whilst not removing the potential for retrograde contamination, 'antimicrobial/anti-biofilm' OFs may, in combination with a flushing regimen, prevent *P. aeruginosa* from continually contaminating the water delivered from the outlet. Retrofitting taps with 'antimicrobial/anti-biofilm' OFs is possible but this may not be easy or appropriate. Some designs would require the replacement of the entire tap assembly, whilst the simplification of other designs has had a negative impact upon the original function of the OFs (i.e. to regulate and straighten the flow of water).

Furthermore, relying on the antimicrobial properties of material (or OF) is an insufficient infection control measure. Flushing and frequent tap use reduces water system stagnation allowing any decontamination agents added to the tank water to reach the tap outlet and OF. It is important to remember that augmented care units, where taps tend to be used very frequently[32], are not the only wards that immunocompromised patients spend time on, and *P. aeruginosa* infections have been acquired from outpatients clinics[33]. Immunocompromised outpatients receiving prolonged treatment, such as oncology and cystic fibrosis patients, visit day wards and clinics regularly, and also make unexpected presentations to emergency departments[34]. Retrograde contamination of sinks, taps and OFs could occur regardless of ward. It is, therefore, important to maintain and flush all hospital taps regularly and essential that factors contributing to retrograde contamination (be it human behaviour or otherwise) be investigated and addressed.

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**Conflict of interest statement**

The views expressed in this publication are those of the authors and not of Public Health England.

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Healthcare Infection Society

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**Figures and Legends:**

**Table I** The number of water samples delivered through contaminated outlet fittings that contained *P. aeruginosa* at levels above (≥10CFU/100mL) or below (1-9CFU/100mL) the hospital alert limit for augmented care, or below the detection limit of the assay (<1CFU/100mL). Samples (n=12) were collected over five consecutive tap usages (i.e. five consecutive 30 second flushes).

**Figure 1. Reduction ($Log_{(10)}$) in cloth-transferred *P. aeruginosa* on outlet fitting types [mean (n=12) ± standard deviation]**

Table

**Table I** The number of water samples delivered through contaminated outlet fittings that contained *P. aeruginosa* at levels above (≥10CFU/100mL) or below (1-9CFU/100mL) the hospital alert limit for augmented care, or below the detection limit of the assay (<1CFU/100mL). Samples (n=12) were collected over five consecutive tap usages (i.e. five consecutive 30 second flushes).

| **OF type** | ***P. aeruginosa* (CFU/100mL)** | **Frequency of tap use** | | | | |
|---|---|---|---|---|---|---|
| | | **1st flush** | **2nd flush** | **3rd flush** | **4th flush** | **5th flush** |
| OF-A | ≥10 | 12 | 11 | 8 | 8 | 6 |
| | 1-9 | 0 | 1 | 2 | 1 | 2 |
| | <1 | 0 | 0 | 2 | 3 | 4 |
| OF-B | ≥10 | 12 | 0 | 0 | 0 | 0 |
| | 1-9 | 0 | 0 | 0 | 0 | 0 |
| | <1 | 0 | 12 | 12 | 12 | 12 |
| OF-C | ≥10 | 12 | 2 | 1 | 0 | 0 |
| | 1-9 | 0 | 0 | 0 | 1 | 0 |
| | <1 | 0 | 10 | 11 | 11 | 12 |
| OF-D | ≥10 | 12 | 11 | 2 | 0 | 0 |
| | 1-9 | 0 | 1 | 4 | 4 | 2 |
| | <1 | 0 | 0 | 6 | 8 | 10 |

Figure

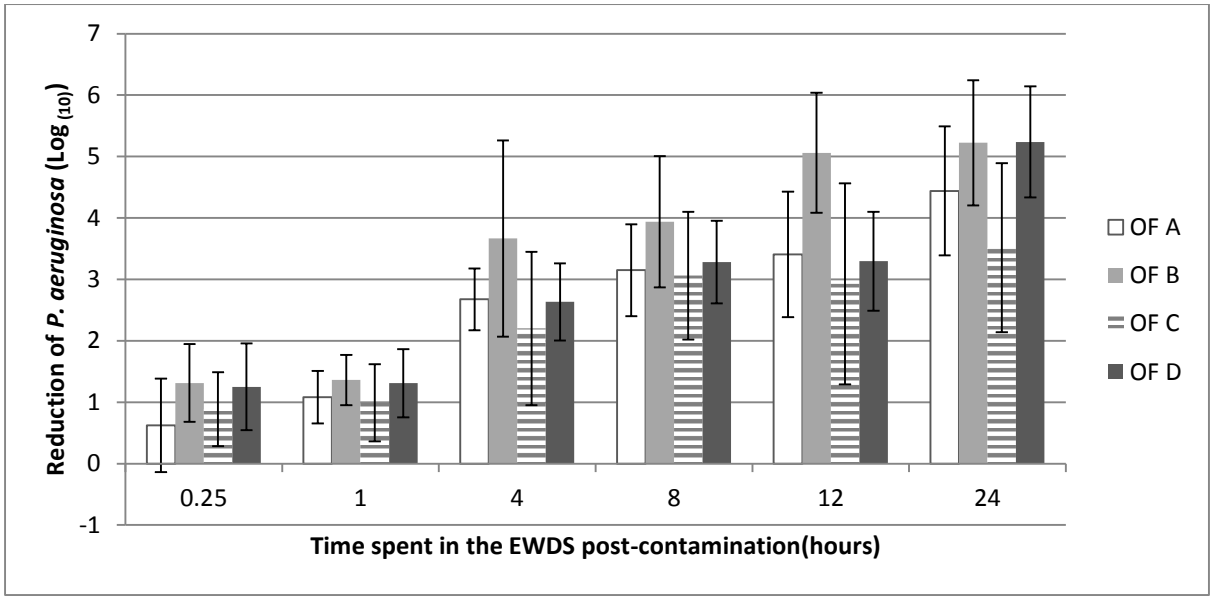


**Figure 1.** Reduction ($Log_{(10)}$) in cloth-transferred *P. aeruginosa* on outlet fitting types [mean (n=12) ± standard deviation]