Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Persistent Colonisation of Antimicrobial Silver-Impregnated Shower Heads and Hoses Presents A Risk for Acquisition of *Pseudomonas Aeruginosa* in Healthcare Settings

Özge Yetiş (ozge.yetis.18@ucl.ac.uk)
University College London
Shanom Ali
University College London Hospitals NHS Foundation Trust
Kush Karia
University College London Hospitals NHS Foundation Trust
Paul Bassett
Statsconsultancy, Ltd
Peter Wilson
University College London Hospitals NHS Foundation Trust

Research Article

Keywords: P. aeruginosa, hospital environment, hospital shower water and drains, antimicrobial resistance

Posted Date: March 29th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1403389/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Pseudomonas aeruginosa in healthcare waters presents an infection-risk to immune-suppressed patients.

We introduced interventions (quarterly antimicrobial silver-impregnated shower head-hose replacement and shower-hose truncation) to determine effect on presence/persistence of *P. aeruginosa* in hospital shower-waters and drains over a seven-month survey (560 samples; 14 time-points) on augmented-care (immune-compromised) and non-augmented (general) wards.

P. aeruginosa occurrence in the shower or drain predisposed to colonisation of the adjacent site/outlet (chi-square; $p<0.001$).

Up to 80% of shower-waters/drains (augmented-care) were heavily-contaminated (>300CFU) before intervention and persisted despite remediation ($P>0.05$). Conversely, for every week elapsed, *P. aeruginosa* in the non-augmented wards increased by ~18% ($P<0.001$) in shower waters (OR=1.19; CI=1.09-1.31) and drains (OR=1.18; CI=1.09-1.30).

Imipenem (showers), and aztreonam/ciprofloxacin-resistance (drains) occurred in up to 75% of sites in augmented-care with cefepime-resistance expressed in the general wards. Resistance-phenotypes declined after remediation despite ciprofloxacin being the only antimicrobial agent prescribed during this period.

The shower environment in non-augmented care settings represents unrecognised reservoirs of *P. aeruginosa.* Once *P. aeruginosa*-bioburden is established, antimicrobial shower/hose materials and shortening of hoses are ineffective options for remediation.

Efficacy-validation of antimicrobial materials and prevention of cross-contamination of the shower environment, plumbing and control-valves is essential to minimise acquisition of *P. aeruginosa* by vulnerable patients.

Introduction

P. aeruginosa is a common cause of bacteraemia in immune-suppressed patients and is associated with a high mortality [1–3]. Hospitals with a large proportion of haematology/oncology patients have correspondingly higher rates of *P. aeruginosa* bacteraemia and are subject to surveillance and peer review by PHE in UK [4]. In hospitals, *P. aeruginosa* is found in sinks and faucets, showers and shower heads, ice machines, humidifiers, sink traps, and tap-outlet flow straighteners, in the distal plumbing but not in the water supply [1,5]. The organism can grow in a wide range of temperatures and in nutrient-depleted environments [6]. Patients with haematological malignancy, tumours, chemotherapy, diabetes, soft tissue, respiratory or urinary infection, or recent surgery are at increased risk of *P. aeruginosa* bacteraemia [7,8]. Antimicrobial resistance is common and the risk of mortality is greater in patients with bacteraemia caused by multiresistant strains [3].

Water quality in hospitals and healthcare settings are regulated through guidelines set by local or national groups in European countries and USA [9]. In the UK, Health Technical Memoranda HTM 04-01C requires regular sampling of water outlets for *P. aeruginosa* and remedial action in augmented care areas (i.e. areas with immune compromised or critically ill patients) [10]. In USA, water system specifications are regulated by the American National Standards Institute (ANSI) in accordance with International Organization for Standardization (ISO) guidelines. Standards are guiding the water quality measurement programs [11,12]. Control of *P. aeruginosa* colonisation of hospital water systems is achieved through chemical disinfection, thermal treatment or point-of-use-filtration [13]. Infection control practitioners are asked to advise hospital engineers both on the clinical context and remedial action when initial measures fail to remove colonisation. Therefore, understanding the mechanisms under which contamination occurs is essential. The presence of biofilm initially in the faucet or showerhead and later in the adjacent plumbing is a more important source than contamination of the mains water supply.

In the case of hospital showers, outlets are likely to be contaminated directly by the users or from the associated drains, for example, by poor cleaning practice. Drains are contaminated by organisms in the shower head and by the users themselves. A shower hose that touches the drain when dropped is liable to contamination, thus shortening the hose may reduce accidental

contamination. Similarly, regular replacement of the shower head and hose (with/without antimicrobial additives) may prevent contaminated biofilm spreading from the shower head to the water supply pipes. Antimicrobial impregnated filters are used widely but restrict flow, and are prone to retrograde contamination and a continuing cost to the ward budget [2].

This study is a longitudinal investigation to determine the effect of reducing the shower hose length and quarterly replacement of shower head and hose on the presence of *P. aeruginosa* in hospital shower water and the antimicrobial susceptibility profiles of the isolates found.

Methods

Prior to the study, the frequency of sampling for *P. aeruginosa* in augmented care wards and remedial action were determined by the hospital Water Safety Group. Where a contaminated outlet was identified on an augmented care ward, disinfection was performed as advised by HTM guidelines [10]. Mitigation included mechanical flushing (opening of the outlet for 2 minutes) before use, chemical disinfection, and replacement of the hose and showerhead, or, if all these failed, a point-of-use filter. Outlets which continued to harbour high level contamination despite mitigation were withdrawn from use until contamination was eradicated or a point of use filter applied; these outlets were flushed daily when out of use. Irrespective of the type of patient, many showers were found to be colonized with *P. aeruginosa* on routine surveillance testing, and standard measures of disinfection and cleaning failed to eradicate the organism, including thermal disinfection, chlorination and local injection of peracetic acid. Flushing (opening the outlet for a set duration) before use was encouraged but compliance was variable.

Clinical Setting and Selection Criteria

Two wards (Ward A and Ward B) on separate floors of a tertiary teaching hospital (London, UK) were selected for detection of *P. aeruginosa* by environmental sampling of shower water and drains. Ten patient beds were selected at random on ward A (a non-augmented care unit; surgical patients) and ten from ward B (augmented care unit; haematology patients). Both wards accommodated adult in-patients. Beds in Ward A were either within a Single-Isolation Room (SIR) or multiple-patient- bay whereas Ward B had SIRs only. All SIRs selected included a dedicated en-suite bathroom while surgical patients in bays used shared patient bathroom facilities. Ethical approval was sought and deemed not required for this service evaluation.

Shower modification and Sampling frequency

Measures to reduce pseudomonal colonisation were determined by the local Water Safety Group and were performed quarterly. They comprised an exchange of the existing shower head (standard plastic, antimicrobial silver-impregnated or point-of-use antimicrobial filtration unit) and hose (ethylene propylene diene monomer (EPDM) or polyvinyl chloride (PVC) (length 1.2m) with an unused replacement head (antimicrobial silver-impregnated) and truncated hose (PVC) of length 0.8m. Signage was placed in shower areas to encourage users to avoid putting the shower head onto its mount after use, i.e. allowing the shower head to drain freely above the shower tray.

The study was performed over seven months and change of the shower and hoses (interventions) was undertaken at weeks 3-4, 13-15 and after week 21. The final shower change in ward B occurred after the end of the study. Environmental sampling of shower waters and surface swabbing of drains were performed at weekly during weeks 1-5, every two weeks between week 6 and 21 and then at week 29. The initial three weeks of the sampling study comprised the pre-intervention phase.

Shower water collection and processing:

Water collection was performed according to Health Technical Memorandum (HTM) 04-01 guidance [10] . Before and after collection of water samples, the entire outer surface of the shower heads was disinfected by wiping the surface with sterile alcohol wipes (Azo Wipes – 70% Isopropyl alcohol). A volume of at least 100 mL shower water was collected in sample bottles pre-loaded with two-mL of a neutraliser solution (0.1% sodium thiosulfate, 0.3% Lecithin, 3% Tween 80) capturing the pre-flush (initial volume of water upon opening the shower valve) flow. To avoid cross contamination of the sample, a sterile water-sample collection bag was secured around the opening of the shower head and the opposite end of the bag was used to direct

the water flow into the sample collection container via an incision to the bag using pre-disinfected scissors. Water was collected from the showers with the shower heads in place regardless of the shower head type (i.e. whether a point-of-use filter-shower was present or absent).

Samples were stored at refrigeration temperatures (2-8°C) and processed by filter-concentration within 24 hours of collection. Water filtration followed ISO standards (BS EN ISO 8199:2018) [14]. The water sample was concentrated by passing 100mL through a 0.45µm pore size nitrocellulose membrane mounted on a filtration manifold and evacuated using a vacuum pump (Max. 65kPa pressure). The membrane was transferred aseptically onto the surface of a pseudomonas C-N selective agar plate (Oxoid Ltd, Basingstoke, UK). Plates were incubated at 37°C for 48 hours under aerobic conditions and inspected at 24 hours to enumerate the presumptive *P. aeruginosa* colonies. The limit of detection of samples assayed in this way was 1CFU/100mL.

Surface sampling shower drains:

Sterile cotton-tipped swabs were pre-soaked by immersing in neutraliser solution (as above) before swabbing the drain exterior ensuring all exposed surfaces were covered, followed by swabbing of the drain interior cavity accessed via the drain holes. The swab contents were transferred immediately onto pseudomonas C-N agar plates using a streaking motion going horizontally, vertically and diagonally on the surface and rotating the swab tip continuously. Plates were incubated aerobically at 37ºC for 24 hours.

Confirmation of *P. aeruginosa*

Presumptive *P. aeruginosa* colonies (based on colony morphology) were enumerated as CFUs (colony forming units). Presumptive *P. aeruginosa* colonies were purified by harvesting a representative sample using a 10uL loop and streaking onto Columbia Blood Agar (CBA) (Oxoid Ltd, Basingstoke, UK) and incubating at 37ºC for 24 hours. Single colonies from the streaks were re-streaked in parallel onto Nutrient Agar (Oxoid Ltd, Basingstoke, UK) and Milk Cetrimide Agar (Oxoid Ltd, Basingstoke, UK) plates. Oxidase test was performed on colonies present on nutrient agar plates using test strips (BioConnections, UK). Casein hydrolysis (translucent zone around the colony) and fluorescence under 253-320nm UV light (using a UV-illuminator viewing chamber) was observed for on colonies present on Milk Cetrimide Agar plates. Isolates exhibiting growth on CN agar, presumptive plate morphology, oxidase-positive reaction, and casein-hydrolysis were considered confirmed *P. aeruginosa*. Isolates exhibiting a positive oxidase reaction and/or hydrolysing casein on MCA plates were selected for MALDI-TOF analysis (Maldi-TOF Biotyper® IVD system Bruker Daltronics) for confirmation. Strains were transferred to cryopreservation beads (Microbank™) and stored below -20 °C.

Antimicrobial susceptibility profiling of *P. aeruginosa* isolates

Susceptibility profiles of *P. aeruginosa* strains were generated by disc-diffusion assay [15] against the following twelve antibiotics (Oxoid): amikacin (30µg), gentamicin (10µg), tobramycin (10µg), aztreonam (30µg), meropenem (10µg), imipenem (10µg), ceftazidime (10µg), cefepime (30µg), ciprofloxacin (5µg), piperacillin (30µg), piperacillin/tazobactam (36µg), and ticarcillin/clavulanic acid (Biorad, 75/10µg).

Antimicrobial susceptibility testing (ASTs) by disc-diffusion and zone-inhibition were performed on a computer generated random 50% sample of the total strains isolated from shower waters and drain surfaces. Overnight cultures of the test *P. aeruginosa* strains were prepared on CBA at 37°C from which a colony was harvested using a cotton-tipped swab pre-moistened with sterile Phosphate Buffered-Saline (PBS; Oxoid, UK) before transferring to glass tubes containing 3mL PBS and re-suspending (vortex-mixing). Further dilutions were performed to adjust the turbidity to 0.5 McFarland standard solution. Resulting suspensions were streak-transferred to prepare a lawn on the surface of a Muller-Hinton (MH) agar plate (90mm diameter; 4mm depth: Oxoid, UK) and the target antibiotic disc arrays. MH plates were incubated at 35°C for 18+/-2 hours prior to reading. Susceptibility patterns were determined by measuring inhibition zone diameters against corresponding EUCAST breakpoints for *P. aeruginosa* (version 11.0) [16].

Statistical Analyses

Differences in results between groups were considered statistically significant when the p-value was <0.05.

i) Environmental P. aeruginosa occurrence

Occurrence of *P. aeruginosa* at a given location and time point during study was treated as a binary outcome (present/absent) and analysed using logistic multilevel (mixed) regression and the association with occurrence described as Odds ratio (OR) and confidence interval (CI).

Differences in occurrence were assessed through two approaches:

1. Changes over time: considering time as a continuous variable to examine occurrence of *P. aeruginosa*;
2. Time in categories: considering occurrence between the three time periods (interventions)

ii) Changes in antibiotic susceptibility profiles

Changes in *P. aeruginosa* susceptibility to each antibiotic between interventions were determined by categorising outcomes on a three-point ordinal scale; sensitive, intermediate or resistant and analysed using the Kruskal-Wallis and Mann-Whitney U-test.

Results

Fourteen sampling sessions were conducted over a seven-month period between 21.11.2018 and 06.06.2019, resulting in 280 shower water samples and 280 surface swabs taken from drains. Seven out of the ten showers on Ward A (non-augmented care unit) were dedicated to bed bays where the showers were shared between four patients while the remaining three showers belonging to SIRs. All ten showers on Ward B (augmented care unit) belonged to single isolation rooms.

Shower water and drain contamination

The presence of *P. aeruginosa* contamination in shower waters and drains during the study are shown in Table I. Where *P. aeruginosa* was isolated, there were >300CFU/100mL in all cases.

At the beginning of the study, all showers from ward A (non-augmented care) had long hoses (EPDM composition-1.2m) except for one (Shower #6) with a short hose (PVC-0.8m). Four showers were fitted with a point-of-use filtration device (Showers #1, #2, #4 and #5), four were plastic-chrome shower heads (Showers #3, #8, #9, #10) and two were antimicrobial silver-impregnated showers (Showers #6 and #7). Although measures against pseudomonas were not stipulated in a general ward, there had been earlier outbreaks of pseudomonal wound infection. All showers in ward B (augmented-care) were antimicrobial silver-impregnated showers with long hoses (PVC-1.2m) and without a filter.

During the course of the study, shower hoses were changed three times in Ward A (non-augmented care unit) (Table I.A) and two times in Ward B (augmented-care unit) (Table I.B). Initial sampling results showed that 4/10 shower outlets (40%) were contaminated with high counts (>300CFU/100mL) of *P. aeruginosa* in Ward A, whereas 8/10 (80%) of them were contaminated in Ward B. By the final sampling week, 5/10 shower outlets (50%) and 9/10 shower outlets (90%) were contaminated with *P aeruginosa* in Ward A and Ward B respectively.

P. aeruginosa was isolated from 5/20 drains at the start of the study and 10/20 drains at the end. *P. aeruginosa* in the drains did not always coincide with shower water contamination (Table I). Drains harboured many bacterial species. Other than *P aeruginosa*, species growing on C-N agar media, were *P. alcaligenes, P. fulva, P. guariconensis, P. mosselii, P. nitroreducens, P. stutzeri; Aeromonas hydrophilia, Aeromonas caviae, Citrobacter braaki, Citrobacter freundii, Achromobacter insolitus, Achromobacter denitrificans* and *Enterobacter cloacae*.

Table I. The effect of introducing unused antimicrobial shower head-hose units (interventions) into non-augmented care (Table I.A) and augmented care (Table I.B) wards on the presence of P. aeruginosa in showers (green) and corresponding drains

(orange) over a 29-week period.

Table I.A. Presence of *P. aeruginosa* in a non-augmented care setting (ward A)

#	Weeks / Room Type	Week 1	Week 2	Week 3	Intervention 1	Week 4	Week 5	Week 7	Week 9	Week 11	Week 13	Intervention 2	Week 15	Week 17	Week 19	Week 21	Intervention 3	Week 29
1	Bay	*	*	*														
2	Bay	*	*	*														
3	Side Room																	
4	Side Room	*	*	*														
5	Bay	*	*	*														
6	Bay																	
7	Bay																	
8	Side Room																	
9	Bay																	
10	Bay																	

Table I.B. Presence of *P. aeruginosa* in an augmented care setting (ward B)

#	Weeks / Room Type	Week 1	Week 2	Week 3	Intervention 1	Week 4	Week 5	Week 7	Week 9	Week 11	Week 13	Intervention 2	Week 15	Week 17	Week 19	Week 21	No Intervention	Week 29
11	Side Room																	
12	Side Room																	
13	Side Room																	
14	Side Room																	
15	Side Room																	
16	Side Room																	
17	Side Room																	
18	Side Room																	
19	Side Room																	
20	Side Room																	

*Denotes POU filter shower heads in-situ

Presence *of P. aeruginosa* is indicated by green (shower) and orange (drain) highlighted cells respectively. Blank cells represent no contamination by *P. aeruginosa*. Quarterly shower hose changes are marked by column.

At the start of the study, shower head and hose types varied between wards. Ward A shower heads: #1,#2,#4 and #5 had point-of-use (membrane) filters with long hose; shower heads #3, #8, #9 and #10 were plastic chrome (non-antimicrobial) with long hose (EPDM); shower heads #6 and #7 were antimicrobial silver-impregnated bodies with short and long hoses (PVC) respectively. All showers on Ward B were antimicrobial silver impregnated shower heads mounted on a long hose (PVC).

P. aeruginosa colonised both shower-waters and drains simultaneously in 79/260 occasions (~30%) with presence in only the drain or shower water on 11/260 (~4%) and 81/260 (~31%) of occasions respectively. In 89/260 occasions (~34%), *P. aeruginosa* did not present in the shower water or drain. A cross-tabulation of *P. aeruginosa* presence in the shower environment showed contamination of one site (shower or drain) correlated with occurrence in the adjacent site/outlet (chi-square: $p < 0.001$).

Associations between ward type and exposure (time)

a) Approach 1 – Occurrence of contamination over time

Logistic multi-level regression modelling demonstrated a significant association between duration of use in the clinical setting and occurrence of *P. aeruginosa* in shower waters (P=0.004) and drains (P=0.03); these are depicted in figure 1 (showers) and figure 2 (drains).

For every week elapsed, the odds for colonisation with *P. aeruginosa* in the non-augmented care setting increased by 19% in shower waters (OR= 1.19; CI= 1.09 - 1.31, P<0.001) and 18% in the drains (OR = 1.18; CI= 1.09 - 1.30, P<0.001). Odds for *P. aeruginosa* colonisation over time remained unchanged in the augmented care wards for showers (OR= 0.95; CI= 0.84 - 1.07, P=0.42) and drains (OR= 1.04; CI= 0.98 - 1.11, P=0.23). Graphical illustrations of the fitted change over time are shown in Figure 1 for showers, and for Figure 2 for drains.

b) Approach 2 – Occurrence of colonisation between interventions

The frequency of contamination of shower waters and drains with *P. aeruginosa* and odds of occurrence between interventions (introducing antimicrobial shower head-hose units) in augmented and non-augmented care wards is shown in table II. The pre-intervention (control) phase comprised the number and percentage of measurements where *P. aeruginosa* was present for each sample site/ward/timepoint combined. The first and second intervention depict the odds of *P. aeruginosa* occurrence in each period relative to the odds for the control period.

Table II: Comparison of frequency and odds of occurrence of *P. aeruginosa* in shower waters and drain surfaces following interventions relative to a pre-intervention (control) phase.

Sample site	Clinical setting (Ward)	*P. aeruginosa* occurrence rate **n/N**(%) and Odds ratio (95% CI)			Time P-value
		Week 1-3 [Pre-intervention / control]	Week 4-13 [First intervention]	Week 15-21 [Second intervention]	
Shower waters	Non-augmented care (A)	**12/20** (40%) 1.00	**25/60** (42%) 1.14 (0.33, 3.91)	**27/40** (68%) 11.4 (2.35, 55.1)	0.003
	Augmented care (B)	**23/30** (77%) 1.00	**44/60** (73%) 0.52 (0.08, 3.62)	**29/40** (73%) 0.45 (0.05, 3.66)	0.73
Drains	Non-augmented care (A)	**4/30** (13%) 1.00	**14/60** (23%) 2.31 (0.61, 8.83)	**23/43** (58%) 18.2 (4.22, 78.6)	<0.001
	Augmented care (B)	**7/30** (23%) 1.00	**24/60** (40%) 2.84 (0.91, 8.88)	**18/40** (45%) 3.79 (1.11, 12.9)	0.09

Relative to the pre-intervention phase, the likelihood of shower water and drains in the non-augmented care setting (ward A) becoming colonised with *P. aeruginosa* increased over time (p<0.05), with the highest density of colonised showers/drains after the second intervention.

Colonisation of showers and drains in the augmented care ward (Ward B) persisted at high frequency regardless of the intervention.

Antimicrobial Susceptibility profiles

Of 274 positive *P. aeruginosa* samples, 117 were isolated from Ward A (69 shower head water; 48 shower drain) and 157 were isolated from Ward B (105 shower water and 52 shower drain). ASTs were performed on a 50% sample (Ward A shower water n=35; Ward A shower drain n=24; Ward B shower water n=53; Ward B shower drain n=26).

In the non-augmented care setting, cefepime resistance in *P. aeruginosa* from shower water declined after the first and second interventions ($P<0.05$) relative to the pre-intervention isolates with no changes observed in drain isolates (see supplementary Table 2). No other resistance profiles changed in relation to interventions on this ward.

The first set of analyses examined changes over the course of the study for measurements taken from showers in Ward A. A summary of the analysis results is shown in Supplementary Table 1.

Approximately 50% of the sample population of *P. aeruginosa* isolated from shower waters in the augmented care wards were resistant to imipenem in the pre-intervention phase. Frequency of imipenem-resistance declined to 12% and 31% after the first and second intervention respectively ($P<0.05$). No other significant differences were observed for the other antibiotics.

The final analyses compared between time periods for data from drains in Ward B. The results are summarised in Supplementary Table 4. Drains in the augmented care wards harboured *P. aeruginosa* with resistance to aztreonam in 75% of occasions during the pre-intervention phase that declined to 20% and 0% after subsequent interventions (first and second intervention respectively). Similarly, ciprofloxacin resistance in drain-derived *P. aeruginosa* declined after the interventions relative to the pre-intervention phase ($P<0.05$). Statistically significant differences between the three time periods were observed for ciprofloxacin, resistant measurements were most frequently seen in period 1, with a fewer in the subsequent time periods.

Discussion

Safeguarding vulnerable patient groups requires appropriate monitoring of shower waters to identify clinical risk and, when *P. aeruginosa* is found, effective remedial interventions. In this study, we demonstrated shower waters and drains were contaminated simultaneously with *P. aeruginosa* on 87/280 occasions (31%). However, changing or shortening the hoses did not provide any long-lasting benefit. Contamination may transfer from shower to drain or vice versa. Once *P. aeruginosa* is attached to the outside of the shower head, it migrates into stagnant water inside the head and then the shower hose itself [10]. Further retrograde spread to the thermostatic mixing valve, O rings and proximal plumbing is no longer remediable by changing the hose or local disinfection of the distal surfaces of the outlet. Some showers became colonized during the study, likely from retrograde contamination. However, the mains supply was free of contamination whenever tested. Hence, additional systemic chlorination or hyperchlorination was not attempted. Water supplies in the area had between 80 - 365 milligrams per litre calcium carbonate (Thames Water) and readily formed scale deposits [17].

The sampling sites adopted in this study did not include sentinel the sampling points used typically for legionella monitoring as presence of *P. aeruginosa* was related to local use not systemic contamination. Sampling of mains supplies during this period was consistently negative.

Sampling the same twenty showers over seven months demonstrated that once the shower environment (hose, showerhead, drain) was colonised with *P. aeruginosa*, it persisted for extended periods and in high numbers, particularly in augmented care wards. Only 8 of 20 showers (40%) were free of colonization at the outset. The type of shower hose and head and periodic replacement did not alter the colonization status of most showers, whether in a single-isolation room or shared-occupancy bay. In only 3 of 20 showers (15%) did change of hose and head result in a temporary loss of colonization. One shower remained free of *P. aeruginosa* contamination after hose change for 24 weeks before it was recolonized. Routine 6-monthly sampling

frequencies as recommended in HTM guidelines is not sufficient to capture intermediate changes in colonisation status of outlets [10,13]. In the non-augmented care ward, one shower was cleared of *P. aeruginosa* completely, suggesting colonization had not reached the thermostatic mixer valve where reseeding of shower head-hose units would occur. Over a third of drains contained *P. aeruginosa* throughout the study period but shortening of the hose had no effect on colonization of the shower head, except in a single shower in non-augmented care.

A strong linear correlation was observed for the probability of *P. aeruginosa* colonisation in the non-augmented care setting. Shower and drain colonisation increased by ~18% for each additional week shower head-hose units were exposed to the environment, despite the introduction of antimicrobial silver-impregnated shower units in a regular (3 monthly) cycle. Antimicrobial silver is active against *P. aeruginosa* [18]. However, eradication of *P. aeruginosa* biofilms may require antimicrobial-silver concentrations 10-100 times greater than that required for planktonic cells, with doses of ~5-10 ug/mL typically required to demonstrate significant reductions [18]. In our study, the antimicrobial-shower head-hose units were commercially-available products. To achieve microbial reductions an antimicrobial material, such as silver-impregnated shower-head-hose units, would require an adequate concentration of silver at the interface between the surface and the microorganism. Thus, silver distributed within the thickness of the shower head-hose material will have no effect on contamination. The concentration of silver in these shower units was not known; this was beyond the scope of the study objectives and not found in manufacturer product specifications. Since dilutions of samples taken were not performed, all positive sites were reported as >300CFU/100mL or >300CFU/swab. However, the absence of reduction in microbial contamination suggests the colonisation was greater than the level against which silver would be effective or activity was inhibited by biofilm.

Drains are a known reservoir of *P. aeruginosa* and contamination of water outlets from drains has been demonstrated[19]. Preventing contact and droplet transmission between shower head and drain by truncating shower hoses did not affect colonization rates in our study. Horizontal-cross transmission of *P. aeruginosa* between the showers and drains, likely via the patient, healthcare staff and/or domestic cleaning personnel, appeared to be more important. The likely route of contamination of shower heads from drains was through contaminated droplets/bioaerosols generated during ablutions and/or handling of the shower head with contaminated hands. In this study, *P. aeruginosa* occurred more frequently in showers than drains; this may be due to daily disinfection of the shower environment suppressing contamination in the drains. Despite this, correlation between these sites suggest that once *P. aeruginosa* colonises a shower or drain, other sites in the same room are likely to be contaminated. The primary reservoir in each shower-drain pairs was not known.

Under HTM 04-01 guidelines, non-augmented care wards do not need routine monitoring for pseudomonas and the contamination-status of the water outlets would not be known. The shower environment in the non-augmented care setting may represent an unrecognised reservoir of *P. aeruginosa* in showers waters and drain surfaces.

Patients in augmented care wards had sole use of their showers whilst 7 of 10 in non-augmented care were shared. There were no differences in cleaning frequency or bed occupancy in the wards and both bays and single rooms were almost fully occupied throughout the period of study. However, the frequency of shower use and the volume of water used per episode was not recorded. In point prevalence surveys, the proportion of patients in haematology (43%) and critical care wards (56%) receiving antibiotics exceeded those in surgical (28%) or medical (23%) wards and this may have influenced type of flora in the patient environment [7] . The presence of identical genotypes of *P. aeruginosa* in water outlets and patients is well described, although the direction of transmission is often unclear [2]. The distance between a thermostatic mixer valve and point of use in a shower greatly increases the volume of stagnant mixed hot and cold water in a shower compared with a tap. Consequently, contaminating organisms in a shower hose will have a large available luminal surface area. Mechanical flushing with water may be less likely to eliminate *P. aeruginosa* from showers, particularly if water pressure is low. To minimise colonisation of the shower hose with biofilm, users had been encouraged to allow the hose to drain under gravity into the shower tray. Either this measure was not effective or users were not compliant.

The direction of transmission between the patient and the shower or drain was not demonstrated in our study. However, patients with mucositis, intravascular catheters or foot wounds would be open to potentially invasive contamination from the

environment. Others have suggested showers and taps represent a significant reservoir of *P. aeruginosa* for patients vulnerable to developing bacteraemia. In a study of outlets in 23 augmented care units over 16 weeks [20] , between 0.9% and 16% of outlets demonstrated colonisation. Whole genome sequencing suggested a single genotype persisted within an outlet, possibly related to contamination in manufacture. Judging by epidemiological links in time and place, indistinguishable isolates suggested acquisition from the environment in 5% of patients. In another study, taps in 10 ICUs were repeatedly sampled and isolates typed by pulsed field electrophoresis [21]. More infections appeared to be transmitted between patients than from the outlets to patients. A tap water source of organisms detected on patient screening was suggested in 17% of patient acquisitions. Strains persisted in taps a median of 5 weeks with longer durations in electronic taps. However, non-augmented care areas were not sampled in either study. In 141 isolates taken from showers in a burns unit, whole genome sequencing showed clustering of isolates by room and outlet and three patients had identical genotypes to their environment [22]. A thermostatic mixer valve was shown to be one source of water contamination.

In the current study, two statistical approaches showed significant increase in *P. aeruginosa* over time in both showers and drains for Ward A but not Ward B. Personal protective equipment (PPE) was worn by staff when entering the single isolation rooms depending on the resident patients' infection status[23]. However, shared bay showers were located on corridors, near bed bays and entry was not restricted.

The diverse microbiome in the clinical water drains may pose a risk of antimicrobial gene transfer between co-existing microbial communities [24,25]. However, among the antibiotic susceptibility tests against twelve antibiotics, there were few statistically significant differences over the period of study and some for antibiotics not used in the wards, such as aztreonam. Resistance to aztreonam has been reported to be persistent, even in the absence of any selective pressure [26].

There are limitations to this study. A single hospital was involved and a small number of outlets were tested repeatedly. Rates of colonization are likely to depend on the patient population and arrangement of en-suite versus shared showers. The results may not be generalisable to older buildings and different patient populations.

Conclusion

Where *P. aeruginosa* colonisation is established in shower systems, shower hose change, installation of antimicrobial shower heads and repeated local disinfection were ineffective in reducing colonisation. Point-of-use filtration of showers was effective in the short term but at significant cost. More effective means of preventing and removing *P. aeruginosa* colonization are required, for example, regular surface disinfection and/or mechanical flushing before every use. For augmented care patients, routine testing of water for *P. aeruginosa* should be continued, even in the presence of a filter. The frequency of testing should be determined locally depending on use, flushing and disinfection regimens and prevalence of colonisation and patient infections.

Non-augmented care wards are exempt from routine microbial-water monitoring but pose potential reservoir for acquisition and the horizontal transfer of *P. aeruginosa* to vulnerable patients in augmented and non-augmented settings. Studies to determine a microbial bio-load threshold-value (CFU/cm^2) beyond which antimicrobial-silver surfaces fail to eradicate *P. aeruginosa* biofilms is required as a standard for manufacturers. Adoption of antimicrobial silver products in healthcare water systems should be supported with in-situ efficacy data against *P. aeruginosa.*

Declarations

Acknowledgements

We thank Samuel Yui and Monika Muzslay for their guidance and help through the study. We also thank Shubha Talwar and Jotika Halai for their assistance with antibiotic susceptibility testing. Part of this work was presented in FIS/HIS International Conference 2020 as an oral presentation.

Conflict of Interest

No conflicts of interest declared.

Funding Source

Author Özge Yetiş is funded by Turkey Ministry of National Education for her doctoral studies. Author Peter Wilson was part funded by supported in part by the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

Data Availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

References

1. Loveday, H. P. *et al.* Association between healthcare water systems and *Pseudomonas aeruginosa* infections: A rapid systematic review. *J. Hosp. Infect.* (2014) doi:10.1016/j.jhin.2013.09.010.
2. Bédard, E., Prévost, M. & Déziel, E. *Pseudomonas aeruginosa* in premise plumbing of large buildings. *MicrobiologyOpen* 5, 937 (2016).
3. Recio, R. *et al.* Predictors of Mortality in Bloodstream Infections Caused by *Pseudomonas aeruginosa* and Impact of Antimicrobial Resistance and Bacterial Virulence. *Antimicrob. Agents Chemother.* 64, (2020).
4. Fingertips. AMR local indicators - Data - OHID. *Fingertips.phe.org.uk*(2021). at <https://fingertips.phe.org.uk/profile/amr-local-indicators/data#page/3/gid/1938132910/pat/158/par/TE_trust/ati/118/are/RGT/iid/93399/age/1/sex/4/cid/4/tbm/1.>
5. Ortolano, G. A. *et al.* Hospital water point-of-use filtration: A complementary strategy to reduce the risk of nosocomial infection. *Am. J. Infect. Control* (2005) doi:10.1016/j.ajic.2005.03.014.
6. Wu, W., Jin, Y., Bai, F. & Jin, S. *Pseudomonas aeruginosa. J. mol. med.* 2–3, 753–767 (2015).
7. Versporten, A. *et al.* Antimicrobial consumption and resistance in adult hospital inpatients in 53 countries: results of an internet-based global point prevalence survey. *Lancet Glob. Health* 6, e619–e629 (2018).
8. Choi, Y., Paik, J. H., Kim, J. H., Han, S. B. & Durey, A. Clinical Predictors of *Pseudomonas aeruginosa* Bacteremia in Emergency Department. *Emerg. Med. Int.* 1–6 (2018).
9. Exner, M. *et al.* Prevention and control of health care-associated waterborne infections in health care facilities. *Am. J. Infect. Control* (2005) doi:10.1016/j.ajic.2005.04.002.
10. Department of Health. *Health Technical Memorandum 04-01: Safe water in healthcare premises. Part C: Pseudomonas aeruginosa – advice for augmented care units* (2016).
11. ASTM D5612-94(2008) - Standard Guide for Quality Planning and Field Implementation of a Water Quality Measurement Program. https://webstore.ansi.org/Standards/ASTM/ASTMD5612942008.
12. How Standards Support National Water Quality Month. *American National Standards Institute - ANSI*(2015). at <https://www.ansi.org/news/standards-news/all-news/2015/08/how-standards-support-national-water-quality-month-11.>
13. Department of Health. *Health Technical Memorandum 04-01: Safe water in healthcare premises Part A: Design, installation and commissioning*. (2016).
14. ISO - ISO 8199:2018 - Water quality — General requirements and guidance for microbiological examinations by culture. https://www.iso.org/standard/64151.html.
15. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. *ASM* 1–13 (2012).

16. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 8.1, valid from 2018-05-15. http://www.eucast.org. (2018).
17. Thames Water Utilities Limited Water Quality Report. 1–12 https://www.thameswater.co.uk/help/water-quality/check-your-water-quality#/search (2021).
18. Bjarnsholt, T. *et al.* Silver against *Pseudomonas aeruginosa* biofilms. *APMIS: acta pathologica, microbiologica, et immunologica Scandinavica* 115, 921–928 (2007).
19. Berrouane, Y. F. *et al.* Outbreak of severe *Pseudomonas aeruginosa* infections caused by a contaminated drain in a Whirlpool Bathtub. *Clin. Infect. Dis* 31, 1331–1337 (2000).
20. Halstead F D , Quick J, Niebel M, Garvey M, Cumley N, Smith R, Neal T, Roberts P, Hardy K, Shabir S, Walker J T, Hawkey P, L. N. J. *Pseudomonas aeruginosa* infection in augmented care: the molecular ecology and transmission dynamics in four large UK hospitals. *J Hosp Infect* 111, 162–168 (2021).
21. Coppry M , Leroyer C , Saly M , Venier A-G , Slekovec C , Bertrand X , Parer S , Alfandari S , Cambau E , Megarbane B , Lawrence C , Clair B , Lepape A , Cassier P , Trivier D , Boyer A , Boulestreau H, Asselineau J , Dubois V , Thiébaut R, R. A.-M. Exogenous acquisition of *Pseudomonas aeruginosa* in intensive care units: a prospective multi-centre study (DYNAPYO study). *J. Hosp. Infect.* 104, 40–45 (2020).
22. Quick, J. *et al.* Seeking the source of *Pseudomonas aeruginosa* infections in a recently opened hospital: an observational study using whole-genome sequencing. *BMJ Open* 4, e006278 (2014).
23. Ballantine, N. Policy For The Use Of Personal Protective Equipment When Handling Chemotherapy, Spillage Of Chemotherapy, Body Waste And / Or Clinical Samples From Patients Receiving Chemotherapy. *Birmingham Children's Hospital* v3.0, (2019).
24. Muzslay, M., Moore, G., Alhussaini, N. & Wilson, A. P. R. ESBL-producing Gram-negative organisms in the healthcare environment as a source of genetic material for resistance in human infections. *J. Hosp. Infect.* 95, 59–64 (2017).
25. Oberauner, L. *et al.* The ignored diversity: Complex bacterial communities in intensive care units revealed by 16S pyrosequencing. *Sci. Rep.* 3, 1–12 (2013).
26. Jorth, P. *et al.* Evolved aztreonam resistance is multifactorial and can produce hypervirulence in *Pseudomonas aeruginosa*. *mBio* 8, (2017).

Figures

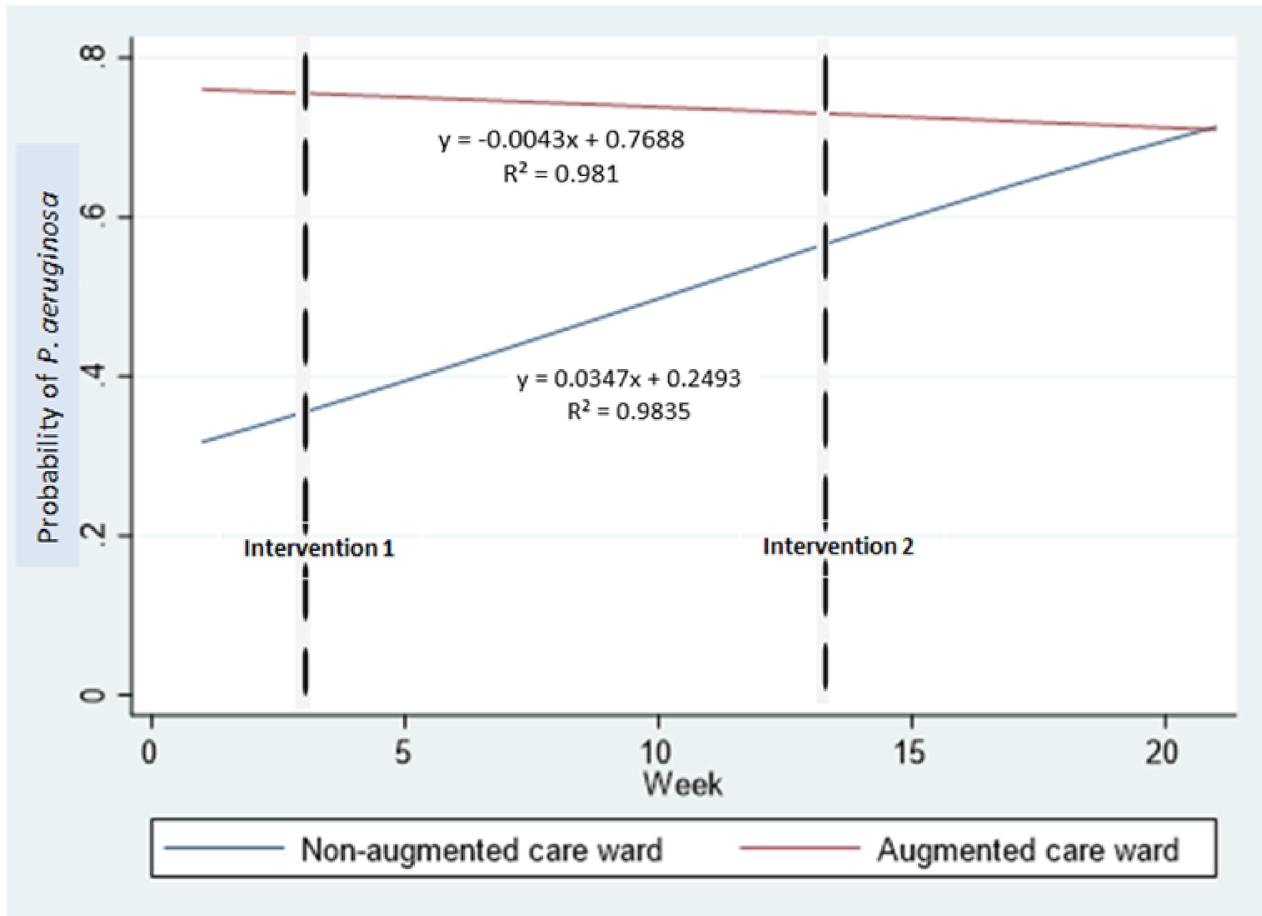

Figure 1

Probability of *P. aeruginosa* contamination of shower waters with time (weeks) in augmented and non-augmented care settings. Y-axis represents the occurrence ratio as a function of duration of exposure (X-axis). No changes were made to drains. Shower head-hose units were replaced with unused antimicrobial silver-impregnated replacements in week 3 and 13 (interventions 1 and 2 respectively).

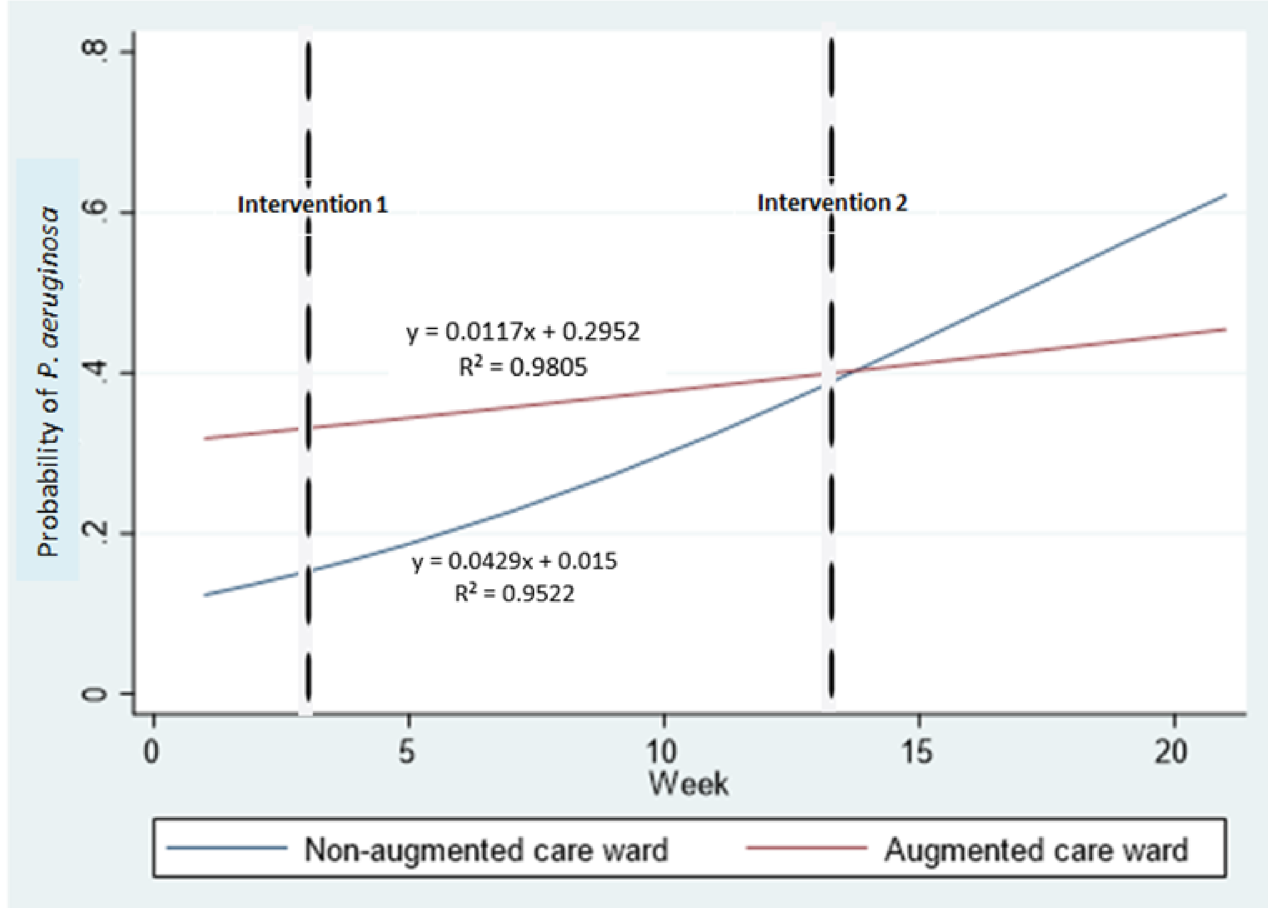

Figure 2

Probability of *P. aeruginosa* contamination of drain surfaces with time (weeks) in augmented and non-augmented care settings. Y-axis represents the occurrence ratio as a function of duration of exposure (X-axis). Shower head-hose units were replaced with unused antimicrobial silver-impregnated replacements in week 3 and 13 (interventions 1 and 2 respectively).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PaperSupplementaryData.docx
- Rawdata.AntibioticSusceptibilityTestResults.xlsx
- Predictedprobabilitiesforregressiongraphs.xlsx