The effects of a prospective sink environmental hygiene intervention on *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* burden in hospital sinks

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Summary

Background Opportunistic premise plumbing pathogens (OPPPs) can establish reservoirs in hospital plumbing and cause healthcare associated infections (HAIs). There is currently no widely accepted protocol for sink drain cleaning to reduce OPPP burden.

Methods We implemented a sink cleaning intervention in 12 intensive care unit (ICU) rooms. At low frequency (1×/week) and high frequency (5×/week) intervals, we wiped sink surfaces with 10% bleach wipes and pumped a foamed preacid disinfectant into sink drains. We also maintained untreated rooms (0×/week). We used E-swabs to sample sink drains and surrounding surfaces during one baseline, two intervention, and two post-intervention periods over 23 months. Samples were selectively cultured for bacterial growth and antimicrobial resistant organism (ARO) isolation. We conducted whole-genome sequencing (WGS) on *Pseudomonas* spp. and *Stenotrophomonas* spp. isolates to track impacts on reservoirs over time. We also collected and analysed clinical isolates from patients occupying the study rooms and information about HAIs that occurred.

Findings The intervention reduced the proportion of sink drains yielding Gram-negative bacteria by up to 85% (95% CI: 56–114%) in high frequency rooms versus the baseline period, but this was not significant in low frequency rooms. It also reduced the proportion of sink drains yielding *Pseudomonas* spp. and *Stenotrophomonas* spp. by up to 100% (95% CI: 79–121%) and 95% (95% CI: 65–125%) versus the baseline period in high frequency rooms and up to 71% (95% CI: 50–92%, $p < 0.001$) and 66% (95% CI: 27–105%, $p < 0.05$) in low frequency rooms, respectively. WGS showed strains of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* that colonised sink drains for over 3 years across two studies. Following the intervention periods, *P. aeruginosa* reservoirs were replaced with new strains, while *S. maltophilia* reservoirs returned with the same strains.

Interpretation This environmental hygiene intervention may be effective in reducing the burden of OPPPs in hospital sinks.

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Research in context

Evidence before this study

Opportunistic pathogens can reside in hospital sink drains long-term and cause patient infections. There are currently no standardised protocols for sink drain cleaning to reduce or remove these organisms. While previous studies have utilised sink cleaning interventions in response to outbreaks, these studies are limited in that the intervention is generally applied over relatively short periods of time and they lack an untreated comparison group. Additionally, it is unclear how effective the interventions may be in a non-outbreak setting.

Added value of this study

We characterised a sink drain cleaning protocol in a non-outbreak setting to study long-term effects and compare with untreated rooms. In intervention rooms, we pumped a foamed disinfectant (Virasept, Ecolab, St. Paul, MN) into hospital sink drains to reduce bacterial burden and used a bleach wipe to disinfect sink surfaces. We tested this protocol at 1×/week (low frequency) and 5×/week (high frequency) frequencies and included untreated rooms that received no intervention. We found significant decreases in Gram-negative bacterial burden from high frequency but not from low frequency rooms, as well as decreases in antimicrobial resistant organisms such as *Pseudomonas* spp. and *Stenotrophomonas* spp. at both intervention frequencies when compared to their pre-intervention states. We found that *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* return to sink drains differently after cessation of the intervention. Finally, we saw no increases in antimicrobial resistance genes or mutations in *P. aeruginosa* or *S. maltophilia* as a result of the intervention.

Implications of all the available evidence

This intervention can successfully reduce hospital sink drain bacterial burden that may cause patient infections. Additional work should examine the feasibility of hospital staff implementing this intervention as part of regular cleaning protocols. The design of this study provides a model for examining interventions of this type prospectively rather than just as an urgent response to outbreaks.

Introduction

Antimicrobial resistant organisms (AROs) can inhabit hospital premise plumbing and have been linked to healthcare associated infections (HAIs).[1–7] These AROs have been dubbed opportunistic premise plumbing pathogens (OPPPs) and can establish reservoirs that resist standard cleaning efforts.[5–8] Numerous outbreaks of OPPPs such as *P. aeruginosa, S. maltophilia,* and carbapenem-resistant Enterobacterales have derived from hospital sink drains.[1,3,5–7,9–11] Moreover, recent work has revealed that infections traced to sink drain colonisation can also occur in non-outbreak settings, suggesting that the true impact of OPPPs may be underestimated.[12]

Despite growing concern over the risk of OPPPs from sink drains to patients, there is currently no widely accepted, standardised protocol for sink drain cleaning in healthcare facilities.[5–7,13] Structural interventions have been proposed as a potential solution for reducing OPPPs in healthcare settings, but these strategies are often difficult and costly to implement.[5,6,14–16] One proposed intervention targets planktonic OPPPs circulating through the water system by installing filters on faucets, but this strategy does not impact OPPPs colonising downstream pipes or sink drain surfaces.[14] Others have implemented fully 'waterless' hospital rooms, but this complicates patient care and requires a large initial investment to restructure rooms.[15,17] As a last resort in an outbreak setting, some facilities have elected to fully replace the sink infrastructure in the affected rooms; however, this option is expensive and impractical in many settings.[7,16]

Numerous chemical interventions have also been piloted, but many disinfectants have lacked efficacy against the biofilms formed by OPPPs.[5,6,18] However, some work has suggested that using a foam versus a liquid disinfectant can increase contact time with sink drain surfaces, increasing the ability of a disinfectant to break down OPPP biofilms.[19]

We sought to investigate the effectiveness of pumping a foamed preacid-based disinfectant (Virasept) into sink drains and wiping surrounding sink surfaces with a bleach wipe for the reduction of OPPPs in hospital sink drains.[20] Our primary goal was to measure the reduction in total bacterial burden and recoverable AROs by our collection and selective culture methods. Next, we utilised bacterial whole-genome sequencing (WGS) to track strains and examine the ability of the intervention to reduce or remove long-term colonising *P. aeruginosa*

and *S. maltophilia*. We included isolate WGS data from a previous study (HM Study) in the same hospital rooms to extend our surveillance in these rooms. Finally, we used genomics analyses to characterise mutations in long-term colonising strains that are correlated with the intervention.[12]

Methods

Study design

This study was conducted at Barnes-Jewish Hospital (BJH), a large tertiary care academic medical centre in St. Louis, Missouri, United States.[21]

The intervention was tested in two distinct units on the same floor that share staff: a stem cell transplant intensive care unit (SCTO-ICU) and a surgical intensive care unit (SICU). Eighteen patient rooms were included in this intervention trial: 8 in the SCTO-ICU housing SCTO-ICU patients, 8 in the SICU housing SICU patients, and 2 in the SICU but housing SCTO-ICU patients. Rooms from both units were sequentially assigned to each of 3 intervention arms: an untreated arm, a low frequency intervention arm (1×/week), and a high frequency intervention (5×/week, Monday-Friday) arm (Fig. 1). In the BMT ICU, rooms were sequentially assigned to intervention groups (1 = low frequency intervention, 2 = high frequency intervention, 3 = untreated, repeat). Rooms in the SICU were randomly assigned to an intervention group using a random number generator. The drains in two shared spaces in each ICU, the housekeeping closet (HC) and soiled utility room (SU), were also included. The SICU shared spaces received the low frequency intervention while the SCTO-ICU shared spaces received the high frequency intervention.

All rooms received routine, standard of care, cleaning throughout the study period, Standard cleaning at this centre involved environmental services wiping down sink surfaces with a bleach wipe daily for 6 days per week, and then applying Brulin Performex (Brulin, Indianapolis, IN, #161052) disinfectant on the 7th day. The standard cleaning practices do not include a process for cleaning sink drains. All patient room sinks shared the same design: gooseneck faucets are centrally positioned behind the sink bowl such that water flows directly into the drain, which sits near the rear of the sink bowl.

This study spanned a total of 23 months. This included 5 periods composed of an initial 16-week baseline period during which no rooms received the intervention, followed by a 27-week intervention period, and then a 16-week post-intervention period. This was followed by another 23-week intervention period and another 16-week post-intervention period (Fig. 1).

Ethics

The study protocol was approved by the Washington University Human Research Protection Office (IRB #202008081). A waiver of the requirement to obtain informed consent was granted because this study involved only environmental sampling and the collection of remnant clinical isolates that were collected for clinical purposes. The HAI data was obtained in accordance with standard National Healthcare Safety Network (NHSN) surveillance protocols.

Intervention design

The intervention was implemented by research study personnel after samples were collected in that room. For sinks in patient rooms, the intervention included 2 steps. First, Sani-Cloth™ Bleach Germicidal Disposable Wipes (PDI™, Woodcliff Lake, NJ, #P84172) were used to clean the faucet, sink bowl, lower counter surrounding the sink, and raised counter next to the sink. A separate wipe was used for each surface, and, after a contact time of 10 min, the surfaces were rinsed with distilled water and then wiped dry with a paper towel. Next, a 1.5 L FOAMit Pump (FOAMit, Grand Rapids, MI, #F1.5L) was used to manually infuse 10 ounces (295 mL) of a preacid based disinfectant (Virasept, Ecolab, St. Paul, MN, #6002314), which is Environmental Protection Agency (EPA)-approved for biofilm disinfection, directly into the sink drains.[20] After a dwell time of 3 min, the faucet was run for 30 s. In the HC and SU, a 10-gallon (38 L), battery-powered FOAMit pump (FOAMit, FI-10N) was used to infuse 32 ounces (946 mL) of Virasept into the primary sink drain. No bleach wipes were used in these shared spaces due to a lack of sink surfaces. Each room was assigned to its own FOAMit pump for the duration of the entire study to reduce potential cross contamination and the pumps were cleaned after each use.

Environmental swabbing and selective culture

Environmental samples were collected from six surfaces in study rooms a total of 31 times during the 23-month study period. Samples were collected immediately prior to application of the intervention. At each sampling time point, 3 E-swabs (Copan, Murrieta, CA, #480C) were moistened with sterile molecular water and held in tandem to sample each of the following surfaces in each patient room: the sink drain, the faucet, the sink bowl, the lower sink counter, and the raised sink counter (Fig. 1). For drains, swabs were inserted through the grate and swiped around the drainpipe. In the shared areas, only drain samples were collected. On dates when the intervention was applied, environmental samples were collected prior to application of the intervention. After collection, all E-swabs were placed into Aimes transport medium (Thermo Fisher Scientific, Lenexa, KS, #CM0425J) and stored at room temperature for up to 4 h before culturing.

During non-intervention periods, samples were collected weekly for three weeks and then monthly for three months. During intervention periods, samples were collected monthly. Full selective culture agar

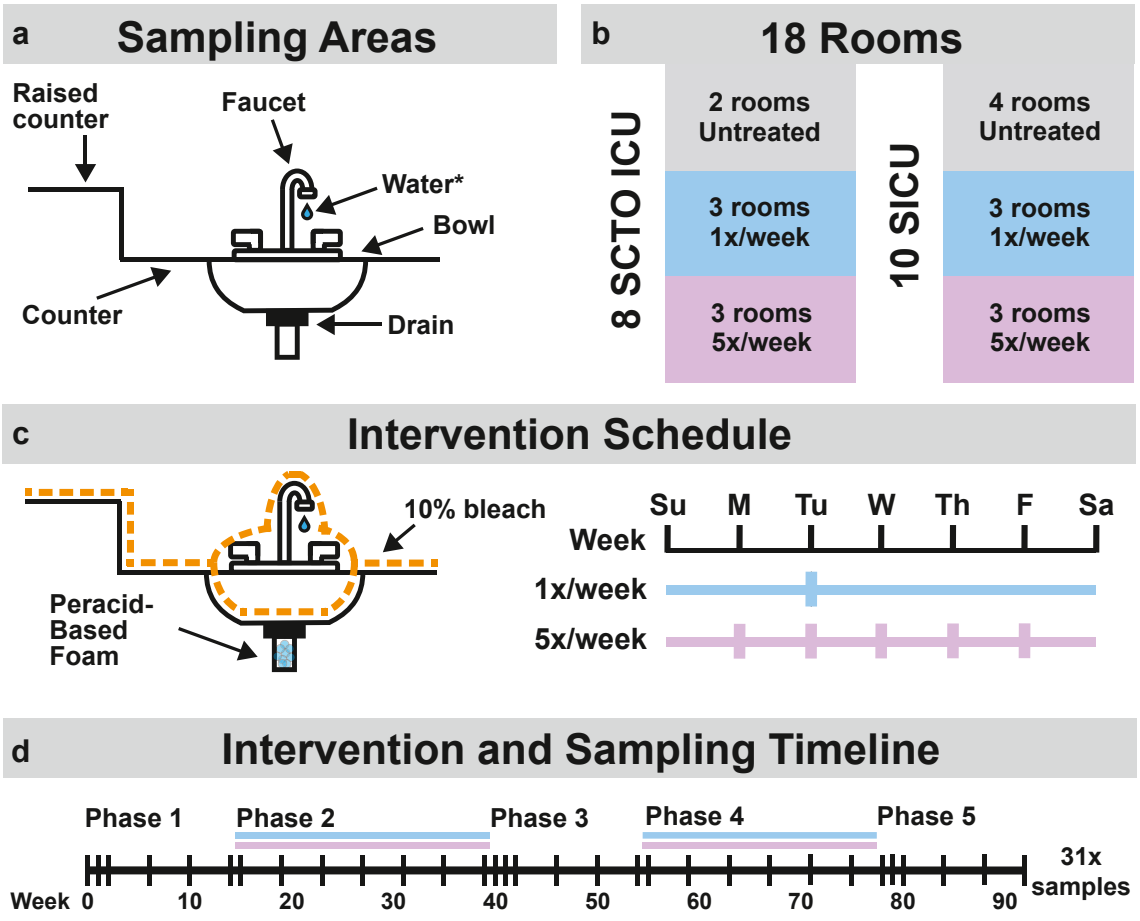

Fig. 1: **Study design and sample processing overview**. a) E-swabs were collected from the raised counter, surrounding counter, faucet, sink bowl, and sink drain at every sampling timepoint. Water was not collected at every sampling timepoint. b) This study included 18 rooms, with eight from the stem cell transplant intensive care unit (SCTO-ICU) and ten from the surgical intensive care unit (SICU). c) The intervention was applied at low (1×/week) and high (5×/week) frequencies. d) This study covered 5 periods of intervention or non-intervention and included 31 sample collection timepoints.

details can be found in Supplementary Table S1. For each surface sample, 100 µL of eluate was cross-streaked onto selective agars. One colony of each unique morphotype was selected and subcultured to a blood agar plate (Hardy, Santa Maria, CA, #A10BX). After isolation, small amounts of selected colonies were placed onto VITEK MS Target Slides (bioMerieux, Durham, NC, #410893) using wooden toothpicks. Isolate taxa were identified using matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry (MS) using the VITEK MS (bioMerieux), an automated system which can identify microbes at the genus or even species level.[22] A stock of each isolate was frozen at −80 °C in tryptic soy broth (TSB) with 10% glycerol (Hardy, #U127). All isolates collected from selective agars were considered antimicrobial resistant organisms (AROs). All culturing, colony selection, and identification were conducted by a trained microbiology team.

Water sampling and selective culture

Water samples from the faucets in patient rooms and shared area rooms were collected 12 times across the study period: twice during each non-intervention period (baseline and two post-intervention periods) and 3 times during each intervention period. Each faucet was run for 10–20 s before 1 L of water was collected directly into a sterile plastic container. Water samples were stored at room temperature for up to 24 h before processing. Samples were then poured through a sterile membrane filter (Cytiva, Marlborough, MA, #4764). The filter was then placed grid side up on a blood agar plate and incubated for up to 48 h at 35 °C. Water samples were considered negative if ≤ 50 colonies grew on a heterotrophic plate, which is 1 order of magnitude below than the Environmental Protection Agency (EPA) standard.[23] Unique colony morphologies were isolated and identified as above. AROs (including *Pseudomonas* spp. and

Stenotrophomonas spp.) were stored as TSB-glycerol stocks at –80 °C.

Clinical isolate collection

Twice per week during the study period, we ran an electronic medical record (EMR) query to identify clinical specimens that had been collected by hospital staff for the purposes of routine patient care from patients admitted to the two study wards, and that, upon analysis by the hospital microbiology laboratory, had tested positive for *P. aeruginosa* or *S. maltophilia*. These isolates were obtained from frozen stocks in the hospital microbiology laboratory, when available.

HM study isolates

The SCTO-ICU in this study had also undergone sampling during a previous surveillance study conducted 2 years earlier, called "Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates" (HM study).[12] The goal of this study was to investigate ARO colonisation on patient room surfaces before and after patients were moved into a new hospital ward, including sink surfaces and drains. In this study, patient room surfaces including sink surfaces and drains were swabbed regularly for 1 year from 2018 to 2019, and ARO isolates cultured from swabs were subjected to WGS as described below. Sequenced reads from *P. aeruginosa* and *S. maltophilia* isolates from environmental, water, and clinical samples collected during the HM study were obtained from BioProject PRJNA741123. These earlier isolates were compared to isolates from this study to evaluate patterns in ARO colonisation over a longer time period (5 years).

Whole-genome sequencing and *de novo* genome assembly

Environmental, water, and clinical isolates identified by MALDI-TOF MS as *Pseudomonas* spp. and *Stenotrophomonas* spp. were subjected to whole-genome sequencing (WGS). Isolate genomic DNA (gDNA) was extracted using the QIAmp BiOstic Bacteremia DNA Kit (Qiagen, Hilden, Germany, #12240-50) and quantified using the PicoGreen dsDNA assay (Thermo Fisher Scientific, Waltham, MA, #P7589). gDNA from each isolate was diluted to 0.5 ng/uL for library preparation using a modified Nextera kit (Illumina, San Diego, CA, #FC-131-1002) protocol and pooled for sequencing.[24] Pooled libraries were sequenced on the NovaSeq 6000 platform (Illumina) to obtain 2 × 150 bp reads. Demultiplexed reads were adaptor sequenced trimmed and quality filtered using Trimmomatic v0.38 and read quality was assessed using FastQC v0.12.1 and MultiQC v1.14.[25–27] Trimmed reads from this study and the HM study were assembled into draft genomes with Unicycler v0.4.7 and assessed for quality with BBMap v35.85, Quast v5.2.0, and CheckM v1.2.2.[28–31] Assemblies were considered high quality and used for further analysis if they had less than 500 contigs greater than 1000 bp in length, greater than 90% coverage, less than 5% contamination, and N50 greater than 10,000 bp.

Isolate species was confirmed first by using Mash Screen v2.3 to compare each assembly to all RefSeq bacterial type strains as done previously.[12,32] Average nucleotide identity (ANI) between the isolate and the top three hits from Mash Screen was calculated using dnadiff v1.3.[33] Species assignment was dependent on >75% alignment and >96% ANI to a type strain; if no match was found, the isolate was classified as genomospecies of the genus level taxonomy call.[34]

Phylogenetic analysis and strain tracking

Multi locus sequencing typing (MLST) type for isolates was determined with mlst v2.23.0.[35] Genomes were annotated for coding sequences using Prokka v1.14.5 and a species-specific annotated reference (*P. aeruginosa*: GCF_000006765.1; *S. maltophilia*: GCF_900475405.1).[36] Annotated genomes of each species were input into Panaroo v1.3.3 to construct a core gene alignment, and phylogenetic relationships between isolates were obtained using Fasttree v2.1.[37,38] Pairwise core gene single nucleotide polymorphisms (core SNPs) were called within each species using snippy-core v4.6.0.[39] A core SNP strain threshold was empirically determined by plotting a histogram of all pairwise core SNPs; the threshold for *P. aeruginosa* was set to 7 core gene SNPs and the threshold for *S. maltophilia* was set to 10 core gene SNPs. All isolates within that threshold from one another were considered to be in the same strain group.

Within-strain evolution characterisation

Antimicrobial resistance (AMR) genes were annotated using the National Center for Biotechnology Information's (NCBI) Antimicrobial Resistance Gene Finder (AMRFinderPlus) v.3.11.20.[40]

Tracking of mutations within strains was conducted as described previously.[41] Strain groups with at least one isolate collected after the baseline period (and thus could have been exposed to the intervention) were included in this analysis. Cleaned reads were subsampled from each isolate in a strain to construct a strain group specific pseudo-reference assembly. Assembly and coding sequence annotation was done as described above. Whole genome SNPs were identified within each strain by aligning reads from each isolate to the pseudo-reference assembly with snippy.[39] The gene location of each SNP was identified using a custom Python script and the Prokka annotations. The number of SNPs identified within each strain group was randomly permuted across the pseudo-reference assembly 1000 times using a custom Python script. Mutations in genes labelled as 'hypothetical protein', 'intron' or 'unknown node' were ignored, and gene alleles were collapsed into

the same gene. Finally, a custom R script was used to identify genes that were mutated more frequently than by chance ($p < 0.05$, empirical distribution function with BH correction). All custom scripts are available at https://doi.org/10.5281/zenodo.15015609.

HAI data collection

Data on HAIs among patients admitted to the SCTO-ICU and SICU during the study period, including central line-associated blood stream infections (CLABSIs), catheter-associated urinary tract infections (CAUTIs), and ventilator-associated events (VAEs), were obtained from BJH Infection Prevention.

Statistics

Statistical analysis relating to the primary outcomes of this study, including reduction in total bacterial burden, unique AROs, and Pseudomonas spp. and Stenotrophomonas spp. was preplanned. A test of proportions power analysis using an n of 600 (the combined number of expected samples for each intervention and their respective baseline and post-intervention periods) indicated a power of 93.4% to detect a conservative effect size of 0.2. With an n of 60, (the number of proposed samples collected per week by intervention) this test yielded 90% power to detect an effect size of 0.6 or greater. Analysis relating to the genomic effects of the intervention was developed as our understanding of the organisms and their responses to the intervention developed.

Significant changes in the proportion of drains yielding growth or number of isolates recovered were determined by t-test with BH correction. We used Shapiro–Wilks tests for non-normality within study periods to confirm growth and isolate results were normally distributed. Changes in the number of unique strain groups found each week and the mean number of HAIs were assumed to be non-normal and were tested using permutation tests with BH correction. All data analysis was conducted using Rstudio and visualised using the ggplot2 package.[42]

Role of funders

Funders and funding sources had no role in study design, data collection, data analyses, interpretation, or writing this report.

Results

Specimen collection and microbial culture

We collected a total of 2766 environmental swabs, and from these cultured 1182 ARO isolates. Selective culture methods targeted vancomycin resistant Enterococci, methicillin-resistant *Staphylococcus aureus*, beta-lactam resistant Gram negative bacilli, and *Pseudomonas* spp. and *Stenotrophomonas* spp (Supplementary Table S1). The most frequently identified genera by MALDI-TOF MS were *Pseudomonas* spp. (447/1182, 37.8%) and *Stenotrophomonas* spp. (236/1182, 20.0%, Supplementary Table S2). We detected other common opportunistic premise plumbing pathogens (OPPPs) less frequently from surfaces, including *Klebsiella* spp. (51/1182, 4.31%) and *Acinetobacter* spp. (13/1182, 1.10%). Four *Pseudomonas* spp. isolates and one *Stenotrophomonas* spp. isolate were collected from water samples throughout the study.

We acquired a total of 114 *P. aeruginosa* and 2 *S. maltophilia* clinical isolates as identified by MALDI-TOF MS during the study period MS (Supplementary Table S2).

Sink drains and surrounding surfaces provide the most frequent growth on blood agar plates

During the baseline period, we recovered viable, cultivatable growth on blood agar plates (BAPs) from a relatively high proportion of patient room sink drains (mean = 0.87, se = 0.3) and shared area drains (mean = 1.00, se = 0) each week (Supplementary Figure S1a). We also recovered growth from high proportions of lower sink counters (mean = 0.80, se = 0.6) and raised sink counters (mean = 0.92, se = 0.2). We recovered growth from a moderate proportion of sink bowls (mean = 0.58, se = 0.8) and faucets (mean = 0.50, se = 0.6). All surface growth results are in Supplementary Table S3.

Sink drains house the most unique ARO isolates, particularly *Pseudomonas* spp. and *Stenotrophomonas* spp.

Isolates collected from the panel of selective agars were considered antimicrobial resistant organisms (AROs). Patient room sink drains yielded a mean 2.12 (se = 0.11) unique ARO isolates per drain, per week. In contrast, we only recovered a mean < 0.3 ARO isolates per surface, per week from other sink surfaces (Supplementary Figure S1b). The shared area drains frequently yielded the most unique ARO isolates, with a mean of 5.13 isolates (se = 0.43) identified per drain, per week.

We recovered *Pseudomonas* spp. and *Stenotrophomonas* spp. from a mean 0.48 (se = 0.03) and 0.52 (se = 0.06) patient room sink drains per week, respectively. The shared area sink drains yielded *Pseudomonas* spp. from nearly all drains (mean = 0.95, se = 0.04), but *Stenotrophomonas* spp. slightly less frequently (mean = 0.38, se = 0.06). All sink drains yielded more of these isolates than all other patient room surfaces, where all these taxa were found in a mean of <0.04 samples (Supplementary Figure S1c and d).

The intervention reduced the proportion of sink drains from which we recovered bacterial growth

During the baseline period, we recovered bacterial growth from fewer sink drains that received the high frequency intervention than those that received the low frequency intervention on BAP ($p < 0.05$, Dunn's test

with BH correction), but not on MacConkey agar plates (MAC) (ns, $p > 0.05$, Dunn's test with BH correction, Fig. 2a and b).

When comparing bacterial growth on BAP during the two intervention periods versus the baseline period, the low frequency intervention rooms showed 35% (95% CI: 9–62%, $p < 0.05$, t-test with BH correction) and 31% (95% CI: 14–48%, $p < 0.01$, t-test with BH correction) decreases in the proportion of drains from which we recovered growth in the first and second intervention periods (Fig. 2a). These decreases extended into the two post-intervention periods, where we recovered growth from 31% (95% CI: 14–48%, $p < 0.01$, t-test with BH correction) and 19% (95% CI: 2–36%, $p < 0.05$, t-test with BH correction) fewer drains per week, as compared to baseline (Fig. 2a). The high frequency intervention also showed significant decreases in the proportion of drains yielding growth on BAP, with 33% (95% CI: 6–60%, $p < 0.05$, t-test with BH correction) and 62% (95% CI: 15–108%, $p < 0.05$, t-test with BH correction) decreases during the intervention periods, as compared to baseline. Untreated room drains showed nonsignificant decreases of <8% relative to baseline during intervention periods (ns, $p > 0.05$, t-test with BH correction). There were no significant decreases in the proportion of surfaces with growth on BAP from non-drain sink surfaces during either intervention period (ns, $p > 0.05$, t-test with BH correction), though there was a significant decrease during the first post-intervention period in the untreated rooms ($p < 0.0001$, t-test with BH correction, Supplementary Figure S2a).

When evaluating Gram-negative yield on MAC plates, we recovered growth from 39% (95% CI: 5–83%, ns, $p > 0.05$, t-test with BH correction) and 42% (95% CI: 9–74%, ns, $p > 0.05$, t-test with BH correction) fewer sink drains in the low frequency intervention rooms during intervention periods as compared to the baseline period; though neither decrease reached statistical

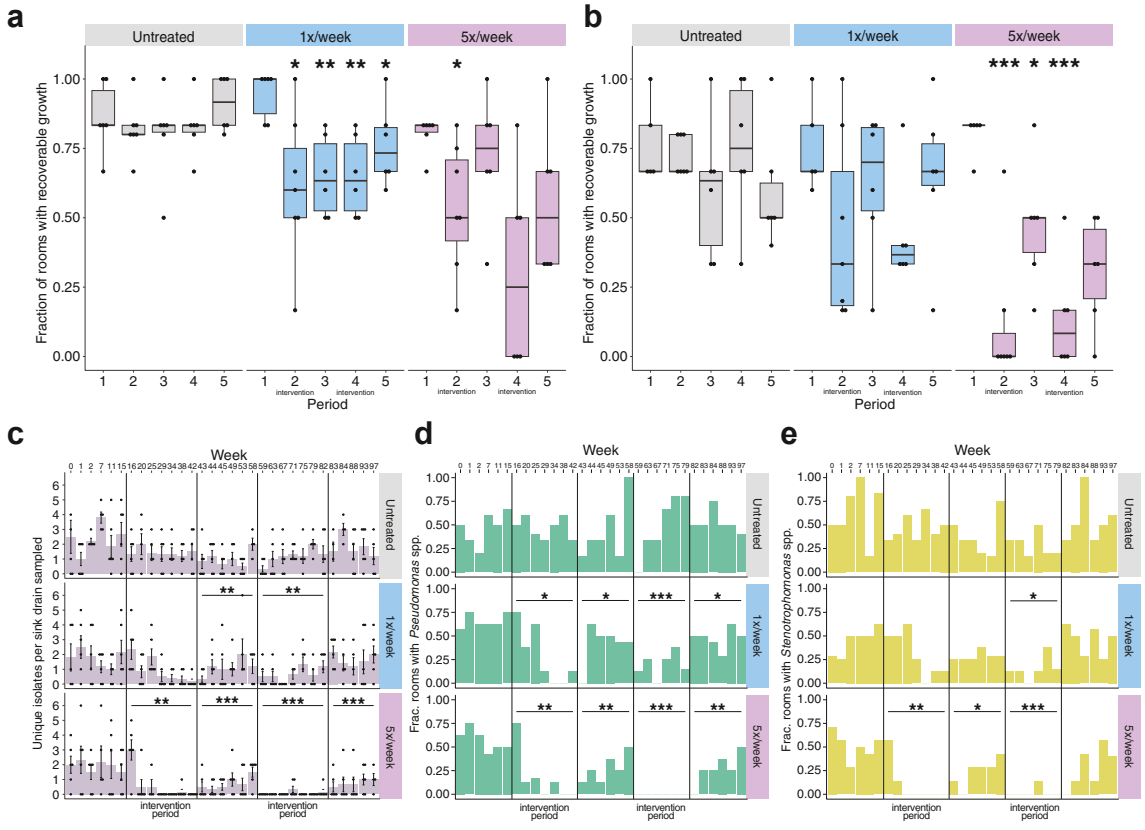

Fig. 2: **Interventions reduce total bacterial growth and specific ARO growth**. Average weekly proportion of sink drains in each intervention group that yielded viable cultivable growth on a) BAP (n = 528 plates from sink drains cultured) and b) MAC plates (n = 550 plates from sink drains cultured). Significant changes in weekly proportion of sink drains yielding growth by period versus the baseline period shown. c) Weekly counts per sink drain of unique AROs (n = 984 AROs collected from sink drains). Average weekly counts across all rooms in each week were calculated, and significant changes in weekly counts by period versus the baseline period are shown. Proportion of rooms yielding d) *Pseudomonas* spp. and e) *Stenotrophomonas* spp. from patient room sink drain. Significant changes in weekly proportion of sink drains yielding growth by period versus the baseline period shown (t-test with BH correction).*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

significance (Fig. 2b). Drains in the high frequency intervention rooms had reductions by 85% (95% CI: 56–114%, p < 0.001, t-test with BH correction) and 83% (95% CI: 57–108%, p < 0.001, t-test with BH correction) versus the baseline period during the two intervention periods, followed by decreases of 41% (95% CI: 12–70%, p < 0.05, t-test with BH correction) and 62% (95% CI: 36–88%, p < 0.01, t-test with BH correction) the post-intervention periods. The untreated rooms, in contrast, had <6% reductions during intervention periods versus the MAC baseline period, neither were significant (ns, p > 0.05, t-test with BH correction).

Both intervention frequencies significantly reduced recovery of AROs from sink drains

In the low frequency intervention rooms, the number of unique ARO isolates recovered from sink drain samples decreased by 45% (95% CI: 15–88%, ns, p > 0.05, t-test with BH correction) and 68% (95% CI: 41–95%, p < 0.01, t-test with BH correction) during the two intervention periods relative to baseline (Fig. 2c). This decrease was maintained into the first post-intervention period, which had a 45% (95% CI: 18–71%, p < 0.01, t-test with BH correction) decrease versus the baseline period. The high frequency intervention room drains yielded 71% (95% CI: 34–107%, p < 0.01, t-test with BH correction) and 96% (95% CI: 75–117%, p < 0.001, t-test with BH correction) fewer unique AROs during the two intervention periods than during baseline. We also observed significant decreases of 64% (95% CI: 36–92%, p < 0.001, t-test with BH correction) and 69% (95% CI: 47–92%, p < 0.001, t-test with BH correction) during the two post-intervention periods. For multiple weeks during both intervention periods, we recovered no AROs from the sink drains in the high frequency intervention rooms. In the untreated rooms, the number of unique AROs did not decrease significantly during any intervention or post-intervention period (Fig. 2c). There were no significant decreases in unique AROs from non-drain surfaces in the untreated rooms or intervention groups (ns, p > 0.05, t-test with BH correction) during the study period (Supplementary Figure S2b).

The low frequency intervention rooms exhibited decreases in *Pseudomonas* spp. by 57% (95% CI: 14–99%, p < 0.05, t-test with BH correction) and 71% (95% CI: 50–92%, p < 0.001, t-test with BH correction) during the two intervention periods, and maintained decreases of 37% (95% CI: 3–71%, p < 0.05, t-test with BH correction) and 28% (95% CI: 9–47%, p < 0.05, t-test with BH correction) into the post-intervention periods (Fig. 2d). The low frequency intervention achieved a significant reduction in *Stenotrophomonas* spp. during the second intervention period, by 66% (95% CI: 27–105%, p < 0.05, t-test with BH correction) versus baseline (Fig. 2e).

In the high frequency intervention rooms, the proportion of drains from which *Pseudomonas* spp. was recovered significantly decreased by 71% (95% CI: 26–115%, p < 0.01, t-test with BH correction) and 100% (95% CI: 79–121%, p < 0.001, t-test with BH correction) during the two intervention periods versus baseline (Fig. 2d). This decrease continued into the two post-intervention periods, which both yielded 53% (95% CI: 23–83%, p < 0.01, t-test with BH correction, and 95% CI: 20–85%, p < 0.01, t-test with BH correction, respectively) decreases versus baseline. Similarly, recovery of *Stenotrophomonas* spp. decreased by 80% (95% CI: 37–123%, p < 0.01, t-test with BH correction) and 95% (95% CI: 65–125%, p < 0.001, t-test with BH correction) during the two intervention periods (Fig. 2e). There was also a significant decrease of 53% (95% CI: 17–90%, p < 0.05, t-test with BH correction) maintained in the first post-intervention period. Untreated rooms experienced no statistically significant reductions in recovery of *Pseudomonas* spp. or *Stenotrophomonas* spp. during the intervention periods (ns, p > 0.05, t-test with BH correction).

P. aeruginosa and *S. maltophilia* were the most frequent species collected from surface samples

We conducted whole-genome sequencing (WGS) on all *Pseudomonas* spp. and *Stenotrophomonas* spp. surface isolates because these were the most frequent AROs collected. Using WGS data, we identified the most common species as *P. aeruginosa* (187/447, 41.8% of all surface *Pseudomonas* spp. isolates) and *S. maltophilia* (90/236, 38.1% of all surface *Stenotrophomonas* spp. isolates). None of the *Pseudomonas* spp. collected from water were identified as *P. aeruginosa* using WGS data.

Phylogenetic clustering between this study and an earlier study in the same SCTO-ICU

We included isolates collected in a previous study in the same rooms (the HM Study, conducted 2 years prior) to expand our surveillance. We included 77 *P. aeruginosa* isolates and 49 *S. maltophilia* isolates from sink surface samples, and 28 *P. aeruginosa* isolates from patient specimens from the HM Study.[12]

We constructed phylogenetic trees for each species to identify clustering among all surface and clinical isolates collected during the two studies (Supplementary Figure S3a). For *P. aeruginosa*, many of the clinical and sink surface isolates collected did not have a defined MLST type (ST); these undefined allele sets were submitted to PubMLST for ST definition. We observed clustering and overlap of environmental *P. aeruginosa* isolates between the two studies within many STs. Across both studies, a plurality of sink surface isolates were identified as ST 1894; 63/187 (33.7%) of *P. aeruginosa* sink surface isolates from this study and 43/77 (55.8%) of *P. aeruginosa* sink surface isolates from the HM study fell within ST 1894. While there were 3 bloodstream infections (BSIs) caused by ST 1894 in

the HM study, no clinical isolates from *P. aeruginosa* ST 1894 were identified in the current study.

Phylogenetic clustering also revealed associations between *S. maltophilia* isolates collected during the HM study and during this study (Supplementary Figure S3b). The isolates clustered primarily by ST, with the most frequent ST group being *S. maltophilia* ST 1 (20/90, 22%). Some *S. maltophilia* ST groups were exclusive to either the HM study (like *S. maltophilia* ST 27) or this study (like *S. maltophilia* ST 365), but others showed overlap between the two studies. There were no clinical isolates identified as *S. maltophilia* in either study.

P. aeruginosa strains can inhabit sink drains across at least 4 years

We defined 49 strain groups of *P. aeruginosa* isolates from both studies using pairwise, core gene single nucleotide polymorphisms (SNPs) to track highly related strains (Supplementary Figure S4a and b). The largest strain group was *P. aeruginosa* Group 62, which contains *P. aeruginosa* ST 1894 (n = 109 isolates, Supplementary Figure S4e). The remaining strain groups ranged in size from 2 to 16 isolates, and 72 isolates did not fall within a strain group.

Longitudinal strain tracking across both studies revealed evidence of continued *P. aeruginosa* colonisation of study sink drains (Fig. 3a) over a period of 52 months (4.3 years). *P. aeruginosa* Group 62 was the most prevalent strain identified in environmental samples collected during the previous HM study and was still present in five of the six patient rooms included in both studies during the baseline period of this study. Group 62 was then regularly collected throughout this study. *P. aeruginosa* Group 82 was also found in the same patient room (Room 24) during both studies. Several other groups were recovered over the course of this study but were largely collected from untreated rooms or during non-intervention periods in intervention rooms.

S. maltophilia strains can inhabit sink drains for at least 3 years, but not all sinks were colonised

As described above, we defined highly related *S. maltophilia* strain groups to track *S. maltophilia* over time (Supplementary Figure S4c and d). The largest of these strain groups was *S. maltophilia* Group 4, which contained 52 isolates of *S. maltophilia* ST 1 (Supplementary Figure S4f).

In contrast to the *P. aeruginosa*, which was found in all study rooms, there were several rooms from which *S. maltophilia* was not isolated during the current study period. Despite this, at least two strain groups (*S. maltophilia* Groups 4 and 6) were recovered in the same rooms across both studies, and Group 4 was collected across 224 weeks of collections in Room 20 (Fig. 3b).

New *P. aeruginosa* strain groups colonised drains after cessation of intervention, but the same *S. maltophilia* strain groups returned during the study period

We found that for both intervention groups, the number of unique strains of *P. aeruginosa* isolated from sink surfaces decreased during the two intervention periods. This decrease was statistically significant versus the baseline levels for the 5×/week intervention during both intervention periods ($p < 0.05$, permutation test, BH corrected, Fig. 4a). During both post-intervention periods, the number of unique strain groups identified in rooms assigned to both intervention frequencies increased back to the levels observed in the untreated rooms (ns, $p > 0.05$, permutation test, BH corrected).

For the intervention and post-intervention periods, we compared the number of unique strain groups that were new to each patient sink (had not been isolated in each room in an earlier period, coloured in pink) or recurring (had been isolated from each room in an earlier period, coloured in navy) (Fig. 4c). Significant differences between the proportions of recurring and new strains in each intervention group and period were compared to the proportions from the untreated group during the same period using a Fisher's exact test with BH correction. During both intervention periods, both the 1×/week and 5×/week rooms showed significantly more new *P. aeruginosa* strains versus recurring strains when compared with untreated rooms during the same study periods ($p < 0.05$, Fisher's exact test, BH corrected).

We found no significant differences in the number of unique *S. maltophilia* strain groups present on sink surfaces in either intervention group in any period when compared to their baseline period (ns, $p > 0.05$, permutation test, BH corrected, Fig. 4b). In contrast with *P. aeruginosa*, the number of new versus recurring *S. maltophilia* strains found during the intervention and follow-up periods in both intervention groups were not significantly different than those in the untreated rooms (ns, $p > 0.05$, Fisher's exact test, BH corrected). Instead of new strains being isolated from sink drains, the same strains that were found prior to the intervention returned after the intervention was stopped.

The interventions did not select for increased antimicrobial resistance in *P. aeruginosa* or *S. maltophilia* recovered during the study period

To ensure that the intervention protocol did not lead to increased AMR among *P. aeruginosa* strains recovered from sink surfaces, we examined AMR gene carriage in all *P. aeruginosa* and *S. maltophilia* isolates collected from sink surfaces (Supplementary Figure S3a and b, Supplementary Table S4). All 187 *P. aeruginosa* isolates collected across this study carried *fosA* and *blaOXA*, and 186/187 (99.5%) carried *catB7* and *blaPDC*. 123/187 (65.8%) carried *crpP*, and we used a Fisher's exact test

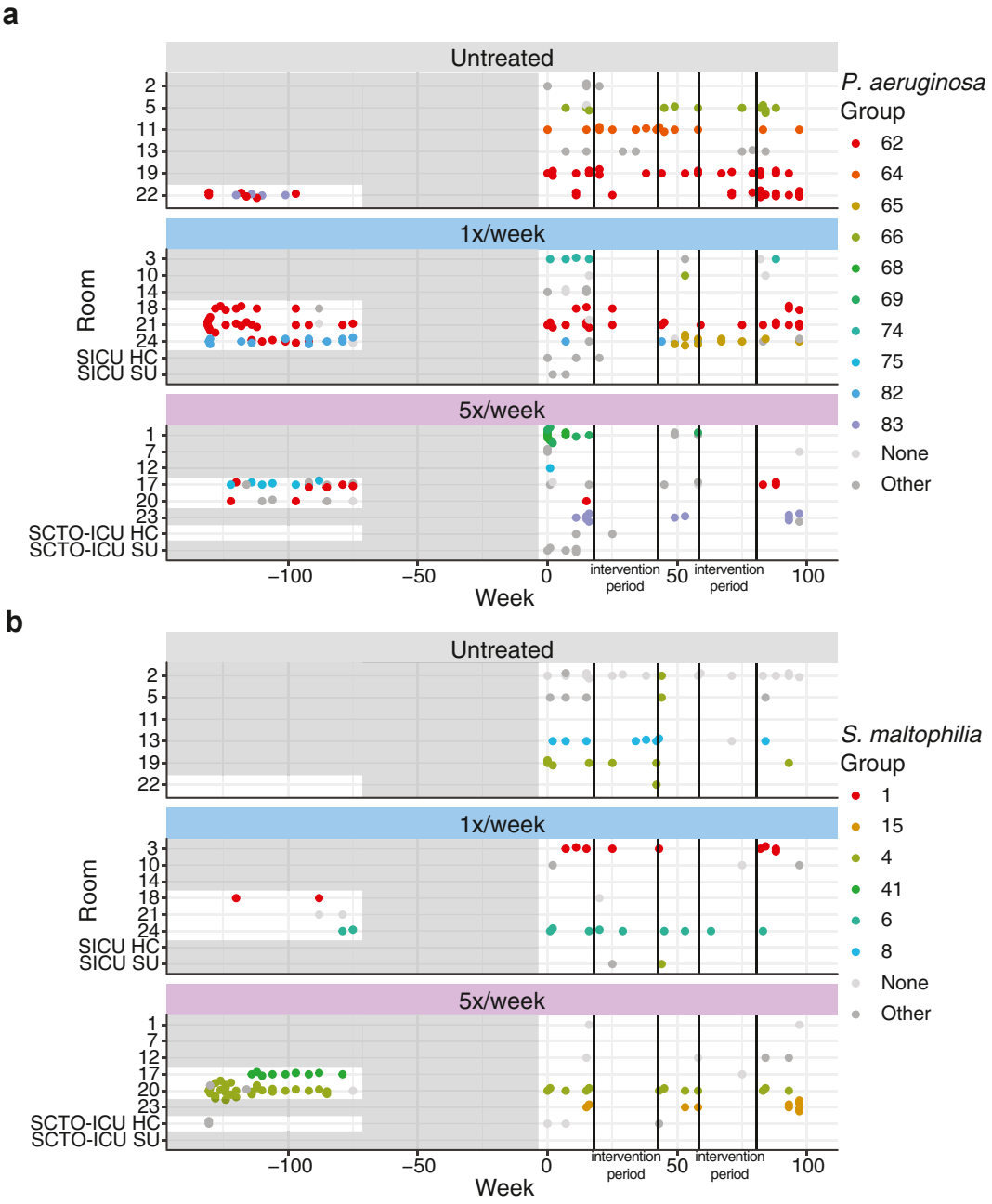

Fig. 3: **Strain tracking of *P. aeruginosa* and *S. maltophilia* reveals long term sink colonisation**. Tracking of core SNP-based strain groups from this study and a previous study in the same ward (HM Study[12]) for a) *P. aeruginosa* (n = 264) and b) *S. maltophilia* (n = 139). Grey boxes mark rooms where samples were not collected in the previous study. Interventions are labelled in the second and fourth periods of this study. SICU = surgical intensive care unit, HC = housekeeping closet, SU = soiled utility room, SCTO-ICU = stem cell transplant intensive care unit.

with BH correction test for changes in proportions of isolates carrying *crpP* versus the baseline period for a given intervention. The proportion of isolates carrying *crpP* did not increase versus baseline in any period or either intervention group, and the proportion of strains carrying *crpP* significantly decreased in the first post-intervention period in the low-frequency intervention rooms ($p < 0.05$, Fisher's exact test, BH corrected, Supplementary Figure S5a).

All 90 *S. maltophilia* isolates carried *aph(3′)*, 89/90 (98.9%) carried *blaL1*, and 81/90 (90.0%) carried aph(6). 45/90 (50.0%) of *S. maltophilia* isolates carried *aac(6′)*-

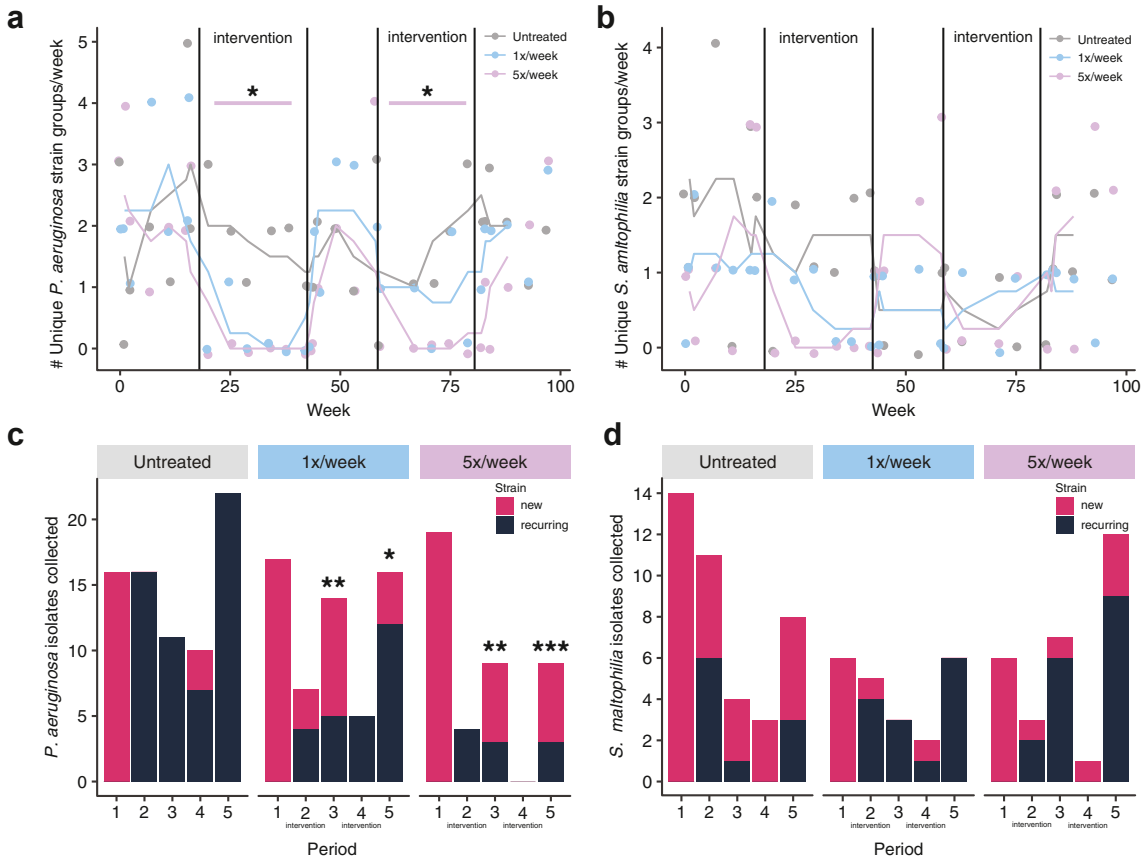

***Fig. 4:* Variation in strain diversity of *P. aeruginosa* and *S. maltophilia* across study periods.** Number of unique strain groups found each week in each intervention group for a) *P. aeruginosa* (n = 49 total strain groups) and b) *S. maltophilia* (n = 12 total strain groups). Isolates not included in strain groups counted as their own unique strains. Lines represent rolling mean across 4 collection timepoints. Significant decreases in mean number of unique strain groups versus the baseline period shown (permutation test with BH correction). Count of sink surface isolates that were c) *P. aeruginosa* or d) *S. maltophilia* strains collected from the same room an earlier period (recurring, blue) or not seen yet (new, red) in that room. Significant changes in proportions of recurring and new strains versus untreated rooms during the same period shown (Fisher's exact test with BH correction). *: $p < 0.05$, **: $p < 0.01$), ***: $p < 0.001$.

Iz, but when compared to the baseline period as above there were no significant changes in proportion of isolates carrying *aac(6′)-Iz* through the periods for any intervention group (ns, $p > 0.05$, Fisher's exact test, BH corrected, Supplementary Figure S5b). One isolate carried 9 additional AMR genes, but these were not seen again in any other isolates (Supplementary Figure S3b).

The intervention did not significantly increase mutations in *P. aeruginosa* or *S. maltophilia* collected during the study period

Next, we investigated genes that acquired mutations within strain groups during the study period. We identified genes that were mutated more frequently than by chance ($p < 0.05$ empirical distribution function with BH correction) in sink surface *P. aeruginosa* and *S. maltophilia* strain groups across three groups of sink surface isolates collected from: HM Study rooms during the HM Study, untreated rooms during the current study, and all intervention rooms during the current study (Supplementary Figure S5c and d). Then, we compared the proportion of strains carrying mutations in genes between intervention groups using pairwise Fisher's exact tests with BH correction. We found no significant differences in the proportion of strain groups that carried mutations in these genes in isolates collected from any of the comparison groups (ns, $p > 0.05$, pairwise Fisher's exact tests with BH correction, only genes with mutations in $n \geq 2$ strains shown).

Like for *P. aeruginosa*, we found no significant differences in proportion of *S. maltophilia* strain groups carrying mutations in any genes between sink surface isolates collected from HM Study rooms, current study untreated rooms, and current study intervention rooms (ns, $p > 0.05$, pairwise Fisher's exact tests with BH correction, Supplementary Figure S5d, only genes with

mutations in n ≥ 2 strains shown). All strain group mutations can be found in Supplementary Table S5.

Few clinical isolates associated with sink colonising OPPPs

Of the 114 *P. aeruginosa* isolates collected from clinical specimens in this study, 96/114 (84.2%) were confirmed to be *P. aeruginosa* by WGS, representing 59 patients and approximately 65 infections. Of these, five infections were associated with the same strain groups as surface *P. aeruginosa* isolates (Supplementary Figure S6a). In four cases, clinical isolates were collected from patients prior to the strain being isolated from sink drains. In one case, strain Group 17 was collected from the Room 13 sink drain for several weeks prior to the isolate being collected from the patient. Neither of the two *S. maltophilia* isolates collected from clinical specimens were confirmed to be *S. maltophilia* by ANI.

The interventions had no quantifiable effects on HAIs in study rooms

During the study period, BJH Infection Prevention identified a total of 69 patient CLABSIs, VAEs, and CAUTIs in the study ICUs that they determined to be healthcare-associated by NHSN standards. There were no significant differences in the rates of HAIs across the 5 periods of the study (ns, $p > 0.05$, permutation test, BH corrected, Supplementary Figure S6b).

Discussion

Previous studies have implicated sinks and sink drains as a reservoir for and the probable source of multiple hospital outbreaks of OPPPs that can cause serious HAIs such as *P. aeruginosa*, *S. maltophilia*, and carbapenem-resistant Enterobacterales.[1,3,5,6,9–11,16] In this study, we identified multiple sink surfaces as a source of bacteria, but only sink drains were a frequent and significant source of OPPPs. Therefore, structured protocols for the cleaning of hospital sink drains are urgently needed to reduce the risk of HAIs.[1,5,6] The intervention evaluated in this study used a readily available foamed disinfectant. We sought to first quantify the effectiveness of this intervention in reduction of total bacterial burden, as well as specific taxa such as Gram-negative bacteria, unique AROs, and common OPPPs such as *Pseudomonas* spp. and *Stenotrophomonas* spp. Then, we used genomic insights to understand how this intervention affected strain colonisation, AMR gene presence, and mutation acquisition.

The intervention protocol reduced the proportion of sink drains from which Gram-negative bacteria were recovered by up to 85% when applied 5 times per week (high frequency). This finding is important because outbreaks associated with sink drain contamination are frequently associated with Gram-negative bacteria.[3,5,6,10,43–45] While we also observed a reduction in this proportion by up to 42% compared to baseline when applied once per week (low frequency), this finding was statistically insignificant. This suggests that the low frequency intervention may not be sufficient to eliminate Gram-negative growth in all sinks, possibly due to differences in specific taxa or biofilm composition. Thus, individual sinks may require more frequent treatments to reduce risk. We found no significant decreases in growth from non-drain surfaces in intervention rooms, though the untreated rooms showed a significant decrease in one period. This could possibly be a result of additional cleaning as part of patient care. The level later returned to baseline levels in other periods.

Both intervention frequencies also resulted in reductions in unique AROs isolated from sink drains. In the high frequency intervention rooms, there were several weeks where no AROs were recovered during intervention periods. These decreases were even maintained into both post-intervention periods in the high frequency rooms. Crucially, the intervention also reduced the proportion of drains with recoverable *Pseudomonas* spp. and *Stenotrophomonas* spp, genera which have been associated with OPPPs.[3,5,10,43] Both intervention frequencies were associated with significant decreases in recovery of *Pseudomonas* spp. during the intervention and post-intervention periods, and resulted in several weeks with no recovery. The high frequency intervention did result in a larger magnitude of decreased *Pseudomonas* spp. recovery, with up to 100% reduction during the second intervention period. Both intervention frequencies also resulted in decreases in *Stenotrophomonas* spp. recovery during the second intervention period, though the high frequency also achieved this during the first intervention and post-intervention periods.

Genomic insights provide an understanding of the mechanisms at play in sink drain reservoirs maintenance and spread.[3,12,46] Strain tracking of *P. aeruginosa* and *S. maltophilia* revealed highly related lineages maintaining reservoirs in sink drains throughout an earlier study and this study in the same rooms, totalling to >4 years of potential colonisation.[12] This work also allowed us to characterise differing responses to the intervention by *P. aeruginosa* and *S. maltophilia*. The former experienced strain group replacement during the post-intervention periods, while the latter maintained the same strain groups throughout the entire study. Future work could help understand whether this is a result of differing effects due to the intervention, or differences in mechanisms of colonisation and reservoir maintenance.

As this intervention protocol involves chemical disinfectants, we sought to ensure that it did not inadvertently cultivate additional AMR or select for mutations that may cause off-target effects. In *P. aeruginosa* and

S. maltophilia, we found no evidence of additional AMR in strains colonising sink surfaces during intervention or post-intervention periods versus the baseline period. We also found no evidence that strains of *P. aeruginosa* or *S. maltophilia* that have been exposed to the intervention acquired mutations in any genes differently than strains that had not been exposed to the intervention. While further work is still needed to quantify these effects, the lack of differences between intervention room strain groups and non-intervention room strain groups suggests that mutations in these genes may be associated with common selection pressures from colonising hospital sink drains, rather than something unique to the intervention protocol. These results suggest that this intervention protocol does not select for strains carrying additional AMR genes or mutations.

Despite our success at reducing microbial burden and ARO carriage in sink drains, we wish to acknowledge study limitations. First, this intervention was performed by trained study personnel, not hospital staff. As such, additional research is needed to evaluate the feasibility and effectiveness of the intervention when performed by hospital staff, including staff training, the time necessary for cleaning, the use of dedicated pumps for each sink, and across multiple and different types of healthcare facilities. In addition, this intervention utilised both bleach wipes and foamed preacid disinfectant. The bleach wipes are already part of routine cleaning but were repeated by study personnel as part of the intervention to ensure they were consistently applied. This limited our ability to disentangle the unique effects of bleach wipes versus foamed disinfectant. However, we assert that the majority of the impact of this intervention was still likely due to the foamed preacid disinfectant, as it targeted the sink drains, where we saw the most impact. Next, we tailored our selective culture methods to isolate bacteria previously associated with HAIs and sink drain-associated outbreaks.[1,3,6,9–11] Such enrichment methods may miss other organisms that may cause HAIs, including fungi. Finally, some of our findings were limited due to the small size of the study. The limited number of strains tracked reduced our ability to observe AMR gene or mutation acquisition over time. The ideal result of this intervention would be a reduction in ARO burden in sink drains such that transmission to patients no longer occurs. Though the small number of rooms sampled likely contributed to our inability to observe impact on patient HAIs or colonisation, the significant reductions observed in Gram-negative growth and specific OPPPs are a promising proof of concept. While complete eradication of AROs from sink plumbing would be exceptional, it is not currently feasible as we lack knowledge of the depth of ARO biofilms in sink drains, or if these strains are being re-seeded from an outside source. Future work implementing this intervention in an outbreak setting would help reveal its effectiveness in preventing HAIs.

Despite these limitations, this study still provides a strong framework for the development of a standardised sink cleaning intervention. While previous studies have tested a variety of potential solutions to reduce ARO burden in hospital sinks, many of these strategies have limited or confounding evidence for their effectiveness as they are often conducted in outbreak settings.[1,3,5,6,17] This study took place prospectively in a non-outbreak setting to characterise the intervention both during and after use. These real-world data indicate that this sink cleaning protocol can successfully reduce the burden of bacterial and specific OPPPs in sink drains versus pre-intervention baseline burden, particularly when applied multiple days per week. The intervention proposed here utilises commercially available products, but does require training, equipment maintenance, and personnel time to implement. While we found that the intervention was most effective against key OPPPs known to cause HAIs when applied 5 times per week with limited off target effects, it also reduced total bacterial burden and key OPPPs when applied once per week, which may be more feasible in a hospital setting. Future work could prioritise testing the feasibility of this intervention as well as the cost versus benefit of more or less frequent application. Future work could also examine other agents to compare their effects on bacterial burden and strain adaptation. Establishing evidence-based methods to reduce the risk of HAIs caused by OPPPs is critical as rates of antimicrobial resistance in clinically-relevant microbes continue to rise globally.[47,48] These results provide critical groundwork for establishing practical and standardised protocols for hospital sink hygiene.

Contributors

Conceptualisation, C.A.O., K.V.S., C.D.B., G.D., J.H.K. Funding acquisition: E.P.N., C.A.O., K.P., K.V.S., C.D.B., G.D., J.H.K. Data curation, C.A.O., L.V., D.M., C.C., M.A.W., T.H., F.Y., C.M., R.G., O.A., H.S., M.A., K.J., K.A., A.V., C.S., K.P., D.P., E.S. Investigation: J.H.K., C.D.B., G.D. Methodology: J.H.K., C.D.B., G.D. Formal analysis: E.P.N., C.A.O., K.P. Supervision: C.D.B., G.D., J.H.K. Visualisation: E.P.N., K.P. Writing—original draft: E.P.N., C.A.O., C.D.B., G.D., J.H.K. Writing—review & editing: E.P.N., C.A.O., F.Y., C.D.B., G.D., J.H.K. E.P.N., C.A.O., and K.P. have accessed and verified the underlying data. All authors read and approved the final version of the manuscript.

Data sharing statement

All custom scripts used are available at https://doi.org/10.5281/zenodo.15015609. Paired reads from WGS data are available under BioProject PRJNA1138263.

Declaration of interests

C.D.B. holds roles with the Journal of Clinical Microbiology, ASM Case Reports, and stock/stock options and other financial/nonfinancial interests with Pattern BioScience. K.V.S. holds a role and receives payments from Seeding Inc (Tiny Health). The other authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105772.

References

1 Volling C, Ahangari N, Bartoszko JJ, et al. Are sink drainage systems a reservoir for hospital-acquired gammaproteobacteria colonization and infection? A systematic review. *Open Forum Infect Dis*. 2021;8(2):ofaa590.
2 Williams MM, Armbruster CR, Arduino MJ. Plumbing of hospital premises is a reservoir for opportunistically pathogenic microorganisms: a review. *Biofouling*. 2013;29(2):147–162.
3 Baranovsky S, Jumas-Bilak E, Lotthe A, et al. Tracking the spread routes of opportunistic premise plumbing pathogens in a haematology unit with water points-of-use protected by antimicrobial filters. *J Hosp Infect*. 2018;98(1):53–59.
4 Baker AW, Lewis SS, Alexander BD, et al. Two-phase hospital-associated outbreak of Mycobacterium abscessus: investigation and mitigation. *Clin Infect Dis*. 2017;64(7):902–911.
5 Bourdin T, Benoit ME, Prevost M, et al. Disinfection of sink drains to reduce a source of three opportunistic pathogens, during Serratia marcescens clusters in a neonatal intensive care unit. *PLoS One*. 2024;19(6):e0304378.
6 Lowe C, Willey B, O'Shaughnessy A, et al. Outbreak of extended-spectrum beta-lactamase-producing Klebsiella oxytoca infections associated with contaminated handwashing sinks(1). *Emerg Infect Dis*. 2012;18(8):1242–1247.
7 Kotsanas D, Wijesooriya WR, Korman TM, et al. "Down the drain": carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks. *Med J Aust*. 2013;198(5):267–269.
8 Falkinham JO 3rd. Common features of opportunistic premise plumbing pathogens. *Int J Environ Res Public Health*. 2015;12(5):4533–4545.
9 Hota S, Hirji Z, Stockton K, et al. Outbreak of multidrug-resistant Pseudomonas aeruginosa colonization and infection secondary to imperfect intensive care unit room design. *Infect Control Hosp Epidemiol*. 2009;30(1):25–33.
10 Gideskog M, Welander J, Melhus A. Cluster of S. maltophilia among patients with respiratory tract infections at an intensive care unit. *Infect Prev Pract*. 2020;2(4):100097.
11 De Geyter D, Blommaert L, Verbraeken N, et al. The sink as a potential source of transmission of carbapenemase-producing Enterobacteriaceae in the intensive care unit. *Antimicrob Resist Infect Control*. 2017;6:24.
12 Sukhum KV, Newcomer EP, Cass C, et al. Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates. *Commun Med (Lond)*. 2022;2:62.
13 CDC. Considerations for reducing risk: water in healthcare facilities 2024. updated October 24, 2024. Available from: https://www.cdc.gov/healthcare-associated-infections/php/toolkit/water-management.html?CDC_AAref_Val=https://www.cdc.gov/hai/prevent/environment/water.html.
14 Barna Z, Antmann K, Paszti J, et al. Infection control by point-of-use water filtration in an intensive care unit - a Hungarian case study. *J Water Health*. 2014;12(4):858–867.
15 Hopman J, Tostmann A, Wertheim H, et al. Reduced rate of intensive care unit acquired gram-negative bacilli after removal of sinks and introduction of 'water-free' patient care. *Antimicrob Resist Infect Control*. 2017;6:59.
16 Knoester M, de Boer MG, Maarleveld JJ, et al. An integrated approach to control a prolonged outbreak of multidrug-resistant Pseudomonas aeruginosa in an intensive care unit. *Clin Microbiol Infect*. 2014;20(4):O207–O215.
17 Low JM, Chan M, Low JL, Chua MCW, Lee JH. The impact of sink removal and other water-free interventions in intensive care units on water-borne healthcare-associated infections: a systematic review. *J Hosp Infect*. 2024;150:61–71.
18 Buchan BW, Arvan JA, Graham MB, et al. Effectiveness of a hydrogen peroxide foam against bleach for the disinfection of sink drains. *Infect Control Hosp Epidemiol*. 2019;40(6):724–726.
19 Jones LD, Mana TSC, Cadnum JL, et al. Effectiveness of foam disinfectants in reducing sink-drain gram-negative bacterial colonization. *Infect Control Hosp Epidemiol*. 2020;41(3):280–285.
20 Nardie-Warner C. Ecolab receives first EPA approval for biofilm disinfection in drains. Available from: https://www.ecolab.com/news/2020/11/ecolab-receives-first-epa-approval-for-biofilm-disinfection-in-drains; 2020.
21 Washington University Physicians. Barnes-jewish hospital. Available from: https://physicians.wustl.edu/places/barnes-jewish-hospital/; 2024.
22 Dubois D, Grare M, Prere MF, Segonds C, Marty N, Oswald E. Performances of the Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry system for rapid identification of bacteria in routine clinical microbiology. *J Clin Microbiol*. 2012;50(8):2568–2576.
23 Services USDoHH. Pseudomonas aeruginosa in healthcare settings. Available from: https://www.cdc.gov/hai/organisms/pseudomonas.html; 2019.
24 Baym M, Kryazhimskiy S, Lieberman TD, Chung H, Desai MM, Kishony R. Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS One*. 2015;10(5):e0128036.
25 Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30(15):2114–2120.
26 Andrews S. FastQC: a quality control tool for high throughput sequence data 2010. Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
27 Ewels P, Magnusson M, Lundin S, Kaller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*. 2016;32(19):3047–3048.
28 Wick RR, Judd LM, Gorrie CL, Holt KE, Unicycler. Resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol*. 2017;13(6):e1005595.
29 Bushnell B. BBMap: a fast, accurate, splice-aware aligner osti.gov2014. Available from: https://www.osti.gov/servlets/purl/1241166.
30 Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013;29(8):1072–1075.
31 Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res*. 2015;25(7):1043–1055.
32 Li W, O'Neill KR, Haft DH, et al. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. *Nucleic Acids Res*. 2021;49(D1):D1020–D1028.
33 Kurtz S, Phillippy A, Delcher AL, et al. Versatile and open software for comparing large genomes. *Genome Biol*. 2004;5(2):R12.
34 Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A*. 2009;106(45):19126–19131.
35 Seemann T. mlst Github. Available from: https://github.com/tseemann/mlst.

36 Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30(14):2068–2069.
37 Price MN, Dehal PS, Arkin AP. FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS One*. 2010;5(3):e9490.
38 Tonkin-Hill G, MacAlasdair N, Ruis C, et al. Producing polished prokaryotic pangenomes with the Panaroo pipeline. *Genome Biol*. 2020;21(1):180.
39 Seemann T. snippy: fast bacterial variant calling from NGS reads Github2015. Available from: https://github.com/tseemann/snippy.
40 Feldgarden M, Brover V, Gonzalez-Escalona N, et al. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci Rep*. 2021;11(1):12728.
41 Thanert R, Choi J, Reske KA, et al. Persisting uropathogenic Escherichia coli lineages show signatures of niche-specific within-host adaptation mediated by mobile genetic elements. *Cell Host Microbe*. 2022;30(7):10347–10347.e6.
42 Wickham H. *ggplot2: elegant graphics for data analysis*. Springer-Verlag New York; 2016.
43 Gbaguidi-Haore H, Varin A, Cholley P, Thouverez M, Hocquet D, Bertrand X. A bundle of measures to control an outbreak of Pseudomonas aeruginosa associated with P-trap contamination. *Infect Control Hosp Epidemiol*. 2018;39(2):164–169.
44 Falkinham JO, Pruden A, Edwards M. Opportunistic premise plumbing pathogens: increasingly important pathogens in drinking water. *Pathogens*. 2015;4(2):373–386.
45 Kizny Gordon AE, Mathers AJ, Cheong EYL, et al. The hospital water environment as a reservoir for carbapenem-resistant organisms causing hospital-acquired infections-A systematic review of the literature. *Clin Infect Dis*. 2017;64(10):1435–1444.
46 Nurjadi D, Scherrer M, Frank U, et al. Genomic investigation and successful containment of an intermittent common source outbreak of OXA-48-producing Enterobacter cloacae related to hospital shower drains. *Microbiol Spectr*. 2021;9(3):e0138021.
47 Dadgostar P. Antimicrobial resistance: implications and costs. *Infect Drug Resist*. 2019;12:3903–3910.
48 Masia MD, Dettori M. Antimicrobial resistance, healthcare-associated infections, and environmental microbial contamination. *Healthcare (Basel)*. 2022;10(2).