Journal of Hospital Infection 159 (2025) 100–105

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Drain decontamination using in-situ-generated ozone

M.Z. Pajak-Zajac [a,c], A. Dowdell [a], H.E. Potts [a], A. Smith [b], D.A. Diver [a,*]

[a] School of Physics and Astronomy, University of Glasgow, Glasgow, UK
[b] University of Glasgow Dental School, Glasgow Dental Hospital and School, Glasgow, UK
[c] Devro Plc, Glasgow, UK

ARTICLE INFO

Article history:
Received 23 October 2024
Accepted 4 February 2025
Available online 15 February 2025

Keywords:
Cold plasma
Disinfection
Drain
Aerosolization

SUMMARY

Background: Sink drains can be a significant source of microbial contamination in healthcare settings due to aerosolization and splashback of pathogens caused by flowing water.

Aim: To demonstrate a method of ozone disinfection of drains using a novel generation process that suppresses pathogen growth in the drain sump, whether planktonic or dwelling in biofilms.

Methods and results: Highly biocidal ozone was generated *in situ* in the drain using the ambient air sealed between the water trap and a portable cold plasma device. Safety mechanisms in the device ensured that the operator was not exposed to ozone. Subsequent bacterial recovery illustrated an approximate bioburden reduction of 5 $\log_{10}$ for biofilms in the drain itself, and 6 $\log_{10}$ for biofilms located in the sink.

Conclusions: Plasma-generated ozone is a safe and effective method for controlling bioburden in periodically wetted, otherwise inaccessible pipework and drains.

Significance and impact of study: The portable ozone disinfection system described has demonstrated potential for controlling the escape of pathogens from drains. Compared with conventional liquid-based disinfection techniques, the portable ozone disinfection system has the following advantages: (i) a gaseous biocide can reach all surfaces inside the treatment target, without any restriction from orientation or surface tension; (b) ozone is effective in reducing planktonic and biofilm bacterial counts; and (c) ozone is generated at the point of use from air, using minimal electrical power, requiring no chemical delivery or storage, and producing no toxic residues.

Introduction

Drains and other (partially or periodically) water-submerged pipework, such as general sanitary plumbing and tap outlets, present a unique challenge to decontamination in that they are difficult to access, and provide ideal conditions for the growth of micro-organisms that produce biofilms, and the consequent aerosolization and splashback of pathogens [1–8].

This article reports the preliminary results from a novel solution that reduces such risks significantly: the safe and controlled deployment of ozone into the sink drainage, resulting in measurable and significant reductions in bioburden when biofilms are present.

The technique uses cold plasma to generate ozone from the ambient air; ozone — O_3, a variant of standard oxygen O_2 — is a gaseous biocide that is effective against both planktonic

* Corresponding author. Address: School of Physics and Astronomy, Kelvin Building, University of Glasgow, Glasgow G12 8QQ, UK.
E-mail address: Declan.Diver@glasgow.ac.uk (D.A. Diver).

https://doi.org/10.1016/j.jhin.2025.02.001

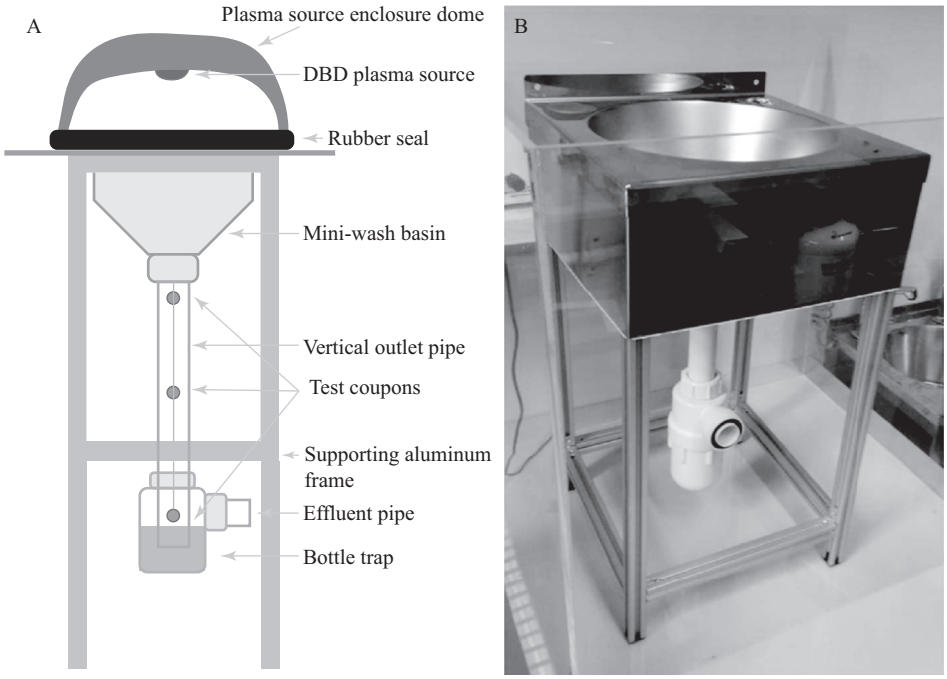

Figure 1. (A) Schematic picture of the model wash basin with the dielectric barrier discharge (DBD) plasma source placed over the basin aperture, and the placement of the coupons in the drain. (B) The model wash basin enclosed in an acrylic tank separating the test rig from the rest of the laboratory.

bacteria and biofilm contamination [9,10]. The novel device allows the safe and practical use of ozone in a sink drain, and has no requirements other than electrical power (which could be delivered by batteries) and ambient air. The advantages of this system are essentially three-fold: (i) proven efficacy against biofilm contaminations, given that biofilms are generally resistant to liquid disinfection [11] but are gas permeable; (ii) all internal drain surfaces are exposed to gaseous ozone — there are no limitations due to surface tension or orientation; and (iii) minimal logistical requirements: no purchasing and storage of hazardous chemicals, and no disposal or waste effluent concerns. The article concludes with a discussion of existing methods for drain decontamination.

Methods

Ozone device and model drain

The cold plasma source is contained within a concave unit that forms a seal against the drain opening in the sink. When the plasma creates the ozone, the latter is contained safely between the impermeable surfaces of the device and the drain plumbing, plus the water layer in the U-bend (into which ozone may dissolve). At all stages, environmental monitoring via an ozone personal monitor (BW GasAlert Extreme; BW Technologies, Schaumburg, IL, USA), placed adjacent to the equipment to alert users to ozone detection beyond the workplace exposure limit of 0.2 ppm in air averaged over 15 min (Health and Safety Executive, 2014), confirmed that no ozone escaped into the wider environment.

A model wash basin and drain assembly was constructed using commercially available parts, consisting of a 290-mm stainless steel square mini-wash bowl (no overflow or tap holes and a central drain), a vertical polypropylene outlet pipe ending with a polypropylene bottle trap (Floplast; ∅ 40 mm inlet and outlet, compression fittings) and an effluent pipe. The system rested on a speed frame (Rexroth aluminium strut 20 mm × 20 mm). The bottle trap was filled with fresh tap water just below the overflow level to the effluent pipe. An experiment diagram and a picture of the model wash basin and drain assembly are shown in Figure 1.

Rather than having to dismantle the plumbing to test efficacy after each experiment, inoculated coupons were placed in various strategic parts of the drain system, and tested for viable colonies after the sink treatment.

Microbial strain and culture media

Pseudomonas aeruginosa NCTC 10662 was used in all experiments. If not otherwise specified, tryptic soy broth (TSB) (Sigma-Aldrich, Gillingham, UK) was used as a rich nutrient medium, and recovered bacteria were cultured on to tryptone soy agar (TSA) plates (E&O Labs, Bonnybridge, UK).

Biofilm formation on coupons

A CDC bioreactor (BioSurface Technologies Corporation, Bozeman, MO, USA) was used to generate a reproducible *P. aeruginosa* biofilm in high shear conditions. The method is described in ASTM Standard E2562-12 [12], with the temperature modified in some of the experiments (20—22 °C instead of 30 °C). The arms of the CDC bioreactor were fitted with ½ inch polycarbonate or polytetrafluoroethylene coupons, and the bioreactor was filled with 35 mL of TSB and 315 mL of sterile deionized water. It was then inoculated with 1—1.6 mL of *P. aeruginosa* culture [approximately 10^9 colony-forming units (CFU)/mL]. The biofilm was grown with agitation (130 rpm) for up to 24 h at 20—22 °C. For more mature biofilms, the

Table I
Inactivation of biofilms grown for up to 72 h on polytetrafluoroethylene coupons

Biofilm maturity (h)	Mean recovery ± SEM (CFU/mL)		Log_{10} reduction	Ozone treatment	
	Untreated control	Ozone		Max (ppm)	Total (mg)
12	$(5.9 \pm 1.6) \times 10^7$	<LOD	>5.9	1589	3.10
24	$(2.6 \pm 1.8) \times 10^8$	<LOD	>6.2	1614	3.20
48	$(2.2 \pm 0.9) \times 10^9$	<LOD	>7.4	1810	3.56
72	$(3.0 \pm 0.2) \times 10^8$	<LOD	>6.7	1888	3.47

SEM, standard error of the mean; CFU, colony-forming unit; LOD, limit of detection.

bioreactor was run for an additional 48 h in the continuous phase, which entailed feeding the bioreactor with diluted medium (16.7 mL of TSB per 5000 mL of sterile deionized water) at 5 rpm. Each arm (eight in total) can accommodate three coupons, allowing for a total of 24 biofilms to be cultured. The temperature is kept consistent across all arms for any given experiment. Upon reaching a defined timepoint for growth, the arms are removed from the bioreactor and gently submerged in a tube with 50 mL of sterile PBS or ¼ strength Ringer's solution with 0.05% polysorbate 80 to dislodge planktonic cells and provide consistently biofouled coupons.

Method of biofilm recovery by sonication

ASTM Standard E2562-12 was modified by replacing scraping as a method of recovery with sonication. Sonication has been demonstrated to be more reproducible, as well as being the most effective method for removal of biofilms from medical devices [13—15]. For this method, treated or untreated biofilms, grown on the specified coupon type and under the desired conditions, as required, were transferred aseptically to glass universal containers filled with 10 mL of a sterile neutralizer (PBS or ¼ strength Ringer's solution with 0.05% polysorbate 80) and sonicated for 10 min in an ultrasonic bath (Medisafe Reliance PC+ 5L; Medisafe UK Ltd, Bishop's Stortford, UK). The resultant bacterial suspension was then diluted as required, plated on TSA plates, incubated at 37 °C overnight and enumerated. Verification of a *P. aeruginosa* biofilm was confirmed by colony morphology and Gram stain.

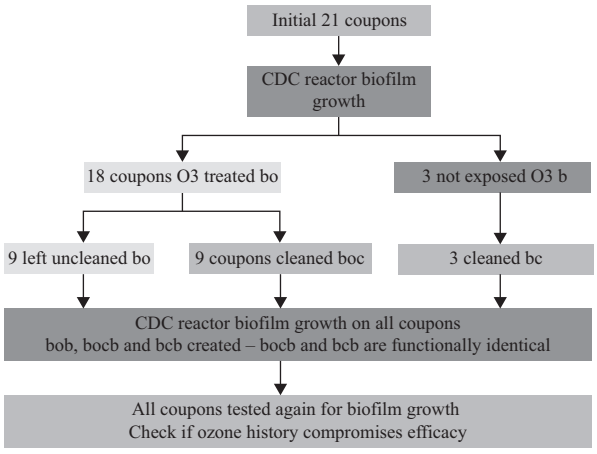

Figure 2. Flow chart illustrating the history of coupon treatment for the regrowth experiments. b, biofilm; o, treated with ozone (O_3); c, cleaned conventionally without O_3.

Experiments

Ozone treatment of biofilms of different maturity

Biofilms were grown as described above ('Biofilm formation on coupons'), on PTFE coupons and at room temperature, with each set of two arms in the bioreactor containing coupons to be retrieved after growth timepoints of 12, 24, 48 and 72 h. At the specified timepoints, coupons were removed aseptically and bagged individually into generic clear polyethylene grip-seal bags. Samples from the first arm in each set were treated using plasma generated *in situ* for 100 s at 4.7 kV. Control samples were left untreated with ozone. Bacterial population growth was quantified as described above ('Method of biofilm recovery by sonication'). The results are shown in Table I.

Ozone treatment of a model wash basin and drain

A 13-h biofilm was grown on several PTFE coupons in the CDC bioreactor, at room temperature, as described above ('Biofilm formation on coupons'). All coupons had a short piece of PTFE tubing (inner ∅ 1 mm, 3—4 mm length) attached to one side using silicone, and placed in a manner that they faced the bioreactor wall. Three coupons were placed in the model wash basin, spaced equally. Another three coupons were suspended on a nylon filament and hung in the vertical outlet pipe: the bottom coupon was suspended just above the surface of the water, the top coupon was suspended just below the drain, and the middle coupon was suspended approximately mid-way between the top and bottom coupons, as shown in Figure 1.

After treatment, all coupons were removed, along with the PTFE tubing and silicone. One set of three sterile PTFE coupons was used as the negative control, and one set of three untreated coupons from the same biofilm production batch was used as the positive control. Bacterial population growth was quantified as described above ('Method of biofilm recovery by sonication'). The treatment protocol consisted of a series of brief plasma pulses, with the resulting ozone measured using an ozone meter.

Biofilm regrowth on ozone-inactivated biofilm

This study investigated whether previously biofouled and ozone-treated coupon surfaces had increased potential for bacterial growth — a vital consideration if drains are to be decontaminated routinely using this technique. The process is captured schematically in Figure 2. Biofilm was initially grown on polycarbonate coupons at 30 °C for 24 h, as described above ('Biofilm formation on coupons'). The methodological process can be described by labelling an episode of biofilm growth as 'b'. Each experimental batch initially comprised 21 coupons with biofilm 'b', of which 18 were placed into individual generic clear polyethylene grip-seal bags and treated with ozone as in previous experiments (plasma generated for 100 s,

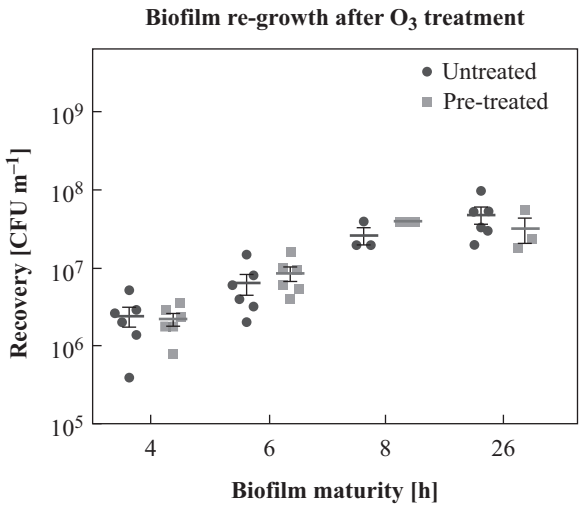

Figure 3. Recovery of total culturable *Pseudomonas aeruginosa* after previous ozone treatment, with statistical error bars. These results show that using ozone on coupons does not enhance biofilm formation; if it did, this would make such an approach to disinfection impractical, as it could worsen contamination. CFU, colony-forming units.

4.7 kV) and then labelled as 'bo' (biofilm and ozone). Three coupons (controls) were not exposed to ozone, and these were labelled 'b' (biofilm alone). From the batch of 18 'bo' coupons, nine had their biofilm quantified as described above ('Method of biofilm recovery by sonication), and were decontaminated by cleaning and steam sterilization; these were labelled 'boc' (biofilm, ozone and cleaning). The three untreated control coupons were also decontaminated and labelled 'bc' (biofilm and clean). These 12 coupons (nine 'boc' and three 'bc') were then fitted back into the bioreactor, along with the remaining nine previously ozone-treated coupons ('bo'). The bioreactor was used to grow biofilms again on these 21 coupons, resulting in three different treatment processes, labelled as 'bob' (biofilm, ozone and biofilm), 'bcb' (biofilm, cleaning and biofilm) and 'bocb' (biofilm, ozone, cleaning and biofilm).

The bioreactor was restarted using the same parameters as before, with the cleaned and ozone-treated coupons arranged in opposing arms. Samples were taken from each at 4, 6, 8 and 26 h. The produced biofilm was recovered and enumerated as described above ('Method of biofilm recovery by sonication'). In this way, biofilms were grown again on to surfaces that were: (i) never exposed to ozone, but cleaned and autoclaved between biofilm growth episodes ('bcb'); (ii) treated with ozone after the first biofilm growth, but cleaned before the second growth stage ('bocb'); and (iii) treated with ozone after the first growth episode, but left with the debris from ozone treatment, and then exposed to a second round of biofilm growth ('bob') – the latter were meant to represent surfaces in practice that have been treated with ozone, but not otherwise scraped and restored to virgin condition, just like the inaccessible regions of a drain. The first two categories are essentially functionally similar, and together serve as a comparator for (iii). The critical test here is: does prior treatment with ozone without scraping off the damaged biofilm predispose a surface to enhanced biofilm growth in a secondary infection phase? The answer is no: no significant difference was detected between untreated and pre-treated surfaces (Figure 3).

Coupon cleaning and biofilm recovery

ASTM Standard E2562-12 was modified by replacing scraping as a method of recovery with sonication. Sonication has been demonstrated to be more reproducible, as well as being the most effective method for removal of biofilms from medical devices [13–16]. For this method, treated or untreated biofilms, grown on the specified coupon type and under the desired conditions, as required, were transferred aseptically to glass universal containers filled with 10 mL of a sterile neutralizer (PBS or ¼ strength Ringer's solution with 0.05% polysorbate 80) and sonicated for 10 min in an ultrasonic bath (Medisafe Reliance PC+ 5L; Medisafe UK Ltd). The resultant bacterial suspension was then diluted as required, plated on TSA plates, incubated at 37 °C overnight and enumerated. Verification of a *P. aeruginosa* biofilm was confirmed by colony morphology and Gram stain.

Results

Ozone treatment of biofilms of different maturity

The treatment of biofilms grown at room temperature on PTFE coupons, sampled at 12–72 h and exposed to ozone rendered no detectable culturable *P. aeruginosa*, as shown in Table I. The limit of detection for biofilm recovery via sonication was calculated based on quantities and dilutions used in a validation of this technique as a method for quantifying bioburden. This value was estimated at 50 CFU/mL.

Ozone treatment of a model wash basin and drain

The bacterial recovery from untreated coupons was $(4.4 \pm 0.2) \times 10^7$ CFU/mL. No culturable organisms were recovered from the coupons in the model wash basin, while the mean number of organisms recovered from the drain was $(1.9 \pm 1.8) \times 10^2$ CFU/mL. This is a $\log_{10}$ reduction of at least 5.9 for the wash basin coupons and at least 5.1 for the coupons suspended in the drain. No ozone was detected escaping from the water trap during this experiment.

Biofilm regrowth on ozone-inactivated biofilm

The number of culturable organisms recovered from PTFE coupons on which a fresh *P. aeruginosa* growth cycle has been induced, after previous biofilm growth and subsequent ozone treatment, is shown in Figure 3. There was no detectable difference in biofilm regrowth between coupons with previous biofilm growth and subsequent ozone treatment ('bob') and previously untreated (i.e. never exposed to ozone) coupons ('bcb').

Discussion

Existing healthcare practice on drain disinfection relies on liquid cleaners such as sodium hypochlorite, sodium dichloroisocyanurate and peracetic acid [17]. Even with three 15-min treatments per day, these record, at best, >4 log reductions in viability in only some sections of a drain [17], even at concentrations of 1000 ppm (4000 ppm for peracetic acid). Given that the standard cleaning cycle in hospital wards suggests that sinks are cleaned, at best, once daily, this suggests that

biocontamination control from drains could be improved significantly using ozone generated as per this study. Indeed, it was found that sodium dichloroisocyanurate did not control biofilms in any part of a drain. The ability of ozone to inactivate micro-organisms lies in its oxidizing effect on the cell wall, particularly of lipids, and the induction of cell lysis [18]. Following treatment of biofilms grown for up to 72 h with gaseous ozone, no culturable *P. aeruginosa* were recovered, and the maximum $\log_{10}$ reduction achieved was $\geq$7.4.

Testing the efficacy of the plasma technology in more challenging conditions — by treating biofilm-covered coupons placed in a model wash basin and drain assembly — was also successful, given the 5 $\log_{10}$ reduction for biofilms in the drain itself, and 6 $\log_{10}$ reduction for biofilms located in the sink. Such experimental set-ups demonstrate the properties of plasma-generated ozone to penetrate small structures of the drain, and to successfully inactivate biofilm above the bottle-trap level, and, most importantly, below the strainer or gasket. This reduces the risk of cross-contamination via back-splatter during use of the wash basin, which has been proven to pose an issue in healthcare facilities [19]. Resident biofilms can promote the recruitment and incorporation of planktonic cells into an existing biofilm structure [20]; from the authors' initial experiments, it is clear that plasma-generated ozone treatment does not promote biofilm regrowth, and so helps to control spread. The biofilm regrowth on ozone-inactivated biofilm was an important series of experiments to undertake if drains are to be decontaminated routinely using this technique. These biofouled and ozone-treated coupons are meant to model drain surfaces in practice that have been treated with ozone, but not otherwise cleaned and restored to pristine condition. Critically, the outcome tested here was whether prior treatment with ozone without scraping off the damaged biofilm would predispose a surface to enhanced biofilm growth in a secondary recolonization phase. The results (see Figure 3) demonstrated no significant different between untreated and pre-treated surfaces in this model.

As an additional guide to the efficacy of the technique, a trial disinfection of a hospital floor drain was executed at Queen Elizabeth University Hospital, with before and after swabs of drain surfaces (but avoiding false-negatives that might arise from repeated swabbing of identical positions on the drain structure), with the following results (see online supplementary material): drain cover contamination reduced from TNTC (too numerous to count) to 7 CFU; internal drain surface contamination reduced from TNTC to 15 CFU; and sump water contamination reduced from approximately 450 CFU to 106 CFU. This outcome has been included in this paper as it is indicative of efficacy in a real-world situation, supplementing the activity presented above.

By controlling the treatment zone to the drain and water trap, preventing transient environmental leakage of ozone, this technique offers a practical and effective way to minimize infections in drains. Furthermore, once the treatment is concluded, any unused ozone reverts rapidly to oxygen, leaving no toxic residues in drain surfaces or the waste water. As the ozone is generated electrically from the ambient air, no hazardous chemicals need to be manufactured, purchased, stored or deployed, making this technique not just effective, but sustainable. Coupled with inherent consistency and ease of use, this technology is a compelling alternative to existing techniques.

Author contributions

MZPZ — experiments, planning, writing. AD — data reduction, writing. AB — experiments, planning. HEP — plasma technology advisor, planning. AS — microbiological advice, planning. DAD — concept, planning, funding, experiments.

Conflict of interest statement

DAD, AD and AS are employees of the University of Glasgow. MZP-Z was also an employee of the University of Glasgow at the time of the study, but has since moved to Devro Ltd. MZP-Z remains an honorary staff member of the University of Glasgow. HEP was formerly CSO at Anacail Ltd, and is also an honorary staff member of the University of Glasgow.

Funding

This work was funded by InnovateUK (via the Medical Research Council) Biomedical Catalyst 1402_BCF_R6 Grant 'Ozone destruction of biofilms for high level decontamination', Project No. 102137 awarded jointly to Anacail Ltd and the University of Glasgow. Additional funding for AD came from EPSRC IAA Award EP/X5257161/1. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Ethical approval

Not required.

Data availability

The data underlying this article are available in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2025.02.001.

References

[1] Wingender J. Hygienically relevant microorganisms in biofilms of man-made water systems. In: Flemming HC, Wingender J, Szewzyk U, editors. Biofilm highlights. Berlin: Springer-Verlag; 2011. p. 189—238.

[2] Inkster T. A narrative review and update on drain related outbreaks. J Hosp Infect 2024;151:33—44.

[3] Charron D, Bédard E, Lalancette C, Laferrière C, Prévost M. Impact of electronic faucets and water quality on the occurrence of *Pseudomonas aeruginosa* in water: a multi-hospital study. Infect Control Hosp Epidemiol 2015;36:311—9.

[4] Gormley M, Aspray TJ, Kelly DA, Rodriguez-Gil C. Pathogen cross-transmission via building sanitary plumbing systems in a full scale pilot test-rig. PLoS One 2017;12:e0171556.

[5] Halstead FD, Quick J, Niebel M, Garvey M, Cumley N, Smith R, et al. *Pseudomonas aeruginosa* infection in augmented care: the molecular ecology and transmission dynamics in four large UK hospitals. J Hosp Infect 2021;111:162—8.

[6] Breathnach AS, Cubbon MD, Karunaharan RN, Pope CF, Planche TD. Multidrug-resistant *Pseudomonas aeruginosa* outbreaks in two hospitals: association with contaminated hospital waste-water systems. J Hosp Infect 2012;82:19—24.

[7] Bedard E, Prevost M, Deziel E. *Pseudomonas aeruginosa* in premise plumbing of large buildings. Microbiologyopen 2016;5:937—56.

[8] Pirzadian J, Souhoka T, Herweijer M, van Heel L, van Wamel WJB, Goossens RHM, et al. Impact of sink design on bacterial transmission from hospital sink drains to the surrounding sink

environment tested using a fluorescent marker. J Hosp Infect 2022;127:39—43.
[9] Akter M, Yadav DK, Ki SH, Choi EH, Han I. Inactivation of infectious bacteria using nonthermal biocompatible plasma cabinet sterilizer. Int J Mol Sci 2020;21:8321.
[10] Barkhade T, Nigam K, Ravi G, Rawat S, Nema SK. Plasma sterilization for bacterial inactivation: studies on probable mechanisms and biochemical actions. Plasma Chem Plasma Process 2023;44:429—54.
[11] Flemming HC, van Hullebusch ED, Neu TR, Nielsen PH, Seviour T, Stoodley P, et al. The biofilm matrix: multitasking in a shared space. Nat Rev Microbiol 2023;21:70—86.
[12] ASTM International. Standard test method for quantification of *Pseudomonas aeruginosa* biofilm grown with high shear and continuous flow using CDC biofilm reactor. Conshohocken, PA: ASTM International; 2012.
[13] Assere A, Oulahal N, Carpentier B. Comparative evaluation of methods for counting surviving biofilm cells adhering to a polyvinyl chloride surface exposed to chlorine or drying. J Appl Microbiol 2008;104:1692—702.
[14] Bjerkan G, Witso E, Bergh K. Sonication is superior to scraping for retrieval of bacteria in biofilm on titanium and steel surfaces in vitro. Acta Orthop 2009;80:245—50.
[15] Webber B, Canova R, Esper LM, Perdoncini G, Pinheiro do Nascimento V, Pilotto F, et al. The use of vortex and ultrasound techniques for the in vitro removal of *Salmonella* spp. biofilms. Acta Sci Vet 2015;43:1332.
[16] Singh BP, Ghosh S, Chauhan A. Development, dynamics and control of antimicrobial-resistant bacterial biofilms: a review. Environ Chem Lett 2021;19:1983—93.
[17] Ledwoch K, Robertson A, Lauran J, Norville P, Maillard JY. It's a trap! The development of a versatile drain biofilm model and its susceptibility to disinfection. J Hosp Infect 2020;106:757—64.
[18] Thanomsub B, Anupunpisit V, Chanphetch S, Watcharachaipong T, Poonkhum R, Srisukonth C. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. J Gen Appl Microbiol 2002;48:193—9.
[19] Kotay S, Chai W, Guilford W, Barry K, Mathers AJ. Spread from the sink to the patient: in situ study using green fluorescent protein (GFP)-expressing *Escherichia coli* to model bacterial dispersion from hand-washing sink-trap reservoirs. Appl Environ Microbiol 2017;83:e03327-16.
[20] Habimana O, Casey E. Biofilm recruitment under nanofiltration conditions: the influence of resident biofilm structural parameters on planktonic cell invasion. Microb Biotechnol 2018;11:264—7.