Carbapenem-resistant Enterobacteriaceae dispersal from sinks is linked to drain position and drainage rates in a laboratory model system

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SUMMARY

Background: Hospital sinks, waste traps and drains can harbour carbapenem-resistant Enterobacteriaceae (CRE).

Aim: To investigate the dispersal of CRE from sinks in which water delivered from the tap flows directly into the drain and from clinical handwash basins with the drain at the rear. The effect of fast and slow drainage rates was also assessed.

Methods: Waste traps, known to be colonized with CRE, were taken from a hospital and installed within a model laboratory system. New waste traps were also installed and artificially inoculated with CRE. The potential for bacteria to be dispersed from sinks was assessed using cyclone air samplers and/or settle plates.

Findings: When the waste traps were artificially contaminated and CRE colonization was confined to the waste trap water, significantly fewer bacteria were dispersed from sinks that drained quickly ($P = 0.004$) and/or from rear-draining sinks ($P = 0.002$). When the waste traps were naturally contaminated and CRE colonized the trap, pipework and drain, there was significant interaction between sink drainage and position of the drain ($P < 0.001$). When drainage was slow, dispersal from rear-draining sinks was almost 30-fold less than from sinks with the drain underneath the tap ($P < 0.001$). When drainage was fast, rear-draining sinks again released comparatively fewer CRE, although, in this case, the difference was not statistically significant ($P = 0.7$). Contaminated splashes travelled up to 1 m from the sink.

Conclusion: Slow drainage rates and sink designs with the drain directly underneath the tap increase the risk of CRE present in waste traps and drains contaminating the ward environment.

Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) have spread globally and, over the last 10—15 years, have become increasingly important causes of healthcare-associated infection [1]. The hospital reservoirs of CRE include infected and colonized patients and patient-to-patient cross-transmission can act as a mechanism of spread, with direct contact between patients and staff widely thought to be the primary means of transmission [2,3]. However, environmental reservoirs have also been identified.

It has been known for over 20 years that hospital sinks and drains can support long-term persistence of multidrug-resistant Enterobacteriaceae [4,5] and other Gram-negative organisms, including *Pseudomonas aeruginosa* [6]. More recently and in several hospitals, sinks, drains and waste traps have been found to harbour identical or very similar CRE strains as those colonizing and infecting patients [7—16]. Based on this genetic association and epidemiological links, some studies have concluded that these environmental reservoirs represent a source for CRE colonization/infection of patients [7,9,11,13,14] or a persistent reservoir between patient-associated outbreaks [16].

The need for environmental interventions targeting sinks to control outbreaks has been recognized by many hospitals. These have included, among others, removal of sinks and pipework [15,16], enhanced cleaning of sinks and modification to sink design [17] and installation of self-disinfecting siphons [18]. Recent intervention studies have also shown that changes in the management of water and waste water in hospital intensive care units (ICUs) can reduce the incidence of Gram-negative bacteria acquisitions [19—21]. In one study, the removal of sinks from ICU rooms was associated with a decrease in infection and colonization rates of multidrug-resistant *P. aeruginosa* and *Klebsiella pneumoniae* [21]. In another study, the installation of covers on in-room hoppers used for waste-water disposal in a number of ICUs in a university hospital decreased the number of infections and colonizations by *K. pneumoniae* carbapenemase-producing organisms [20].

To date, despite all the epidemiological evidence available, investigation of the mechanisms and determinants of transmission from contaminated sinks and drains to patients has been scarce [9,22,23]. In this study, a large-scale laboratory model sink system was used to investigate the effects of two different factors on the dispersal of CRE from sinks: the position of the drain in relation to tap outlet and draining conditions.

Methods

Model sink system design

A laboratory model system, incorporating 12 individual sinks, associated pipework and bottle waste traps was designed and built to simulate a clinical setting (Supplementary material: Figure A). Five of the sinks were clinical handwash basins (CHWB) with the drain located at the rear of the unit and seven were small stainless-steel utility sinks (SSUS), with the drain located directly underneath the tap outlet (Markwik 21 Wall Mounted Sequential Thermostatic Basin Mixer; Armitage Shanks, Kingston upon Hull, UK) (Supplementary material: Figure A). Rigid partitions (40 × 57.5 cm) were installed between each sink to prevent cross-contamination from one sink to another. The distance between each tap outlet and the surface of the sink was ~30 cm. Water was delivered at a temperature of ~40°C at a flow rate of ~4 L/min. For the purposes of the current study, two different drainage conditions were simulated. Operating the tap and waste pump concurrently provided 'fast drainage' conditions. Delaying operation of the waste pump by 10 s allowed water to accumulate in the basin whilst the tap was operating simulating 'slow drainage'. No routine cleaning of the sinks took place.

Dispersal of CRE from an artifically contaminated waste trap

In order to investigate whether CRE present within waste trap water can be dispersed into the surrounding environment, new clean waste traps were artificially seeded with CRE. Before each experiment, a colony of a carbapenem-resistant *Citrobacter freundii* strain isolated from a hospital waste trap and grown from a -80°C stock was inoculated in 10 mL nutrient broth (Oxoid Ltd, Basingstoke, UK). After overnight incubation at 37°C, the entire culture volume was added to the waste trap. This was carried out by feeding sterile tubing through the drain hole and injecting the inoculum into the trap or by opening the waste trap and adding the culture directly. This (regardless of inoculation method) resulted in an average concentration of $2 \times 10^7 \pm 7 \times 10^6$ cfu/mL in the waste trap water. The sink basin was cleaned with a cloth soaked in 70% isopropanol (VWR International, Leicestershire, UK) and swabbed to ensure contamination was confined to the waste trap. Immediately after contamination of the waste trap, the sink tap was operated and water allowed to flow for 30 s directly into the sink. CRE dispersed in droplets were collected using settle plates (diameter 90 mm; MacConkey Agar No.3;

Oxoid) placed around the perimeter of the sink and which, on culture (18–24 h at 37°C) formed splash-forming units (sfu) (Supplementary material: Figure B).

Active air samping, using a cyclone air sampler (operating at 650 L/min) [24] was also carried out. The sampler was placed as close as possible to the drain (~10 cm above and, depending on sink design, 2 cm or 23 cm away) and operated for ~38 s to capture the duration of the flush. The position and design of the sampler meant that it would collect both aerosolized particles (<10 μm in diameter) and large droplets/splashes (>10 μm in diameter). The collecting fluid (phosphate buffer containing manucol and antifoam) was withdrawn and cultured using MacConkey Agar No.3, Brilliance™ *E. coli*/coliform agar (Oxoid) and chromID™ CARBA (Biomérieux, Basingstoke, UK). To enhance detection, these samples were also enriched in tryptone soya broth (TSB; Oxoid) before sub-culture on chromID™ CARBA.

Samples from the waste trap water and swabs of the sink surface were collected before and after the 30-s flush to confirm CRE concentration and to investigate surface contamination, respectively. Samples were diluted as required and plated on MacConkey No.3 and/or Brilliance™ *E. coli*/coliform agar, chromID™ CARBA, and Pseudomonas selective agar CN (Oxoid). All plates, were incubated at 37°C for 18–24 h. Experiments were carried out in triplicate and dispersal from both types of sink under both drainage conditions was assessed. Control samples were taken in the absence of seeding.

Hospital waste trap installation

In January 2018 (4 months before commencement of the current study), eight waste traps known to be colonized with CRE were collected from a UK hospital that had been experiencing a protracted outbreak of *K. pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae since 2009 in which waste-water sites had been implicated [16]. Waste traps were transported to the laboratory at room temperature and were installed in the model system within 4 days. Upon installation, all waste traps received a daily dose (~3 mL) of TSB. Dosing with nutrients was required to maintain the Enterobactericeae population at similar levels to those found at installation.

Dispersal of CRE and other Enterobacteriaceae from naturally contaminated waste traps and sinks

Two sinks (one SSUS with the drain directly underneath the tap and one CHWB with its drain at the rear) were used. Two consecutive 30-s flushes were carried out. Droplet dispersal was assessed using settle plates (MacConkey Agar No.3; Oxoid) placed on the surfaces immediately surrounding the sink and on the floor up to 1 m away from the sink. During these experiments, no surface disinfection was carried out. Surface swabs were taken before and after each 30-s flush and samples from the waste trap water collected before and after each experiment. Once each set of experiments had been completed, a sample of the biofilm colonizing the drain was also collected with a swab. All samples were diluted as required and cultured as before. Isolates of interest were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonik MALDI biotyper;

Bruker, Bremen, Germany) using the direct transfer method. Enterobacteriaceae isolates were compared by antibiotic resistance profiling using the disc diffusion method (ampicillin, gentamicin, amikacin, meropenem, ceftazidime and ciprofloxacin) following EUCAST guidelines. Experiments were carried out in triplicate and dispersal from both types of sink under both drainage rates was assessed. Samples taken when the taps were not operated provided an indication of any background contamination.

Statistical analysis

The results were analysed using STATA version 14.2. For the experiment detecting dispersal from the artifically contaminated waste trap, a dichotomous outcome (zero (i.e. no dispersal) vs greater than zero (i.e. dispersal observed)) was created and analysed using logistic regression. The covariates in the model were drainage type, position of the drain and their interaction. The interaction was tested for significance and, if not significant at the 5% level, removed from the model. The main effects were then tested.

For the experiment measuring dispersal from hospital waste traps, the total number of sfu across the two consecutive flushes was used for statistical calculations. Normality was tested using the skewness and kurtosis test with correction. The test for Normality gave a *P*-value of 0.1, hence the null hypothesis for Normality was not rejected at the 5% level and a normal linear regression was used to analyse the data. The covariates in the model were as for the model described above with the addition of the logarithm of the initial concentration in the waste trap. The appropiate function of this logarithm was explored by beginning with a cubic function and successively simplifying to quadratic and linear if not statistically significant at each step. Testing was performed by means of the likelihood ratio testing and a linear function of the logarithm appeared adequate. The number of plates was used as exposure or offset, respectively, in the two models.

Results

Dispersal of CRE from a sink with an artifically contaminated waste trap

After seeding, a high concentration of CRE ($2 \times 10^7 \pm 7 \times 10^6$ cfu/mL) was present in the waste trap water. The sink tap was operated for 30 s and active and passive air sampling techniques were used to detect dispersal of CRE. Results are expressed as cfu/volume of air sampled (~400 L) and sfu/agar plate, respectively (Table I); sfu comprised one to tens of cfu (Supplementary material: Figure B).

When the sinks had fast drainage, very little dispersal occurred regardless of drain position with low dispersal detected only on one of three occasions from a sink with the drain directly under the tap (Table I). However, if draining was impaired, the position of the drain in relation to the tap determined whether dispersal would occur. When the drain was positioned directly underneath the tap, CRE were released from the waste trap. In contrast, there was no dispersal from the waste trap when the drain was positioned at the rear of the sink (Table I). Statistical analysis confirmed that the interaction between drainage and position of the drain was not

Table I
Dispersal of carbapenem-resistant Enterobacteriaceae (CRE) from waste traps artificially seeded with CRE

	Fast drainage		Slow drainage	
	Active sampling Cyclone sampler (cfu/400 L air)	Passive sampling Settle plates (total sfu)	Active sampling Cyclone sampler (cfu/400 L air)	Passive sampling Settle plates (total sfu)
Drain underneath faucet	0[a]	0	$2.3 \times 10^3 \pm 1.8 \times 10^3$	$1.4 \times 10^2 \pm 6.1 \times 10^1$
Drain at rear	0	0	0[a]	0

The sink tap was operated for 30 s and the effect of drain position and drainage condition was assessed. Cyclone samples are expressed as mean ± standard deviation colony forming units (cfu) and the settle plates as mean ± standard deviation splash-forming units (sfu) ($N = 3$).
[a] One out of three samples positive after enrichment (i.e. <30 × cfu/volume of air sampled).

significant ($P > 0.999$) but both drainage conditions ($P = 0.004$) and position of the drain ($P = 0.002$) were determined to be highly significant, with good drainage and sinks with the drain at the rear having lower odds of producing splashes and dispersing CRE than poor drainage and sinks with the drain directly underneath the tap.

No surface contamination was detected when the sinks drained fast. However, when the sinks had slow drainage, strainers associated with drains positioned directly below the tap did become contaminated with CRE (mean $6 \times 10^1 \pm 6 \times 10^1$ cfu/swab).

Dispersal of CRE and other Enterobacteriaceae from naturally contaminated waste traps and sinks

Waste traps taken from hospital sinks were installed within the model system and were dosed with nutrients on a daily basis. Over time, and as observed previously [23], biofilm present within the waste trap and/or associated pipework were able to migrate to the drain. The number of CRE recovered in the drain from rear-draining sinks and those directly underneath the faucet were 3×10^7 cfu/swab and 9×10^5 cfu/swab, respectively, which might reflect differences in material and design. The strainer of the sink with the drain directly underneath the faucet had an average of 1×10^6 CRE/swab. There was no strainer associated with the rear-draining sink used in this investigation.

Under these conditions, the mean concentration of CRE recovered from the waste trap water was 4×10^7 cfu/mL. Two consecutive 30-s flushes were carried out, with the first flush always resulting in greater dispersal (Table II). The interaction of drainage rate and sink type was found to be significant ($P < 0.001$). When the drain was directly underneath the tap, CRE dispersal occurred regardless of draining conditions, but

was almost eight-times greater when draining was impaired ($P < 0.001$ by the Wald test). In both cases, sfu were detected up to 1 m from the sink but they were more numerous in the area immediately surrounding the sink (Table II).

When the drain was at the rear of the unit and the sink was allowed to drain rapidly, CRE dispersal was minimal (mean: 0.3 sfu). Although more CRE (mean: 18.5 sfu) were detected when drainage was slow, this difference did not reach statistical significance ($P = 0.9$ by the Wald test). As before, whilst sfu were more numerous on surfaces surrounding the sink, they were also detected up to 63 cm away from the sink (Table II).

When drainage was impaired, dispersal from sinks with the drain located directly underneath the tap was almost 30-times greater than sinks with the drain at the rear of the unit ($P < 0.001$ by the Wald test). There was no corresponding statistical difference between the different drain positions when drainage was fast ($P = 0.7$ by the Wald test), even though sinks with the drain directly underneath the faucet produced a higher number of sfu (Table II).

Representative isolates recovered from waste trap water and from settle plates were identified using MALDI-TOF and matched by comparing antibiotic resistance profiles. From sink A, a carbapenem-resistant *Enterobacter cloacae* was dispersed. From sink B, a carbapenem-resistant *C. freundii* was dispersed, along with a *C. freundii* strain sensitive to carbapenems and *Pseudomonas* spp. Therefore, in some cases, sfu from sink B contained Enterobacteriaceae sensitive to carbapenems.

Discussion

In this study, a laboratory model system incorporating artificially and naturally contaminated waste traps was used to investigate dispersal of CRE from sinks, with particular

Table II
Dispersal from sinks known to be colonized with carbapenem-resistant Enterobacteriaceae (CRE) in the waste trap and drain

Distance from sink (cm)		Number of CRE detected using settle plates (Total sfu)									
		Fast drainage					Slow drainage				
		Around sink	0–27	27–54	54–100	Total	Around sink	0–27	27–54	54–100	Total
Drain underneath faucet (Sink A)	Flush 1	30.3	18.3	6.3	4	69.5	224	96	36.6	17	536.5
	Flush 2	2.7	1	6.6	0.3		106	34.3	17.3	5.3	
Drain at rear (Sink B)	Flush 1	0	0	0	0.3	0.3	14.3	0.6	0	0	18.5
	Flush 2	0	0	0	0		3	0.3	0.3	0	

Mean ($N = 3$ replicate experiments) number of splash-forming units (sfu) detected on settle plates placed immediately around the sink and at distances up to 1 m from the sink during two consecutive 30-s flushes.

focus on the position of the drain in relation to the tap and drainage rate.

Active air samplers have been previously used to detect Gram-negative organisms in aerosols released from the drain during flushing [9,25]. In the current study, the use of a cyclone sampler was combined with settle plates to assess dispersal from artificially contaminated waste traps. The results suggest that dispersal is largely droplet mediated and that splashes are multidirectional. This has been recently observed by others [25] and implies that the use of settle plates alone can provide a good indication of spread and range of dispersal.

Disruption of biofilm present within a sink drain can transfer viable organisms to surrounding surfaces and the dispersal of Gram-negative organisms, resulting from tap water directly hitting the drain, has been implicated in a number of nosocomial outbreaks [10,11,22]. UK guidelines for the design of handwash basins in healthcare facilities state that the 'tap outlet flow should not discharge directly into the waste aperture' [26]. However, whilst rear-draining handwash basins are commonly installed on hospital wards, adherence to guidelines is not mandatory and sink design may differ depending on, for example, hospital, location and/or usage. Sinks in clinical and non-clinical areas can harbour CRE [16] and sink drains, regardless of position, might provide a potential route of entry into the hospital environment.

During the current study, when waste traps were artificially contaminated with CRE (i.e. the contamination was confined to the waste trap water only and was not part of an established biofilm) and when the sinks were draining correctly, there was no dispersal from the sink regardless of drain position. Kotay *et al.* [23] carried out a similar study using sinks incorporating p-traps (as opposed to bottle traps) inoculated with either fluorescent microspheres or GFP-expressing *Escherichia coli*. As in the current study, no dispersal was detected when the markers were confined to the waste trap. However, in contrast, previous experiments using fluorescent markers have suggested that when the drain is positioned directly underneath the tap, droplets containing the marker and originating from the waste trap can be dispersed up to 1 m from the sink [22]. This discrepancy might be due to a number of factors, including differences in bacteria/fluorescent markers, tap design, sink depth and/or water pressure. Drainage is also likely to be an important factor. In the current study, when slow drainage was simulated, significant dispersal from the sinks was detected, but only when the drain was directly underneath the tap. When the drain was located at the rear of the sink dispersal from an artificially (i.e. transiently) contaminated waste trap was largely prevented (Table I).

Although the experimental design does not allow for direct statistical comparison between the experiments incorporating artificially and naturally contaminated waste traps, the results suggest that the formation and presence of biofilm within and/or beyond the waste trap is associated with increased risk of dispersal, which would be consistent with other data [23].

CRE are able to persist in sinks and drains in spite of chemical disinfection, with only two out of 10 outbreak studies involving CRE from a systematic review of carbapenem-resistant organisms causing hospital-acquired infections reporting that environmental elimination was achieved [27]. Rapid re-colonization of drains and other pipework after replacement of all plumbing infrastructure in a ward, back to

the drainage stacks, has also been reported [16]. However, because many studies report the presence/absence of CRE rather than actual counts, the level of CRE contamination present in hospital sinks is largely unknown [11,13,15], as is the significance of this burden as a risk factor for transmission to patients. The waste traps removed from the hospital for installation within our model system were heavily contaminated with visible biofilms resulting in high concentrations of CRE in the waste trap water (mean: 3×10^5 cfu/mL, $N = 4$). The addition of supplementary nutrients was required to maintain this level of contamination and over time, the biofilm spread to the inner surfaces of the drain and, when present, the strainer. Kotay *et al.* [23] made similar observations in their model system which incorporated waste traps containing GFP-expressing *E. coli*; in this context, after adding nutrients, bacterial growth extended upward at an average rate of 1 inch/day, reaching the strainer after 7 days [23]. Disposal of various forms of nutrition including leftover beverages and foodstuffs from patients, surplus medication, including antibiotics, and the contents of intravenous fluid administration bags down hospital sinks has been regularly observed in a recent study characterizing sink activities [28] and had previously been reported by others [9,10]. Therefore, the addition of nutrients and the CRE levels in this study are likely to reflect conditions found in healthcare settings.

When assessing dispersal from sinks colonized with a CRE-containing biofilm, the number of sfu and the distance these splashes travelled was greatest when the drain was positioned directly underneath the faucet. However, rear-draining sinks can still disperse CRE when draining conditions are suboptimal. Problems with sink drainage are common in hospitals. Between 2005 and 2010, Breathnach *et al.* [29] reported an average of 391 notifications of blocked sinks, toilets or sluices per year in a hospital and determined that an outbreak of multidrug-resistant *P. aeruginosa* resulted from sewage backflow and the slow drainage of water from showers [29]. Kotay *et al.* [23] reported dispersal to the basin from a p-trap when water accumulated in the sink basin during flushing. In the current study, differences in drainage also influenced dispersal rates with slow draining conditions allowing dispersal from rear-draining sinks and increasing dispersal from sinks with the drain under the tap. However, whilst it has been demonstrated that draining conditions play an important role in determining the extent of CRE dispersal, there are other parameters, such as water pressure, basin type, waste trap design and the distance between waste trap and drain, that have not been considered and may also be of significance. More research is warranted to account for all variables, which also need to be taken into account when the infectious risk of sinks is assessed in clinical settings.

A further limitation of this study is the inability to quantify the CRE present in all parts of the sink and associated pipework. Sampling could have disrupted the biofilm and, therefore, it is possible that differences in the level of contamination present within the two sink types may have affected dispersal. However, CRE levels in the waste trap water were measured and accounted for in the statistical model and, when measured at the end of the experiment, drain colonization was found to be at similar levels in both sinks if slightly higher in the sink with the drain at the rear. It was also not possible to simulate clinical practice in this study and no sink activities were carried out during flushing (e.g., handwashing)

which may affect dispersal. Nonetheless, the use of a novel model system incorporating naturally contaminated waste traps taken from a hospital has provided a more realistic representation of the healthcare environment. This work and that of others [23,25] is proving that the use of laboratory model systems can produce valuable data to be considered when designing and testing infection prevention and control guidelines regarding sink management.

In all our experiments most dispersed bacteria were concentrated on surfaces immediately surrounding the sink, where the risk for cross-contamination is highest. In a recent study carried out in the Medical Intensive Care Unit of a US hospital, it was estimated that medical and/or personal items were placed around the sink located in a patient's room at least once but up to five times per day per room [28]. Others have reported inappropriate storage of clean items close to sluices [29]. These results highlight the risk of such items becoming contaminated and how medical and personal items could act as a vehicle of transmission from sinks to patients. Kitchen sinks have also been implicated in outbreaks [16], suggesting that the placement of plates, cutlery and water jugs around and within sinks should be avoided. However, the current study has also demonstrated that CRE-containing droplets originating from a sink could be dispersed up to 1 m and this should be considered when designing hospital rooms, both patient and utility (i.e. ward kitchens).

In conclusion, CRE can be dispersed from hospital sinks, particularly if inappropriate disposal of waste facilitates biofilm formation and colonization of the drain and/or if sink drainage is impaired. This has implications for onward transmission of any colonizing organism from these reservoirs, with multidrug-resistant Gram-negative organisms being of particular relevance and concern. Whilst rear-draining sinks can help prevent dispersal, appropriate sink usage and maintenance of the water and drainage system are also essential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2018.12.007.

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