

Article

Migration of *Escherichia coli* and *Klebsiella pneumoniae* Carbapenemase (KPC)-Producing *Enterobacter cloacae* through Wastewater Pipework and Establishment in Hospital Sink Waste Traps in a Laboratory Model System

Paz Aranega-Bou ^{1,*}, Nicholas Ellaby ², Matthew J. Ellington ³ and Ginny Moore ¹

¹ Biosafety, Air and Water Microbiology Group, National Infection Service, Public Health England, Manor Farm Rd, Porton Down, Salisbury SP4 0JG, UK; Paz.AranegaBou@phe.gov.uk

² Antimicrobial Resistance and Health Care Associated Infections, National Infection Service, Public Health England, 61 Colindale Avenue, London, NW9 5EQ, UK; Nicholas.Ellaby@phe.gov.uk

³ National Infection Service Laboratories, Public Health England, 61 Colindale Avenue, London, NW9 5EQ, UK; Matthew.Ellington@phe.gov.uk

* Correspondence: Paz.AranegaBou@phe.gov.uk; Tel.: +441980612630

Abstract: Sink waste traps and drains are a reservoir for multi-drug resistant Gram-negative bacteria in the hospital environment. It has been suggested that these bacteria can migrate through hospital plumbing. Hospital waste traps were installed in a laboratory model system where sinks were connected through a common wastewater pipe. Enterobacterales populations were monitored using selective culture, MALDI-TOF identification and antibiotic resistance profiling before and after a wastewater backflow event. When transfer between sinks was suspected, isolates were compared using whole-genome sequencing. Immediately after the wastewater backflow, two KPC-producing *Enterobacter cloacae* were recovered from a waste trap in which Carbapenemase-producing Enterobacterales (CPE) had not been detected previously. The isolates belonged to ST501 and ST31 and were genetically indistinguishable to those colonising sinks elsewhere in the system. Following inter-sink transfer, KPC-producing *E. cloacae* ST501 successfully integrated into the microbiome of the recipient sink and was detected in the waste trap water at least six months after the backflow event. Seven weeks and three months after the backflow, other inter-sink transfers involving *Escherichia coli* ST5295 and KPC-producing *E. cloacae* ST501 were also observed.

Keywords: CPE; drains; hospital plumbing; environmental contamination; infection control

1. Introduction

Carbapenemase-producing Enterobacterales (CPE) are becoming increasingly common causes of hospital-acquired infections and are often associated with outbreaks. Hospital reservoirs include colonized patients and wastewater [1]. Shower and sink drains can harbour identical or highly similar strains to patients and are considered a potential source of transmission [2]. Contamination of these sites might not be localised and might not remain contained. Migration of Gram-negative bacteria through wastewater plumbing to colonize nearby sink and shower drains has been reported [3, 4]. Here we describe the propagation of two strains of KPC-producing *Enterobacter cloacae* and one strain of *Escherichia coli* through pipework in a laboratory model sink system containing hospital waste traps.

2. Materials and Methods

2.1. Waste trap installation in the hospital sink laboratory model system

The model system design has been described previously [5]. Five waste traps were collected from three different wards at the same hospital in England, transported to the

laboratory and fitted to sinks (numbered 1 to 5) connected via a common waste pipe. The associated taps were automatically flushed 4 times a day for 30 seconds, simulating a low usage sink. Approximately 3mL of nutritious broth was poured into each sink on a daily basis to maintain Enterobacterales populations at similar levels in the waste trap water. Water and biofilm samples from waste traps were collected pre-installation and cultured for coliform bacteria and for CPE using Brilliance™ *E.coli*/coliform agar (Oxoid Ltd, Basingstoke, UK) and chromID™ CARBA (Biomérieux, Basingstoke, UK) respectively. All plates were incubated at 37°C for 18-24h. Waste trap water populations were regularly monitored using the methods previously described [5].

2.2. Wastewater backflow event

Water was drained from the sinks into a 124 L collecting vessel. Drainage was automatically controlled by means of a pump. Approximately three weeks after the waste traps were installed, prevention of the normal operation of the pump resulted in wastewater backflow through common pipework and re-entry into the sinks, simulating a major blockage. After remaining stagnant for four hours, the water was drained. After 24 hours, sinks and the taps were allowed to operate again. The waste trap water of each sink continued to be collected and cultured on a regular basis.

2.3. Phenotypical characterisation of isolates

Presumptive Enterobacterales were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry (Bruker Daltonik MALDI biotyper; Bruker, Bremen, Germany) using the direct transfer method. Isolates were compared by antibiotic resistance profiling using the disk diffusion method (ampicillin, gentamicin, amikacin, meropenem, ceftazidime and ciprofloxacin) (Oxoid) following EUCAST guidelines. Isolates were stored in cryobeads (Technical Service Consultants, Heywood, UK) at -80°C.

2.4. Genotypical characterisation of isolates

Isolates were recovered from beads and DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Manchester, UK). Whole-genome sequencing (WGS) was carried out on an Illumina HiSeq 2500 (Illumina, San Diego, CA). Reads were submitted to the European Nucleotide Archive (number PRJEB43840). Genome assemblies were generated by SPAdes [6] and compared using Mash [7]. MLST profile was determined by mapping reads against publicly available species databases (<https://pubmlst.org/>) using the tool MOST [8].

3. Results

3.1. KPC-producing *Enterobacter cloacae* transfer events

When hospital waste traps were first installed in our system, carbapenemase-producing *Enterobacter cloacae* was isolated from sinks 1 and 3. Using disc diffusion assays it was determined that the strain originating in sink 1 was resistant to ampicillin, ceftazidime and meropenem (Antibiotic resistance profile A). The strain from sink 3 also showed intermediate resistance to ciprofloxacin and gentamicin (Antibiotic resistance profile B). No CPE were recovered from sinks 4 and 5. However, immediately following the backflow event, *E. cloacae* isolates exhibiting antibiotic resistance profiles A and B were detected in the waste trap water of sink 5. Subsequent culture results implied that *E. cloacae* with antibiotic resistance profile A had become part of the microbial community colonising the waste trap and isolates were recovered from sink 5 for at least 6 months after the initial transfer (Table 1). In contrast, *E. cloacae* with antibiotic resistance profile B was not detected in this sink during subsequent sampling. Approximately 7-weeks after the backflow event, *E. cloacae* isolates exhibiting antibiotic resistance profile A were detected in sink 4, implying another transfer event (Table 1). Nine isolates

recovered from the affected sinks were sequenced and compared. WGS confirmed that two different strains (ST501 originating from sink 1 (antibiotic resistance profile A) and ST31 originating from sink 3 (antibiotic resistance profile B)) had migrated through the system. According to Mash, isolates belonging to ST501 shared over 98.6% similarity within the group while isolates belonging to ST31 shared over 99.2% (Supplementary table 1).

Table 1. Isolates characterised in this study. The *E. coli* transfer event was detected three months after the water back-flow.

Bacteria	Antibiotic re- sistance profile	Sequence type	Isolate ID	Date of isolation	Sink
<i>Enterobacter cloacae</i>	A	ST501	A	Pre-installation	1
			B	10 days before water backflow	1
			C	Immediately after wa- ter backflow	5
			D	7 weeks after water backflow	4
			E	3 months after water backflow	5
			F	6 months after water backflow	5
<i>Enterobacter cloacae</i>	B	ST31	G	Pre-installation	3
			H	10 days before water backflow	3
			I	Immediately after wa- ter backflow	5
<i>Escherichia coli</i>	No resistance detected by disk diffusion	ST5295	J	Pre-installation	4
			K	5 days before ob- served transfer	4
			L	Observed transfer	3
			M	Observed transfer	5
			N	10 weeks after ob- served transfer	3
			O	10 weeks after ob- served transfer	5
			P	5 months after ob- served transfer	5
			Q	6 months after ob- served transfer	5

3.2. *Escherichia coli* transfer events

Following installation of the waste traps, *E. coli* was detected in sink 4 but none of the other sinks. No immediate changes regarding *E. coli* distribution in the system were observed immediately after the backflow event. However, three months later, *E. coli* was detected in the two sinks either side of sink 4 (i.e. sinks 3 and 5). *E. coli* isolates continued to be recovered from sinks 3 and 5 for a further ten weeks and six months respectively (Table 1). WGS identified all eight isolates as *E. coli* ST5295 with over 99.4% shared similarity (Supplementary table 2).

4. Discussion

The results presented in this study suggest that CPE contamination within a single waste trap might not remain localised and transfer of Enterobacterales between sinks connected through common pipework is possible and perhaps linked to drainage problems and the backflow of wastewater. Although rarely reported in the literature, plumbing problems such as blockages are frequent in hospitals [9].

Here, we found bacterial transfer occurred between waste traps separated by up to 2 metres. Panels between each sink prevented above drain cross-contamination (e.g. via splashback [5]) implicating pipework migration as the most likely pathway. Our system also incorporates sinks that drain to individual wastewater pipes; bacterial transfer between these sinks has not been observed. Recent work looking into the ecology of *E. coli* and *Klebsiella* spp. populations in hospital waste traps has shown that different wards and even different sinks harbour distinct ecosystems, with few shared lineages [10]. It seems therefore unlikely that identical strains were found in different waste traps by chance.

It is interesting to notice that while some transfers were identified immediately after the backflow event (*E. cloacae* ST31 and ST501 from sinks 1 and 3 to sink 5), others were not identified until some months after the event (*E. cloacae* ST501 from sink 1 or 5 to sink 4 and *E. coli* ST5295 from sink 4 to sinks 3 and 5). One possible explanation is that small numbers of bacteria transferred during the backflow were incorporated into the existing biofilm and took time to increase to detectable levels. Another possible explanation is that migrations occurred later and were due to unrelated transfer events.

In a recent study, Hopman et al. [3] described the environmental investigation that followed a fatal hospital-acquired infection cause by a carbapenemase-producing *Pseudomonas aeruginosa*. The same strain was recovered from shower and sink drains in seven different rooms which shared pipework to a common sewage collection point but not from the sinks and showers in nearby rooms that drained into a different sewage collection point. The authors suggested that the strain was introduced into a drain and then spread via plumbing to proximate rooms. However, as no environmental cultures were available prior to the positive cultures, it is unknown when and how the introduction took place and how long it took for it to spread to the other rooms. Other reports have also suggested migration of Gram-negative bacteria through pipework [11, 12] but alternative explanations (e.g. introduction via hand-washing) could not be ruled out. A key aspect of the work described in this report is the availability of pre-installation samples (Table 1) which provide information about the bacterial populations before the transfer.

Some of the transfer events observed in this study led to a stable colonisation lasting for at least 6 months (*E. cloacae* ST501 and *E. coli* ST5295 in sink 5) while in others the colonisation was more transient lasting from days to a few months (*E. cloacae* ST501 in sink 4, *E. cloacae* ST31 in sink 5 and *E. coli* ST5295 in sink 3). It is interesting that a strain that successfully colonized one sink was unable to do so in another, suggesting that the microbial environment can exclude certain strains that could otherwise be successful, or at least prevent their growth to detectable levels. Utilising a similar model sink system, Kotay et al. [4] showed that, in the absence of competing microorganisms, a GFP-expressing *E. coli* strain inoculated on a sink waster trap could be detected in other sinks connected by a common waste pipe, after just a few days. Rapid colonization of newly replaced pipework and sinks with carbapenem-resistant organisms has also been observed

in the hospital setting [2]. However, these systems, while not being sterile, lacked the extensive biofilm coverage of old, existing hospital wastewater plumbing. The lack of microbial competition might have facilitated the colonisation of sinks in these scenarios.

5. Conclusions

This work supports previous studies that have demonstrated migration of Gram-negative pathogens through plumbing [4] and provides evidence of CPE migration through contaminated wastewater sites. This implies that once a sink is colonised with CPE, the contamination might not remain localised and could spread to other sites via plumbing, particularly when drainage problems and stagnation of wastewater occur.

Supplementary Materials: Table S1: MinHash similarity matrix representing the proportion of kmers shared by the different *Enterobacter cloacae* isolates characterised in this study, Table S2: Supplementary table 2. MinHash similarity matrix representing the proportion of kmers shared by the different *Escherichia coli* isolates characterised in this study.

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Data Availability Statement: The data presented in this study are available in the article and supplementary material. Sequencing reads are available on the European Nucleotide Archive (number PRJEB43840).

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Conflicts of Interest: Armitage Shanks (UK) provided the washbasins incorporated within the model system free of charge.

References

1. van Loon K, Voor In 't Holt AF, Vos MC. A Systematic Review and Meta-analyses of the Clinical Epidemiology of Carbapenem-Resistant Enterobacteriaceae. *Antimicrobial agents and chemotherapy* **2017**; 62(1): e01730-17.
2. Carling PC. Wastewater drains: epidemiology and interventions in 23 carbapenem-resistant organism outbreaks. *Infection Control & Hospital Epidemiology* **2018**; 39(8): 972-9.
3. Hopman J, Meijer C, Kenters N, et al. Risk Assessment After a Severe Hospital-Acquired Infection Associated With Carbapenemase-Producing *Pseudomonas aeruginosa*. *JAMA network open* **2019**; 2(2): e187665.
4. 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(8).
5. Aranega-Bou P, George RP, Verlander NQ, Paton S, Bennett A, Moore G. Carbapenem-resistant Enterobacteriaceae dispersal from sinks is linked to drain position and drainage rates in a laboratory model system. *The Journal of hospital infection* **2019**; 102(1): 63-9.
6. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* **2012**; 19(5): 455-77.
7. Ondov BD, Treangen TJ, Melsted P, et al. Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biology* **2016**; 17(1): 132.
8. Tewolde R, Dallman T, Schaefer U, et al. MOST: a modified MLST typing tool based on short read sequencing. *PeerJ* **2016**; 4: e2308.
9. 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. *Journal of Hospital Infection* **2012**; 82(1): 19-24.

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10. Constantinides B, Chau KK, Quan TP, et al. Genomic surveillance of *Escherichia coli* and *Klebsiella* spp. in hospital sink drains and patients. *Microbial genomics* **2020**; 6(7).
 11. Buchan BW, Graham MB, Lindmair-Snell J, et al. The relevance of sink proximity to toilets on the detection of *Klebsiella pneumoniae* carbapenemase inside sink drains. *American Journal of Infection Control* **2019**; 47(1): 98-100.
 12. Moloney EM, Deasy EC, Swan JS, Brennan GI, O'Donnell MJ, Coleman DC. Whole-genome sequencing identifies highly related *Pseudomonas aeruginosa* strains in multiple washbasin U-bends at several locations in one hospital: evidence for trafficking of potential pathogens via wastewater pipes. *Journal of Hospital Infection* **2019**.