Infection and Drug Resistance

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SHORT REPORT

Intensive Care Unit Sluice Room Sinks as Reservoirs and Sources of Potential Transmission of Carbapenem-Resistant Bacteria in a South African Tertiary Care Hospital

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Purpose: Carbapenem-resistant bacteria (CRB) pose a major health risk to patients in intensive care units (ICU) across African hospitals. There are hardly any data about the role of hospital sinks as reservoirs of CRB in resource-poor African settings. Furthermore, the specific within-sink location of the highest concentration of pathogens and the role of splash back as a transmission mechanism remains poorly clarified.

Methods: We swabbed ICU sluice room sinks in a tertiary hospital in Cape Town, South Africa. Swabs were taken from four different parts of the sluice room sinks (tap-opening, trap, below the trap, and u-bend). Dilutions were prepared and plated on carbapenem-infused agar. Colonies were identified and drug resistance profiles were determined using a biochemical analyser. To evaluate the potential transmission from the sink, similar plates were placed at fixed distances from the sink when the tap was turned on and off.

Results: CRB were isolated from the trap, water interface below the trap, and the u-bend (the latter harboured the highest density of CRB species). Five CRB, resistant to at least 7 antibiotic classes, were isolated including *Pseudomonas, Klebsiella, Citrobacter, Serratia, and Providencia*. CRB could be cultured from droplets that fell on agar-containing plates placed at a varying distance from the trap.

Conclusion: There is a higher density of CRB in the u-bend of ICU sluice room sinks which can act as a potential source of transmission. The data inform targeted CRB transmission-interruption strategies in resource-poor settings.

Keywords: carbapenem-resistant bacteria, multi drug resistant bacteria, sluice room sink, intensive care units, antimicrobial resistance

Introduction

Antimicrobial resistance is considered by the WHO as one of the top ten public health challenges facing humankind in the 21st century.[1] Nosocomial infections involving highly drug-resistant or pan drug-resistant bacteria are now common globally.[2–8] Outbreaks occur frequently, mortality is higher in affected persons, and the outcomes are worse in immunocompromised patients.[2] Interrupting transmission of drug-resistant pathogens is a priority.

However, the reservoirs and transmission linkages involving hospital-based drug-resistant bacteria are only partially understood. Several mechanisms and processes have been implicated; in particular, hospital sinks have been singled out as harbouring drug-resistant bacteria via a biofilm mechanism (reviewed in[9]). Indeed, several global reports have documented sinks being reservoirs of pan-resistant organisms.[10–12] However, there is a paucity of data regarding the role of hospital sinks as reservoirs for drug-resistant bacteria in resource-poor African settings. There are hardly any data

Received: 24 April 2023
Accepted: 25 July 2023
Published: 21 August 2023

about the specific location within sinks where drug-resistant bacteria reside. This information is critical for implementing transmission interrupting interventions. Finally, whether sink-based reservoirs of drug-resistant pathogens can serve as sentinels for onward transmission remains largely unclarified.

To address these knowledge gaps, swabs were taken from different locations or areas of two surgical intensive care unit (ICU) sluice room sinks in a South African tertiary hospital and the samples plated on carbapenem-infused agar plates. Unique colonies were isolated, identified and their drug resistance profile determined. Finally, carbapenem-infused agar plates were placed at varying distances away from the sink, whilst the tap was turned on, and subsequently cultured.

Methods

Sample Collection

Two sluice room sinks in the surgical ICU unit at Groote Schuur Hospital in Cape Town, South Africa, were swabbed on different days at the opening of the tap, the trap (drain grate), 10 cm below the trap, and at the u-bend (overview-figure Figure 1). Nurses on duty at the ICU were also interviewed to determine sink cleaning protocols, which revealed that the sinks were being surface scrubbed with 0.06% (w/v; 250 ppm) sodium dichloroisocyanurate (Hygenie Capricide Extra, Caprichem, South Africa) 3 times a day, and in addition when biological material was discarded. There was no specific cleaning of the sink pipe below the trap and leading to the u-bend. The study was approved by the University of Cape Town Research Ethics Committee (HREC approval number 153/2019) and the Groote Schuur Hospital research approvals board.

Swabs were placed in Stuart's transport medium. In the laboratory (intervening transport time of ~15 minutes) the swabs were cut and placed in 1mL Dulbecco's phosphate-buffered saline (DPBS; Lonza, Germany) and vortexed for 1 minute. Five serial 1 in 10 dilutions were prepared, and 100µL of each dilution was spread on to carbapenem-infused agar plates (ChromID Carba Smart Agar Plates; BioMérieux; Marcy-l'Étoile, France; Figure 1C). The plates were incubated aerobically at 30°C for 5 days, after which individual colonies were streaked onto MacConkey agar plates (Sigma, South Africa) and incubated for an additional 5 days.

Splashback Experiments

Four carbapenem-infused agar plates were placed at different locations (varying distances of 1cm to 30cm from the trap) inside one of the sinks in the ICU unit (Figure 1D). The last remaining plate was placed on the countertop on the edge of the basin. The tap was turned on for 30 seconds. The plates were incubated as outlined above.

Identification and Susceptibility Testing

The bacterial species cultured on the MacConkey agar plate were identified and antibiotic resistance profile determined using the automated biochemical analyser, VITEK® 2 (BioMérieux, South Africa), using the GN identification and AST-255 susceptibility testing cards. Clinical Laboratory Standards Institute clinical breakpoints (M100) were applied.[13]

Results

A total of 4 swabs were taken from the various areas of the sink depicted in Figure 1, of which 3 yielded growth on the carbapenem-infused agar. Eight unique carbapenem-resistant bacterial species (resistant to 7 antibiotic classes; Table 1), with potential to cause outbreaks in the ICU, were identified from 8 chromogenically and morphologically distinct colonies (Table 1). The greatest concentration of carbapenem-resistant bacteria (CRB; plate growth density and number of species) were found at the bottom of the u-bend though CRB could also be cultured from the trap and the water interface below the trap (but not from the tap opening). One *K. pneumonia* isolate was resistant to almost all 18 antibiotics tested. Splash back experiments isolated colonies of *P. fluorescens* from the plate within the sink and closest to the drain trap but not from plates placed outside the basin (Figure 1).

The greatest concentration of CRB (by plate growth density and number of species) were found in the u-bend: these included *S. marcescens, K. pneumoniae*, and *P. rettgeri*. While no CRB were isolated from the tap-opening, *C. freundii* and *P. aeruginosa* were cultured from the water-level and *S. marcescens* and *P. aeruginosa* were cultured from the trap.

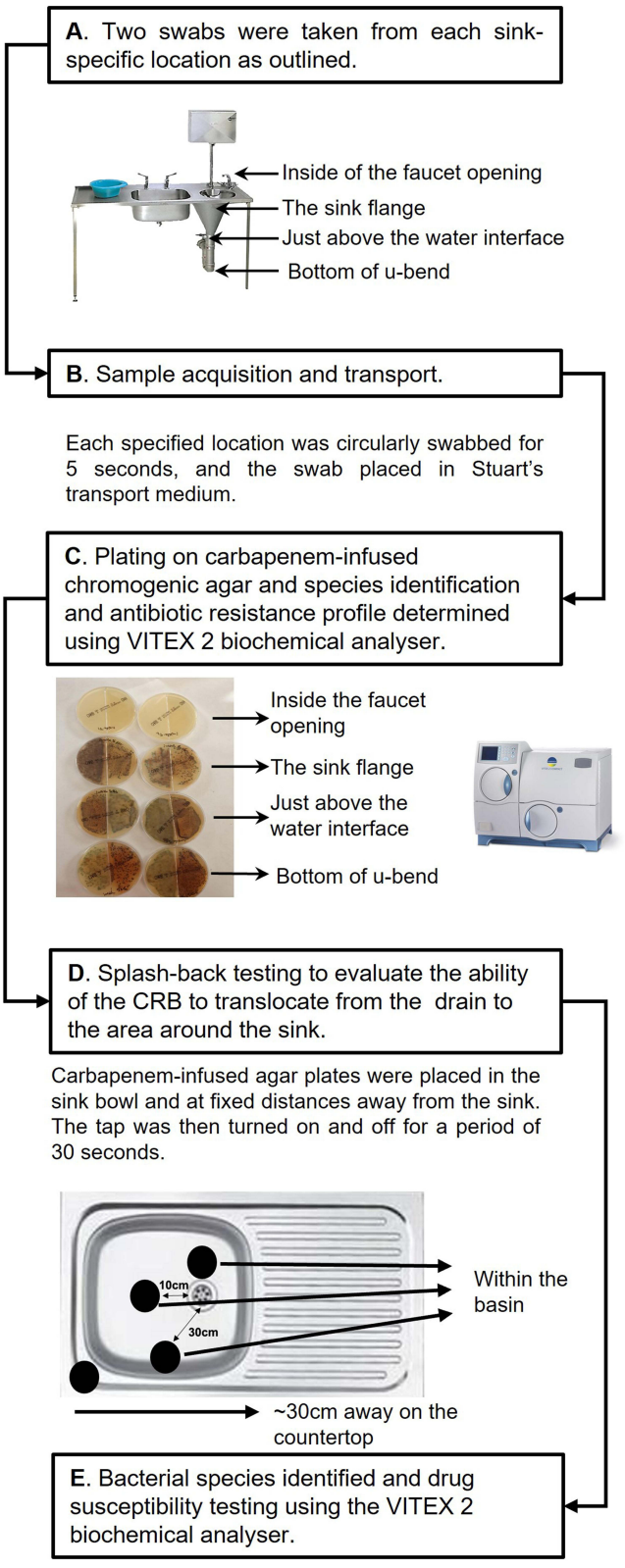

Figure 1 Schematic showing the study overview and the sampling methods used. (**A**) The sluice drain was swabbed inside the tap opening, the area around the trap, at the water interface below the trap, and at the bottom of the u-bend. (**B**) The swabs were transported to the laboratory and dilutions were plated on to carbapenem-impregnated agar plates as detailed in the materials and methods. (**C**) After incubating the plates for 5 days at 30°C individual colonies were selected for phenotypic identification and antibiotic susceptibility testing (VITEX 2). (**D**) To study the transmission dynamics of the CRB, carbapenem-infused agar plates were placed around various areas of the sluice sink as outlined. The tap was turned on and off for 30 seconds, and the agar plates were incubated at 30°C for 5 days. (**E**) The bacteria were identified, and antimicrobial susceptibility testing performed as above.

Table 1 Carbapenem-Resistant Bacteria Isolated from the Sluice Drain of an ICU at a Tertiary Hospital in Cape Town, South Africa. The Drug Susceptibility Profile of Each Organism is Shown, and the Organisms are Ordered by Isolation Location from the Trap to the u-bend

Antibiotic	**CRB Isolated from the ICU Sink**							
	S. marcescens	***P. aeruginosa***	***C. freundii***	***P. aeruginosa***	***S. marcescens***	***K. pneumoniae***	***P. rettgeri***	***P. fluorescens***
Trimethoprim-sulfamethoxazole	Resistant	N/A	Resistant	N/A	Resistant	Resistant	Resistant	Resistant
Amoxicillin and other penicillins	N/A	Resistant	Resistant	Resistant	N/A	Resistant	Resistant	Resistant
Cefoxitin and cefuroxime (2nd generation cephalosporins)	Resistant	N/A	Resistant	N/A	Resistant	Resistant	N/A	N/A
Ciprofloxacin (2nd generation cephalosporin)	Intermediate	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Cefotaxime / Ceftriaxone (3rd generation cephalosporins)	Resistant	N/A	Resistant	N/A	Resistant	Resistant	Resistant	N/A
Ceftazidime (3rd generation cephalosporin)	Resistant	Resistant	Resistant	Susceptible	Resistant	Resistant	Intermediate	Resistant
Cefepime (4th generation cephalosporin)	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	N/A	Resistant
Gentamicin	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Amikacin	Resistant	Resistant	Resistant	Resistant	Resistant	Susceptible	Resistant	Susceptible
Ertapenem	Resistant	N/A	Resistant	N/A	Resistant	Resistant	Resistant	N/A
Imipenem	Data not available	Resistant	Intermediate	Resistant	Data not available	Resistant	Resistant	Resistant
Meropenem	Resistant	Resistant	Intermediate	Resistant	Resistant	Resistant	Intermediate	Resistant
Tigecycline	Resistant	Resistant	Intermediate	Resistant	Resistant	Resistant	N/A	N/A

Notes: Susceptible: The organism is susceptible to the tested antibiotic. Resistant: The organism is resistant to the tested antibiotic. Intermediate: The organism is intermediately susceptible to the tested antibiotic. N/A: The organism is intrinsically resistant to the specific antibiotic, and hence was not tested.

Discussion

The key findings of this preliminary study were that (i) CRB could be cultured from ICU sluice room sinks in a resource-poor African setting, (ii) the sample location within the sink that cultured the highest taxonomic density of CRB was the u-bend, and (iii) CRB could be cultured from agar plates placed at the sink trap when the tap was turned on suggesting cross-contamination and potential transmission via this route may occur. It is often assumed that multi-drug antibiotic resistance is uncommon in Africa.[14] However, we have demonstrated that this assumption is untrue. Clearly, further epidemiological studies are required from resource-poor settings to add to the limited existing data.[14]

Overall, there are hardly any data about CRB density from different parts of the sink. In most studies samples were only taken from the sink trap (reviewed in[15]) though several studies documented CRB growth from the u-bend of the sink.[15] Our data, incorporating sampling of the tap opening, trap, at water level below the trap, and the u-bend suggest that simply sampling from the sink trap is insufficient in determining the extent of CRB colonisation, and that better decontamination strategies are required to remove bacterial biofilms deeper within the draining pipe. Such strategies may include pressured steam in conjunction with chemical disinfectants though there are no comprehensive comparative studies.[15] Another method to prevent CRB colonisation may involve installing sinks without a u-bend and only a trap, where the contents are discarded into biohazardous drums. We did not ascertain the quantitative microbiological load at each of the drain locations because of funding constraints, the preliminary nature of the study, and the high cost of the carbapenem-infused agar plates (US$ 125/plate). Another limitation was that confirmation of the mechanism of carbapenem resistance and detection of carbapenemases, either phenotypic, antigenic, or genotypic, was not performed, nor was molecular typing of isolates (for the same reasons as outlined above).

Our droplet-related spread data are in keeping with that of other studies using fluorescent-labelled fluid or whole-genome sequencing[16,17] showing the potential of CRB to be transmitted to patients and healthcare workers. A limitation of all these methods and studies is lack of data about the clinical impact of these findings. Thus, there is a need for further studies of improved splash-resistant sink design and transmission-minimising protocols for health care workers and those who clean in health care facilities.[18]

In conclusion, we isolated a variety of CRB from sluice room sinks in an African ICU. The u-bend of ICU sluice room sinks are reservoirs for CRB with transmission potential. These data have implications for infection control interventions and limiting antimicrobial resistance in resource-poor settings. Such interventions may include implementation of regular approved and effective sink cleaning protocols, regularly swab monitoring of hospital sinks, and using sink designs that minimise splashback or do not have conventional trap systems that perpetuate microbiological reservoirs.

Acknowledgments

The authors would like to thank the ICU Head Nurse, Sr Jennifer Abrahams, who generously helped to facilitate the sampling and interviews, and Dr Jason Limberis, who provided valuable intellectual input.

Funding

This work was supported by Antrum Biotech, a University of Cape Town spin off company, through an unrestricted student support grant. The research and development company does not work in the field of antibiotics, antimicrobial resistance, or decontamination products, and its employees played no role in the study design, collection, analysis and interpretation of data, writing of the report, or the decision to submit the article for publication. AE was supported by funding received from the European and developing Countries Clinical Trials Partnership (EDCTP TMA-2015CDF-1052). Some of work was supported by the South African MRC (RFA-EMU-02-2017). AP was supported by funding received from the European and developing Countries Clinical Trials Partnership (EDCTP TMA-2019CDF-2780).

Disclosure

The authors report no conflicts of interest in this work.

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