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Short Report

Increased detection of carbapenemase-producing Enterobacteriaceae on post-clean sampling of a burns unit's wet surfaces

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SUMMARY

Wet surface biofilms are a potential reservoir for multidrug-resistant Gram-negative organisms, including carbapenemase-producing Enterobacteriaceae (CPE). Recognition of environmental sources is important in reducing secondary patient transmission. We report the increased detection of *bla*IMP-4^{+} CPE in environmental samples from floor drains in burns unit shower rooms, when collected following cleaning as compared to pre-cleaning. We propose that disruption of biofilms during cleaning may account for the increased detection of multi-resistant organisms. The results highlight the role of the wet environment as an under-recognized potential source of CPE transmission. Environmental screening focusing on pre-cleaning samples alone will likely underestimate environmental contamination.

Introduction

The persistence of multidrug-resistant organisms (MDROs) in environmental niches such as drains and sinks is widely recognized [1–5]. Multiple interplaying factors are thought to contribute, and the presence of biofilm is noted to be an important determinant of persistent bacterial colonization [1,5,6]. However, there are few descriptions of the repeated isolation of Gram-negative MDROs, including carbapenemase-producing Enterobacteriaceae (CPE), immediately post cleaning from sites which previously tested negative for MDROs. The present study reports the consistent observation of the post-cleaning isolation of Gram-negative organisms carrying extended-spectrum β-lactamases (ESBLs) and *bla*IMP-4 over a four-year period, from surfaces in a burns unit, some of which were negative for MDROs on pre-cleaning screens.

In September 2006, *bla*IMP-4^{+} metallo-β-lactamase was first identified in the burns unit at Concord Hospital at the time of an inter-hospital transfer of a patient known to be colonized with *bla*IMP-4^{+} *Enterobacter cloacae*. Since then, *bla*IMP-4^{+} Enterobacteriaceae have been consistently isolated from

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environmental sampling despite targeted, enhanced environmental disinfection [7]. In regular sampling of environmental sites in the Concord Hospital burns unit, we have consistently observed that certain Gram-negative MDROs, especially *bla*IMP-4^{+} Enterobacteriaceae, are more frequently isolated from specific sites after, rather than before, environmental cleaning.

Methods

We completed a study of environmental isolates collected from the burns unit at Concord Hospital over the period of 2012–2015. The burns unit is an enclosed unit, receiving statewide referrals for severe burns injury. It contains eight single rooms and separate access ante-rooms for mandatory pre- and post-entry handwashing and donning of contact precautions. Five shower facilities and one bathroom containing a specialized 'Arjo' burn bath are located within the burns unit and shared by all patients.

Over four years, from 2012 to 2015, environmental swabs from pre-defined sites were collected by burns unit nursing staff immediately before and within 30 min after vigorous physical cleaning with a chlorine-based product. The predefined environmental surfaces included high-touch surfaces such as bed bases, call bells, monitors and furniture surrounding the patient zone. In addition, samples were collected from 'wet areas' including sinks, floor drains and sluices located within shower and bath facilities.

Pre-moistened sterile gauze swabs were used for sampling and were immediately inoculated into 10 mL of brain–heart infusion (BHI) broth upon collection. The inoculated BHI broth was incubated overnight in 5% CO_2 at 35–37°C and subcultured after 24 h on to ESBL chromogenic media which was incubated in 5% CO_2 at 35–37°C for 24 h.

For Enterobacteriaceae cultured on the ESBL screening media, phenotypic susceptibility testing and DNA amplification tests, using an in-house IMP-4- and VIM-specific SYBR Green reverse transcription–polymerase chain reaction, were performed to detect production of carbapenemases. If this targeted IMP-4/VIM assay was negative, a multiplex in-house CPE DNA amplification molecular assay, with broader carbapenemase targets (KPC, NDM, VIM, IMP-4, OXA-48), was run on the isolate.

In addition, over the period February to July 2014, a more intensive (monthly) parallel environmental surveillance study was also completed in the burns unit. This sub-study again compared pre- and post-cleaning environmental sampling; however, more samples were collected at shorter time intervals than in the primary surveillance study. This sub-study identified Gram-negative organisms producing ESBLs in addition to CPE.

Results

In the four-year period of data collection, 1901 environmental swabs were collected from the pre-defined areas in the burns unit at regular time-intervals. In total 73 (4%) CPE were isolated from specified pre-clean and post-clean surfaces (Table I). Of these, 64.4% were isolated only from post-cleaning screens, and these rates were consistent when assessed per two-year intervals over the study period. Pre-cleaning screen alone detected 24.6% of the MDROs and a further 11% were detected on both pre- and post-clean sampling from the same site. The greater proportion of MDROs identified on post-clean sampling only was echoed in a parallel intensified sampling study conducted over six months in 2014 which detected both CPE and ESBLs (Table II). Of importance, following disinfection, all CPE isolated from post-clean sampling alone were only isolated from 'wet surfaces', that is from floor drains and sluices (Table I).

All CPE detected were found to be *bla*IMP-4 positive on molecular testing. The predominant Enterobacteriaceae species were *Citrobacter freundii* (36.9%), *Enterobacter cloacae* (32.9%), *Enterobacter hermannii* (15.1%), and *Leclercia adecarboxylata* (9.6%). Each of these organisms was isolated in greater numbers in sampling performed post cleaning. Other organisms encountered were *Raoutella planticola* (1.3%) and *Klebsiella oxytoca* (1.3%) pre cleaning, and *Klebsiella aerogenes* (1.3%) and *Enterobacter asburiae* (1.3%) post cleaning.

Discussion

The critical role of environmental cleaning in reducing the bacterial bioburden of surfaces and minimizing rates of hospital-acquired infections is well established [6,8]. Equally, however, the potential for survival of MDROs through the cleaning process is extensively reported [1,5,8]. In addition, the rebound contamination of surfaces at 24 h post-cleaning, at levels exceeding original colony counts, has been demonstrated [9]. Several reasons have been proposed for this, including carry-over and spread from poorly used cleaning wipes; however, the majority pertain to the presence of biofilm and the negating impact this can have on cleaning processes and the efficacy of detergents [1,5,6,8]. Unlike dry biofilms, which are overwhelmingly associated with Gram-positive bacteria, wet surface biofilms are more likely to harbour Gram-negative bacteria [10]. In this study, we describe the increased isolation of multidrug-resistant Gram-negative bacteria

Table I
Isolation of *bla*IMP-4^{+} CPE in pre- and post-clean sampling over four years, 2012–2015

Site	No. of CPE isolated from environmental sampling of pre-specified environmental sites			
	Pre cleaning only	Post cleaning only	Pre and post cleaning	Total CPE
Arjo bathroom drains + sluice	2	7	0	9
En suite drain	1	1	0	2
Shower 2 Drains + sluice	4	14	0	18
Shower 3 Drains + sluice	5	16	6	27
Shower 4 Drains + sluice	4	9	2	15
Shower 5 Drains + sluice	1	0	0	1
Room 7 Visitor's chair	1	0	0	1
Total	18 (24.6%)	47 (64.4%)	8 (11%)	73

CPE, carbapenemase-producing Enterobacteriaceae.

Table II
Isolation of environmental MDR Gram-negative bacteria (*bla*IMP-4 and EBSL) in pre- and post-clean sampling over six months, February to July 2014

Site	No. of environmental sites on the burns unit from which MDR Gram-negative bacteria were isolated		
	Pre cleaning only	Post cleaning only	Total MDR Gram-negative bacteria
Patient room surface ($N = 129$)	1 (IMP-4)	0	1
Shower room surface ($N = 170$)	0	6 (4 IMP-4, 2 ESBL)	6
Shower room drains ($N = 52$)	7 (5 IMP-4, 2 ESBL)	18 (15 IMP-4, 1 IMP-4/ESBL, 2 ESBL)	25
Patient room sink ($N = 16$)	1	0	1
Total ($N = 367$)	9 (2.5%)	24 (6.5%)	33 (9.0%)

MDR, multidrug-resistant; ESBL, extended-spectrum β-lactamase.

following environmental cleaning, and we hypothesize that the disruption of biofilm during manual environmental cleaning of wet surfaces underpins this observation.

The formation of biofilm on wet surfaces not subject to frequent physical disruption has been extensively described [2,4,6]. Biofilm forms a sanctuary site for sessile and planktonic bacteria which require disinfectant concentrations of up 1000 times the standard to be destroyed [6,11]. MDROs, including carbapenemase-producing *Klebsiella pneumoniae*, meticillin-resistant *Staphylococcus aureus*, and multidrug-resistant *Acinetobacter baumannii*, may survive for months in biofilm reservoirs. Protracted CPE outbreaks, including KPC-producing Enterobacteriaceae, have been associated with MDROs harboured in the biofilm of toilets and sinks [4].

Bacteria sequestered within biofilms are known to be difficult to culture [1,11]. Sonication techniques and enrichment broth are often required to aid recovery in culture; however, even when visible on electron micrography, bacteria in biofilms may be non-viable using conventional culture techniques [1]. Hence, it is likely that the consistent observation of lower MDRO rates before cleaning is due to the impaired ability of pre-cleaning swabs to disrupt biofilm and to adequately recover bacteria present within a biofilm. Other reasons for such findings include the sessile state of growth in biofilm, lack of appropriate culture conditions, and non-viability of organisms [1,11]. However, the improved growth of MDROs following biofilm disruption in our study suggests that these organisms persist in a viable state in the wet environment. Further to this, as exposure to chlorine-based cleaning products is known to induce some organisms to enter a viable but non-culturable state, it is possible that the proportion of post-cleaning MDROs in our study, though higher than the pre-clean MDRO proportion, remains under-reported.

During the process of vigorous physical cleaning, the polymer matrix of biofilm is disrupted. Manual cleaning results in the mechanical disruption of the exopolymeric substances of biofilm and may result in the intermittent release of planktonic bacteria [1]. Hence, as seen in our study, immediate post-clean sampling will isolate MDROs with greater susceptibility than those performed pre cleaning. Such results are consistent with the findings of Lesho *et al.* who also reported increased isolation of MDR Gram-negative bacteria post cleaning [12]. That study differs from our observations, in that increased rates of MDR Gram-negative bacteria were detected on both wet and dry surfaces, and MDR was defined as non-susceptible to at least one agent in three antimicrobial classes without testing for carbapenemase or ESBL production [12].

The hospital water environment forms an ideal complex niche [2,4]. The low-pressure intricate network of hospital plumbing with stagnant, temperate waste water, engenders the growth of bacteria and formation of biofilm [2]. Multi-resistant organisms from a range of sources, including patient waste, may cause the initial inoculation of the plumbing system with MDROs [4]. In our study, except for one isolate, MDR Gram-negative bacteria were only isolated from 'wet areas' — drains and sluices. No MDROs were detected from post-cleaning samples of dry surfaces or patient rooms. This is not surprising as dry surfaces in rooms may be easier to clean and disinfect as part of routine hospital measures for terminal disinfection, compared to wet surfaces. Moreover, wet surfaces, bathrooms and drains may be an under-recognized source of patient MDRO transmission, despite what may otherwise be assumed to be high standards of disinfection.

The reported CPE transmission rate to patients from a contaminated water environment is in the range of 1.6–26.7% [4]. Aerosolization of MDROs in the water supply or direct contact with MDRO-contaminated faucets, drains, or aerates are the most likely routes of transmission [2,4]. Over the period of 2006–2012, before the commencement of this study, it was estimated that 1.5% of patients in the burns unit became colonized with *bla*IMP-4^{+} CPE which were concurrently isolated in the burns unit environment [3]. Significant clinical infection with *bla*IMP-4^{+} CPE occurred in 30% of those colonized patients [7]. Although *bla*IMP-4^{+} CPE continues to be isolated from wet surface environmental samples, following introduction of more systematic disinfection with a specific focus on regular physical cleaning of bathroom drains, patient colonization and infection has become a rare occurrence.

We have demonstrated that disruption of wet surface biofilms results in increased detection of MDROs. In our study, *bla*IMP-4^{+} CPE were detected more frequently on post-cleaning environmental sampling. Wet surface biofilms from sinks, sluices, and drains may be a frequent source of MDRO persistence in the environment, leading to secondary patient colonization and infection. Our data support recent studies and recommendations that highlight the role of water-related sources in CPE transmission and emphasize their elimination in the setting of localized outbreaks due to ESBL and CPE [4]. Pre-disinfection microbial environmental surveillance is likely to underestimate the level of contamination with selected MDROs, including CPEs.

Conflict of interest statement
None declared.

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References

[1] Vickery K, Deva A, Jacombs A, Allan J, Valente P, Gosbell IB. Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. J Hosp Infect 2012;80:52—5.
[2] Decker BK, Palmore TN. The role of water in healthcare-associated infections. Curr Opin Infect Dis 2013;26:345—51.
[3] Betteridge T, Merlino J, Natoli J, Cheong EY, Gottlieb T, Stokes HW. Plasmids and bacterial strains mediating multidrug-resistant hospital-acquired infections are coresidents of the hospital environment. Microb Drug Resist 2013;19:104—9.
[4] Kizny Gordon AE, Mathers AJ, Cheong EYL, Gottlieb T, Kotay S, Walker AS, 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;64:1435—44.
[5] Hu H, Johani K, Gosbell IB, Jacombs AS, Almatroudi A, Whiteley GS, et al. Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: combined results of conventional culture, pyrosequencing, scanning electron microscopy, and confocal laser microscopy. J Hosp Infect 2015;91:35—44.
[6] Otter JA, Vickery K, Walker JT, deLancey Pulcini E, Stoodley P, Goldenberg SD, et al. Surface-attached cells, biofilms and biocide susceptibility: implications for hospital cleaning and disinfection. J Hosp Infect 2015;89:16—27.
[7] Leung GH, Gray TJ, Cheong EY, Haertsch P, Gottlieb T. Persistence of related bla-IMP-4 metallo-beta-lactamase producing Enterobacteriaceae from clinical and environmental specimens within a burns unit in Australia — a six-year retrospective study. Antimicrob Resist Infect Control 2013;2:35.
[8] Gavalda L, Pequeno S, Soriano A, Dominguez MA. Environmental contamination by multidrug-resistant microorganisms after daily cleaning. Am J Infect Control 2015;43:776—8.
[9] Stewart M, Bogusz A, Hunter J, Devanny I, Yip B, Reid D, et al. Evaluating use of neutral electrolyzed water for cleaning near-patient surfaces. Infect Control Hosp Epidemiol 2014;35:1505—10.
[10] Ledwoch K, Dancer SJ, Otter JA, Kerr K, Roposte D, Maillard JY. Beware biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; a multicentre study. J Hosp Infect 2018;100(3):e47—56.
[11] Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. Trends Microbiol 2005;13:34—40.
[12] Lesho E, Carling P, Hosford E, Ong A, Snesrud E, Sparks M, et al. Relationships among cleaning, environmental DNA, and healthcare-associated infections in a new evidence-based design hospital. Infect Control Hosp Epidemiol 2015;36:1130—8.