

Introduction

Gram negative bacteria are increasingly associated with several avoidable Healthcare Associated Infections. These include Catheter Associated Urinary Tract Infection (CAUTI), Hospital Acquired Pneumonia (HAP) and peripheral or central venous catheter (CVC or PVC) related bloodstream infections. These infections particularly affect patients requiring numerous invasive healthcare interventions such as in a critical care environment. Such areas often have high levels of antibiotic consumption adding selection pressure for multidrug resistant (MDR), extremely drug resistant (XDR) and Carbapenem Resistant Gram negative bacteria to proliferate, seed into the immediate environment and then be transmitted to equipment, staff or patients leading potentially to further spread.

Contemporary strategies being explored to prevent transmission of highly drug resistant Carbapenem Resistant Organisms (CRO) include the use of hydrogen peroxide (H₂O₂) in liquid and vapour forms. H₂O₂ remains effective even at low concentrations, and acts as a biocide through strong oxidising properties, eventually breaking down into harmless by-products, making it ideal for use in an active healthcare environment. The utilisation of H₂O₂ in its vapour phase ensures complete access of the biocidal agent to exposed surfaces providing greater coverage⁽¹⁾. This is of particular importance given the prevalence of multi-species biofilms throughout the hospital environment and the limitations of conventional cleaning in reaching all such areas. Effective decontamination though could be hampered in closed spaces where vapour penetration is reduced, such as in clinical wash hand basin drains and other narrow openings.

Here we present a case study charting the recovery of viable bacteria from two hospital rooms after discharge of a carrier of several CRO (*Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Escherichia coli*).

Methodology

Sampling Procedure

Adapted and modified from Blazejewski *et al.*, 2015. Three time points were selected; the first after standard twice daily cleaning with chlorine releasing agent, the second post terminal cleaning and the third after H₂O₂ fogging (HPV, Bioquell®). For the second and third time point, an equivalent area 5cm² directly adjacent to the original swabbing location was swabbed to prevent sampling the same area multiple times.

Room description and furniture

Ten individual sampling locations were identified through discussion with Infection Prevention and Control staff and through assessment of common touch points throughout the patient rooms. Two rooms were made available for the study following patient discharge.

Room 1 is a critical care single patient room of approximately 60m³ containing a patient bed, monitoring unit, commode, adjustable table and hand wash basin. There is an anteroom containing a hand wash basin at the room entrance which was not sampled in this study.

Room 2 is a single patient room in a general ward which measures approximately 50m³ with an en-suite bathroom (20m³). The patient room contains a patient bed, monitoring unit, adjustable table and hand wash basin. The en-suite bathroom contains a toilet (including disabled handrails) and hand wash basin. Only the basin in the main patient room was sampled in this study. The patient room is entered via an anteroom of approximately 10m³ containing a hand wash basin which was not sampled in this study.

Room Decontamination Methodologies

Standard cleaning of an isolation room hosting a CRO affected patient: twice daily domestic cleaning with chlorine releasing agent (ChlorClean® 1:1000ppm), ensuring frequent touch surfaces are cleaned effectively whilst the patient is resident in the room.

Terminal clean of an isolation room hosting a CRO affected patient: on transfer or discharge, the room is cleaned with chlorine releasing agent (ChlorClean® 1:1000ppm), including curtain change once the patient has left the room and it is empty.

H₂O₂ fogging: terminal clean was followed by decontamination with H₂O₂ vapour (HPV, Bioquell®) using standardised protocols before the room was occupied by a new patient.

Sample Processing

Swabs were inoculated directly into transport medium and kept at room temperature for transport. The swabs were used to inoculate Columbia Blood Agar (CBA) plates and then placed directly into Brain Heart Infusion (BHI) broth. Broth enrichment cultures were grown overnight at 37°C. Those cultures that exhibit growth in BHI were plated in dilutions on CBA and frozen down for storage.

All enrichment cultures that showed evidence of growth were diluted by a factor of 10⁻⁶ and plated onto CBA for a further overnight incubation, those that did not exhibit growth in BHI were not plated out on CBA. Plates were assessed for viable counts of the different strains recoverable from each location.

Table 1 Gram-negative organism and their susceptibilities placing the test room in risk of CRO contamination
Carbapenemase enzymes present: *K. pneumoniae* - OXA-48; *A. baumannii* - OXA-23; *E. coli* - KPC;
Using EUCAST breakpoints for interpretation of susceptibilities.
Susceptibilities carried out by AMRHAJ Reference Unit, Public Health England, Colindale

Antibiotic	MIC (mg/L)	S/I/R	MIC (mg/L)	S/I/R	MIC (mg/L)	S/I/R
	<i>K. pneumoniae</i>		<i>A. baumannii</i>		<i>E. coli</i>	
Amikacin	16	I	>64	R	8	I
Gentamicin	>32	R	>32	R	<=1	S
Tobramycin	>32	R	>32	R	>=16	R
Amoxicillin/Clavulanate	>64	R	-	-	-	-
Ampicillin	>64	R	-	-	-	-
Aztreonam	>64	R	-	-	>=64	R
Cefepime	>64	R	-	-	16	R
Cefotaxime	>256	R	-	-	>=64	R
Cefoxitin	>64	R	-	-	16	R
Ceftazidime	8	R	256	R	>=64	R
Ertapenem	>16	R	-	-	4	R
Imipenem	8	I	32	R	>=16	R
Meropenem	16	R	>32	R	>=16	R
Piperacillin/Tazobactam	>64	R	>64	R	>=128	R
Temocillin	>128	R	-	-	16	I
Ceftolozane/tazobactam	>16	R	-	-	-	-
Ceftazidime/Avibactam	0.5	S	-	-	-	-
Colistin	<=0.5	S	<=0.5	S	<=0.5	S
Ciprofloxacin	>8	R	>8	R	>=4	R
Tigecycline	1	S	-	-	<=0.5	S
Minocycline	-	-	8	I	-	S

Results and Discussion

Routine room cleaning with a chlorine releasing agent represents the standard level of cleanliness expected during the day-to-day cleaning of the hospital isolation rooms while occupied by patients carrying CRO (time point 1). Viable bacteria may be recovered from sites such as the mattress, bedrails, commode seat, door handle and clinical wash hand basin drain. These are classed as "high-frequency" touch-points, and correspondingly harbour a higher level of bacterial load. The interior of the clinical wash hand basin drain is characterised as a "difficult to clean" area and as such it is a site also mostly likely to harbour microorganisms.

Time point 2 represents the level of cleanliness expected after patient discharge and a terminal clean with a chlorine releasing agent including curtain change, to minimise as far as possible any risk of persistence of transmissible organisms remaining in the environment before a new patient is placed in the room.

Table 2 demonstrates the need for effective terminal cleaning of rooms that have been inhabited by patients who are carriers of CRO. Gram negative organisms were recoverable from a variety of surface sites in room 2 prior to its terminal clean. Unfortunately sampling of Room 1 was not possible prior to its conventional terminal clean.

Environmental sampling after conventional terminal cleaning of both rooms demonstrated that the frequently touched surfaces had been effectively decontaminated and were now free of bacterial contamination. However in both rooms, the clinical wash hand basin drain sampling continued to culture colonies of *Escherichia coli* and *Klebsiella pneumoniae* after disinfection with a chlorine releasing agent. A further round of decontamination using vaporised H₂O₂ vapour was unable to further reduce the bacterial burden at this site in both rooms. This suggests that either penetration of the vapour into the drain was insufficient or unable to influence eradication of bacterial growth potentially in biofilm formation⁽³⁾.

The persistence of Enterobacteriaceae in clinical wash hand basin drains potentially could function as a reservoir of CRO. Splashing of water from this site could then potentially allow transmission of CRO back into the room environment and potentially to a new resident of the room as has been observed with *Pseudomonas aeruginosa* transmission events⁽⁴⁾.

Table 2: Recovery Rates
†1000 remaining colonies of Gram negative bacteria were identified as *E. coli* and *K. pneumoniae* which were meropenem susceptible.
GNO: Gram negative organism Cfu: colony forming unit HPV: H₂O₂ vapour

Description	Post-standard twice daily clean (GNO cfu 10 ⁻⁶)	Post HPV (cfu)	Description	Pre-terminal clean (GNO cfu 10 ⁻⁶)	Post-terminal clean, pre-HPV (cfu)	Post HPV (cfu)
Mattress	30	0	Mattress	217	0	0
Patient bedrail	106	0	Patient bedrail	0	0	0
Patient monitor buttons	0	0	Patient monitor buttons	20	0	0
Commode seat	19	0	Toilet seat	331	0	0
Commode handle	0	0	Toilet handrail	254	0	0
Patient table – underside	0	0	Patient table – underside	1000 (neat)	0	0
Door handle (inner)	<10	0	Door handle (inner)	48	0	0
Clinical wash hand basin tap handles	0	0	Clinical wash hand basin tap handles	341	0	0
Clinical wash hand basin drain	38	1000 (neat)	Clinical wash hand basin drain	1000†	1000†	1000†
Keyboard and number pad	0	0	Nurse call button	86	0	0

Conclusion

We observe that clinical wash hand basin drains are reservoirs of viable Enterobacteriaceae which could potentially lead to recontamination of the healthcare environment with resistant Enterobacteriaceae even after H₂O₂ vapour decontamination.

References

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