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Comparison of Nylon Flocked Swab and Cellulose Sponge Method for Carbapenem-resistant *Enterobacteriaceae* and Gram-negative Organism Recovery from High Touch Surfaces in Patient Rooms

Clare Rock, MD, MS[1,2,3], **Michael Anderson, BS**[1], **Shawna Lewis, BA**[1], **Verna Scheeler, MLA**[1], **Elaine Nowakowski**[3], **Yea-Jen Hsu, PhD, MHA**[4], **Aaron M. Milstone, MD, MHS**[1,2,3], **Karen C Carroll, MD**[1], **Lisa L Maragakis, MD, MPH**[1,2,3], **Patricia J Simner, PhD, D(ABMM)**[1], and **CDC Prevention Epicenters Program**

[(1)]Johns Hopkins University School of Medicine, Baltimore, MD, United States

[(2)]Johns Hopkins Medicine Armstrong Institute for Patient Safety and Quality, Baltimore, MD, United States

[(3)]Department of Hospital Epidemiology and Infection Control, The Johns Hopkins Hospital, Baltimore, MD, United States

[(4)]Health Policy and Management, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Abstract

The ideal sampling method and benefit of qualitative versus quantitative culture, for Carbapenemase-resistant *Enterobacteriaceae* recovery in hospitalized patients' rooms/bathrooms is unknown. Although nylon-flocked swab improved overall Gram-negative organism recovery compared with cellulose sponge, both were similar for Carbapenemase-resistant *Enterobacteriaceae*. Quantitative culture was inferior and unrevealing beyond the qualitative results.

Background:

Carbapenem-resistant *Enterobacteriaceae* (CRE) are important healthcare-associated pathogens with high mortality rates.[1,2] CRE recovery from the patient environment may be informative for evaluation of efficiency of cleaning and disinfection in routine and outbreak setting and infection prevention research studies[3]. Although the rayon-tipped swab has better sensitivity than cellulose sponge (CS) methods for detection of *Acinetobacter* in the near patient environment, the ideal sampling method for CRE detection from high touch surfaces (HTS) in the patient room is unknown.[4] We compared two sampling methods (nylon-flocked swab [NFS] and CS) and two culturing methods (qualitative and quantitative) for detection

Corresponding Author: Clare Rock, MD, MS, Johns Hopkins University School of Medicine, Division of Infectious Diseases, 600 North Wolfe Street, Halsted 831, Baltimore, MD 21287-5425, Clare.Rock@jhmi.edu, T: 410-955-8384, F: 410-614-0888.

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of CRE, non-CRE carbapenem-resistant organisms and all other Gram-negative organisms in rooms where the occupant harbored CRE.

Methods:

We prospectively identified patients at the Johns Hopkins Hospital, a 1,145 patient bed tertiary academic center in Baltimore, Maryland, with recent (past 3 days) clinical or surveillance culture(s) growing CRE who had occupied the same hospital room for the most recent 2 days. High Touch Surfaces (HTS) from patient room were sampled. One half of each HTS was sampled using NFS (eSwab™; Copan Diagnostics, Murrieta, CA) dipped in neutralizer buffer (Hardy Diagnostics; Santa Maria, CA) using a previously published method.[4,5] An individual NFS was used per half HTS. The other half of the HTS was sampled using CS with neutralizer (3M; Maplewood, MN), where up to 5 HTS were sampled with the specific sides of the CS (e.g. a composite).[4,6] Due to right hand dominance, bacteria may have been more likely to be removed from that side of the HTS during cleaning. To avoid introducing a systematic bias, alternating sides of the HTS were sampled by each method.

Qualitative (PBS with tween broths were held for up to 3 days and subcultured if turbid) and quantitative cultures (including positive and negative controls) were performed following CDC protocols using MacConkey agar for selection of Gram-negative organisms, incubated overnight at 35°C.[5,6] Identification and antimicrobial susceptibility testing of recovered organisms were performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics Inc.) and via the BD Phoenix™ Automated Microbiology System, respectively.[4] *Enterobacteriaceae* resistant to at least ertapenem were identified as CRE. If a CS was culture positive, then all HTS of the composite were deemed positive. Limit of Detection was determined by preparing a 0.5 McFarland standard, plating 100 μl aliquots of 10-fold dilution series of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* ATCC BAA-1705 onto sterile Formica slabs and sampled by CS and NFS in a similar manner to HTS sampling.

The frequency of Gram-negative organism recovery for each method was compared to a gold standard, defined as recovery using either NFS or CS method. Each HTS was categorized according to frequency of Gram-negative organism recovery by either method: 0–10%, 10–20%, and >20%. Descriptive statistical analysis was conducted using SAS 9.4.

This study was acknowledged by the Johns Hopkins University institutional review board as non-human subjects research.

Results:

There were 229 HTS sampled in 17 unique patient rooms from May to December 2016. Eight of 17 (47%) patient rooms had Gram-negative bacteria detected from at least 1 HTS by either method; 2/17 (12%) had CRE recovered, 2/17 (12%) had a non-CRE carbapenem-resistant organism recovered; 7/17 (41%) had other or additional Gram-negative organisms (Table 1). For the two rooms where CRE was detected, one was by NFS and one by CS (see Table 1, Patient 3, Patient 5). Due to low overall recovery of CRE and non-CRE

carbapenem-resistant organisms, we grouped these with all other Gram-negative organism recovery to define the gold standard. The sensitivity for detection of any Gram-negative organisms in the environment was 100% for NFS and 21% for CS. Seven of the eight (88%) positive rooms were identified using qualitative culture, and four of eight (50%) by quantitative culture. The Limit of Detection for NFS and CS methods was ~2×10^7 CFU/ml. Figure 1 shows frequency of Gram-negative organism recovery from HTS in patient room and bathroom.

Discussion

We infrequently recovered CRE in the room and bathroom of in-patients known to be infected or colonized with CRE. Although we were unable to ascertain if NFS method or CS method was superior at CRE recovery from environmental surfaces we did find some practical advantages of NFS method. With NFS the specific positive HTS is known, rather than the CS composite where detected organisms could have been recovered from up to 5 HTS. In addition, NFS method requires less microbiologist time and expertise, and less specialized equipment (stomacher or large centrifuge) than CS, making is less costly. Although, it took less time to process a single NFS (~ 8 mins) compared to the sponge (~ 35 mins), the additional NFS's per patient room (up to 23) were more time intensive than the CS approach (up to 5 CS per room) due to the higher number of samples collected. We found qualitative cultures had a higher sensitivity for Gram-negative organism recovery than quantitative. NFS are likely readily available in many healthcare facilities where they are used for patient multidrug-resistant organism surveillance programs, making it a feasible option when sampling the environment in a CRE outbreak situation, or in research studies when assessing cleaning practices. Some studies have favored different sampling methods over NFS for CRE recovery. An Israeli study compared recovery of *Klebsiella pneumoniae* carbapenemase -Carbapenemase-producing *Enterobacteriaceae* in the hospital setting using contact plates and NFS with either direct plating to *Klebsiella pneumoniae* carbapenemase selective agar or broth enrichment, and found enhanced recovery with contact plates (contact plate 32% vs NFS with direct plating 24% vs 16% NFS with broth enrichment).[7] However, these studies did not use neutralizer prior to sampling with NFS. Use of NFS with neutralizer rather than phosphate-buffered saline has been found to be superior at recovery of *Staphylococcus aureus,* and neutralizer with NFS in this study may have helped with bacteria recovery.[8] Another potential strength of our study design was accounting for the important confounding variable of right hand dominance during cleaning whereby we alternated which half of HTS was sampled by each method.

We found the environment of patients known to harbor CRE frequently contaminated with other Gram-negative organisms. We are not aware of any other studies looking at all Gram-negative organism recovery, however MDR Gram-negative organism recovery can range from 1.8 to 30% of surfaces.[9,10] Shams et al found 34% of high touch surfaces contaminated with MDROs post-daily cleaning, although mostly with Gram-positive organisms.[6]

Our study has some limitations. The Limit of Detection for CRE was ~2×10^7 CFU/mL, therefore it is possible that HTS with lower Gram-negative organism burden may have given negative results by sampling methods. Although we sampled a small number of patient

rooms, this study supports the use of NFS when recovering Gram-negative organisms in the patient environment. The NFS method is more feasible, due to decreased cost, increased availability, and less lab expertise necessary, and may be advantageous during outbreak investigations as the specific contaminated HTS is identified.

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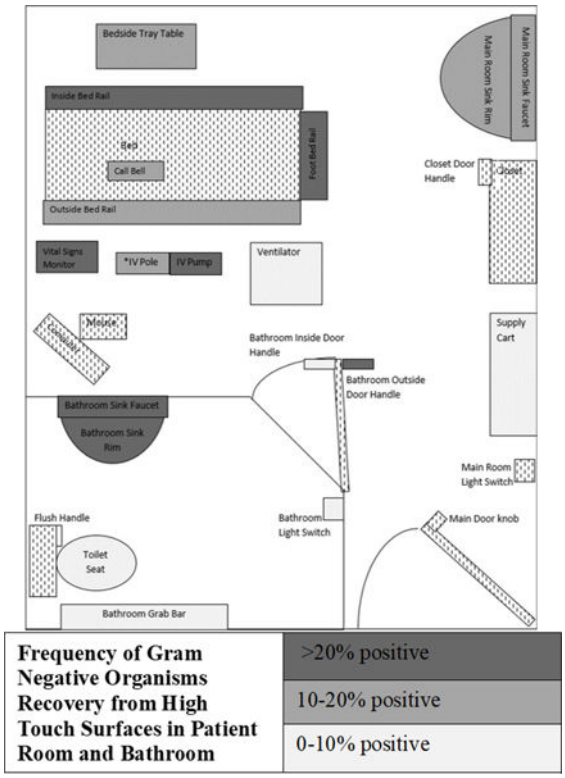

Figure 1.

Frequency of Gram-Negative Organism Recovery from High Touch Surfaces in Patient Room and Bathroom

Table 1:

Patient Carbapenemase-resistant *Enterobacteriacea* (CRE) culture and Environmental Culture Results for Nylon flocked swab and Cellulose Sponge Methods

Patient[a]	**Patient CRE Culture**		**# days patient had occupied room on day of sampling**	**Environmental Culture Results**			
	Source	**Organism**		**Nylon flocked swab: Qualitative**	**Nylon flocked swab: Quantitative**	**Cellulose Sponge: Qualitative**	**Cellulose Sponge: Quantitative**
1	Urine	New Delhi metallo-β lactamse-producing *Escherichia coli*	37				
2	Sputum	*Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*	80				
3[a]	Sputum	KPC-producing *K. pneumoniae*	66	IV pump: KPC-producing *K. pneumoniae* Bathroom sink: *Pseudomonas putida, Acinetobacter* species			
4	Blood	KPC-producing *K. pneumoniae*	28	Bathroom light switch, side bed rail, foot bed rail, over bed table & toilet seat: *Stenotrophomonas maltophilia* Bathroom sink: *Pseudomonas aeruginosa* & *Enterobacter cloacae*	Grab bar, vital sign monitor, bathroom sink, bathroom light switch, bathroom door knob, bedroom inside knob, side bed rail, foot bed rail, over bed table IV pump/pole: 1.6 - >3 × 10^2 CFU/mL *S. maltophilia*	Composite 4 -Room and bathroom sink and faucet: *E. cloacae*	Composite 1 - Bed rails/ vital signs monitor/ call bell >9× 10^3 CFU/Sponge *S. maltophilia*
5[b]	Sputum	Non-Carbapenemase producing-CRE (Non-CP-CRE) *E. cloacae*	50			Composite 1 - Bed rails/vital signs monitor/ call bell & Composite 4 - Room and bathroom sink and faucet: *E. cloacae*	
6	Sputum	KPC-producing *Citrobacter freundii* complex & KPC-producing *Citrobacter amalonaticus*	31				Composite 2 – Bedside table/ IV pole/ IV pump/ bathroom outside door handle 1.5 CFU/sponge

Patient[a]	Patient CRE Culture		# days patient had occupied room on day of sampling	Environmental Culture Results			
	Source	Organism		Nylon flocked swab: Qualitative	Nylon flocked swab: Quantitative	Cellulose Sponge: Qualitative	Cellulose Sponge: Quantitative
							Enterobacter aerogenes
7[c]	Blood	KPC-producing *K. pneumoniae*	18	Patient room sink: Non-Carbapenemase resistant *K. pneumoniae* Bathroom sink: *P. aeruginosa*	Patient room sink: 70 CFU/mL *P. aeruginosa,* 10 CFU/mL *P. putida,* 64 CFU/mL *Klebsiella oxytoca* Bathroom sink: 75 CFU/mL *P. aeruginosa*, 2.5×10^2 CFU/mL *Pseudomonas fluorescence*		
8	Rectal Swab	New Delhi metallo-β lactamse-producing *K. pneumoniae*	5	Patient bathroom sink: *P. aeruginosa*	Patient bathroom sink: 29 CFU/mL *P. aeruginosa*, 8 CFU/mL *C. freundii*		
9	Blood	Non-CP-CRE *E. cloacae* complex	7				
10	Sputum	Non-CP-CRE *E. cloacae* complex	17				
11	Urine	Non-CP-CRE *E. cloacae* complex	8	Patient bathroom sink: *S. maltophilia*			
12	Urine	KPC-producing *K. pneumoniae*	10				
13	Rectal Swab	Non-CP-CRE *E. aerogenes*	21				
14	Blood	OXA-48-like and New Delhi metallo-β lactamse -producing *K. pneumoniae*	30				
15	Tissue	Non-CP-CRE *K. pneumoniae*	22				
16	Tissue	KPC-producing *C. freundii* complex	94	Bedside inside rail/grip, beside outside rail/grip, vital sign monitor and bedside tray: *E. coli*			
17	Urine	Non-CP-CRE *E. cloacae*	14				

[a] Patient 3: The clinical and environmental KPC-producing *K. pneumoniae* were identical strains confirmed by pulsed-field gel electrophoresis.

[b] Patient 5: The clinical carbapenem resistant *E. cloacae* and environmental carbapenem susceptible *E. cloacae* were identical strains confirmed by pulsed-field gel electrophoresis.

[c] Patient 7: The clinical and environmental *K. pneumoniae* were different strains confirmed by pulsed-field gel electrophoresis.