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Short Communication

Evaluation of Environmental Sampling Methods for Detection of *Staphylococcus aureus* on Fomites

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Abstract

We evaluated a variety of methods to recover *S. aureus* from inanimate surfaces. Two contact agar plates and three swab sampling methods were tested on porous and non-porous surfaces and bar soap. The cost and ease of use of each method was also evaluated. *S. aureus* was recovered using all methods on both porous and non-porous surfaces. *S. aureus* could not be detected on three of four brands of soap.

ABBREVIATIONS

MRSA: methicillin-resistant *Staphylococcus aureus.*

INTRODUCTION

Staphylococcus aureus is an important and versatile pathogen with the ability to colonize individuals and cause superficial and invasive infection. *S. aureus* can survive on environmental surfaces for prolonged periods of time and can be transferred to skin by fomites [1,2]. Thus, environmental surfaces are potential reservoirs for *S. aureus* transmission [3-5]. Bar soap that has been in contact with human skin has been demonstrated to harbor microorganisms [6]. A paucity of data exists regarding optimal sampling techniques to recover *S. aureus* from environmental surfaces. A recent review of studies evaluating environmental *S. aureus* contamination found a lack of consistency in sampling methods as well as limited information regarding specific techniques utilized in these investigations [7]. Additionally, the typical bioburden of *S. aureus* surface contamination has not been well described [8] and no "gold standard" method for environmental sampling exists. Thus, we performed a qualitative assessment of five sampling methods to detect serial dilutions of *S. aureus* applied to multiple surface types. Our primary objective was to determine effective and efficient methods to recover *S. aureus* from porous and non-porous surfaces in addition to multiple brands of bar soap while also considering the practicality of use and cost of sampling. Secondarily, we were interested in evaluating the ability of *S. aureus* to persist on bars of soap. The results from this investigation can inform future epidemiologic studies of environmental reservoirs of *S. aureus*.

MATERIALS AND METHODS

Three surface types were tested: a laboratory countertop (Trespa Toplab, New York, NY) representing a non-porous surface; cotton washcloths representing a porous, textured surface; and four common brands of bar soap (a moisturizing bar, an antibacterial soap, and two deodorant soaps) placed in sterilized plastic boxes to mimic soap in dishes. Five sampling methods were tested: the Baird Parker Agar contact plate (Hardy, Santa Maria, CA), the RODAC (replicate organism detection and counting) trypticase soy agar (TSA) + lecithin and polysorbate 80 contact plate (Becton Dickinson [BD], Franklin Lakes, NJ), the Eswab (BD) with and without enrichment in trypticase soy broth (TSB) with 6.5% NaCl (BBL, BD), and the Enviroswab (3M, St. Paul, MN).

Suspensions of a strain of USA300 methicillin-resistant *S. aureus* (MRSA) recovered from a human buttock abscess at St. Louis Children's Hospital (St. Louis, Missouri, USA) were prepared to a density of 0.5 McFarland Standard in normal saline. From this, six ten-fold dilutions were prepared to create ultimate colony counts ranging from 0 to 10^5 colony forming units (CFU)/mL. Dilutions were verified by plating directly to TSA with sheep blood (blood agar plates, BAPs; BBL, BD) and performing colony counts after overnight incubation. Before inoculation, the countertop surface was decontaminated with ethanol and

rinsed with sterile water, and the washcloths and soap dishes were autoclaved. Soap bars were new (i.e., unused) and placed into the dish in a manner which did not introduce contamination. A unique area of bench top, washcloth, or soap bar was used for each dilution and each sampling method. Each surface was cultured initially to ensure the absence of *S. aureus* at baseline.

After an initial pilot evaluation of different volumes for *S. aureus* inoculation of surfaces, dilutions were delivered to surfaces in 15 mL volumes, as this amount allowed uniform delivery of inocula to each surface. Immediately following preparation, ten-fold dilutions (from 0 to 10^5 CFU/mL) were applied evenly to a 6 x 12 inch (15.2 x 30.5 cm) area of laboratory countertop and 6 x 12 inch washcloths and allowed to dry overnight. After 24 hours, contact plates were stamped for five-second intervals over each surface in six non-overlapping locations. Swabs were swiped back and forth across the entire surface in two perpendicular directions. All soap bars were of approximately equal size. Dilutions (0, 10^3-10^5 CFU/mL) were applied to each bar of soap and allowed to incubate at room air overnight. Contact plates were uniformly stamped twice each on the top (dry side) of the soap bars in the location that the suspensions were applied, and again on the bottom (wet side) of each soap bar. Swabs were swiped back and forth across the entire top and bottom of each bar. The soap dishes were then sampled with a separate set of contact plates and swabs.

Contact plates were incubated overnight at 35°C in ambient air. Growth on contact plates was sub cultured to BAPs. For Eswabs, 100 µL of eluate was inoculated to each of a BAP and TSB with 6.5% NaCl and incubated overnight. Following incubation, broth cultures were plated to BAPs and incubated overnight. Enviroswabs were inoculated directly onto BAPs which were subsequently incubated overnight. Beta-hemolytic colonies characteristic of our parent strain recovered on BAPs were confirmed as *S. aureus* with catalase and Staphaurex (Remel, Lenexa, KS) tests. The limit of detection (LOD) was defined as the lowest dilution of *S. aureus* (CFU/mL) applied to each surface that could be detected by each method. Three independent replicates of each experiment were performed. The ultimate goal of this investigation was to determine qualitatively whether *S. aureus* could be detected from the surface sampled by each method.

RESULTS

From the non-porous surface, the limit of detection for four of five methods (i.e., all methods with the exception of the Enviroswab) was an inoculum of 10^2 CFU/mL (Table 1). From the porous surface, the RODAC contact plate and Eswab with broth enrichment were able to detect an inoculum of 10 CFU/mL. *S. aureus* was not detected at any inoculum using any of the sampling techniques from the antibacterial or deodorant soaps (or their corresponding "soap dishes"). *S. aureus* was detected on the moisturizing bar (and its corresponding "soap dish") using four of five methods (i.e., all methods with the exception of the Eswab without broth enrichment), at an inoculum of 10^3-10^5 CFU/mL, dependent upon method used (Table 1). The reproducibility of *S. aureus* detection over three replicates of the experiment is reported in (Table 1).

In addition to recovery of *S. aureus* if present, other important factors including cost, ease of use, and minimum days to obtain final results were also considered (Table 2).

Contact plates: The list price of the Hardy contact plate, which is supplied with a locking lid, is $3.17 USD. The list price of the RODAC contact plate is $3.80 USD and is supplied with a non-locking lid, which was more difficult to transport and could contribute to contamination (although a locking lid is available for an additional fee). Contact plates require a second day of processing (subculture to BAP and overnight incubation) prior to *S. aureus* verification.

Swabs: The 3M Enviroswab is $1.80 USD; the BD Eswab is $0.81 USD, and the additional step of broth enrichment results in a cost of $1.94 USD as well as an additional day of incubation/processing. Notably, direct plating of the Enviroswab to BAPs resulted in gouging and deterioration of the agar, which could compromise results. Eswabs (when plated directly to BAP) require only 1 day for processing and *S. aureus* verification.

DISCUSSION

The existing literature is inconsistent and incomplete regarding the optimal method to detect *S. aureus* on environmental surfaces [9-13]. We employed a systematic approach to evaluate the recovery of different concentrations of MRSA from common environmental surface types using a variety of sampling techniques, including contact plates and swabs, with and without broth enrichment. Similar to other studies, while variation in *S. aureus* detection was noted using different sampling methods, all methods, studied qualitatively, recovered *S. aureus* from both porous and non-porous surfaces [9]. While several studies have demonstrated superiority of contact plates for recovery of microorganisms from environmental surfaces, the contour of the surface to be cultured is an important consideration; contact plates are limited to flat surfaces while swabs are able to sample uneven surfaces and larger surface areas [9-11]. A study by Claro and colleagues investigated Petrifilm (3M) for environmental sampling, which provided the benefit of contact methods and could adapt to the contour of surfaces [12].

Bar soap has been epidemiologically associated with MRSA transmission. In a study by Nguyen and colleagues, football players with MRSA skin or soft tissue infection (SSTI) were 15 times more likely to have shared bar soap with teammates than players without recent SSTI [14]. Thus patients with recurrent infections are often discouraged from using and sharing bar soaps. However, in a laboratory setting, this risk has not been demonstrated. In a study by Desai and colleagues, MRSA could be transferred to skin from all tested fomites (e.g. toys, towels, razors), with the exception of soap bars [1]. Bannan and colleagues determined that, though bacteria (*Serratia marcescens*) could be transmitted from skin to bar soap, the bacteria was not transmitted to subsequent users of the same soap bar [15]. Similarly, in our qualitative study, recovery of *S. aureus* from bars of soap was limited compared to other surfaces. Even with broth enrichment (which potentially dilutes soap deposited on the sampling device which could enhance organism recovery), *S. aureus* was detected on only one brand tested, a moisturizing bar, using multiple sampling methods that successfully recovered the same dilutions of *S. aureus* from other non-soap surfaces. As

Table 1: Detection of *S. aureus* dilutions applied to environmental surfaces by sampling method.

	Sampling Method																													
	Hardy Baird Parker Agar Contact Plate						BD RODAC Contact Plate						3M Enviroswab						BD Eswab						BD Eswab with Broth Enrichment					
Surface Type	Applied dilution (CFU/mL)																													
	10^5	10^4	10^3	10^2	10	0	10^5	10^4	10^3	10^2	10	0	10^5	10^4	10^3	10^2	10	0	10^5	10^4	10^3	10^2	10	0	10^5	10^4	10^3	10^2	10	0
Non-porous	**D3**	**D3**	**D3**	**D2**	ND	ND	**D3**	**D3**	**D3**	**D3**	ND	ND	**D3**	**D2**	**D3**	ND	ND	ND	**D2**	**D1**	**D1**	**D1**	ND	ND	**D1**	**D1**	**D1**	**D2**	ND	ND
Porous	**D3**	**D2**	**D2**	ND	ND	ND	**D3**	**D1**	ND	ND	**D1**	ND	**D3**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**D3**	**D1**	**D1**	**D2**	**D1**	ND
Soap	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moisturizing bar	**D2**	ND	ND	-	-	ND	**D2**	**D1**	ND	-	-	ND	ND	**D1**	**D1**	-	-	ND	ND	ND	ND	-	-	ND	**D1**	**D1**	**D1**	-	-	ND
Antibacterial soap	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND
Deodorant soap A	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND
Deodorant soap B	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND
Soap dish	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Moisturizing bar	**D3**	ND	ND	-	-	ND	ND	**D2**	ND	-	-	ND	**D1**	**D1**	**D1**	-	-	ND	ND	ND	ND	-	-	ND	ND	**D1**	**D1**	-	-	ND
Antibacterial soap	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND
Deodorant soap A	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND
Deodorant soap B	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND	ND	ND	ND	-	-	ND

Note: D1 = *S. aureus* detected in 1 of 3 replicates; D2 = *S. aureus* detected in 2 of 3 replicates; D3 = *S. aureus* detected in all 3 replicates; ND = *S. aureus* not detected in any of the replicates; = Dilution not performed for soap surfaces; 15 mL of each dilution were applied to each surface.

Table 2: Cost, ease of use, and minimum days to results for each *S. aureus* environmental sampling method.

Factor	Sampling Method				
	Hardy Baird Parker Agar Contact Plate	BD RODAC Contact Plate	3M Enviroswab	BD Eswab	BD Eswab with Broth Enrichment
Cost[a]	$3.17	$3.80	$1.80	$0.81	$1.94
Ease of use[b]	+++	+	+	+++	++
Days to results	2	2	1	1	2

[a] List price for each product from manufacturer/distributor (USD)
[b] Ease of use scale: + = most difficult to use, +++ = easiest to use

the potential for bar soap to harbor and transmit MRSA appears limited, clinicians may reconsider advising against the use of bar soap until additional epidemiologic studies of MRSA transmission via bar soap are performed.

While our study has several unique strengths, and represents one of the first efforts to directly compare multiple sampling methods for *S. aureus* simultaneously, this study is not without its limitations. First, the *S. aureus* contamination burden and recovery could be altered in community or hospital settings due to the presence of organic material, other microorganisms, cleaning methods, or disinfectant residues at sampling sites on hospital or community surfaces and thus our *in vitro* study may not accurately recapitulate all these variables [6,12,15]. In addition, while we only tested one MRSA strain type, we selected a strain representative of a contemporary MRSA epidemic clone that is common in both community and hospital settings.

CONCLUSION

We compared the performance of five sampling methods to detect MRSA in the environment. We determined that both contact plates and swabs provided adequate *S. aureus* recovery from porous and non-porous environmental surfaces, while MRSA was infrequently recovered from bar soap. Environmental sampling protocols for large epidemiologic studies must balance cost (which may vary by institutional contracts), time to results, ease of use, and the contour of the surface to be sampled. The importance of each of these metrics may vary depending on the objective of a particular investigation.

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REFERENCES

1. Desai R, Pannaraj PS, Agopian J, Sugar CA, Liu GY, Miller LG. Survival and transmission of community-associated methicillin-resistant *Staphylococcus aureus* from fomites. Am J Infect Control. 2011; 39: 219-25.
2. Oller AR, Mitchell A. *Staphylococcus aureus* recovery from cotton towels. J Infect Dev Ctries. 2009; 3: 224-228.
3. Uhlemann AC, Knox J, Miller M, Hafer C, Vasquez G, Ryan M, et al. The environment as an unrecognized reservoir for community-associated methicillin resistant *Staphylococcus aureus* USA300: a case-control study. PLoS One. 2011; 6: e22407.
4. Eells SJ, David MZ, Taylor A, Ortiz N, Kumar N, Sieth J, et al. Persistent environmental contamination with USA300 methicillin-resistant *Staphylococcus aureus* and other pathogenic strain types in households with *S. aureus* skin infections. Infect Control Hosp Epidemiol. 2014; 35: 1373-1382.
5. Miller LG, Eells SJ, Taylor AR, David MZ, Ortiz N, Zychowski D, et al. *Staphylococcus aureus* colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. Clin Infect Dis. 2012; 54: 1523-1535.
6. McBride ME. Microbial flora of in-use soap products. Appl Environ Microbiol. 1984; 48: 338-341.
7. Davis MF, Iverson SA, Baron P, Vasse A, Silbergeld EK, Lautenbach E, et al. Household transmission of meticillin-resistant *Staphylococcus aureus* and other staphylococci. Lancet Infect Dis. 2012; 12: 703-16.
8. Weese JS. Environmental surveillance for MRSA. Methods Mol Biol. 2007; 391: 201-208.
9. Dolan A, Bartlett M, McEntee B, Creamer E, Humphreys H. Evaluation of different methods to recover meticillin-resistant *Staphylococcus aureus* from hospital environmental surfaces. J Hosp Infect. 2011; 79: 227-230.
10. Lemmen SW, Häfner H, Zolldann D, Amedick G, Lütticken R. Comparison of two sampling methods for the detection of gram-positive and gram-negative bacteria in the environment: moistened swabs versus Rodac plates. Int J Hyg Environ Health. 2001; 203: 245-248.
11. Obee P, Griffith CJ, Cooper RA, Bennion NE. An evaluation of different methods for the recovery of meticillin-resistant *Staphylococcus aureus* from environmental surfaces. J Hosp Infect. 2007; 65: 35-41.
12. Claro T, Galvin S, Cahill O, Fitzgerald-Hughes D, Daniels S, Humphreys H. What is the best method? Recovery of methicillin-resistant *Staphylococcus aureus* and extended-spectrum ß-lactamase-producing Escherichia coli from inanimate hospital surfaces. Infect Control Hosp Epidemiol. 2014; 35: 869-871.
13. Davis MF, Baron P, Price LB, Williams DL, Jeyaseelan S, Hambleton IR, et al. Dry collection and culture methods for recovery of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* strains from indoor home environments. Appl Environ Microbiol. 2012; 78: 2474-2476.
14. Nguyen DM, Mascola L, Brancoft E. Recurring methicillin-resistant *Staphylococcus aureus* infections in a football team. Emerg Infect Dis. 2005; 11: 526-532.
15. Bannan EA, Judge LF. Bacteriological Studies Relating to Handwashing. 1. The Inability of Soap Bars to Transmit Bacteria. Am J Public Health Nations Health. 1965; 55: 915-922.

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