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Dry Collection and Culture Methods for Recovery of Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus* Strains from Indoor Home Environments

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***Staphylococcus aureus* in home environments may serve as a reservoir for human colonization, making sampling of indoor surfaces relevant to exposure assessment. Using laboratory experiments and application to homes of asthmatic children in Barbados, we characterize microbiological methods adapted for settings with transportation delays between sampling and initiation of culture.**

Staphylococcus aureus is an environmentally hardy pathogen (3, 10) found widely in hospital and community environments (15). Home environmental contamination with methicillin-resistant *S. aureus* (MRSA) has been implicated as a source for recurrent human colonization (1, 7), making evaluation of environmental reservoirs relevant for exposure assessment linked to clinical outcomes.

Most methods for environmental sampling of *S. aureus* use wet-cloth or medium-based methods adapted from health care settings for use in home environments (1, 11, 14). However, these methods often require special handling for transportation, such as refrigeration, and prompt progression from sample collection to initiation of culture in the laboratory. The ability to use wet or medium-based methods may be constrained where access to laboratory resources is limited.

In this paper, we present a novel strategy for parallel culture enrichment of MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates from dry environmental dust wipe samples. We provide laboratory quantification of method limit of detection (LOD) and describe strains in terms of survival characteristics relevant for sample transport. We adapted this protocol from published sampling and culture methods for *S. aureus* (2, 4, 8, 15) and applied it to indoor surface sampling in the homes of children with asthma in Barbados, West Indies, during a pilot study involving long lag times between sample collection and culture.

For sampling, we used autoclaved dry electrostatic cloths (Swiffer; Proctor & Gamble) placed in sterile stomacher bags for transport. For culture, 60 to 120 ml Mueller-Hinton broth containing 6.5% NaCl (MHB+) was added initially to the bags. For MRSA only, after a standard 16 to 20 h of incubation at 35°C, 1 ml of MHB+ was inoculated into 9 ml tryptic soy broth with 2.5% NaCl, 3.5 mg/liter cefoxitin, and 10 mg/liter aztreonam (TSB+). MHB+ overnight growths (for MSSA) or TSB+ overnight growths (for MRSA) were plated onto tryptic soy with 5% blood agar. After incubation, distinct colonies based on phenotype were subcultured to Baird Parker agar (2) for 24 to 48 h of incubation. Colonies phenotypic for *S. aureus* were selected for further testing.

To estimate an LOD, sterilized cloths were spiked with 1-ml inoculations (10^8 to 10^{-1} CFU/ml) of ATCC 43300 (MRSA), ATCC 25923 (MSSA), and a field ST80 strain (MRSA) collected from a Barbadian home. To determine strain survival on sterilized electrostatic cloths over time, replicates of 1-ml inoculations (10^8 to 10^{-1} CFU/ml) were performed, and spiked cloths were cultured at 3 days and then weekly for 4 weeks. The supplement provides a detailed description of the culture methods and laboratory experiments.

During 2010, 26 asthmatic children were enrolled from a hospital outpatient population in a pilot study investigating household environmental exposures in Barbados. In January (visit one), microbiological samples were collected to assist method develop-

TABLE 1 MSSA and MRSA isolates recovered from household environmental surfaces by visit, PCR and MLST results, and location within the household

House identifier	Visit[a]	PCR result	MLST type	Location within house
A	1	MSSA (*femA*)	ST8	Top of wardrobe (bedroom)
	2	MSSA (*femA*)	ST6	Child's pillow (bedroom)
H[b]	1	MSSA (*femA*)	ST72	Living room shelf
B	2	MSSA (*femA*)	ST72	Child's pillow (bedroom)
F	2	MSSA (*femA*)	ND[c]	Child's pillow (bedroom)
C	2	MSSA (*femA*)	ST5	Child's pillow (bedroom)
G	2	MRSA (*femA mecA*)	ST5	Child's pillow (bedroom)
I	2	MRSA (*femA mecA*)	ST39	Child's pillow (bedroom)
D	2	MRSA (*femA mecA*)	ST80	Top of refrigerator
E	1	MRSA (*femA mecA*)	ST80	Top of refrigerator
	2	MRSA (*femA mecA*)	ST80	Top of refrigerator
	2	MRSA (*femA mecA*)	ST80	Child's pillow (bedroom)

[a] No pillows were sampled at visit 1.
[b] Household H was not sampled at visit 2.
[c] MLST sequence type was not determined.

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TABLE 2 Associations between household and bedroom factors and odds of *S. aureus* recovery from household surfaces and pillows at the second visit

Factor	Value		OR[a] for *S. aureus*	95% CI	*P* value
	S. aureus ($n = 8$)	No *S. aureus* ($n = 11$)			
Household					
Mean no. of rooms	4.4	4.8	0.73	0.32–1.65	0.45
No. (%) of households with conditions that were[c]:					
Above avg	3 (37.5)	3 (27)	2.00	0.24–16.61	0.52
Avg (reference)	3 (37.5)	6 (55)			
Below avg	2 (25)	2 (18)	2.00	0.18–22.06	0.57
No. (%) of homes with:					
Any mold or leaks	5 (63)	7 (64)	0.95	0.14–6.28	0.96
No mold or leaks (reference)	3 (37)	4 (36)			
Mean temp (°C)	28.4	26.7[b]	1.22	0.87–1.71	0.25
Mean relative humidity (%)	63.4	63.5[b]	0.86	0.30–2.51	0.79
No. (%) of homes with:					
Any rodent evidence	3 (38)	4 (36)	1.05	0.16–6.92	0.96
No rodent evidence (reference)	5 (62)	7 (64)			
Any roach evidence	6 (75)	8 (73)	1.13	0.14–8.99	0.91
No roach evidence (reference)	2 (25)	3 (27)			
Bedroom					
No. (%) of homes with:					
Two or more children in one bedroom	4 (50)	4 (36)	1.75	0.27–11.15	0.55
Single child room (reference)	4 (50)	7 (64)			
No. (%) of bedrooms that were[c]:					
Highly cluttered	2 (25)	2 (18)	1.50	0.16–13.75	0.72
Less cluttered (reference)	6 (75)	9 (82)			
No. (%) of bedrooms with:					
Any mold or leaks	4 (50)	1 (9)	10.0	0.84–119.3	0.07
No mold or leaks (reference)	4 (50)	10 (91)			

[a] Odds ratios were obtained from logistic regression modeling.
[b] $n = 10$; one home missing temperature and relative humidity data.
[c] Determinations of household conditions were subjective.

ment and sampling strategy. Nineteen children remained in the study for a second visit in July 2010 (visit two), which was used to test the adapted culture method.

Field staff wearing nonsterile nitrile gloves sampled the asthmatic child's pillow and a single ~30-cm^2 dusty surface in a common room, typically the top of the refrigerator or entertainment unit. Temperature and relative humidity were determined using a HOBO data logger (Onset; Cape Cod, MA). Field staff conducted interviews and home inspections for household environmental characteristics. Cloths were cultured as described, and suspect *S. aureus* colonies were confirmed by real-time PCR for *femA* (MSSA) and *mecA* (MRSA) genes (Pathogene, LLC). *S. aureus* isolates were typed by multilocus sequence typing (MLST) as described previously (5). All appropriate institutional review boards in Barbados and at Johns Hopkins University approved the Barbados pilot study in which this project was nested.

For the laboratory experiments, the average LOD for recovery of *S. aureus* at time zero was 2.3 CFU/ml (95% confidence interval [CI], 0.04 to 160). This field ST80 strain had a lower average LOD (0.3 CFU/ml) than either of the reference *S. aureus* strains, which had average LODs of 17.8 CFU/ml each ($P = 0.01$). No LOD attrition was noted for strains between days 0 and 3. On average, 1.7 fewer log CFU (95% CI, 1.5 to 2.0) were detectable per week of the study, controlling for correlation within strains over time ($P < 0.001$). The supplemental material provides detailed findings.

At the first field visit, 3/51 (6%) samples from 26 homes were *S. aureus* positive. From the MRSA protocol, one MRSA isolate and one MSSA isolate were cultured. From a subset of 16 samples cultured using the parallel MSSA protocol, one MSSA was isolated. Table 1 provides strain type by household location for isolates from both visits.

At the second visit, MRSA was cultured from 2/19 (11%) common room surface samples (repository site) and from 3/19 (16%) pillow samples (site touched by the child). MSSA was cultured from 4/19 (21%) pillow samples. The child's pillow was contaminated with *S. aureus* at 7 of 8 positive homes (88%). MRSA ST80 was cultured from the top of the refrigerator in the same household 6 months apart. Lag time between collection and culture ranged from 2 to 24 days and was not significantly associated with *S. aureus* recovery. Generally, *S. aureus* recovery was not strongly associated with household characteristics (Table 2), except for bedrooms with mold/leaks. When analysis was restricted to *S. aureus* recovery in bedrooms, this estimate of association strengthened (odds ratio [OR], 14.7; 95% CI, 1.16 to 185.2; $P = 0.04$). No households reported indoor pets, corroborated by visual inspection. Five households reported outdoor dogs, and *S. aureus* was not recovered from the indoor environments of these homes.

Barbadian strains were consistent with pandemic clones circulating in Caribbean (13), Latin American (9), and global human populations (12). Finding such strains in home environments on Barbados may indicate that pandemic clones have established circulation patterns on the island.

This study is the first to report on culturing MRSA and MSSA from the home environments of children with asthma. For standard transport within 3 days without refrigeration, this method detected as few as 1 to 100 CFU/cloth. LOD attrition was noted with longer delays. Our research suggests that, even with delays of several weeks, if the initial cloth sample collects at least 10^8 viable CFU, *S. aureus* detection is likely given no inhibition or microbial competition. This study is limited by small sample size and scope, and these results may not be generalizable to households in other settings. Considering the potentially intermittent nature of human colonization (6), future studies should explore indoor household environmental sampling to serve as a measure of short-term chronic exposure for human household members in the context of clinical outcomes, including asthma.

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