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Comparison of Culture-Based Methods for Identification of Colonization with Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* in the Context of Cocolonization

Meghan F. Davis,[a] Baofeng Hu,[b] Karen C. Carroll,[c] Warren B. Bilker,[d] Pam Tolomeo,[e] Valerie C. Cluzet,[f] Patrick Baron,[a] Jacqueline M. Ferguson,[a] Daniel O. Morris,[g] Shelley C. Rankin,[h] Ebbing Lautenbach,[f] Irving Nachamkin,[b] for the CDC Prevention Epicenters Network

Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA[a]; Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA[b]; Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA[c]; Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA[d]; Center for Clinical Epidemiology and Biostatistics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA[e]; Division of Infectious Diseases, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA[f]; Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA[g]; Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA[h]

Two screening methods to detect staphylococcal colonization in humans were compared. Direct plating to CHROMagar (BD Diagnostics) was compared to a broth preenrichment followed by plating to Baird-Parker agar. The broth-enrichment method was comparable to CHROMagar for methicillin-resistant *Staphylococcus aureas* (MRSA) detection, but the enrichment method was optimum for recovery of coagulase-positive *Staphylococcus* spp.

Patients with community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and their household companions often develop recurrent episodes of MRSA infection. Cyclical reinfection within households, potentially driven by environmental or animal reservoirs, contributes to the burden of MRSA infection (1). Culture of specific organisms from the environment typically requires enrichment or other selection methods to address contamination with nontarget bacteria (2, 3). Methods that allow culture of *Staphylococcus* spp. other than *S. aureus* are critical to assess cocolonization outcomes and the presence of animal-associated staphylococci, given the potential role for these microbiota to modulate colonization by pathogens (4). Currently, the culture-based screening methods to identify *S. aureus* from human samples are generally different from methods for environmental or animal samples. Use of the same culture method for all types of samples (human, environmental, animal) would enhance the comparability of results. However, methods designed for environmental and animal specimens may not be optimal for use on human samples. The aim of this study was to compare a method to screen for MRSA and methicillin-susceptible *S. aureus* (MSSA) using CHROMagar media (5) with a broth-enrichment method, used on the same specimens, that was optimized to detect methicillin-susceptible and methicillin-resistant staphylococci from environmental and animal specimens (2, 6).

(Portions of this work were presented at the Consortium of Universities for Global Health conference [7] and the 2015 ASM-ESCMID Conference on Methicillin-Resistant Staphylococci in Animals.)

Index participants with MRSA skin or soft tissue infection (SSTI) and their household members were recruited as part of a three-arm nonblinded, randomized, controlled trial (NCT00966446), i.e., the Commonwealth Universal Research Enhancement (CURE) trial (8). This trial evaluated the effect of two similar householdwide decolonization protocols using nasal mupirocin ointment and chlorhexidine body wash versus education control on human MRSA colonization. A subset of these households participated in a nested evaluation of home environments and companion animals, i.e., the Pets and Environmental Transmission of Staphylococci (PETS) study (6, 9). Two home visits were conducted at a 3-month interval; randomization and treatment occurred between these visits. People sampled themselves using Copan ESwabs (Copan Diagnostics, Murrieta, CA) at (i) both nares and (ii) axillae and groin creases (pooled, referred to as the skin site). Index patients submitted a third ESwab from the site of the original MRSA SSTI lesion. Self-swabbing has been validated for use in this context (10).

Figure 1 illustrates the protocols and timing of inoculation for the two methods. For the CHROMagar method, swabs transported in Copan Amies medium were cultured onto BBL CHROMagar MRSA and CHROMagar Staph aureus (BD Diagnostics, Sparks, MD) per manufacturer's guidelines (5). Isolates

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Address correspondence to Meghan F. Davis, mdavis65@jhu.edu, or Irving Nachamkin, nachamki@upenn.edu.

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FIG 1 Protocols for the two culture-based methods (CHROMagar and broth enrichment) compared in this analysis. Additional incubation time (24 to 48 h) before reading of the inoculated agar media plates (CHROMagar or Baird-Parker) is not depicted but was comparable between the methods. For the broth-enrichment method, multiple colonies were selected from the blood agar plates based on distinct colony morphology, and these were subcultivated as needed before pure colony growth was inoculated onto Baird-Parker agar; this may result in an additional day or (rarely) days to complete the protocol.

presumptively identified as MRSA based on colony morphology (mauve colonies) were confirmed as MRSA by *mecA* PCR. For the broth-enrichment method, to isolate *S. pseudintermedius* and other animal-associated staphylococci from the same human swabs, residual medium (typically at least 100 μl) from the Copan swabs was then subjected to broth-enrichment culture as previously described (2), modified to use Columbia CNA blood agar. This protocol was designed for environmental samples and included parallel arms for nonselective and methicillin-resistance-selective culture. Isolates that demonstrated tellurite reduction and lecithinase activity on Baird-Parker agar were presumptively identified as coagulase-positive staphylococci (CPS) (11). Personnel performing the CHROMagar and broth-enrichment protocols were blinded to results from the other protocol. For additional details, see the supplemental material.

All index participants who submitted swabs for the joint

TABLE 1 Prevalence of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* colonization and correlation of results according to the CURE CHROMagar and PETS broth-enrichment study protocols

Study group	*S. aureus* prevalence (no. positive [%])			Test sensitivity (%)		Test agreement		Sample size
	Joint[a]	CHROMagar	Broth enrichment	CHROMagar	Broth enrichment	Kappa (95% confidence interval)[b]	Kappa interpretation[c] (agreement)	
MRSA								
Enrollment visit								
Subset[d,e]	37 (40.7)	27 (29.7)	29 (31.9)	73.0	78.4	0.54 [0.35–0.72]***	Moderate	91
Index only	32 (40.5)	26 (32.9)	24 (30.4)	81.3	75	0.59 [0.40–0.78]***	Moderate	79
3-mo visit								
Subset[d,e]	12 (48.0)	12 (48.0)	10 (40.0)	100	83.3	0.84 [0.63–1.00]***	Almost perfect	25
Index only	29 (53.7)	22 (40.7)	26 (48.2)	75.9	89.7	0.63 [0.42–0.83]***	Substantial	54
MSSA								
Enrollment visit								
Subset[d,e]	20 (22.0)	8 (8.8)	15 (16.5)	40	75	0.17 [−0.08–0.42]*	Slight	91
Index only	12 (15.2)	7 (8.9)	8 (10.1)	58.3	66.7	0.34 [0.00–0.67]**	Fair	79
3-mo visit								
Subset[d,e]	1 (4.0)	1 (4.0)	1 (4.0)	100	100	1.00 [1.00–1.00]***	Almost perfect	25
Index only	8 (14.8)	3 (5.6)	8 (14.8)	37.5	100	0.50 [0.15–0.86]***	Moderate	54

[a] Joint prevalence combines test results from the CHROMagar and broth-enrichment protocols and is positive if either protocol yielded the target organism (gold standard), and the sensitivity is calculated by dividing the number positive for the individual tests by the number positive according to the gold standard (17).
[b] *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.
[c] According to criteria by Landis and Koch (14).
[d] Subset was all household members from the first 25 homes enrolled, of which 14 continued in the study at the 3-month visit.
[e] No lesion swabs were included in the subset analysis; the subset includes index participants in order to mimic whole-household testing.

CURE/PETS study were selected for complete identification of all species of CPS isolated from the broth-enrichment culture protocol. A subset of their household members (25 households), identified *a priori*, were also selected for evaluation (6). All presumptive CPS isolates identified from these swabs were subjected to species identification by PCR using *nuc* (*S. aureus*, *S. pseudintermedius*, *S. schleiferi*) and *mecA/C* genes (12, 13). Any nasal CPS isolates that were not identified by PCR were identified biochemically to species using the BD Phoenix system (BD Diagnostics). Results of the CHROMagar-based and the broth-enrichment culture methods were compared using the kappa statistic (14) and chi-squared analysis in Stata 13 (College Station, TX).

The University of Pennsylvania and Johns Hopkins University Institutional Review Boards and Institutional Animal Care and Use Committees approved this study. Participants provided written informed consent.

Swabs were obtained from 79 (90%) of the 88 index participants evaluated in the broth-enrichment study and from an additional 68 household members in the subset of 25 households, for a total of 147 participants. Of these, 54 (68% of 79) index participants and 16 household members remained in the study and were evaluated at the subsequent 3-month visit. At enrollment, 9 households failed to provide swabs concurrently with the home visit and were excluded from this analysis. Dropout following enrollment was due to noncompliance with the protocol ($n = 18$), censoring for incomplete data ($n = 10$), or participant withdrawal ($n = 5$); these participants are included only for enrollment data.

Table 1 compares MRSA and MSSA results obtained by the CHROMagar method with the results obtained by the broth-enrichment method, demonstrating almost perfect strength of agreement for the kappa statistic for MRSA (14) but weaker agreement for MSSA results. Results were similar for the aggregated person-level analysis and the swab-level analysis considering nares, skin, and lesion sites separately (swab-level data not shown). The weaker agreement for MSSA results was driven by more frequent MSSA detection from nasal swabs using the broth-enrichment protocol. Broth enrichment has been shown to enhance test sensitivity for *S. aureus* (15, 16). Combined prevalence (designating a person as positive for MRSA or MSSA if either protocol yielded a confirmed isolate) was higher than prevalence for either study alone. Although these tests were completed sequentially, they were performed independently (i.e., simultaneous testing); hence, this increase in net sensitivity over individual test sensitivity was expected (17).

To demonstrate the utility of the data on *Staphylococcus* spp. from the broth-enrichment protocol, we next assessed cocolonization between MRSA and MSSA, methicillin-susceptible *Staphylococcus pseudintermedius* (MSSP), and other presumptive CPS. Table 2 provides these findings, demonstrating that only one participant colonized nasally with MRSA was also colonized nasally with *Staphylococcus epidermidis* and *Staphylococcus lugdunensis*. Identification of MRSA and other staphylococci together occurred more frequently with skin swabs; however, these swab samples were pooled from axillae and groin creases. Thus, cocolonization at the same skin site could not be determined, which is a limitation of this study. Table 3 lists the identified *Staphylococcus* spp.; *S. lugdunensis* and *S. epidermidis* were the most common. Many of these isolates, testing positive for lec-

TABLE 2 Methicillin-resistant *Staphylococcus aureus* cocolonization among index participants and household members from 25 PETS study homes and all PETS study index participants from whom swabs were obtained

Group, sample location, and isolate[a]	Cocolonization (*n* [%]) in people: Colonized with MRSA	Not colonized with MRSA	Chi-squared *P* value[d]
All participants from subset of 25 households			
Enrollment ($n = 91$; 181 sites, nares and skin only)			
Nares only	19 (21)	72 (79)	
MSSA	0	13	0.04
MSSP	0	4	0.29
Other CPS	0	15	0.03
Nares or skin	37 (20)	144 (80)	
MSSA	3	15	0.68
MSSP	0	4	0.30
Other CPS	0	33	0.001
3mo visit ($n = 25$; 50 sites, nares and skin only)			
Nares only	7 (28)	18 (72)	
MSSA	0	1	0.52
MSSP	0	0	NE
Other CPS	0	6	0.08
Nares or skin[b]	11 (22)	39 (78)	
MSSA	0	1	0.59
MSSP	0	0	NE
Other CPS	1	17	0.04
All index participants			
Enrollment ($n = 79$; 236 sites, nares, skin, lesion site)			
Nares only	10 (13)	69 (87)	
MSSA	0	7	0.29
MSSP	0	2	0.58
Other CPS	0	9	0.23
Nares, skin or lesion[b]	41 (17)	195 (83)	
MSSA	1	10	0.45
MSSP	0	3	0.42
Other CPS	1	33	0.02
3-mo visit ($n = 54$; 162 sites, nares, skin, lesion site)			
Nares only	16 (30)	38 (70)	
MSSA	0	6	0.09
MSSP	0	0	NE
Other CPS	1[c]	8	0.18
Nares, skin or lesion[b]	40 (25)	122 (75)	
MSSA	1	9	0.27
MSSP	0	0	NE
Other CPS	6	36	0.07

[a] MSSA, methicillin-susceptible *S. aureus*; MSSP, methicillin-susceptible *S. pseudintermedius*; CPS, coagulase-positive *Staphylococcus*.
[b] Anatomical-site-level analysis.
[c] *S. epidermidis* and *S. lugdunensis* cocolonization.
[d] NE, not estimable.

TABLE 3 *Staphylococcus* spp., identified as lecithinase positive on Baird-Parker agar, other than *S. aureus* that were cultured from the nares of 147 participants in the PETS study

Participants	Species (no. positive isolates) identified at:	
	Enrollment[a]	3-mo visit[b]
Index group[c]	*S. epidermidis* (2) *S. pseudintermedius* (2) *S. lugdunensis* (1) *S. kloosii* (1)[e] *S. haemolyticus* (1)[f] *S. warneri* (1) *S. chromogenes* (1) Unidentified (2)[j]	*S. lugdunensis* (4)[i] *S. epidermidis* (2)[i] *S. kloosii* (2)[e] *S. haemolyticus* (1)[f] *S. sciuri* (1)
Household members[d]	*S. epidermidis* (3) *S. lugdunensis* (3)[g] *S. haemolyticus* (2)[h] *S. pseudintermedius* (2)[g] *S. warneri* (2)[h] *S. equorum* (1) Unidentified (2)[j]	*S. lugdunensis* (2) *S. haemolyticus* (2)

[a] $n = 11$ (12%) for index group and 13 (19%) for household members.
[b] $n = 9$ (17%) for index group and 4 (25%) for household members.
[c] $n = 91$ at enrollment and 54 at 3 mo.
[d] $n = 68$ at enrollment and 16 at 3 mo. While all index participants in the PETS study were evaluated fully, only household members from the first 25 homes were included in this analysis.
[e] One index participant *S. kloosii* positive at both visits.
[f] One index participant *S. haemolyticus* positive at both visits.
[g] One household member cocolonized with *S. pseudintermedius* and *S. lugdunensis*.
[h] One household member cocolonized with *S. haemolyticus* and *S. warneri*.
[i] One index participant cocolonized with *S. epidermidis*, *S. lugdunensis*, and MRSA.
[j] Unidentified isolates were cultured and were confirmed not to be *S. aureus*, *S. pseudintermedius*, or *S. schleiferi* using PCR but were not further identified to the species level using the BD Phoenix instrument, due to isolate loss.

ithinase on Baird-Parker agar, were identified as coagulase-negative *Staphylococcus* (CNS) species instead of CPS. This finding was unexpected and may indicate poor correlation between lecithinase and coagulase activity in this context or may indicate coagulase and/or lecithinase positivity by another mechanism in the cultured strains (see further discussion in the supplemental material).

This study demonstrates similar recovery of MRSA from a CHROMagar method and a broth-enrichment method optimized for the culture of staphylococci from environmental samples. Despite being more resource and time intensive, the broth-enrichment method provided optimum recovery of all coagulase-positive staphylococci, including MSSA. Given its benefits, researchers should consider the use of the broth-enrichment method to reduce bias in comparisons among human, animal, and environmental samples.

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