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BRIEF REPORT

Reliability of *mecA* in Predicting Phenotypic Susceptibilities of Coagulase-Negative Staphylococci and *Staphylococcus aureus*

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The *mecA* gene is commonly used to identify resistance in *Staphylococcus aureus*, but historically is not used for coagulase-negative staphylococci (CoNS). Analysis of 412 staphylococcal blood cultures (2014–2018) revealed that the absence of *mecA* had high concordance (100%) with oxacillin susceptibility for *S. aureus* and CoNS alike.

Keywords. *mecA*; genotypic susceptibility; phenotypic susceptibility; *Staphylococcus*.

Historically *mecA*, the gene that encodes penicillin-binding protein 2a (PBP2a), has been used to predict methicillin resistance in *Staphylococcus aureus*, leading to early clinical treatment decisions before phenotypic testing returns [1, 2]. This *mecA* gene is also harbored by many coagulase-negative staphylococci (CoNS), and with the advent of rapid blood culture identification platforms (BCIDs) that include testing for the *mecA* gene, laboratories now have the possibility of reporting *mecA* for CoNS species, thereby potentially aiding in early therapeutic decision-making [3].

Concordance between genotype and phenotype in relation to *mecA* has not been examined in CoNS species. *mecA* encodes PBP2a, which, unlike other PBPs, is not bound by beta-lactam antibiotics (with the exception of ceftaroline) and thus leads to both methicillin-resistant *S. aureus* (MRSA) and oxacillin-resistant CoNS. PBPs (or more specifically transpeptidases) crosslink the peptidoglycan in the bacterial cell wall. The absence of *mecA* in *S. aureus* (MSSA) has long been used to exclude MRSA and allow the use of narrower, less toxic agents before phenotypic susceptibility is available. Per our hospital antibiogram, 71% of *S. aureus* and 53% of CoNS isolates are susceptible to oxacillin, and as we use the absence of *mecA* to predict this susceptibility in *S. aureus*, we wondered if the absence of *mecA* could also be used to predict oxacillin susceptibility in CoNS.

METHODS

Setting

Children's Hospital Colorado (CHCO) is a freestanding, quaternary care pediatric hospital in Aurora, Colorado, with 444 beds and ~15 000 admissions a year. As a level 1 trauma center, CHCO includes a neonatal intensive care unit, pediatric intensive care unit, cardiac intensive care unit, and a hematology and oncology unit. The hospital provides both liquid (eg, bone marrow) and solid (eg, heart/liver/kidney) transplants. The hospital has a robust antimicrobial stewardship program that partners with the microbiology laboratory onsite to analyze genotypic and phenotypic resistance patterns of *mecA* in staphylococci [1]. All patients at CHCO with a staphylococci-positive blood culture were included in this study. We did not examine or adjust for patient complexity.

Laboratory Methods

Blood specimens were processed as described in Messacar et al. [1]. Briefly, our laboratory uses standardized blood culturing methods (Plus Aerobic/F and PedsPlus/F [Becton Dickinson and Co., Sparks, MD, USA] bottles on a BacTec 9120/9240 automated system [Becton Dickinson and Co.] with positivity followed by a gram stain and organism identification by Biofire Film Array BCID version 1 [BioMérieux, BioFire Diagnostics, Salt Lake City, UT, USA]). This platform includes targets for the *mecA* gene that are resulted if a staphylococcal species is also detected. BCID is not run for every blood specimen collected; instead, it is run with the first positive blood, then subsequently only with positives displaying a different gram stain morphology or every subsequent 4 days. Inducible resistance for oxacillin is not routinely performed in our laboratory, nor is *mecA* testing done on CoNS isolates outside the BCID platform.

For susceptibility testing of staphylococcal isolates, our laboratory uses automated microdilution (Microscan Walkaway 96 Plus System, Beckman Coulter, Brea, CA, USA). The Microscan PC33 panel is used for all staphylococci and contains 24 antimicrobial agents. Clinical Laboratory Standards Institute (CLSI M100, 27th ed, Wayne, PA, USA) breakpoints are used by the Microscan LabPro Software system to determine susceptibility

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interpretations. *S. aureus* isolates with oxacillin minimum inhibitory concentrations (MICs) of ≤2 μg/mL are susceptible, and those with MICs ≥4 μg/mL are resistant. CoNS species with oxacillin MICs ≤0.25 μg/mL are susceptible, and those with MICs ≥0.5 μg/mL are resistant.

Data Methods

We extracted all positive blood culture results from our data warehouse for the period of June 2014 to December 2018. These data included specimen collection date and time, result date and time, blood culture source (central line, peripheral, unknown/other), results of BCID, results of *mecA* testing, ultimate species identification on traditional culture, and reported susceptibilities. Only isolates containing at least 1 staphylococcal species, with both phenotypic and BCID results available, were included in the analysis (n = 456). Cultures with an "unknown/other" source were excluded (n = 44). If the specimens were polymicrobial, those pathogens and susceptibilities were also recorded and confirmed through chart review in our electronic medical record, Epic (Epic Systems, Verona, WI, USA).

Genotypic and phenotypic results were compared for both *S. aureus* and CoNS, including any co-cultured pathogens. Pathogens were categorized according to their BCID result: *Staphylococcus aureus* (MRSA, MSSA) or CoNS. As cultures with both *S. aureus* and CoNS are called *S. aureus* by BCID, those polymicrobial cultures were categorized under *S. aureus*. Pathogens were further categorized by genotypic–phenotypic concordance. Results that were *mecA* positive/oxacillin resistant and *mecA* negative/oxacillin susceptible were considered concordant. Discordant results were divided into the following categories: *mecA* positive/oxacillin susceptible (unexplained discordance), *mecA* negative/oxacillin resistant (unexplained discordance), and mixed oxacillin susceptible/resistant, which was considered explained discordance as multiple staphylococcal pathogens were present with different susceptibility patterns that explained the genotypic results.

Patient Consent Statement

This research was reviewed and approved by the Colorado Multiple Institutional Review Board (submission #20-1549), and informed consent was waived. This study does not include factors necessitating patient consent.

RESULTS

Of the 412 specimens included in this study, 145 had a reported BCID result of *S. aureus* and 267 had a reported BCID result of CoNS. Of the 145 *S. aureus* specimens, 48 (33%) were drawn from a central line source and 97 (67%) were peripheral. The source locations for CoNS specimens were 184 (69%) from a central line and 83 (31%) peripheral. Genotypic–phenotypic resistance was concordant (ie, *mecA* positive/oxacillin resistant or *mecA* negative/oxacillin susceptible) in 93% (385 of 412) of the specimens.

Among the 145 *S. aureus* specimens, all monomicrobial results (138, 95%) were concordant (107 MSSA and 31 MRSA). When BCID results of *S. aureus* were polymicrobial, 3 (2.1%) were concordant, 2 (1.4%) were explained discordant (including 1 MSSA mixed with MRSA and 1 MSSA mixed with oxacillin-resistant CoNS), and 2 (1.4%) were unexplained discordant.

Among the 267 CoNS specimens, all monomicrobial results that were *mecA* negative (n = 61, 22.8%) were concordant with an oxacillin-susceptible phenotype. If *mecA* was positive in monomicrobial culture (n = 169), it concorded with oxacillin resistance in 92.9% (n = 157) of cultures; the other 7.1% (n = 12) were all unexplained discordance (*mecA* positive/oxacillin susceptible). For CoNS cultures mixed with other organisms (n = 37, 13.8%,), *mecA* negativity again concorded with oxacillin susceptibility (100%, n = 7). If instead the *mecA* was positive in polymicrobial culture (n = 30), it concorded with an oxacillin-resistant phenotype in 51.3% (n = 19) of cultures. Of the remaining 48.7% (n = 11) of *mecA*-postive polymicrobial cultures, 5 were explained by the susceptibilities of the other organisms (other CoNS), and 6 were not explained by the susceptibilities of co-pathogens.

Details of these results are displayed in Figure 1 and Table 1.

DISCUSSION

The genetic absence of gene-encoding methicillin resistance, *mecA*, in staphylococcal species is a reliable predictor of phenotypic oxacillin susceptibility in clinical isolates and may be used to narrow therapy. At our quaternary care pediatric facility, none of the 178 (43.2%) *mecA*-negative isolates were oxacillin resistant.

Instead, we describe that among the *mecA*-positive isolates (n = 234, 56.8%), 27 (11.5%) were *mecA* positive but phenotypically oxacillin susceptible. Of these 27 discordant specimens, 7 were considered "explained" as they had an oxacillin-resistant co-pathogen that explained the detection of *mecA* (2 *S. aureus* and 5 CoNS)—a well-described phenomenon [4–7]. The remaining 20 (18 CoNS and 2 *S. aureus*) were categorized as "unexplained discordance," meaning without a resistant co-pathogen. Of these, 12 were monomicrobial CoNS cultures. This phenomenon may be explained by inducible resistance or the presence of *mecC* [8] (which is detected by BCID and reported as mecA by the instrument [9]) and represents true resistance or by the presence of an "empty" cassette lacking a functional *mecA* gene [10, 11]. Another explanation could be mutations in genes known as factors essential for methicillin resistance (FEM) or auxiliary factors. These genes, which are scattered throughout the staphylococcal chromosome and are distinct from *mec*, are responsible for the full expression of methicillin resistance in staphylococci [12]. Transposon-mediated inactivation of these genes [13],

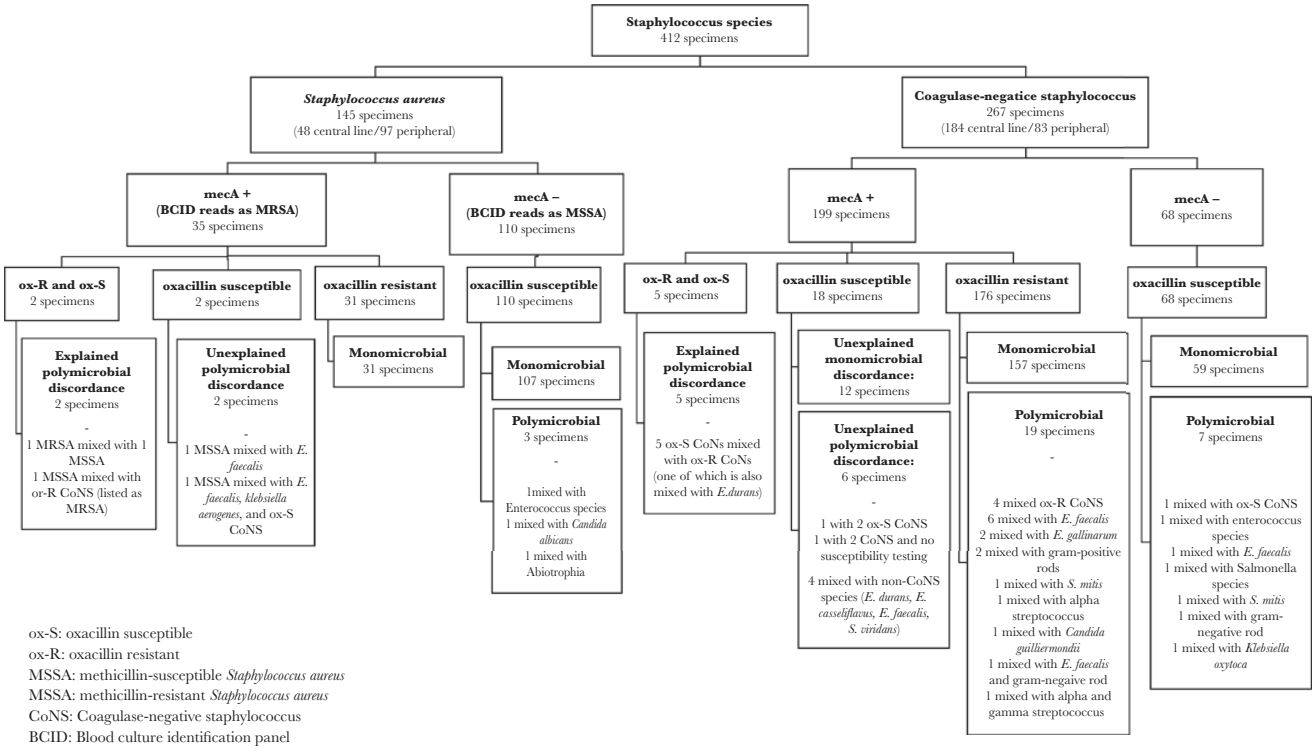

Figure 1. Genotypic and phenotypic susceptibilities for *Staphylococcus* species. Abbreviations: BCID, blood culture identification panel; CoNS, coagulase-negative *Staphylococcus*; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; ox-R, oxacillin resistant; ox-S, oxacillin susceptible.

most notably *femAB*, results in beta-lactam hypersusceptibility, while the production of PBP2a and other PBPs remains unaltered [14]. It is important to note that while a mutation to *femC* or *femD* reduces the basal resistance to methicillin, the remaining mutants are capable of forming highly resistant subclones [14]. Regrettably we do not have the isolates to examine these hypotheses. The clinical significance of treating a *mecA*-positive, phenotypically susceptible CoNS with an antistaphylococcal penicillin

Table 1. Concordance and Discordance in *Staphylococcus* Species

BCID Result	Culture Result	Total (number in polymicrobial culture)	Concordant		Discordant		
			mecA+ Oxacillin Resistant	*mecA*- Oxacillin Susceptible	*mecA*- Oxacillin Resistant	*mecA*+ Oxacillin Susceptible	*mecA*+ Mixed Susceptibilities
Coagulase-negative *Staphylococcus*		267 (37)	176 (19)	68 (7)	0	18 (6)	5 (5)
	Unspecified CoNS	6 (2)	2 (1)	1	0	3 (1)	0
	S. capitis	6	0	5	0	1	0
	S. hominis	50 (8)	30 (6)	17 (2)	0	3	0
	S. epidermidis	184 (15)	135 (8)	40 (4)	0	9 (3)	0
	S. haemolyticus	2	2	0	0	0	0
	S. simulans	3	1	2	0	0	0
	S. ureolyticus	3	1	2	0	0	0
	S. warneri	1	1	0	0	0	0
	Multiple CoNS species	12 (12)	4 (4)	1 (1)	0	2 (2)	5 (5)
Staphylococcus aureus		145 (7)	31	110 (3)	0	2 (2)	2 (2)
	Methicillin-susceptible	112 (5)	0	110 (3)	0	2 (2)	0
	Methicillin-resistant	32 (1)	31	0	0	0	1 (1)
	Multiple *S. aureus* pathogens	1 (1)	0	0	0	0	1 (1)

Abbreviations: BCID, blood culture identification panel; CoNS, coagulase-negative *Staphylococcus*.

or cephalosporin is not clear, and our data set is not sufficiently powered to address this question.

The remaining 8 "unexplained" discordant isolates (2 *S. aureus*, 6 CoNS) were from polymicrobial cultures without a co-pathogen traditionally known to harbor *mecA*. However, the presence of *mecA* is described in environmental isolates for various bacteria, including the prevalent co-pathogens in our study, namely *E. faecalis, E. durans, E. casseliflavus, S. viridans,* and *Klebsiella aerogenes* [15, 16]. For these 8 polymicrobial cultures, it may be possible that the source of *mecA* is one of these co-pathogens. It is unknown if the *mecA* gene is transcribed or translated in these environmental species.

A limitation to our study is that it is single-center; there may clinically exist other resistance mechanisms that confer oxacillin resistance despite the absence of *mecA* in other centers. While we feel that the results of this study are generally translatable to other pediatric and adult settings, some species of *Staphylococcus* are not well represented within this study, and local microbiology should be considered when interpreting these findings. Of particular mention is the absence of *Staphylococcus lugdunensis*. Additionally, we no longer have the isolates and therefore are unable to test isolates for the presence of *mecA* in co-pathogens or inducible resistance/empty cassettes in CoNS. If it is possible to investigate this discrepancy in the future with existing isolates, efforts will be made to explore the influence of bicarbonate on resistance expression [17, 18], as well as performing inducibility studies. Also, our center uses Biofire Film Array for detection of the *mecA* gene; other platforms may perform differently, and BCID2 (recently Food and Drug Administration–approved version of the Biofire test) may not report *mecA* for CONS. Our laboratory interprets results (eg, if targets for *S. aureus* and *mecA* are positive, this is reported as MRSA rather than reporting targets alone), and thus we have the opportunity to interpret the absence of *mecA* in CoNS for clinicians as oxacillin susceptible; other centers that do not interpret results may not benefit from this work, as the implications of *mecA* in CoNS are not common knowledge.

In conclusion, the absence of *mecA* is a reliable predictor of phenotypic susceptibility and may be used to narrow therapy for both *S. aureus* and CoNS. The presence of *mecA* generally corresponds with an oxacillin-resistant phenotype and was very reliable for *S. aureus* monomicrobial cultures, though the clinical significance remains unclear in polymicrobial cultures and CoNS monomicrobial cultures.

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