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COMMENTARY

mecC-Harboring Methicillin-Resistant *Staphylococcus aureus*: Hiding in Plain Sight

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ABSTRACT Previously there was scant data on the performance of laboratory testing to detect *mecC*-mediated beta-lactam resistance in *Staphylococcus aureus*. Kriegeskorte and colleagues (J Clin Microbiol 56:e00826-17, 2018, https://doi.org/10.1128/JCM.00826-17) report the performance of various clinical tests for the detection of *mecC*-harboring methicillin-resistant *S. aureus* (MRSA), which failed to identify from 0 to 41% of tested *mecC*-harboring MRSA isolates. Changes in practice and new test development are necessary to address the challenge of *mecC*-harboring MRSA.

The history of beta-lactam resistance in *Staphylococcus aureus* is long enough that microbiology and clinical practice are well adapted to anticipate it. After Alexander Fleming's accidental discovery of penicillin in 1928, penicillin resistance emerged rapidly in *S. aureus*. By 1944, William Kirby had purified the staphylococcal penicillinase and was using it as a reagent to increase the yield of diagnostic culture in patients treated with penicillin. Subsequent development of penicillinase-stable penicillins such as methicillin was rapidly countered, and by 1961 *S. aureus* strains were discovered that were methicillin resistant (so-called methicillin-resistant *Staphylococcus aureus* [MRSA], distinguished from methicillin-susceptible *S. aureus* [MSSA]) by virtue of acquiring the staphylococcal cassette chromosome *mec* (SCC*mec*), most variants of which carry a penicillin-binding protein, PBP 2a, that has a low affinity for all beta-lactam drugs other than those specifically designed to target it. New diagnostics such as PCR and antigen assays followed to detect PBP 2a or its parent gene, *mecA*, as well as traditional susceptibility testing to detect resistance to methicillin and proxy drugs such as oxacillin.

With the addition of beta-lactam drugs such as ceftaroline and ceftobiprole to directly target PBP 2a, a stalemate was reached that was broken by the arrival of *mecC*, which was described by two groups in 2011 (1, 2). The gene sequence of *mecC* is about 70% identical to that of *mecA*, and its gene product is a penicillin-binding protein with about 63% identity to PBP 2a at the amino acid level. This renders both *mecA* PCR primers and PBP 2a-specific antigen assays unable to detect *mecC*-harboring MRSA, which has been found in wastewater, domestic animals, pets, and several percent of human patients with MRSA infections in northern Europe, suggesting that *mecC*-harboring MRSA isolates have become ubiquitous.

A further diagnostic complication arises when considering the regulation of *mecC*. There are two common inducer/repressor systems regulating the *mec* operon in *Staphylococcus aureus*: *mecI-mecR1*, which is associated with the *mec* operon in the staphylococcal cassette chromosome (SCC), and *blaI-blaR1*, which are associated with the near-ubiquitous staphylococcal penicillinase and can cross-regulate the *mec* system if *mecI* and/or *mecR* is absent (reviewed in reference 3 [see Fig. 1 therein]). *mecR1* can sense cefoxitin but does not effectively sense oxacillin, making cefoxitin a good candidate for derepression of the MRSA phenotype. *mecR* is often nonfunctional, inviting cross-regulation by the *bla* system, which produces the common oxacillin-resistant, cefoxitin-resistant phenotype. "Oxacillin-susceptible" strains containing low-

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level but inducible PBP 2a therefore comprise about 1% of MRSA isolates in the United States (4). Given the prevalence of MRSA, this is a significant problem, but the majority of these isolates can be detected by cefoxitin screening, PCR for *mecA*, or a PBP 2a antigen assay, making this issue manageable by clinical laboratories with a variety of available tools. Unfortunately, all *mecC*-harboring MRSA isolates contain a type XI SCC with similar genetic organization (including an intact *mecR* and *mecI*) to "oxacillin-susceptible" *mecA*-harboring MRSA, suggesting the alarming possibility that a prevalent type of MRSA might be misclassified as MSSA by phenotypic susceptibility testing or chromogenic agars. SCC*mec* types, including the type XI SCC*mec*, can be reviewed at the website of the International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements (5). An early paper by Cartwright and colleagues (6) placed 89% of *mecC*-harboring MRSA isolates as "oxacillin susceptible" but suggested that induction by cefoxitin was a useful adjunct as it classified all isolates as resistant. This finding was later reinforced by Skov and colleagues (7).

THE LABORATORY VERSUS *mecC*

At present, few studies have been done to define the prevalence of *mecC*-harboring MRSA in most geographic regions, and diagnostic testing for "MRSA" is essentially synonymous with that for *mecA*-harboring MRSA. Development of *mecC*-harboring MRSA-specific diagnostic testing is sorely needed to define prevalence and to accurately diagnose disease. In the meantime, laboratories using a variety of PCR, chromogenic agar, and susceptibility testing approaches to MRSA diagnosis are left to wonder whether they are capable of diagnosing *mecC*-harboring MRSA infection.

The present study by Kriegeskorte and colleagues (8) attempts to fill this knowledge gap. Kriegeskorte et al. used a large number (111) of diverse, genetically characterized *mecC*-harboring MRSA isolates to establish the performance of essentially all common phenotypic approaches to MRSA detection. Drawing from the principle that these "oxacillin-susceptible" MRSA isolates might be induced by cefoxitin, Kriegeskorte et al. evaluated susceptibility testing using disks containing 30 μg cefoxitin and interpreted inhibition zones according to both CLSI and EUCAST criteria. Despite CLSI criteria for resistance requiring a 1-mm-smaller zone, all 111 isolates were considered resistant by both standards. In contrast, oxacillin broth microdilution classified 39% of the same isolates as susceptible. These results directly validated the superiority of a widely available and inexpensive approach while invalidating oxacillin as a standalone screen for *mecC*-harboring MRSA.

Automated systems varied widely in their ability to classify *mecC*-harboring MRSA isolates as resistant. The WalkAway system performed best, with 97% of 111 isolates testing as resistant. The Vitek 2 system performed relatively well (92% testing as resistant), although a rate of 8% false susceptibility is still discouraging. By comparison, oxacillin broth microdilution performed poorly, classifying only 61% of isolates correctly. The Phoenix system performed no better (only 65% classified correctly), even though the Phoenix panel included both cefoxitin and oxacillin screens.

Kriegeskorte and colleagues also evaluated 5 chromogenic agars, 3 of which showed reduced growth at rates of 5 to 11%, but none of which showed lack of growth. Furthermore, low inocula similar to what might be encountered from clinical screening samples resulted in acceptable growth, positioning these currently available agars as a promising tool to screen for *mecC*-harboring MRSA carriage in colonized patients or from low-inoculum culture material.

On balance, this study is encouraging as it places cefoxitin disk diffusion testing as simultaneously the most effective, simplest, and least expensive method for detecting both *mecA*- and *mecC*-harboring MRSA isolates. Chromogenic agars also performed well and may be useful alternatives. To the extent that laboratories rely heavily on automated susceptibility testing, this study is cause for concern and, for many laboratories, may demand a change in practice to include supplemental testing for *mecC*.

FUTURE DIRECTIONS

This study illustrates what progress needs to be made to address the challenge of *mecC*-harboring MRSA. Automated susceptibility instruments should recalibrate algorithms developed for *mecA*-harboring MRSA to better select for the kinetics of *mecC*-harboring MRSA resistance. In the United States, barriers exist to Food and Drug Administration (FDA) approval of new systems and breakpoints, such that automated instruments come with interpretive criteria and MIC ranges that often lag many years behind current best practices as defined by the Clinical and Laboratory Standards Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Streamlined regulatory consideration of new and modified testing would give appropriately rapid consideration to what is likely to become a major public health problem—if it is not already. A 2013 study by Petersen et al. (9) illustrates what may happen when countries start looking for *mecC*-harboring MRSA. Having banked *S. aureus* bloodstream isolates from 1958 onward, Peterson et al. revealed that *mecC*-harboring MRSA comprised 2.8% of all MRSA isolates in 2011 and found one isolate dating to 1975. A first step for most laboratories, then, will be to use both clinical and research tools to establish the prevalence of *mecC*-harboring MRSA. Optimized automated susceptibility testing systems would be the most convenient tool for this purpose for most frontline laboratories.

CLSI and EUCAST are often more nimble than the FDA, and this article suggests that cefoxitin disk screening is ready for their recommendation as a screening or confirmatory test for *mecA*- and *mecC*-harboring MRSA isolates. Chromogenic agars might also be recommended, as well as the use of automated systems given a conditional, platform-specific warning, ideally pending performance-improving revision by their respective manufacturers. Cefoxitin disk diffusion testing is essentially ubiquitously available and may be used as a supplemental test to survey the proportion of *S. aureus* isolates classified as MRSA in comparison to those so classified by other systems, providing an estimate of the "oxacillin-susceptible" MRSA burden in a given laboratory.

In any case, the uneven performance demonstrated here puts many laboratories in a difficult position. Several approaches recommend themselves, but the use of any of these as laboratory-developed, off-FDA-label detection systems for *mecC*-harboring MRSA demands their validation at the level of individual laboratories. Most laboratories, however, have no access to strains that are genetically confirmed to be *mecC*-harboring MRSA. The FDA, in the form of the FDA-CDC Antimicrobial Resistance Bank (10), already provides borderline oxacillin-resistant *mecA*-harboring MRSA strains and vancomycin-intermediate strains to laboratories seeking to optimize testing for these difficult to detect phenotypes. A publically available *mecC*-harboring MRSA bank would go a long way to meeting the immediate need for a laboratory-level response to *mecC*-harboring MRSA.

In conclusion, *mecC*-harboring MRSA presents a challenge that is new yet very similar in character to "oxacillin-susceptible" *mecA*-harboring MRSA, with a notable absence of the PCR and antigen testing that have made the latter a tractable clinical diagnostic problem. Kriegeskorte and colleagues present a comprehensive assessment of the performance of currently available testing, highlighting the need for test optimization and the development of new testing, as well as standard setting to define what currently available phenotypic testing methods are acceptable for clinical testing.

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