

## Rethinking the Molecular Diagnostics for Methicillin-Resistant *Staphylococcus aureus*

*To the Editor*—Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) strains are a common clinical problem that causes a high burden of disease and that often requires long-term treatment.<sup>1</sup> MRSA rates vary in different countries, with rates being high in the United States, Middle and Far Eastern countries, and Southern Europe but relatively low in, for example, Northern Europe.

Encoded by different alleles of the *mecA* gene, an alternate penicillin-binding protein (PBP2a) is expressed in MRSA conferring resistance to  $\beta$ -lactam antibiotics (except ceftazidime and ceftibiprole).<sup>2</sup> *mecA* is situated on a mobile genetic element named staphylococcal cassette chromosome *mec* (SCC*mec*). Currently, this mobile element occurs as 12 different types in *S. aureus* as well as in other clinically relevant staphylococci such as *S. haemolyticus* and *S. epidermidis*.<sup>3,4</sup>

In 2011, a divergent *mecA* homologue was discovered and subsequently designated *mecC*. *mecC* MRSA has been isolated from patients from Western and Central Europe.<sup>3–5</sup> It has also been detected in different domestic and wild animals including cattle, sheep, hedgehogs, storks, and fox.<sup>6</sup> The reservoir of *mecC* strains is still unknown, although zoonotic transmission from cows or sheep is believed to play a major role.

The *mecC* gene was detected in various rare lineages of *S. aureus* in multilocus sequence type (MLST) clonal complexes (CC) 49, 130, 425, 599, and 1943.<sup>7</sup> The amino acid identity between *mecC*- and *mecA*-encoded PBP2a is only 63%. While the *mecC* gene itself does not mediate resistance to penicillin, it is frequently accompanied by the  $\beta$ -lactamase *blaZ*, which is part of the SCC*mec* XI element that does confer resistance to penicillin.<sup>8</sup> Previous studies found that *mecC* also mediates resistance to oxacillin and ceftazidime.<sup>9</sup>

We report a 59-year-old male patient with a postthrombotic ulcer on his right lower leg measuring 3 × 3 cm. This patient had no other concomitant disease and no prior hospital admission. The bacterial culture of the wound swab was analysed with Vitek 2 (bioMérieux, Nürtingen, Germany) and broth microdilution according to EUCAST break points. The isolate was further analyzed using the *S. aureus* Genotyping Kit 2.0 (Alere Technologies, Jena, Germany).

After repetitive wound debridement, bacterial culture of a wound swab revealed an *S. aureus* isolate resistant to benzylpenicillin, oxacillin, and ceftazidime in the routine susceptibility test. Other tested antibiotics typically used for treatment of *S. aureus* (eg, glycopeptides and chinolones) were found to be susceptible.

The MRSA isolate carried the *mecC* gene was negative for Pantone-Valentine leukocidin (PVL) and was assigned to the CC130 lineage. The isolate belonged to *agr* group III and capsule type 8, and the *mecC* gene was present as a part of the SCC*mec* XI element together with the distinct *blaZ*. Despite the association

of the *mecC* gene to zoonotic transmission, the patient reported no contact with livestock or any other potential animal source.

The patient was admitted to the hospital for treatment of the leg ulcer and was simultaneously MRSA sanitized according to the German guidelines on 5 consecutive days with an octenidin-containing wound compresses for 20 minutes per day, mupirocin nasal ointment, throat flushing with chlorhexidine, and skin washing with octenidin. After waiting for 3 days, control swabs were taken on 3 consecutive days from the nose, throat, skin, and wound. None of these 12 swabs were positive for *S. aureus*, which was interpreted as evidence for successful sanitization exclusively with topical treatment. During the following 2 years, all 10 swabs taken for diagnostic and screening purposes remained negative for *S. aureus*.

The prevalence of *mecC* in humans and animals is still low: the value reported in a meta-analysis was 0.009% (95% confidence interval = 0.055–0.013%) of MRSA,<sup>10</sup> which corresponds to an order of magnitude range of 1:100 to 1:1000 of typed MRSA.<sup>5</sup> While *mecC* strains in humans do not appear to be particularly virulent, cohort studies are missing and case reports are sparse.

Currently, there is no recommendation on treatment of *mecC* MRSA. With regard to treatment options, minimum inhibitory concentrations (MICs) for methicillin and ceftazidime are usually lower than in conventional MRSA, but these compounds might not be effective. Penicillin is an inhibitor for the *mecC* gene product, but because *mecC* is accompanied by a specific penicillinase (also carried on the SCC*mec* XI element), monotherapy is not a treatment option. However, *in vitro* and *in vivo* models have shown an effective combination with a  $\beta$ -lactamase inhibitor.<sup>8</sup> The patient was treated as an *mecA* MRSA case to overcome the risk of treatment failures in case of susceptible or intermediate oxacillin MICs.

The current setup to diagnose *mecA/mecC* of MRSA is multifaceted and time-consuming. Because of its divergent sequence, *mecC* and its gene product cannot be detected by all assays designed to identify *mecA*/PBP2a,<sup>4</sup> and it has been suggested that *mecC* is largely underreported. The discrepancy between phenotypic resistance and *mecA*-negative molecular or protein-based confirmatory tests might delay reporting to the physician and, thus, also delay the administration of an appropriate treatment. Hence, the discovery of *mecC* has resulted in the recognition of the need to redesign diagnostic tests.

In summary, clinicians and microbiologists should be aware of the changing facets of MRSA infections, particularly the emergence of *mecC* MRSA-conferred resistance against oxacillin. MRSA resistance warrants further investigation, especially in cases of discrepant testing results. Because the clinical experience is limited to case reports (probably due to the underreporting of *mecC* MRSA), a *mecC* MRSA infection should be treated as an *mecA* MRSA infection to avoid treatment failure.

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**Claudia Stein, PhD,<sup>1,4</sup>**  
**Jörg Tittelbach, MD,<sup>2</sup>**  
**Stefan Monecke, MD,<sup>3,4</sup>**  
**Sebastian Weis, MD,<sup>1,5,6</sup>**  
**Oliwia Makarewicz, PhD,<sup>1,4</sup>**  
**Ralf Ehrlich, PhD,<sup>3,4</sup>**  
**Mathias Pletz, MD<sup>1,4</sup>**

Affiliations: 1. Center for Infectious Disease and Infection Control, Jena University Hospital, Jena, Germany; 2. Department of Dermatology, Jena University Hospital, Jena, Germany; 3. Abbott Rapid Diagnostics, Alere Technologies GmbH, Jena, Germany; 4. InfectoGnostics Research Campus; 5. Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany; 6. Klinik für Anästhesiologie und Intensivmedizin, Jena University Hospital, Jena, Germany.

Address correspondence to Claudia Stein, Center for Infectious Disease and Infection Control, Jena University Hospital, Am Klinikum 1, Jena, Germany (claudia.stein@med.uni-jena.de).

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## Outbreak of ST395 KPC-Producing *Klebsiella pneumoniae* in a Neonatal Intensive Care Unit in Palermo, Italy

*To the Editor*—The spread of carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) is an emerging concern worldwide. Italy is a country endemic for *Klebsiella pneumoniae* carbapenemases (KPCs).<sup>1,2</sup> KPC spread in neonatal intensive care units (NICUs) may represent a major safety issue for critical infants and a challenge in managing new admissions.<sup>3</sup> In February 2014, we started an active surveillance program for colonization by multidrug-resistant organisms in 5 NICUs in Palermo, Italy. Inclusion criteria for patients were hospitalization for at least 48 hours and collection of at least 1 rectal swab. Samples were collected monthly in each NICU and cultured on selective media. A gram-negative bacterium resistant to at least 3 different groups of antimicrobial agents (eg, penicillins, cephalosporins, aminoglycosides, and/or carbapenems) was defined as a multidrug-resistant gram-negative (MDRGN). Colonization was defined as the isolation of an MDRGN without evidence of infection.

Swabs were inoculated onto MacConkey agar plates with 4 antibiotic disks containing gentamicin, amoxicillin-clavulanic acid, meropenem, and ceftazidime and were then incubated. Colonies growing into each antibiotic inhibition halo were subcultured, and biochemical identification of isolated strains was performed using the API 20E system (BioMérieux, Marcy-l’Etoile, France). Antibiotic susceptibility testing was performed using a disk diffusion (DD) method on Mueller-Hinton agar plates with a panel of antimicrobials (ie, netilmicin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, cefepime, imipenem, aztreonam, and gentamicin) according to the Clinical and Laboratory Standards Institute guidelines.<sup>4</sup> Colonies growing within meropenem and imipenem inhibition zones were subjected to E-test strips (BioMérieux, Marcy-l’Etoile, France) for minimum inhibitory concentration (MIC) determination. Antibiotic susceptibility testing and extended spectrum β-lactamase (ESBL) detection were performed using DD and a double-disk synergy test.

In December 2016, rectal swabs from 6 patients in 1 NICU identified these patients as colonized by CR-Kp, and 8 isolates were collected. *Klebsiella pneumoniae* strains showing an extended drug-resistant phenotype were screened by