Comparison of Different Phenotypic Approaches To Screen and Detect *mecC*-Harboring Methicillin-Resistant *Staphylococcus aureus*

André Kriegeskorte,[a]* Evgeny A. Idelevich,[a] Andreas Schlattmann,[a] Franziska Layer,[b] Birgit Strommenger,[b] Olivier Denis,[c] Gavin K. Paterson,[d] Mark A. Holmes,[e] Guido Werner,[f] [ID]Karsten Becker[a]

[a]Institute of Medical Microbiology, University Hospital Münster, Münster, Germany
[b]Robert-Koch-Institute, Berlin, Germany
[c]Laboratoire de Microbiologie, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium
[d]Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom
[e]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom
[f]Robert-Koch-Institute, Wernigerode Branch, Wernigerode, Germany

ABSTRACT Similar to *mecA*, *mecC* confers resistance against beta-lactams, leading to the phenotype of methicillin-resistant *Staphylococcus aureus* (MRSA). However, *mecC*-harboring MRSA strains pose special difficulties in their detection. The aim of this study was to assess and compare different phenotypic systems for screening, identification, and susceptibility testing of *mecC*-positive MRSA isolates. A well-characterized collection of *mecC*-positive *S. aureus* isolates ($n = 111$) was used for evaluation. Routinely used approaches were studied to determine their suitability to correctly identify *mecC*-harboring MRSA, including three (semi)automated antimicrobial susceptibility testing (AST) systems and five selective chromogenic agar plates. Additionally, a cefoxitin disk diffusion test and an oxacillin broth microdilution assay were examined. All *mecC*-harboring MRSA isolates were able to grow on all chromogenic MRSA screening plates tested. Detection of these isolates in AST systems based on cefoxitin and/or oxacillin testing yielded overall positive agreements with the *mecC* genotype of 97.3% (MicroScan WalkAway; Siemens), 91.9% (Vitek 2; bioMérieux), and 64.9% (Phoenix, BD). The phenotypic resistance pattern most frequently observed by AST devices was "cefoxitin resistance/oxacillin susceptibility," ranging from 54.1% (Phoenix) and 83.8% (Vitek 2) to 92.8% (WalkAway). The cefoxitin disk diffusion and oxacillin broth microdilution assays categorized 100% and 61.3% of isolates to be MRSA, respectively. The chromogenic media tested confirmed their suitability to reliably screen for *mecC*-harboring MRSA. The AST systems showed false-negative results with varying numbers, misidentifying *mecC*-harboring MRSA as methicillin-susceptible *S. aureus*. This study underlines cefoxitin's status as the superior surrogate *mecC*-positive MRSA marker.

KEYWORDS MRSA, *Staphylococcus aureus*, broth microdilution, cefoxitin, chromogenic media, disk diffusion, *mecC*, methicillin resistance, oxacillin, susceptibility testing

The still worrying occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) in many parts of the world poses a major challenge to health care systems by increasing the burden of disease. Rapid and effective MRSA identification and susceptibility testing are paramount to prevent further dissemination and to adapt antimicrobial treatment. In 2011, a novel PBP 2a-encoding *mecA* homologue designated *mecC* (originally *mecA*$_{LGA251}$) was reported with homologies on the nucleotide and protein levels of only 70% and 63%, respectively (1, 2). Later *mecC* was confirmed as the genetic

determinant that confers methicillin resistance in *S. aureus* for those isolates (3). Farm and wildlife animals have been revealed as reservoirs for *mecC*-harboring MRSA (4, 5), and the zoonotic potential of these livestock-associated MRSA has been shown (6–8).

The limited homology of *mecC* to *mecA* and their respective proteins led to major diagnostic challenges in identification and susceptibility testing of *mecC*-harboring MRSA (9). In addition to obvious but easily resolved difficulties in targeting the divergent *mecC* nucleotide sequence by DNA-based diagnostic tests (10, 11), phenotypic approaches exhibited considerable difficulties due to comparatively low oxacillin MICs (1, 7, 8), which may be caused by differences in the *mecA* and *mecC* promoters (3). Moreover, low homology between the encoded PBP 2a proteins is the reason for the failure of existing PBP 2a agglutination tests to detect *mecC*-positive isolates (5, 7, 8).

In this study, we compared several routinely applied diagnostic approaches in their capability to identify *mecC*-harboring MRSA strains from a comprehensive, heterogeneous, and representative collection. In detail, we compared (i) three (semi)automated susceptibility testing (AST) systems, (ii) five selective chromogenic agar plates (MRSA screening plates), (iii) a cefoxitin disk diffusion test, and (iv) oxacillin broth microdilution.

MATERIALS AND METHODS

A large set of *mecC*-harboring MRSA isolates (*n* = 111) from human and animal specimens isolated in Germany, the United Kingdom, and Belgium were included in the study. All isolates were confirmed as *mecC* positive by PCR (12) and characterized by *spa* typing (t843, *n* = 51; t6292, *n* = 13; t1736, *n* = 6; t1535, *n* = 4; t3391, *n* = 3; t978, t9165, t742, t6902, t6521, t6220, t5930, t1773, and t11706, *n* = 2 each; t9910, t9738, t9280, t9123, t8842, t7914, t7603, t7189, t6300, t524, t13233, t1207, t11702, t11290, t11120, and not typeable, *n* = 1 each). Isolates were of human (*n* = 80), unknown (*n* = 24), bovine/bulk milk (*n* = 4), sheep (*n* = 2), and environmental (*n* = 1) origins. No copy isolates were included.

The following selective chromogenic agar plates were inoculated with a single colony from overnight blood agar plate cultures: (i) Oxoid Brilliance MRSA 2, (ii) bioMérieux chromID MRSA, (iii) BD BBL CHROMagar MRSA II, (iv) Bio-Rad MRSA Select, and (v) MAST Diagnostica CHROMagar MRSA. To simulate potentially low inocula of clinical specimens, nine isolates with different *spa* types (t843, t978, t1207, t1535, t1736, t391, t5930, t6292, and t6902) were each adjusted to 0.5 McFarland standard turbidity, and serial dilutions with the final dilution factor of 10^5 were prepared. Subsequently, 100 μl of the final dilutions was used to inoculate all chromogenic media (except MRSA Select from Bio-Rad due to supply constraints) and blood agar plates for growth control in triplicate. *S. aureus* strains USA300 and ATCC 29213 were used as positive and negative controls, respectively. Growth was evaluated after 24 h and 48 h. Automated systems were inoculated from the same plates as chromogenic media. Automated systems for susceptibility testing were used according to the manufacturers' recommendations: i.e., BD Phoenix (Becton Dickinson, Heidelberg, Germany) was executed with test panel PMIC-72, Vitek 2 (bioMérieux, Marcy l'Etoile, France) with test panel AST P580, and MicroScan WalkAway 96 Plus (Siemens Healthcare Diagnostics, Eschborn, Germany) with test panel Pos MIC 28.

Cefoxitin disk diffusion assays (cefoxitin discs, 30 μg; bestbion dx, Cologne, Germany) were performed according to EUCAST and using *S. aureus* ATCC 29213 as control. The EUCAST guidelines (version 7.0, valid from 1 January 2017 [inhibition zone of <22 mm, resistant]) and CLSI criteria (M100-S27, 27th ed., January 2017 [inhibition zone of ≤21 mm, resistant]) were followed in the interpretation of the results.

Oxacillin (Sigma-Aldrich, Taufkirchen, Germany) susceptibility was determined by broth microdilution, using a final inoculum of approximately 5 × 10^5 CFU/ml and *S. aureus* ATCC 29213 as quality control. MICs were interpreted according to EUCAST guidelines (version 7.0, valid from 1 January 2017 [MIC of >2 μg/ml]) and CLSI criteria (M100-S27, 27th ed., January 2017 [MIC of ≥4 μg/ml]).

RESULTS

Applicability of AST systems to detect *mecC*-positive isolates. Analyzing resistance toward cefoxitin and oxacillin by AST systems, different susceptibility patterns were observed. For all systems, the most frequently detected pattern was the combination of the categorization "cefoxitin-resistant, but oxacillin-susceptible," ranging from 54.1% (Phoenix) and 83.8% (Vitek 2) to 92.8% (WalkAway) of all tested isolates (Table 1). In the WalkAway system, three isolates (2.7%) were categorized as cefoxitin and oxacillin susceptible, whereas in the Vitek 2 and the Phoenix system, 9 isolates (8.1%) and 39 isolates (35.1%), respectively, were categorized as susceptible to both. One isolate was categorized as cefoxitin susceptible and oxacillin resistant by the Phoenix system.

The MIC$_{90}$ values for oxacillin were >2 μg/ml (Phoenix), 2 μg/ml (MicroScan), and 2 μg/ml (Vitek 2). The MIC$_{90}$ values for cefoxitin were >8 μg/ml (Phoenix) and

TABLE 1 Susceptibility pattern testing of cefoxitin and oxacillin for *mecC*-positive *S. aureus* isolates

Cefoxitin/oxacillin susceptibility pattern[a]	No. of isolates (% agreement) tested by[b]:		
	Phoenix	MicroScan WalkAway	Vitek 2
R/R	11 (9.9)	5 (4.5)	9 (8.1)
R/S	60 (54.1)	103 (92.8)	93 (83.8)
S/R	1 (0.9)	0 (0.0)	0 (0.0)
Total R[c]	72 (64.9)	108 (97.3)	102 (91.9)
S/S	39 (35.1)	3 (2.7)	9 (8.1)

[a]R, resistant; S, susceptible.
[b]*S. aureus* ATCC 29213 (MSSA) and *S. aureus* ATCC 43300 (MRSA) were used as quality control strains. Both were correctly categorized by all three systems. A total of 111 isolates were examined.
[c]Positive agreement based on resistance to at least one of the compounds tested (cefoxitin or oxacillin).

>4 μg/ml (WalkAway); Vitek 2 detected 91.9% of isolates as resistant to cefoxitin without reporting a MIC value. Less than 10% of isolates were tested resistant to both cefoxitin and oxacillin (Phoenix, 9.9%; MicroScan, 4.5%; Vitek 2, 8.1%).

Applicability of chromogenic MRSA screening plates for detection of *mecC*-positive isolates. The vast majority of isolates showed typical growth on all tested cefoxitin-containing chromogenic MRSA screening plates. Reduced growth (i.e., smaller colonies, but with characteristic MRSA-indicating color) was observed for a small fraction of isolates (Table 2). Oxoid Brilliance MRSA 2 plates showed a mixed phenotypic appearance with blue (presumptive for MRSA) and white colonies for all isolates.

Additionally, a subset of nine isolates and positive control strain *S. aureus* USA300, tested in triplicate, showed growth on screening plates from four manufacturers using an inoculum of 100 μl from a 10^{-5} dilution of a 0.5 McFarland standard suspension (approximately 100 CFU/plate). MRSA Select agar plates (Bio-Rad) were not tested in this additional experiment due to supply unavailability. Negative control *S. aureus* ATCC 29213 exhibited no growth on chromogenic agar plates.

Applicability of cefoxitin disk diffusion and oxacillin broth microdilution test for detection of *mecC*-positive isolates. The cefoxitin disk diffusion test detected *mecC*-encoded methicillin resistance in 111/111 isolates (i.e., 100%). The oxacillin broth microdilution resulted in a categorization of 43 susceptible (38.7%) and 68 resistant (61.3%) isolates.

DISCUSSION

The occurrence of *mecC*-harboring MRSA has been described in humans, companion animals, and livestock in several European countries (13). While the overall prevalence of these isolates seems to be low, it has been suspected that *mecC* prevalence might be underestimated because of its misidentification as methicillin-susceptible *S. aureus* (MSSA) due to its borderline resistant phenotype. Additionally, negative results in MRSA PCR and agglutination assays if only the *mecA* gene (i.e., the gene encoding PBP 2a) is targeted, hamper *mecC*-harboring MRSA detection efforts. Furthermore, it has been shown that the prevalence of *mecC*-positive *S. aureus* isolates increased at least in Denmark and that *mecC*-positive MRSA isolates are also capable of causing infections

TABLE 2 Growth on selective chromogenic agar media

Chromogenic agar[a]	No. of isolates (% agreement) with:		
	Normal growth[b]	Reduced growth[c]	No growth
Brilliance MRSA 2	111 (100)	0 (0.0)	0 (0.0)
chromID MRSA	111 (100)	0 (0.0)	0 (0.0)
BBL CHROMagar MRSA II	101 (91.0)	10 (9.0)	0 (0.0)
MRSA Select	105 (94.6)	6 (5.4)	0 (0.0)
CHROMagar MRSA	99 (89.2)	12 (10.8)	0 (0.0)

[a]*S. aureus* ATCC 29213 (MSSA) and *S. aureus* ATCC 43300 (MRSA) were used as quality control strains.
[b]According to the respective manufacturer's instructions.
[c]Colonies that were smaller but with the color change indicated for MRSA.

in humans (4). A reliable detection of these isolates is important to ensure both an adequate treatment of *mecC*-harboring MRSA infections and the use of the same prevention measures as already established for *mecA*-harboring MRSA. This study revealed that all chromogenic media and the cefoxitin disk diffusion test were able to categorize all *mecC*-positive MRSA strains properly. Additionally, we were able to show for a subset of strains that inocula as low as approximately 100 CFU per plate result in growth on chromogenic media, indicating that a recovery from clinical swab samples with low MRSA loads can likely be achieved. However, these findings are limited because they could mimic the usual clinical specimen as encountered in the laboratory only partially. To various degrees, all three AST systems displayed limitations in the ability to detect *mecC* MRSA. While the detection rate of WalkAway (97.3%) was also high, Vitek 2 (91.9%) and particularly the Phoenix system (64.9%) showed considerably lower rates. A study by Cartwright et al. showed a detection rate of 88.7% ($n = 62$ *mecC*-positive MRSA isolates) for the cefoxitin-resistant/oxacillin-susceptible pattern using the Vitek 2 (14); similarly, this AST device detected this pattern in 83.8% of the tested isolates in our study. The oxacillin broth microdilution performed poorly, showing a detection rate of only 61.3%. This is in accordance with previous studies (15).

In conclusion, automated systems may fail to detect *mecC*-encoded methicillin resistance, while all chromogenic screening media displayed colonies presumptive for MRSA growth. In comparison to oxacillin, cefoxitin was confirmed as superior surrogate marker to detect *mecC*-harboring MRSA isolates. Discrepancies between positive screening results based on the use of chromogenic media and categorization as methicillin susceptible by AST systems should be verified by molecular assays or disk diffusion.

ACKNOWLEDGMENTS

REFERENCES

1. García-Álvarez L, Holden MT, Lindsay G, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov LR, Peacock SJ, Maskell DJ, Holmes MA. 2011. Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11:595–603. https://doi.org/10.1016/S1473-3099(11)70126-8.
2. Shore AC, Deasy EC, Slickers P, Brennan G, O'Connell B, Monecke S, Ehricht R, Coleman DC. 2011. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical clonal complex 130 methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 55:3765–3773. https://doi.org/10.1128/AAC.00187-11.
3. Ballhausen B, Kriegeskorte A, Schleimer N, Peters G, Becker K. 2014. The *mecA* homolog *mecC* confers resistance against beta-lactams in *Staphylococcus aureus* irrespective of the genetic strain background. Antimicrob Agents Chemother 58:3791–3798. https://doi.org/10.1128/AAC.02731-13.
4. Petersen A, Stegger M, Heltberg O, Christensen J, Zeuthen A, Knudsen LK, Urth T, Sorum M, Schouls L, Larsen J, Skov R, Larsen AR. 2013. Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin Microbiol Infect 19:E16–E22. https://doi.org/10.1111/1469-0691.12036.
5. Paterson GK, Morgan FJ, Harrison EM, Peacock SJ, Parkhill J, Zadoks RN, Holmes MA. 2014. Prevalence and properties of *mecC* methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine bulk tank milk in Great Britain. J Antimicrob Chemother 69:598–602. https://doi.org/10.1093/jac/dkt417.
6. Kerschner H, Harrison EM, Hartl R, Holmes MA, Apfalter P. 2015. First report of *mecC* MRSA in human samples from Austria: molecular characteristics and clinical data. New Microbes New Infect 3:4–9. https://doi.org/10.1016/j.nmni.2014.11.001.
7. Cuny C, Layer F, Strommenger B, Witte W. 2011. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany. PLoS One 6:e24360. https://doi.org/10.1371/journal.pone.0024360.
8. Kriegeskorte A, Ballhausen B, Idelevich EA, Köck R, Friedrich AW, Karch H, Peters G, Becker K. 2012. Human MRSA isolates with novel genetic homolog, Germany. Emerg Infect Dis 18:1016–1018. https://doi.org/10.3201/eid1806.110910.
9. Becker K, Ballhausen B, Köck R, Kriegeskorte A. 2014. Methicillin resistance in Staphylococcus isolates: the "mec alphabet" with specific consideration of *mecC*, a mec homolog associated with zoonotic S. aureus lineages. Int J Med Microbiol 304:794–804. https://doi.org/10.1016/j.ijmm.2014.06.007.
10. Becker K, Larsen AR, Skov RL, Paterson GK, Holmes MA, Sabat AJ, Friedrich AW, Köck R, Peters G, Kriegeskorte A. 2013. Evaluation of a modular multiplex-PCR methicillin-resistant *Staphylococcus aureus*

(MRSA) detection assay adapted for *mecC* detection. J Clin Microbiol 51:1917–1919. https://doi.org/10.1128/JCM.00075-13.

11. Becker K, Denis O, Roisin S, Mellmann A, Idelevich EA, Knaack D, van Alen S, Kriegeskorte A, Köck R, Schaumburg F, Peters G, Ballhausen B. 2016. Detection of *mecA*- and *mecC*-positive methicillin-resistant *Staphylococcus aureus* (MRSA) isolates by the new Xpert MRSA Gen 3 PCR assay. J Clin Microbiol 54:180–184. https://doi.org/10.1128/JCM.02081-15.

12. Cuny C, Wieler LH, Witte E. 2015. Livestock-associated MRSA: the impact on humans. Antibiotics (Basel) 4:521–543. https://doi.org/10.3390/antibiotics4040521.

13. Paterson GK, Harrison EM, Holmes MA. 2014. The emergence of *mecC*

methicillin-resistant *Staphylococcus aureus*. Trends Microbiol 22:42–47. https://doi.org/10.1016/j.tim.2013.11.003.

14. Cartwright EJP, Paterson GK, Raven KE, Harrison EM, Gouliouris T, Kearns A, Pichon B, Edwards G, Skov RL, Larsen AR, Holmes MA, Parkhill J, Peacock SJ, Török ME. 2013. Use of Vitek 2 antimicrobial susceptibility profile to identify *mecC* in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 51:2732–2734. https://doi.org/10.1128/JCM.00847-13.

15. Skov R, Larsen AR, Kearns A, Holmes M, Teale C, Edwards G, Hill R. 2014. Phenotypic detection of *mecC*-MRSA: cefoxitin is more reliable than oxacillin. J Antimicrob Chemother 69:133–135. https://doi.org/10.1093/jac/dkt341.