

G.I. Brennan *et al.*

## Original article

### Evaluation of commercial chromogenic media for the detection of meticillin-resistant *Staphylococcus aureus*

G.I. Brennan<sup>a,b,\*</sup>, C. Herra<sup>c</sup>, D.C. Coleman<sup>b</sup>, B. O'Connell<sup>a,d</sup>, A.C. Shore<sup>a,b</sup>

<sup>a</sup>National MRSA Reference Laboratory, St. James's Hospital, Dublin, Ireland

<sup>b</sup>Microbiology Research Unit, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Ireland

<sup>c</sup>School of Biological Sciences, Dublin Institute of Technology, Dublin, Ireland

<sup>d</sup>Department of Clinical Microbiology, School of Medicine, University of Dublin, Trinity College, St. James's Hospital, Dublin, Ireland

---

\*Corresponding author. Address: National MRSA Reference Laboratory, St. James's Hospital, James's St., Dublin 8, Ireland. Tel.: +353 1 4103662; fax: +353 1 4103666. E-mail address: gbrennan@stjames.ie (G.I. Brennan).

## SUMMARY

**Background:** Selective chromogenic media allowing one-step meticillin-resistant *Staphylococcus aureus* (MRSA) isolation and identification are widely used. However, the changing epidemiology of MRSA means that the suitability of these chromogenic media requires investigation.

**Aim:** To evaluate the following chromogenic media – Colorex MRSA, MRSA Select II, ChromID MRSA, and MRSA Brilliance 2 – for the detection of divergent strain types.

**Methods:** We used a diverse collection of *S. aureus*, including strains harbouring the *mecC* gene, strains expressing varying levels of meticillin resistance, and isolates recovered from patient samples.

**Findings:** MRSA Select II, Colorex MRSA, and ChromID each grew at a density of  $1.5 \times 10^1$  cfu/mL for each SCC*mec* type investigated. Brilliance 2 demonstrated growth at  $1.5 \times 10^1$  cfu/mL for *mecC* MRSA but at a higher density ( $1.5 \times 10^4$  cfu/mL) for the three *mecA* MRSA strains. All four media demonstrated excellent sensitivity for MRSA detection ( $\geq 99\%$ ), but reduced levels of specificity (85–73%) when challenged with a range of meticillin-susceptible *S. aureus* (MSSA) isolates. High levels of false positives (~50%) were also obtained with all chromogenic media when tested with *mec*-negative borderline oxacillin-resistant *S. aureus* (BORSA) isolates.

**Conclusion:** Although false positives may be obtained with some strains of MSSA and BORSA, the high sensitivity of these media and their ability to recover almost all MRSA tested (including oxacillin-susceptible and *mecC*-positive strains) confirm the value of chromogenic agar in MRSA detection.

*Keywords:*

Borderline oxacillin-resistant *S. aureus* chromogenic agar

*mecC*

Meticillin-resistant *Staphylococcus aureus*

## Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) are major healthcare-associated pathogens frequently associated with serious and sometimes life-threatening conditions. Meticillin resistance is mediated by an altered penicillin binding protein PBP2a encoded by *mec* and located on the staphylococcal cassette chromosome *mec* (SCC*mec*) element. To date, 11 different SCC*mec* elements have been described in staphylococci corresponding to the emergence of a wide range of MRSA strains with different genetic backgrounds.<sup>1</sup>

In the last two decades the epidemiology of MRSA has changed significantly with an increasing prevalence of MRSA infections outside the healthcare environment in the community, and more recently among livestock.<sup>2,3</sup> In Ireland, MRSA is endemic in hospitals, and, as in many countries throughout Europe, the sequence type-SCC*mec* ST22-MRSA-IV clone predominates.<sup>4</sup> In addition, a diversity of other strains including community-associated *pvl* toxin-positive and -negative MRSA along with a small number of livestock-associated strains have also been reported in Ireland.<sup>2,3</sup>

Just as the epidemiology of MRSA has changed, so too has the level of meticillin resistance among MRSA. Traditionally MRSA are defined as having an oxacillin minimum inhibitory concentration (MIC)  $\geq 4.0$  mg/L or as harbouring the *mecA* gene encoding PBP2a.<sup>1</sup> However, few MRSA isolates express homogeneous oxacillin resistance. Oxacillin-susceptible *mecA*-positive *S. aureus* isolates have been reported worldwide.<sup>5</sup> Similarly, low-level oxacillin-resistant *mecA*-negative strains known as borderline oxacillin-resistant *S. aureus* (BORSA) isolates have further complicated the definition of MRSA.<sup>6</sup>

Superimposed on this heterogeneous expression of meticillin resistance, recent reports have also identified a variety of MRSA strains of probable animal origin that encode a highly divergent meticillin-resistance gene termed *mecC*. Where once the detection of *mecA* was considered the gold standard in laboratory confirmation of MRSA, the emergence of *mecC*-

encoding strains in infection in both humans and animals adds to the challenge of defining and detecting an MRSA-positive patient.<sup>7-9</sup>

Regardless of the changing epidemiology of MRSA, rapid detection remains essential for the implementation of infection control procedures and effective patient management. The use of selective chromogenic culture media, which allow for one-step MRSA isolation and identification, has now become widespread practice.<sup>10,11</sup> With the frequent application of chromogenic media in diagnostic practice, the suitability of these media to ensure the correct detection of divergent MRSA strain types has come under review. However, whereas many studies have evaluated the use of chromogenic media for the direct recovery of MRSA from patient specimens, few have undertaken a comparative evaluation of all currently available commercial media using a comprehensive collection of diverse *S. aureus* strains, including those with the novel *mecC* gene and those expressing varying levels of meticillin resistance.<sup>11-14</sup>

The purpose of this study was to evaluate the performance of widely used chromogenic MRSA media using a diverse collection of *S. aureus* isolates recovered in Ireland and Europe. The limits of detection (LOD) of four commercial chromogenic media were determined using MRSA strains representative of four SCC*mec* types, i.e. II, IV, V, and XI.<sup>2,3,7</sup> The performance of the media was also evaluated against a collection of genotypically diverse MRSA strains from hospitals, communities, and livestock and representative of SCC*mec* types I–XIII, X, and XI as well as meticillin-susceptible *S. aureus* (MSSA) and BORSA strains isolated from healthcare and community sources. An evaluation of the media was also undertaken using patient samples collected as part of the routine infection prevention and control procedures in a large teaching hospital.

## Methods

### *Limits of detection*

Four MRSA isolates, representative of SCC*mec* types II, IV, and V (carrying *mecA*) and SCC*mec* XI (carrying *mecC*) (Table I) were selected to investigate the LOD of the following four commercial MRSA chromogenic agars: MRSA Select II (BioRad, Hercules, CA, USA), MRSA Brilliance 2 (Oxoid, Basingstoke, UK), Colorex MRSA (E & O Laboratories, Bonnybridge, UK), and ChromID MRSA (bioMérieux, Marcy l'Etoile, France). In each case, isolates were subcultured overnight on Columbia blood agar (Oxoid) at 37°C and then suspended in saline to a density equivalent to 0.5 McFarland standard. A ten-fold dilution series was prepared from  $1.5 \times 10^8$ – $10^0$  colony-forming units (cfu)/mL and a standard volume (100 µL) of each dilution was inoculated on to each of the MRSA chromogenic agars

using a spiral plater (Don Whitley Scientific, Shipley, UK). This application was performed in triplicate for each isolate and plates were incubated as per the manufacturer's instructions. In each case, MRSA recovery was observed in accordance with the manufacturer's description of MRSA colony type, i.e. pink colonies on MRSA Select II, Colorex, and ChromID, or blue colonies on MRSA Brilliance 2. The LOD was recorded as the lowest bacterial density to give detectable growth on the chromogenic agar.

#### *Evaluation of chromogenic media using a diverse collection of S. aureus isolates*

The ability of the media to detect MRSA among a diverse collection of *S. aureus* isolates was also investigated. This included: (i) MRSA isolates representing 10/11 SCCmec types (I–VIII, X and XI); (ii) *mecA*-positive ( $n = 148$ ) and *mecC*-positive ( $n = 13$ ) MRSA isolates representative of a range of genotypes and comprising 149 MRSA isolates with oxacillin MICs ranging from 4 to >256 mg/L and 12 MRSA isolates with an oxacillin MIC  $\leq 2.0$  mg/L (range: 0.125–2.0 mg/L); (iii) 34 MSSA isolates that lacked *mec* genes and were susceptible to oxacillin (MIC range: 0.5–2.0 mg/L); (iv) 20 BORSA isolates which were *mec* negative but which exhibited oxacillin MICs between 4 and 8 mg/L (Supplementary Table I). In each case, isolates were suspended to a density equivalent to 0.5 McFarland standard. A 20  $\mu$ L volume was inoculated on to each of the four commercial MRSA chromogenic agars, and plates were incubated and read as above. Quality control testing was performed on each medium using *S. aureus* control strains ATCC43300 (MRSA) and ATCC25923 (MSSA).

#### *Evaluation of chromogenic media using patient samples*

The ability of the chromogenic media to detect MRSA directly from patient samples was investigated using 228 swabs recovered from the nose, throat, and groin of 76 inpatients at a 936-bed tertiary referral hospital in Dublin, Ireland. Specimens were collected as part of routine screening practices within the hospital. The 228 samples were initially inoculated on to MRSA Select (the earlier formulation of MRSA Select II) in accordance with the routine diagnostic procedures. The specimens were then inoculated on to the test chromogenic agars, changing the order in which the media were inoculated for each sample. All suspect colonies recovered from the screening swabs that were consistent with the manufacturer's description of MRSA were tested for oxacillin susceptibility by disc diffusion and investigated for the presence of *mec* and *nuc* genes using an in-house real-time polymerase chain reaction assay.<sup>15</sup>

#### *Statistical analysis*

The ability of each agar to correctly identify MRSA (sensitivity) and to exclude MSSA (specificity) was determined based on the number of correct results realized among the MRSA and MSSA isolates.

## Results

### *Limits of detection assay*

The results of the LOD evaluation for the four MRSA strains on the four different chromogenic agars tested are shown in Table I. The MRSA Select II, Colorex MRSA, and ChromID each yielded growth at a density of  $1.5 \times 10^1$  cfu/mL for each SCC*mec/mec* gene type investigated. Brilliance 2 also demonstrated growth at  $1.5 \times 10^1$  cfu/mL for the *mecC* MRSA but at a higher density ( $1.5 \times 10^4$  cfu/mL) for each of the three *mecA* MRSA strains tested. For each strain and each agar tested, the results from the three experiments were in agreement with each other.

### *Performance of the chromogenic media using a diverse collection of S. aureus isolates*

The MRSA strains representative of the different SCC*mec* types demonstrated good recovery with typical MRSA morphology on all four media tested. The performance data for the MRSA and MSSA isolates on the four chromogenic media are shown in Table II. For the 161 MRSA isolates, Colorex MRSA and ChromID MRSA were found to be 100% sensitive whereas MRSA Select II and MRSA Brilliance 2 demonstrated slightly lower sensitivity rates of 99% and 98%, respectively. MRSA Select II and MRSA Brilliance 2 failed to detect one *mecA*-positive MRSA isolate, exhibiting an oxacillin MIC of only 0.125 mg/L (*spa* type t2235), and Brilliance 2 failed to detect an additional two MRSA isolates, exhibiting susceptible MICs of 1.0 and 2.0 mg/L (*spa* types t002 and t3500, respectively). When compared with the predominant ST22-MRSA-IV *mecA* strain, *mecC* isolates included in this study yielded colonies equivalent in number and morphology on three of the four chromogenic agars tested and yielded a greater number of colonies on the Brilliance 2 agar.

When tested with the 34 MSSA isolates, the specificity of the four chromogenic agars ranged between 73% and 85% (Table II). The MRSA Select II exhibited the highest number of false-positive results (9 out of 25) whereas other chromogenic agars yielded five or six false positives (Table III). All isolates that generated false positives on the chromogenic agars exhibited oxacillin susceptibilities representative of the range of oxacillin MIC values in the MSSA collection tested and belonged to a range of genotypes (Table III).

As expected, due to their higher oxacillin MICs (4–8 mg/L), the BORSA isolates also proved challenging for the chromogenic agars. Once again MRSA Select II exhibited the highest number of false-positive results, with 13 out of 20 BORSA isolates yielding suspect MRSA colonies. The other three chromogenic agars also produced a high number of false positives with Brilliance 2 and Colorex demonstrating growth for 11 of the BORSA whereas eight were recovered on the ChromID (Table III).

*Performance of the chromogenic media using patient samples*

Of the 228 swabs investigated from 76 patients, six swabs from four patients were positive for MRSA (one patient nose and groin swab positive; two patients groin swab only positive; one patient throat swab only positive). These results were in agreement with the clinical microbiology laboratory results and were detected with all four chromogenic agars, with suspect colonies growing in sufficient numbers and with typical colony morphology that allowed ready detection despite the high number of plates inoculated. All isolates were confirmed as oxacillin resistant and carried the *mecA* gene.

**Discussion**

Although the decreasing level of invasive healthcare-associated MRSA infections in Europe and the USA is encouraging, the changing epidemiology of this pathogen and the emergence of virulent strains in the community require rapid and sensitive laboratory detection.

For the detection of MRSA all media performed well in the evaluation. With the exception of MRSA Brilliance 2, which demonstrated growth at a higher density (15,000 cfu/mL) for the three *mecA* MRSA, the other three chromogenic agars – MRSA Select II, Colorex MRSA, and ChromID – recovered all strains (*mecA* and *mecC* MRSA) when challenged with lower densities at 15 cfu/mL. Previously reported studies of *mecC* isolates suggested that difficulties may arise in the laboratory detection of *mecC*-positive MRSA isolates due to the low oxacillin MIC exhibited by some of these strains.<sup>9,16,17</sup> However, the *mecC* isolates included in this study grew well on each of the chromogenic media investigated, and showed even improved recovery on MRSA Brilliance 2 compared to *mecA*- and healthcare-associated MRSA strains, e.g. ST22-MRSA-IV. The underlying reasons for this disparity between *mecA* and *mecC* MRSA strains on Brilliance 2 agar are unclear but may reflect differential interactions between the *mec* gene products and constituents of the medium.<sup>18</sup> This requires further study.

The chromogenic agars demonstrated excellent sensitivity ranging from 98% to 100%, with Colorex media and ChromID detecting the full collection of MRSA isolates investigated. The high sensitivities recorded here are in agreement with other MRSA chromogenic agar evaluation studies.<sup>11–13</sup> However, a challenge arose for the MRSA Select II and MRSA Brilliance 2 media: each failed to detect a small number of MRSA isolates (one and three isolates, respectively) exhibiting susceptible oxacillin MICs. A small increase in the prevalence of oxacillin-susceptible *mecA*-positive isolates has been previously reported elsewhere and similar isolates have been recovered in the National MRSA Reference

Laboratory in Ireland.<sup>5</sup> As these strains can cause problems in the routine diagnostic laboratory, their successful recovery using the chromogenic media is essential.

When challenged with MSSA isolates the chromogenic agars demonstrated reduced levels of specificity, ranging from 73% to 85%. This specificity rate is low in comparison to other studies where specificity rates of 95–99% have been reported for chromogenic agars.<sup>11–13</sup> However, these other studies confined investigations to clinical specimens only, whereas the current study included a diverse collection of MSSA strains. The recovery of these MSSA strains as presumptive MRSA isolates has implications for the diagnostic laboratory in terms of increasing the turnaround time and resource management.<sup>11</sup>

The reduced specificity of the chromogenic agars was once again demonstrated by the high recovery of BORSA with almost 50% of isolates being recovered on all four of the media. This high rate of false positivity can be expected with the BORSA isolates due to their high oxacillin MICs. Although a previous epidemiological investigation of BORSA isolates indicated that they are not implicated in patient-to-patient spread, their growth on this medium further complicates the detection, prevention, and control of genuine MRSA in the healthcare environment.<sup>19</sup>

Despite the investigation of a relatively large number of patient samples (nose, throat, and groin from 76 patients, i.e. 228 samples) the rate of detection of MRSA was low (5.6%). However, this was similar to previous studies of patient samples from hospitalized patients in Ireland which showed MRSA prevalence rates of 8.5% and 7.6%.<sup>20,21</sup> Additionally, the results correlated with the clinical microbiology laboratory results for these samples, indicating that no false positives or false negatives were detected using any of the four chromogenic agars.

The early identification of MRSA and implementation of infection prevention and control procedures has been shown to reduce healthcare-associated infections. The high sensitivity of the chromogenic agars evaluated in this study confirms the usefulness of this medium in the one-step detection and presumptive identification of MRSA. Whereas the recovery of a high number of MSSA and BORSA isolates is a concern, the ability of the medium to recover almost all MRSA, including oxacillin-susceptible and *mecC*-positive strains, ensures appropriate management and treatment for MRSA-positive patients.

### **Acknowledgements**

We thank Serosep for providing MRSA SelectII media at no cost; Dr R. Skov, the Statens Institute, Denmark, for *mecC*-positive MRSA strains; and Dr S. Monecke, Alere Technologies, Germany, for providing BORSA strains for inclusion in the evaluation. We

also thank the staff of the Irish National MRSA Reference Laboratory for their support with this project and for the routine processing of isolates.

**Conflict of interest statement**

None declared.

**Funding source**

This study was supported by the Microbiology Research Unit, Dublin Dental University Hospital.

**References**

1. Shore AC, Coleman DC. Staphylococcal cassette chromosome *mec*: recent advances and new insights. *Int J Med Microbiol* 2013;**303**:350–359.
2. Kinnevey PM, Shore AC, Brennan GI, *et al.* Extensive genetic diversity identified among sporadic methicillin-resistant *Staphylococcus aureus* isolates recovered in Irish hospitals between 2000 and 2012. *Antimicrob Agents Chemother* 2014;**58**:1907–1917.
3. Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC. Pantone–Valentine leukocidin-positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant *S. aureus* clones, and increasing multiresistance. *J Clin Microbiol* 2014;**52**:859–870.
4. Grundmann H, Schouls LM, Aanensen DM, *et al.* The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: results of a second structured survey. *Eurosurveillance* 2014;**19**:1–10.
5. Kumar VA, Steffy K, Chatterjee M, *et al.* Detection of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* isolates by use of chromogenic medium MRSA ID. *J Clin Microbiol* 2013;**51**:318–319.
6. Livermore DM. beta-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995;**8**:557–584.
7. Shore AC, Deasy EC, Slickers P, *et al.* Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecR1*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2011;**55**:3765–3773.
8. Paterson GK, Larsen AR, Robb A, *et al.* The newly described *mecA* homologue, *mecA<sub>LGA251</sub>*, is present in methicillin-resistant *Staphylococcus aureus* isolates from a diverse range of host species. *J Antimicrob Chemother* 2012;**67**:2809–2813.



9. Paterson GK, Harrison EM, Holmes M. The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*. *Trends Microbiol* 2014;**22**:42–47.
10. Cunningham R, Jenks P, Northwood J, Wallis M, Ferguson S, Hunt S. Effect on MRSA transmission of rapid PCR testing of patients admitted to critical care. *J Hosp Infect* 2007;**65**:24–28.
11. Morris K, Wilson C, Wilcox MH. Evaluation of chromogenic methicillin-resistant *Staphylococcus aureus* media: sensitivity versus turnaround time. *J Hosp Infect* 2012;**81**:20–24.
12. Denys GA, Renzi PB, Koch KM, Wissel CM. Three-way comparison of BBL CHROMagar MRSA II, MRSASelect, and spectra MRSA for detection of methicillin-resistant *Staphylococcus aureus* isolates in nasal surveillance cultures. *J Clin Microbiol* 2013;**51**:202–205.
13. Veenemans J, Verhulst C, Punselie R, Van Keulen PHJ, Kluytmans JAJW. Evaluation of brilliance MRSA 2 agar for detection of methicillin-resistant *Staphylococcus aureus* in clinical samples. *J Clin Microbiol* 2013;**51**:1026–1027.
14. Malhotra-Kumar S, Abrahantes JC, Sabiiti W, *et al.* Evaluation of chromogenic media for detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2010;**48**:1040–1046.
15. European Committee for Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0. *Break tables Interpret MICs Zone diameters Version 4.0*. 2013. Available at: <http://www.eucast.org> [last accessed October 2015].
16. Skov R, Larsen AR, Kearns A, *et al.* Phenotypic detection of *mecC*-MRSA: cefoxitin is more reliable than oxacillin. *J Antimicrob Chemother* 2014;**69**:133–135.
17. Cartwright EJP, Paterson GK, Raven KE, *et al.* Use of Vitek 2 antimicrobial susceptibility profile to identify *mecC* in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2013;**51**:2732–2734.
18. Kim C, Milheiriço C, Gardete S, *et al.* Properties of a novel PBP2A protein homolog from *Staphylococcus aureus* strain LGA251 and its contribution to the  $\beta$ -lactam-resistant phenotype. *J Biol Chem* 2012;**287**:36854–36863.
19. Leahy TR, Yau YCW, Atenafu E, Corey M, Ratjen F, Waters V. Epidemiology of borderline oxacillin-resistant *Staphylococcus aureus* in pediatric cystic fibrosis. *Pediatr Pulmonol* 2011;**46**:489–496.

20. Rajan L, Smyth E, Humphreys H. Screening for MRSA in ICU patients. How does PCR compare with culture? *J Infect* 2007;**55**:353–357.
21. Rossney AS, Herra CM, Brennan GI, Morgan PM, O’Connell B. Evaluation of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the GeneXpert real-time PCR platform for rapid detection of MRSA from screening specimens. *J Clin Microbiol* 2008;**46**:3285–3290.
22. Shore A, Rossney AS, Keane CT, Enright MC, Coleman DC. Seven novel variants of the staphylococcal chromosomal cassette *mec* in methicillin-resistant *Staphylococcus aureus* isolates from Ireland. *Antimicrob Agents Chemother* 2005;**49**:2070–2083.
23. Ma XX, Ito T, Tiensasitorn C, *et al.* Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2002;**46**:1147–1152.
24. Ito T, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome. *Antimicrob Agents Chemother* 2004;**48**:2637–2651.

**Table I**

Limits of detection of MRSA isolates representative of four SCC*mec* types as determined using four chromogenic media

Isolate no.	Genotype	<i>mec</i> gene	Lowest bacterial density (cfu/mL) at which growth was recorded <sup>a</sup>				Reference
			MRSA Select II	ChromID	Colorex	MRSA Brilliance 2	
AR07.4/0 237	ST5- MRSA-II	<i>mecA</i>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>4</sup>	22
CA05	ST22- MRSA-IV	<i>mecA</i>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>4</sup>	23
WIS	ST8- MRSA-V	<i>mecA</i>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>4</sup>	24
M10/0061	ST130- MRSA-XI	<i>mecC</i>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	1.5×10 <sup>1</sup>	7

MRSA, methicillin-resistant *Staphylococcus aureus*.

<sup>a</sup>The limit of detection was recorded as the lowest bacterial density to give detectable growth on chromogenic media.

**Table II**

Sensitivity and specificity of the four chromogenic media using the MRSA and MSSA isolates

Variable	MRSA SelectII	ChromID	Colorex	Brilliance2
Sensitivity	99%	100%	100%	98%
Specificity	73%	85%	85%	82%

MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-susceptible *Staphylococcus aureus*.

**Table III**

Genotypes and oxacillin MIC values of *mecA*- and *mecC*-negative MSSA and BORSA isolates yielding false-positive results using the four chromogenic media

Isolate no.	Genotype <sup>a</sup>	Oxacillin MIC (mg/L)	MRSA Select II	ChromID	Colorex	MRSA Brilliance 2
<b>MSSA</b>						
M11/0175	t306	2	pos	pos	pos	pos
M12/0147	ST80-t088	0.5	pos	pos	pos	neg
M12/0272	ST7-t091	0.5	pos	neg	neg	neg
M14/0220	ST45/46-t065	2	pos	neg	neg	neg
M14/0248	t608	0.5	pos	pos	pos	pos
M14/0249	ST1-t127	0.5	pos	pos	pos	pos
M14/0250	t2828	1	pos	neg	neg	neg
M14/0258	ST8-t008	0.5	pos	neg	neg	neg
M14/0366	ND	2	pos	pos	pos	pos
M11/0281	t11018	0.5	neg	neg	neg	pos
M14/0254	ST8-t008	1	neg	neg	neg	pos
Total			9	5	5	6
<b>BORSA</b>						
M05/0294	ND	4	pos	neg	neg	neg
M07/0138	ND	4	pos	pos	pos	pos
M07/0376	ND	4	neg	neg	pos	neg
M07/0377	ND	4	neg	neg	pos	neg
M08/0079	ND	8	pos	pos	pos	pos
M12/0306	ST8-t008	8	pos	neg	neg	pos
M12/0355	ST8-t008	8	pos	neg	pos	pos
M13/0626	t078	4	pos	neg	neg	neg
M13/0629	ND	4	pos	pos	pos	pos
M14/0178	ST9-t100	4	pos	pos	pos	pos
M14/0179	ST9-t100	4	pos	neg	neg	pos
M14/0188	ST7-t091	8	pos	pos	pos	pos
M14/0260	ND	4	pos	pos	pos	pos
M14/0282	ST15/18-t084	4	pos	neg	neg	neg

M14/0604	ST398-t571	8	pos	pos	pos	pos
M14/0784	ND	4	neg	pos	pos	pos
Total			13	8	8	11

MIC, minimum inhibitory concentration; MSSA, meticillin-susceptible *S. aureus*; BORSA, borderline-oxacillin resistant *S. aureus*; MRSA, meticillin-resistant *Staphylococcus aureus*; pos; positive, neg; negative; ND, not determined.

<sup>a</sup>Where available genotypes are indicated by the multi-locus sequence type (prefix ‘ST’) and/or the *spa* type (prefix ‘t’). The STs were inferred from the *spa* type using the Ridom *spa* server (<http://www.spaserver.ridom.de/>) and/or based on previous experience at the Irish National MRSA Reference Laboratory (NMRSARL).

**Supplementary Table I***Staphylococcus aureus* isolates used for the evaluation of the chromogenic media

Meticillin resistance phenotype	Genotype (n) <sup>a</sup>	<i>spa</i> types <sup>a</sup>	Reference <sup>a</sup>
MRSA	ST1 (5)	t2246, t8698, t127	This study
	ST5-II (1)	ND	Shore <i>et al.</i> <sup>1</sup>
	ST5-IV (15)	t045, t1340, t002, t311, t688,	This study
	ST5-VI (1)	ND	Oliveira <i>et al.</i> <sup>2</sup>
	ST5-VII (1)	ND	Berglund <i>et al.</i> <sup>3</sup>
	ST8-IV (7)	t008, t064	This study
	ST8-V (1)	ND	Ito <i>et al.</i> <sup>4</sup>
	ST8-VIII (1)	ND	Zhang <i>et al.</i> <sup>5</sup>
	ST22-IV (90)	t513, t849, t032, t852, t022, t2235	This study
	ST30-IV (2)	t012	This study
	ST45-IV (6)	t620, t344, t727, t065, t230	This study
	ST39-II (1)	t007	This study
	ST53-IV (1)	t4545	This study
	ST59 (3)	t437	This study
	ST59-V (1)	t316	This study
	ST80 (3)	t044, t088	This study
	ST88-IV (2)	t2622, t692	This study
	CC130-XI (13)	t1535, t978, t2345, t3218, t1736, t3570, t3391, t3256, t843, t8835, t267, t12399, t843, t373, t355	Shore <i>et al.</i> <sup>6</sup> Petersen <i>et al.</i> <sup>7</sup>
	ST246-IV (1)	ND	Ma <i>et al.</i> <sup>8</sup>
	ST250/247 (1)	t051	This study

Meticillin resistance phenotype	Genotype (n) <sup>a</sup>	<i>spa</i> types <sup>a</sup>	Reference <sup>a</sup>
MSSA	ST250-I (1)	ND	Shore <i>et al.</i> <sup>1</sup>
	ST398-V (3)	t011	This study Kinnevey <i>et al.</i> <sup>9</sup>
	ST398-X (1)	ND	Li <i>et al.</i> <sup>10</sup>
	ST772-V (3)	t657	Brennan <i>et al.</i> <sup>11</sup>
	ST779 (1)	t878	This study
	ST981-III (1)	ND	Shore <i>et al.</i> <sup>12</sup>
	ND (4)	t671, t6419, t2277, t579	This study
	ST1 (3)	t127	This study
	ST7 (1)	t091	This study
	ST8 (12)	t008, t190, t024	This study
	ST15 (1)	t279	This study
	ST20 (1)	t164	This study
	ST22 (2)	t005, t8281	This study
	ST45/46 (1)	t065	This study
BORSA	ST80 (1)	t088	This study
	ST2229 (1)	t701	This study
	ND (10)	t2365, t306, t1778, t334, t11018, t2658	This study
	ND (1)	ND	This study
	ST7 (1)	t091	This study
	ST8 (3)	t008	This study
	ST9 (1)	t100	This study
	ST15 (1)	t084	This study
	ST398 (1)	t571	This study
	ND (2)	t164, t9222	This study
ND (11)	ND	This study	



MRSA, meticillin-resistant *S. aureus*; MSSA, meticillin-susceptible *S. aureus*;  
BORSA, boarderline oxacillin-resistant *S. aureus*; ND, not determined.

<sup>a</sup>Isolates that were not part of previously published studies were identified as *S. aureus* and underwent susceptibility testing as described previously as part of routine work at the Irish National MRSA Reference Laboratory (NMRSARL).<sup>6</sup> The genotypes i.e. the MLST clonal complex (CC) or sequence type (ST) and, for MRSA, the SCC*mec* types (indicated with a Roman Numeral), were either identified as described in the previous publications or, where possible, were inferred from the *spa* type and/or the antibiogram-resistogram pattern based on previous experience at the NMRSARL. The presence or absence of *mecA* and *mecC* were determined as described in the previous publications or using an in-house polymerase chain reaction assay. The CC130-MRSA isolates carried *mecC* and the remaining MRSA isolates carried *mecA*.

1. Shore A, Rossney AS, Keane CT, Enright MC, Coleman DC. Seven novel variants of the staphylococcal chromosomal cassette *mec* in methicillin-resistant *Staphylococcus aureus* isolates from Ireland. *Antimicrob Agents Chemother* 2005;**49**:2070–2083.
2. Oliveira DC, Milheiriço C, De Lencastre H. Redefining a structural variant of staphylococcal cassette Chromosome *mec*, SCC*mec* type VI. *Antimicrob Agents Chemother* 2006;**50**:3457–3459.
3. Berglund C, Ito T, Ikeda M, Xiao XM, Söderquist B, Hiramatsu K. Novel type of staphylococcal cassette chromosome *mec* in a methicillin-resistant *Staphylococcus aureus* strain isolated in Sweden. *Antimicrob Agents Chemother* 2008;**52**:3512–3516.
4. Ito T, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome. *Antimicrob Agents Chemother* 2004;**48**:2637–2651.
5. Zhang K, McClure JA, Elsayed S, Conly JM. Novel staphylococcal cassette chromosome *mec* type, tentatively designated type VIII, harboring class A *mec* and type 4 *ccr* gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009;**53**:531–540.
6. Shore AC, Deasy EC, Slickers P, *et al.* Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA*, *mecI*, *mecRI*, *blaZ*, and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-

- resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2011;**55**:3765-3773.
7. Petersen A, Stegger M, Heltberg O, *et al*. Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mecC* gene in Denmark corroborates a zoonotic reservoir with transmission to humans. *Clin Microbiol Infect* 2013;**19**:E16–E22.
  8. Ma XX, Ito T, Tiensasitorn C, *et al*. Novel type of staphylococcal cassette chromosome *mec* identified in *Staphylococcus aureus* strains novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2002;**46**:1147–1152.
  9. Kinnevey PM, Shore AC, Brennan GI, *et al*. Extensive genetic diversity identified among sporadic methicillin-resistant *Staphylococcus aureus* isolates recovered in Irish hospitals between 2000 and 2012. *Antimicrob Agents Chemother* 2014;**58**:1907–1917.
  10. Li S, Skov RL, Han X, *et al*. Novel types of staphylococcal cassette chromosome *mec* elements identified in clonal complex 398 methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2011;**55**:3046–3050.
  11. Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O’Connell B. Emergence of hospital- and community-associated Panton–Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. *J Clin Microbiol* 2012;**50**:841–847.
  12. Shore AC, Rossney AS, O’Connell B, *et al*. Detection of staphylococcal cassette chromosome *mec*-associated DNA segments in multiresistant methicillin-susceptible *Staphylococcus aureus* (MSSA) and identification of *Staphylococcus epidermidis ccrAB4* in both methicillin-resistant *S. aureus* and MSSA. *Antimicrob Agents Chemother* 2008;**52**:4407–4419.