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

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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 1.5×10^1 cfu/mL for each SCC*mec* type investigated. Brilliance 2 demonstrated growth at 1.5×10^1 cfu/mL for *mecC* MRSA but at a higher density (1.5×10^4 cfu/mL) for the three *mecA* MRSA strains. All four media demonstrated excellent sensitivity for MRSA detection (≥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 *mec*C-positive strains) confirm the value of chromogenic agar in MRSA detection.

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

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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.[1]

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.[2,3] In Ireland, MRSA is endemic in hospitals, and, as in many countries throughout Europe, the sequence type-SCC*mec* ST22-MRSA-IV clone predominates.[4] 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.[2,3]

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.[1] However, few MRSA isolates express homogeneous oxacillin resistance. Oxacillin-susceptible *mecA*-positive *S. aureus* isolates have been reported worldwide.[5] Similarly, low-level oxacillin-resistant *mecA*-negative strains known as borderline oxacillin-resistant *S. aureus* (BORSA) isolates have further complicated the definition of MRSA.[6]

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.[7–9]

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.[10,11] 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.[11–14]

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.[2,3,7] 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–VIII, 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 1.5×10^8–10^0 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 SCC*mec* types

Table I
Limits of detection of MRSA isolates representative of four *SCCmec* types as determined using four chromogenic media

Isolate no.	Genotype	*mec* gene	Lowest bacterial density (cfu/mL) at which growth was recorded[a]				Reference
			MRSA Select II	ChromID	Colorex	MRSA Brilliance 2	
AR07.4/0237	ST5-MRSA-II	*mecA*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^4	16
CA05	ST22-MRSA-IV	*mecA*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^4	17
WIS	ST8-MRSA-V	*mecA*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^4	18
M10/0061	ST130-MRSA-XI	*mecC*	1.5×10^1	1.5×10^1	1.5×10^1	1.5×10^1	7

MRSA, meticillin-resistant *Staphylococcus aureus*.
[a] The limit of detection was recorded as the lowest bacterial density to give detectable growth on chromogenic media.

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(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 ≤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 μL 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.[15]

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 1.5×10^1 cfu/mL for each SCC*mec*/*mec* gene type investigated. Brilliance 2 also demonstrated growth at 1.5×10^1 cfu/mL for the *mecC* MRSA but at a higher density (1.5×10^4 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.

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

Variable	MRSA Select II	ChromID	Colorex	Brilliance 2
Sensitivity	99%	100%	100%	98%
Specificity	73%	85%	85%	82%

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

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

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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[a]	Oxacillin MIC (mg/L)	MRSA Select II	ChromID	Colorex	MRSA Brilliance 2
MSSA						
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
BORSA						
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.

[a] 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).

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.[9,19,20] 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.[21] 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.[11–13] 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.[5] 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

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85%. This specificity rate is low in comparison to other studies where specificity rates of 95–99% have been reported for chromogenic agars.[11–13] 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.[11]

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.[22]

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%.[23,24] 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.

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Conflict of interest statement
None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jhin.2015.10.019.

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