Journal of Hospital Infection xxx (2015) 1–6

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

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

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ARTICLE INFO

Article history:
Received 7 August 2015
Accepted 27 October 2015
Available online xxx

Keywords:
Borderline oxacillin-resistant *S. aureus* chromogenic agar
mecC
Meticillin-resistant *Staphylococcus aureus*

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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http://dx.doi.org/10.1016/j.jhin.2015.10.019
0195-6701/

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.

Please cite this article in press as: Brennan GI, et al., Evaluation of commercial chromogenic media for the detection of meticillin-resistant *Staphylococcus aureus*, Journal of Hospital Infection (2015), http://dx.doi.org/10.1016/j.jhin.2015.10.019