Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-011-1265-3

ARTICLE

Performance evaluation of a modified chromogenic medium, ChromID MRSA New, for the detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens

F. Van Hoecke · N. Deloof · G. Claeys

Received: 27 December 2010 / Accepted: 2 April 2011

Abstract A novel chromogenic medium for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA), ChromID MRSA New, was evaluated and compared with the original ChromID MRSA agar, using 355 consecutive screening specimens from nose (120), throat (121) and perineum (114). The specimens were collected with an E-swab and inoculated within 24 hours onto both ChromID MRSA New and on ChromID MRSA. ChromID MRSA New was more sensitive than ChromID MRSA in detecting MRSA after 24 hours of incubation (94.3% versus 81.4%; $p < 0.05$). With the ChromID MRSA New, processing time is reduced from 48 h to 24 h and confirmation of the resistance to methicillin is redundant.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen and is becoming an increasingly frequent cause of community-acquired infections, causing significant morbidity and mortality [1]. The major sources are either the patient's own flora, causing an endogenous infection, acquisition from another person, causing a cross-infection, or from items recently contaminated by a human source, causing an infection from exogenous source [2, 3]. It is therefore recognized that screening of hospitalized patients to detect MRSA colonisation and subsequent isolation of carriers is a cost-effective infection control measure to reduce the risk of transmission [4, 5]. The screening results must therefore be readily available and reliable in terms of sensitivity and specificity for effective hospital infection control, as well as to reduce costs [3, 5]. Traditionally, MRSA screening includes mainly the culturing of nasal swabs. However, up to 35% of MRSA carriers may only be colonized from other sites, like throat or rectum [6]. Culture techniques using selective media including penicillinase-resistant penicillins oxacillin or methicillin to differentiate MRSA from methicillin-sensitive *S. aureus* (MSSA) are predominantly used [7]. However, cefoxitin, a cephamycin, shows better selectivity and is currently preferred for MRSA screening [2, 4, 5]. The use of broth culture enhancement may improve the yield of MRSA, but it is more laborious and may delay identification of the organism [4]. Although PCR-based methods have recently been developed for the direct detection of MRSA in specimens, these methods remain expensive and cannot be performed in every laboratory [8]. For these reasons, the use of chromogenic media has become a key method for the rapid identification of MRSA in clinical samples [3, 7]. These media detect key microbial enzymes as diagnostic markers for pathogens through the use of chromogenic substrates incorporated into a solid-agar-based matrix [3]. The objective of this prospective study with matched design was to evaluate the modified version of ChromID MRSA, named ChromID MRSA New (bioMérieux, Marcy l'Etoile, France), and to compare this new chromogenic medium with the current version of the ChromID MRSA (bioMérieux) in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

F. Van Hoecke · N. Deloof · G. Claeys
Department of Clinical Chemistry,
Microbiology and Immunology, Ghent University Hospital,
Ghent, Belgium

F. Van Hoecke (✉)
Clinical Biology, University Hospital Ghent – 2P8,
De Pintelaan 185,
9000 Ghent, Belgium
e-mail: Frederik.VanHoecke@Ugent.be

Published online: 16 April 2011

Material and methods

A total of 355 MRSA screening samples from 128 newly admitted patients with a high-risk profile or from follow-up patients previously detected as MRSA-carrier were used to evaluate the MRSA colonisation status. All 355 consecutive samples coming from those patients from Sunday till Thursday were incorporated in the study. Samples of the nose (120), throat (121) and perineum (114) were collected with the liquid amies E-swab® system (Copan, Brescia, Italy) and processed separately. Although from most patients three specimens from different sources were taken, from some patients only one or two samples were taken.

The E-swab was stored after collection and before inoculation in its transport medium at room temperature or at 4–8°C. There was no enrichment. Each specimen was inoculated in parallel onto the two chromogenic media by plating 100 μl sample broth onto the agars with an automated volumetric pipette and spread out onto the agar using the streak dilution method. This was done in batch within 24 hours after collection. The chromogenic media evaluated were ChromID MRSA (bioMérieux) and the modified version of the ChromID MRSA, named ChromID MRSA New (bioMérieux).

The ChromID MRSA was interpreted after 24±1 and 48±1 hours while the ChromID MRSA New was interpreted after 18±1 and 24±1 hours of incubation, at 37°C in ambient air, in agreement with the manufacturer's specifications.

On the ChromID MRSA New the colony appearances considered suspicious were tiny white with a green shine (TWG), pale blue-green (PBG), blue green (BG) or dark blue-green (DBG) after 18 hours of incubation and/or BG or DBG after 24 hours of incubation. On the ChromID MRSA the colonies considered suspicious were pale green (PG), green (G) or dark green (DG) after 24 hours of incubation and/or G or DG after 48 hours. Suspicious colonies were confirmed as *S. aureus* with a latex agglutination test (Pastorex Staph plus, Bio-Rad, Redmond, Washington). Resistance to methicillin was confirmed by disk diffusion on BBL® Mueller-Hinton II agar (BD, Franklin Lakes, NJ, USA), using a 30-μg cefoxitin paper disk (Oxoid, Cambridge, UK) [9].

If both requirements were met, namely, positive agglutination and resistance to cefoxitin, the species was considered MRSA. This strategy was used for suspicious colonies on both chromogenic agars. When confirmation tests showed that the suspicious colony was no MRSA, the medium was considered false positive. When confirmation tests showed an MRSA only on one type of agar, the sample was considered positive, the negative medium was considered false negative and the positive medium was considered true positive. No growth or the absence of

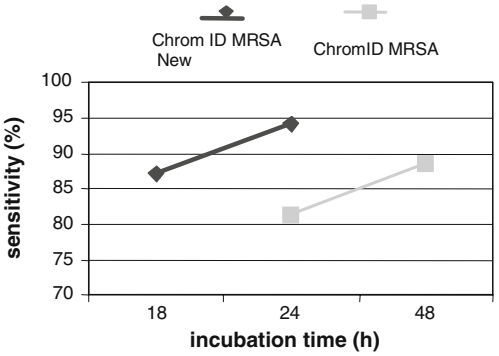

Fig. 1 Sensitivity for methicillin-resistant *Staphylococcus aureus* (MRSA) detection at different incubation intervals on the ChromID MRSA and the ChromID MRSA New

Table 1 Contingency tables for ChromID methicillin-resistant *Staphylococcus aureus* (MRSA) after 24 hours of incubation and 48 hours of incubation

Medium	Incubation	Positive (+) / negative (-)	ChromID MRSA 24 h		
			+	–	Total
ChromID MRSA New	18 h	+	53	8	61
		–	4	5	9
		Total	57	13	70
	24 h	+	55	11	66
		–	2	2	4
		Total	57	13	70
Medium	Incubation	Positive (+) / negative (-)	ChromID MRSA 48 h		
			+	–	Total
ChromID MRSA New	18 h	+	55	6	61
		–	7	2	9
		Total	62	8	70
	24 h	+	58	8	66
		–	4	0	4
		Total	62	8	70

suspicious colonies were considered negative. No samples were re-tested in case of discrepancy between the two media.

Quality control testing was successfully performed on each lot of chromogenic media before and during the use in the study using a standardized inoculum of *S. aureus* ATCC 43300 and *S. aureus* ATCC 29213.

For the sensitivity and specificity analysis only results of the first MRSA identification was used. The calculation and interpretation of sensitivities, specificities, PPV and NPV was done irrespective of the sample type. The statistics were calculated with the MacNemar's test.

Results

MRSA was detected in 70 samples (19.7%) from 37 patients (28.9%). Twenty-six nose samples (21.7%), 20 perineum samples (13.9%) and 24 throat samples (19.8%) were positive for MRSA. The sensitivity of the ChromID MRSA New was 87.1% and 94.3% after 18 h and 24 h of incubation, respectively. The sensitivity of the ChromID MRSA was 81.4% and 88.6% after 24 h and 48 h of incubation, respectively (Fig. 1). The sensitivity after 24 h of incubation was significantly higher for the ChromID MRSA New ($p<0.05$). No other statistically significant differences could be demonstrated (Table 1). The specificity was 94.7% and 95.4% for the ChromID MRSA New after respectively 18 h and 24 h of incubation. The specificity of the ChromID MRSA was 97.9% and 95.8% after, respectively, 24 h and 48 h of incubation. There were no statistically significant differences between the specificities of both media. False positive colonies could mostly be identified as enterobacteriaceae or yeasts. Each colony that could be identified as *S. aureus* was a MRSA. The PPV of ChromID MRSA New was 80.3% and 83.5% after, respectively, 18 h and 24 h of incubation. The PPV of ChromID MRSA was 90.5% and 83.8% after, respectively, 24 h and 48 h of incubation. There were no statistically significant differences between the PPVs. The NPV of ChromID MRSA New was 96.8% and 98.6% after, respectively, 18 h and 24 h of incubation. The NPV of the chromID MRSA was 95.6% and 97.2% after, respectively, 24 h and 48 h of incubation. There were no statistically significant differences between the NPVs.

Discussion

The ChromID MRSA New performs with a significantly higher sensitivity compared with the ChromID MRSA after 24 hours of incubation. An incubation time of 24 hours is sufficient to detect or exclude the presence of MRSA in the sample, resulting in a significantly shorter processing time. The specificity is acceptable with enterobacteriaceae and yeasts being the most frequent cause of a false positive result. Therefore, identification of suspicious colonies such as *S. aureus*, which can easily be done with an agglutination test, remains necessary. However, confirmation of methicillin-resistance of *S. aureus* isolates is unnecessary. The learning time of the technicians for a correct interpretation of the colony morphologies is comparable to that of the original ChromID MRSA. The prescribed incubation time of the modified agar has to be respected because the colony morphology of both MRSA and non-MRSA tends to become less characteristic if the incubation time exceeds 24 hours.

The shorter time to detection and the possibility to omit methicillin-resistance confirmation are two major advantages of the ChromID MRSA New, making it possible to confirm or to exclude MRSA within 24 hours.

Acknowledgments We thank the team of hospital hygiene of Ghent University Hospital for their efforts.

Transparency declaration We acknowledge the support of bioMérieux by supplying the modified ChromID MRSA, the ChromID MRSA New, and for the financial support which made this study possible.

Conflicts of interest Nothing to declare.

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