Comparison of four chromogenic media for culture-based screening of meticillin-resistant *Staphylococcus aureus*

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Meticillin-resistant *Staphylococcus aureus* (MRSA) are responsible for nosocomial and community-acquired infections. Detection of MRSA is of utmost importance for the adaptation of infection control and therapeutic strategies. To date, selective agar plates constitute the standard routine method for reliable detection of this worldwide infectious problem. The performance of four different chromogenic media was evaluated in this study for MRSA detection and identification on >240 consecutive swab samples. The results indicate that primary plating on MRSASelect or MRSA ID is more sensitive than screening with oxacillin-based culture media. In addition, the utilization of cefoxitin- or cephamycin-containing plates reduces significantly the number of required confirmatory tests. Several selective agar plates allowing the identification of *S. aureus* are commercially available. However, their respective performances under real conditions of utilization are heterogeneous, underlining the absence of a gold standard medium for MRSA screening.

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INTRODUCTION

Staphylococcus aureus is one of the most commonly identified pathogens in human medicine and is a major cause of nosocomial infections (Boyce *et al.*, 1983) and community-acquired infections (Naimi *et al.*, 2003; Said-Salim *et al.*, 2003). Resistance to meticillin, reported for the first time in 1961 (Barber, 1961), is now widespread in hospitals all over the world (Doebbeling, 1995), and recently decreased susceptibility to vancomycin was reported (Kuroda *et al.*, 2001; Samra *et al.*, 2005). The rapid and reliable identification of meticillin-resistant *S. aureus* (MRSA) appears nowadays to be essential for appropriate patient care, control of strain spreading and use of adapted empirical antimicrobial guidelines. Active surveillance for MRSA revealed an efficient and recommended strategy to control nosocomial MRSA infections (Arnold *et al.*, 2002; Boyce, 2001), but requires robust and rapid identification. To date, several molecular assays have been developed and evaluated (Cuny & Witte, 2005; Francois *et al.*, 2003; Huletsky *et al.*, 2004; Warren *et al.*, 2004). Despite recent commercialization of some molecular methods, the standard procedure used in routine laboratories for reliable MRSA identification remains based on cultures using selective agar media. Numerous reports

in the literature have described screening media for MRSA identification showing variable performance. Phenotypic methods based on oxacillin-containing medium are not satisfactory in terms of sensitivity and specificity, and are sensitive to incubation temperature (Apfalter *et al.*, 2002; Skov *et al.*, 2006), time (Blanc *et al.*, 2003; Simor *et al.*, 2001) and inoculum density (Smyth *et al.*, 2001). Therefore, MRSA identification using cefoxitin- or cephamycin-containing media appears more robust (Felten *et al.*, 2002; Skov *et al.*, 2003) and is now recommended by the CLSI (Clinical and Laboratory Standards Institute, 2005).

The purpose of our study was to compare the performance of four chromogenic media for the screening of MRSA. We evaluated oxacillin-resistance screening agar base (ORSAB; Oxoid); MRSA ID (bioMérieux); Chromogen oxacillin *S. aureus* (Axon Lab) and MRSASelect (Bio-Rad) on 247 consecutive swab samples collected in a tertiary university hospital. Our results show that the performances of these media vary in terms of sensitivity, and often warrant further confirmatory testing.

METHODS

Study population and specimens. Two hundred and forty-seven consecutive patients were screened for the presence of MRSA. For

Abbreviation: MRSA, meticillin-resistant *Staphylococcus aureus*.

Table 1. Sensitivity and specificity of chromogenic media for detecting MRSA isolates among 247 clinical specimens

Overall, 70 MRSA isolates were identified in our population, equating to 28 % prevalence.

Medium	No. of strains isolated at 24 h (and 48 h)	Relative sensitivity (%) at 24 h*	Relative sensitivity (%) at 48 h*	Specificity (%) at 24 h	Specificity (%) at 48 h
All†	70	–	100	–	100
ORSAB (Oxoid)	53 (61)	76	87	67	68
MRSA ID (bioMérieux)	53 (63)	76	90	97	95
Chromogen oxacillin <i>S. aureus</i> (Axon Lab)	1 (37)	1	53	5	80
MRSASelect (Bio-Rad)	54 (64)	77	91	96	79

*Relative sensitivity compared to MRSA identified in any of the four tested media.

†Total number of MRSA strains isolated.

each patient, nasal and inguinal swabs were obtained and subjected to analysis (Copan Italia).

Culture media. MRSASelect (Bio-Rad) is composed of a selective base medium containing a cephamycin derivative (proprietary formulation) and characterizes *S. aureus* colonies by a specific mauve colour. MRSA ID (bioMérieux) similarly combines chromogenic identification, based on the spontaneous green colouration produced by a specific enzyme of *S. aureus*, with the use of 4 mg cefoxitin l⁻¹. ORSAB (Oxoid) uses aniline blue to detect mannitol fermentation, resulting in intense blue colonies of *S. aureus*. The formula of the agar base is: 11.8 g peptone l⁻¹, 9.0 g yeast extract l⁻¹, 10.0 g mannitol l⁻¹, 55.0 g NaCl l⁻¹, 5.0 g LiCl l⁻¹, 0.2 g aniline blue l⁻¹ and 12.5 g agar l⁻¹. This agar base medium is supplemented with selective compounds to detect oxacillin resistance: 50 000 IU polymyxin B l⁻¹ and 2 mg oxacillin l⁻¹. Chromogen oxacillin *S. aureus* (Axon-Lab) is composed of a base medium characterizing *S. aureus* colonies by a specific pink-mauve colour. Colistin-Salt (CS) broth consists of a brain heart infusion broth supplemented with 10 mg colistin l⁻¹ and 25 g NaCl l⁻¹. MHO broth consists of Mueller–Hinton broth supplemented with 6 mg oxacillin l⁻¹ and 45 g NaCl l⁻¹.

Bacteriological procedures. Particular attention was paid to the design of this study. Each of the four technologists had to evaluate every tested media, to prevent user-related bias. To inoculate equivalent sample volumes on the different media, swabs were extracted with dedicated Eppendorf tubes (Swab Extraction Tube System; Roche), following the manufacturer's instructions. For each patient, collected fluids from the two swabs were pooled and supplemented with sterile NaCl (0.9 %) to a final volume of 250 µl. An identical volume of 50 µl was used to inoculate each of the four selective media. The remaining 50 µl were inoculated into CS broth as a final backup evaluation procedure. All cultures were incubated aerobically at 35 °C. For all samples showing negative results at 24 h on the four media, CS broth was used to re-inoculate another set of selective media, in order to evaluate the utility of an enrichment step before plating onto selective agar.

Identification of MRSA. Mauve colonies on MRSASelect, green colonies on MRSA ID, pink-mauve colonies on Chromogen oxacillin *S. aureus* and blue colonies on ORSAB were considered as presumptive MRSA isolates and subcultured onto Columbia blood agar. Any colony of the expected colour was considered as a positive result, to benefit from optimal assay sensitivity. Confirmation of MRSA isolates was achieved by a combination of tests, including growth in MHO broth, Staphychrom II (International Microbio) (Fonsale *et al.*, 2004) and a duplex quantitative PCR to evaluate the

presence in similar abundance of the *mecA* gene and that of the *femA* gene specific for *S. aureus* (Francois *et al.*, 2003).

RESULTS AND DISCUSSION

A total of 247 consecutive samples submitted to the bacteriology laboratory were screened for the presence of MRSA, each sample consisting of two separate swabs (nostril and groin). All samples were obtained from adults admitted to our institution: 50 % of samples originated from patients hospitalized in long-term healthcare facilities, 40 % from various medical wards, whereas the remaining 10 % were referred by the surgical intensive care unit. Pooled liquid obtained by swab centrifugation was equally inoculated on the four selective media and in a CS enrichment broth. From the 247 specimens tested in this study, a total of 70 MRSA strains were isolated by the combination of all the culture-based media (Table 1). MRSA prevalence was 28 % in our collection, encompassing samples collected during admission screening, reflex testing of neighbouring patients of MRSA carriers and decontamination controls. After 24 h of incubation, CS enrichment broth was subcultured onto the four media. Relative sensitivity was calculated based on positive cultures observed on any of the four media, directly, or including the use of CS broth enrichment (Table 1). When solid culture media were directly inoculated, relative sensitivities at 24 h were 76, 76, 1 and 77 %, and reached 87, 90, 53 and 91 % at 48 h, for ORSAB, MRSA ID, Chromogen oxacillin *S. aureus* and MRSASelect, respectively. When combined with the CS enrichment broth, the sensitivities achieved were 91, 94, 53 and 96 %, respectively. Thus, CS enrichment broth marginally contributed to improve screening sensitivity. The additional reporting delay imposed by its use revealed it was of questionable benefit, when considering the different media tested in this study.

To evaluate the discrimination provided by each medium and quantify its impact on the workload, we determined the number of subcultures required to assess MRSA

identification. Subcultures refer to the process of strain isolation and confirmation testing for any colony morphology of the expected colour that grows on selective medium. Specificity was determined according to the results of these confirmatory tests.

Two hundred and forty-one pink-mauve (positive) colonies that grew on Chromogen oxacillin *S. aureus* were subsequently identified as methicillin-resistant coagulase-negative staphylococci, reflecting insufficient concentrations of the selecting agent, oxacillin. The poor specificity of this medium precludes its use for the rapid detection of MRSA. ORSAB performed better than Chromogen oxacillin *S. aureus*, but was revealed as slightly less sensitive and much less specific than MRSA ID and MRSASelect. Values obtained in this study are in accordance with another evaluation (Apfalter *et al.*, 2002). ORSAB medium presents limitations for surveillance applications (Becker *et al.*, 2002) not only due to lower sensitivity, but also because some coagulase-negative staphylococci (mainly *Staphylococcus haemolyticus*, a frequent skin colonizer) appear blue (Becker *et al.*, 2002). Thus, utilization of this plate warrants confirmatory tests for MRSA identification and should rather be considered in high prevalence settings (Becker *et al.*, 2002; Simor *et al.*, 2001). Sensitivities of MRSA ID and MRSASelect were comparable and outperformed that of ORSAB, likely due to the substitution of oxacillin by a cephamycin (Perry *et al.*, 2004). The few MRSA strains that failed to be detected by MRSASelect and MRSA ID can be explained by shortcomings of selective media when inocula are very low. The good discrimination of green colonies on MRSA ID and mauve colonies on MRSASelect facilitated the early recognition of potential MRSA isolates. However, the evaluation of cost-benefit performances should also integrate the number of required subcultures. The number of subcultures at 24 and 48 h reached 118 and 51, 71 and 22, 170 and 71, and 66 and 51, for ORSAB, MRSA ID, Chromogen oxacillin *S. aureus* and MRSASelect, respectively. Interestingly, MRSA ID grew larger colonies at 24 h and maintained specificity >95 % after 48 h incubation, thus providing the best balance in terms of sensitivity and costs in our hands.

This study has several limitations. First, collecting fluids from the two swabs and supplementing them with sterile saline provides fair comparison of the media, but slightly differs from routine protocols when swabs are directly streaked on agar. Second, such results should be formally compared to other chromogenic assays, including ChromAgar/MRSA (ChromaAgar). This medium, where MRSA colonies appear mauve while other bacteria display different colours, has been extensively tested and shows appreciable sensitivity and specificity (Diederer *et al.*, 2005; Hedin & Fang, 2005; Kluytmans *et al.*, 2002), fulfilling routine laboratory needs in terms of rapidity and sensitivity (Flayhart *et al.*, 2005). Third, the selectivity of each chromogenic medium is based on a distinct and different biochemical feature of *S. aureus*. Thus, the performance of each medium depends also on the possession and

expression of the respective enzyme that is assessed by the chromogenic assay.

Taken together, our results indicate that primary plating on MRSASelect or MRSA ID is convenient and time-saving for the presumptive identification of MRSA in routine microbiology. Interpretation of colony colours is easy and false-positive detection is rare when plates are incubated for 48 h. Combined use of MRSASelect or MRSA ID with a confirmatory MRSA identification assay (e.g. Staphychrom II or *mecA* determination) appears optimal in terms of results. Such a strategy is now routinely implemented in our institution, in combination with our rapid molecular screening assay (Francois *et al.*, 2003), which is employed whenever carriage status has to be known within a few hours. In the present study, this rapid molecular assay allowed us to rapidly identify for confirmatory purpose the totality of MRSA isolates.

Conclusion

Despite similar principles of identification and equivalent reagent costs, selective agar-based media revealed great differences in terms of performance, affecting both specificity and sensitivity. Among the four commercial products tested in our study, MRSASelect and MRSA ID appeared superior but still require confirmatory testing to assess the identification of MRSA in clinical samples.

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REFERENCES

- Apfalter, P., Assadian, O., Kalczyk, A., Lindenmann, V., Makristathis, A., Mustafa, S., Rotter, M. & Hirschl, A. M. (2002). Performance of a new chromogenic oxacillin resistance screen medium (Oxoid) in the detection and presumptive identification of methicillin-resistant *Staphylococcus aureus*. *Diagn Microbiol Infect Dis* **44**, 209–211.
- Arnold, M. S., Dempsey, J. M., Fishman, M., McAuley, P. J., Tibert, C. & Vallande, N. C. (2002). The best hospital practices for controlling methicillin-resistant *Staphylococcus aureus*: on the cutting edge. *Infect Control Hosp Epidemiol* **23**, 69–76.
- Barber, M. (1961). Methicillin-resistant staphylococci. *J Clin Pathol* **14**, 385–393.
- Becker, A., Forster, D. H. & Kniehl, E. (2002). Oxacillin resistance screening agar base for detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **40**, 4400–4401.
- Blanc, D. S., Wenger, A. & Bille, J. (2003). Evaluation of a novel medium for screening specimens from hospitalized patients to detect methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **41**, 3499–3502.
- Boyce, J. M. (2001). MRSA patients: proven methods to treat colonization and infection. *J Hosp Infect* **48** (Suppl. A), S9–S14.

- Boyce, J. M., White, R. L. & Spruill, E. Y. (1983). Impact of methicillin-resistant *Staphylococcus aureus* on the incidence of nosocomial staphylococcal infections. *J Infect Dis* **148**, 763.
- Clinical and Laboratory Standards Institute (2005). *Performance Standards for Antimicrobial Susceptibility Testing*, 15th informational supplement, M100–S15. Wayne, PA: Clinical and Laboratory Standards Institute.
- Cuny, C. & Witte, W. (2005). PCR for the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains using a single primer pair specific for SCCmec elements and the neighbouring chromosome-borne *orfX*. *Clin Microbiol Infect* **11**, 834–837.
- Diederer, B., van Duijn, I., van Belkum, A., Willemse, P., van Keulen, P. & Kluytmans, J. (2005). Performance of CHROMagar MRSA medium for detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **43**, 1925–1927.
- Doebbeling, B. N. (1995). The epidemiology of methicillin-resistant *Staphylococcus aureus* colonisation and infection. *J Chemother* **7** (Suppl. 3), 99–103.
- Felten, A., Grandry, B., Lagrange, P. H. & Casin, I. (2002). Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. *J Clin Microbiol* **40**, 2766–2771.
- Flayhart, D., Hindler, J. F., Bruckner, D. A., Hall, G., Shrestha, R. K., Vogel, S. A., Richter, S. S., Howard, W., Walther, R. & Carroll, K. C. (2005). Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. *J Clin Microbiol* **43**, 5536–5540.
- Fonsale, N., Bes, M., Verdier, I., Carricajo, A., Ploton, C., Aubert, G., Etienne, J., Vandenesch, F. & Freydiere, A. M. (2004). Specific identification of *Staphylococcus aureus* by Staphychrom II, a rapid chromogenic staphylocoagulase test. *J Clin Microbiol* **42**, 1962–1964.
- Francois, P., Pittet, D., Bento, M., Pepey, B., Vaudaux, P., Lew, D. & Schrenzel, J. (2003). Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. *J Clin Microbiol* **41**, 254–260.
- Hedin, G. & Fang, H. (2005). Evaluation of two new chromogenic media, CHROMagar MRSA and *S. aureus* ID, for identifying *Staphylococcus aureus* and screening methicillin-resistant *S. aureus*. *J Clin Microbiol* **43**, 4242–4244.
- Huletsky, A., Giroux, R., Rossbach, V., Gagnon, M., Vaillancourt, M., Bernier, M., Gagnon, F., Truchon, K., Bastien, M. & other authors (2004). New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* **42**, 1875–1884.
- Kluytmans, J., van Griethuysen, A., Willemse, P. & van Keulen, P. (2002). Performance of CHROMagar selective medium and oxacillin resistance screening agar base for identifying *Staphylococcus aureus* and detecting methicillin resistance. *J Clin Microbiol* **40**, 2480–2482.
- Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., Cui, L., Oguchi, A. & Aoki, K. (2001). Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* **357**, 1225–1240.
- Naimi, T. S., LeDell, K. H., Como-Sabetti, K., Borchardt, S. M., Boxrud, D. J., Etienne, J., Johnson, S. K., Vandenesch, F., Fridkin, S. & other authors (2003). Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* **290**, 2976–2984.
- Perry, J. D., Davies, A., Butterworth, L. A., Hopley, A. L., Nicholson, A. & Gould, F. K. (2004). Development and evaluation of a chromogenic agar medium for methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **42**, 4519–4523.
- Said-Salim, B., Mathema, B. & Kreiswirth, B. N. (2003). Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging pathogen. *Infect Control Hosp Epidemiol* **24**, 451–455.
- Samra, Z., Ofer, O. & Shmueli, H. (2005). Susceptibility of methicillin-resistant *Staphylococcus aureus* to vancomycin, teicoplanin, linezolid, pristinamycin and other antibiotics. *Isr Med Assoc J* **7**, 148–150.
- Simor, A. E., Goodfellow, J., Louie, L. & Louie, M. (2001). Evaluation of new medium, oxacillin resistance screening agar base, for the detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens. *J Clin Microbiol* **39**, 3422.
- Skov, R., Smyth, R., Clausen, M., Larsen, A. R., Frimodt-Moller, N., Olsson-Liljequist, B. & Kahlmeter, G. (2003). Evaluation of a cefoxitin 30 µg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **52**, 204–207.
- Skov, R., Smyth, R., Larsen, A. R., Bolmstrom, A., Karlsson, A., Mills, K., Frimodt-Moller, N. & Kahlmeter, G. (2006). Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and Etest on Mueller-Hinton agar. *J Clin Microbiol* **44**, 4395–4399.
- Smyth, R. W., Kahlmeter, G., Olsson, L. B. & Hoffman, B. (2001). Methods for identifying methicillin resistance in *Staphylococcus aureus*. *J Hosp Infect* **48**, 103–107.
- Warren, D. K., Liao, R. S., Merz, L. R., Eveland, M. & Dunne, W. M., Jr (2004). Detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swab specimens by a real-time PCR assay. *J Clin Microbiol* **42**, 5578–5581.