Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-006-0144-9

BRIEF REPORT

J. Loulergue · C. de Gialluly · V. Morange · A. Holstein · N. van der Mee-Marquet · R. Quentin

Evaluation of a new chromogenic medium for isolation and presumptive identification of methicillin-resistant *Staphylococcus aureus* from human clinical specimens

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen, especially in intensive care units in which nasal carriage of MRSA by patients is associated with a high risk of MRSA bacteremia [1]. Early detection of the reservoir of resistant strains, so that appropriate isolation precautions can be taken, is essential for a successful infection control strategy [2]. Thus, an accurate test to screen patients for nasal MRSA colonization would be valuable. Several media have been used for screening nasal carriers of MRSA, but their sensitivity has been poor, especially for detecting non-multidrug resistant MRSA [3–6]. Recently, a new chromogenic and selective medium, CHROMagar MRSA (CHROMagar Microbiology, Paris, France), which is produced by adding a proprietary supplement containing a cephamycin antibiotic to dehydrated CHROMagar MRSA powder, has been tested on a collection of *S. aureus* strains [7]. This medium is sensitive and specific, but its ability to reliably identify MRSA directly from clinical samples remains unproven. Therefore, we tested this medium directly with samples from 831 nasal swabs collected from 321 patients.

Samples were suspended in 0.5 ml physiological (0.85%) saline for homogenization and three media were streaked for isolation in the following order: (a) on Trypticase soy agar supplemented with 5% horse blood (HB; bioMérieux, Marcy-l'Etoile, France); (b) on CHROMagar MRSA; and (c) on Mueller-Hinton agar supplemented with 10 mg/l of tobramycin (MHT; Becton Dickinson, Le Pont de Claix, France). Swabs were reloaded between distributions to each plate. All of the plates were examined after 24 and 48 h of incubation at 37°C. The putative MRSA colonies are recognized on CHROMagar MRSA by their characteristic mauve color. Each type of mauve colony appearing on CHROMagar MRSA and of colonies suspected to be staphylococcal on HB and MHT were identified using Gram stain, catalase test and positive agglutination with Pastorex Staph Plus (BioRad, Marnes-la-Coquette, France). Identity was confirmed using the API ID32 STAPH system (bioMérieux) and, if necessary, with a tube coagulase test. When different colony aspects were observed, each colony type was investigated. The number of colonies was noted when it was low (≤5).

Antimicrobial susceptibility testing was performed using the disk-diffusion method on Mueller–Hinton agar according to the guidelines of the Comité Français de l'Antibiogramme (http://www.sfm.asso.fr/). Methicillin susceptibility was determined using the following three methods: (a) testing with a 30 μg cefoxitin disk, a 10^6 cfu/ml inoculum and incubation at 37°C for 24 h [8]; (b) the MRSA-Screen latex agglutination test (bioMérieux) to confirm the presence of the penicillin-binding protein 2a; and (c) PCR for detecting the *mecA* gene as described previously [9]. These three tests gave concordant results. Isolated MRSA strains were typed using pulsed-field gel electrophoresis (PFGE) as described previously for studying genetic diversity of strains [10].

Of the 831 samples, 219 contained *S. aureus* strains recovered by at least one method, and 113 of these contained MRSA. MRSA was detected by CHROMagar MRSA in 108 samples (sensitivity 95.6%), MHT in 77 samples (sensitivity 68.1%) and HB in 94 samples (sensitivity 83.2%). The lower sensitivity of HB medium may be due to an inhibitory effect of the abundant commensal flora present on this medium. Two different *S. aureus* colony types corresponding to two different MRSA were isolated from six samples. One methicillin-susceptible *S. aureus* (MSSA) and one MRSA were isolated from four additional samples (on HB). Thus, the 219 samples that contained *S. aureus* strains yielded 229 *S. aureus* strains, and 119 of these were MRSA.

J. Loulergue · C. de Gialluly · V. Morange · A. Holstein
Service de Bactériologie-Hygiène, CHRU Trousseau,
37044 Tours Cedex 9, France

N. van der Mee-Marquet · R. Quentin (✉)
IFR 136-EA 3854 "Bactéries et risque maternofoetal" UFR Médecine, Université François-Rabelais de Tours,
2 bis Boulevard Tonnellé,
37032 Tours Cedex, France
e-mail: quentin@med.univ-tours.fr
Tel.: +33-247-478114
Fax: +33-247-478530

After 24 h of incubation, 114 of the 119 MRSA isolates yielded mauve colonies on CHROMagar MRSA, mostly 0.7–1.9 mm in diameter (sensitivity 95.8%) (Fig. 1a). CHROMagar MRSA detected MRSA in 103 of the 105 samples that contained more than five colonies (sensitivity 98.1%) and 11 of the 14 samples that contained 1–5 colonies (sensitivity 78.6%).

After 48 h of incubation, the mean size of the mauve colonies of the 114 MRSA strains was 2–2.2 mm, and three additional MRSA had been recovered (sensitivity 98.3%). These three additional MRSA strains were from two samples obtained from patients receiving antibiotics and from one sample that produced only one colony. Smaller purple colonies (about 0.5 mm) were observed after 48 h of incubation in 80 of the 831 samples; 62 of these were identified as *Corynebacterium* spp (Fig. 1b) and 18 as coagulase-negative staphylococci (CoNS). These colonies are easily distinguished from the MRSA colonies, which are large and mauve. Therefore, further testing is usually not necessary. The two remaining MRSA strains not detected by CHROMagar MRSA produced only two and five colonies on HB. All MSSA were inhibited by CHROMagar MRSA (specificity 100%, positive and negative predictive values 100 and 95.6%, respectively).

Eight MRSA phenotypes were identified on the basis of kanamycin (K), tobramycin (T), gentamicin (G), erythromycin (E), and pefloxacin (Pef) susceptibility. CHROM agar MRSA detected 46 of 47 strains with a KTGPefE resistance profile (sensitivity 97.9%), 31 of 32 strains with a KTPefE resistance profile (sensitivity 96.9%), and all strains of the other phenotypes, i.e., four KTGPef-resistant strains, 14 KTPef-resistant strains, one KT-resistant strain, seven PefE-resistant strains, 12 Pef-resistant strains and two strains susceptible to KTGPefE (sensitivity 100%). This medium thus detected all 21 MRSA that were susceptible to aminoglycosides, thereby overcoming one of the major problems observed with the CHROMagar Staph aureus medium supplemented with oxacillin [6].

After 48 h of incubation, a matte halo surrounded some MRSA colonies (Fig. 1c). This phenomenon varied with aminoglycoside susceptibility. It was observed for 14 of 21 aminoglycoside-susceptible MRSA, 21 of 46 KT-phenotype strains and only 2 of 50 strains with the KTG phenotype.

Eleven PFGE patterns were identified in strains isolated using CHROMagar MRSA in this study (Fig. 1d). When compared to the PFGE patterns we described recently for MRSA isolated from bloodstream infections, these 11 PFGE patterns represent each of the four major MRSA PFGE divisions in which MRSA strains were at least 60% similar [10]. This suggests that CHROMagar MRSA is able to detect the most frequent genogroups of invasive MRSA.

In this study CHROMagar MRSA screening for MRSA nasal colonization provided accurate results in 24 h without requiring any complementary tests. A 48-h incubation period may be useful to detect persistent nasal carriage of MRSA in patients receiving antibiotic therapy.

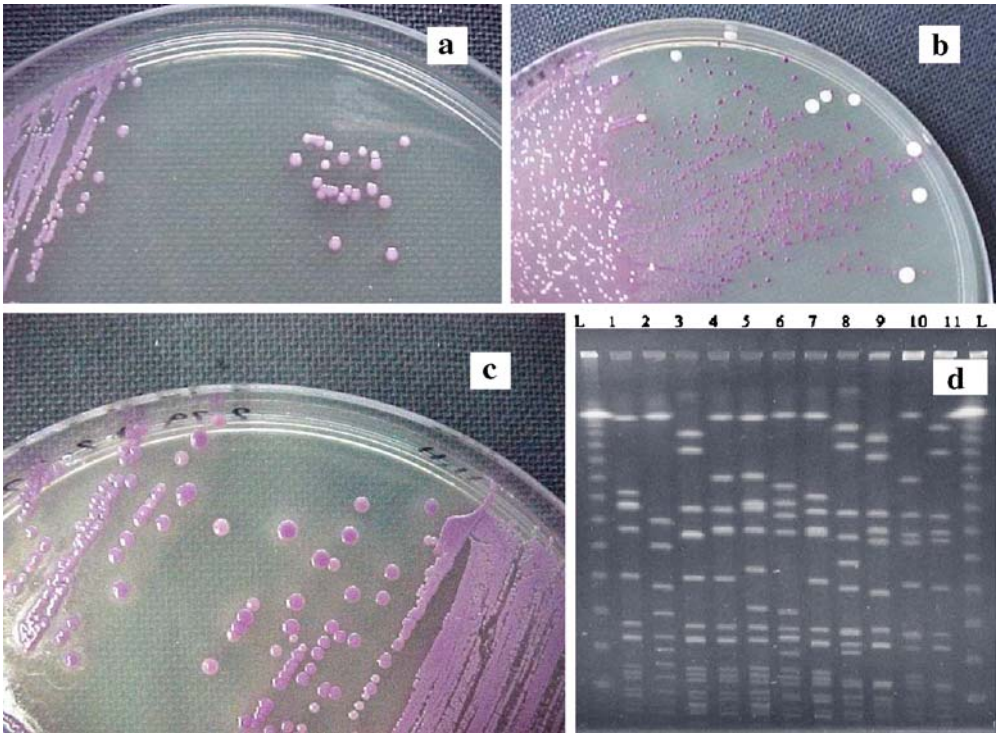

Fig. 1 **a** Appearance of MRSA colonies on CHROMagar MRSA after 24 h of incubation; **b** *small purple* colonies of *Corynebacterium* spp after 48 h of incubation; **c** sample with two MRSA colony types after 48 h of incubation, a *matte halo* surrounds some colonies; **d** 11 PFGE patterns of MRSA strains detected by CHROMagar MRSA (*lanes* 1 to 11). *L* ladder

References

1. Pujol M, Pena C, Pallares R, Ariza J, Ayats J, Dominguez MA, Gudiol F (1996) Nosocomial *Staphylococcus aureus* bacteremia among nasal carriers of methicillin-resistant and methicillin-susceptible strains. Am J Med 100:509–516
2. Pittet D, Waldvogel FA (1997) To control or not control colonization with MRSA. That's the question! Q J Med 90:239–241
3. Apfalter P, Assadian O, Kalczyk A, Lindenmann V, Makristathis A, Mustafa S, Rotter M, Hirschl AM (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
4. Girou E, Pujade G, Legrand P, Cizeau F, Brun-Buisson C (1998) Selective screening of carriers for control of methicillin-resistant *Staphylococcus aureus* (MRSA) in high-risk hospital areas with a high level of endemic MRSA. Clin Infect Dis 27:543–550
5. 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
6. Merlino J, Leroi M, Bradbury R, Veal D, Harbour C (2000) New chromogenic identification and detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus*. J Clin Microbiol 38:2378–2380
7. Diederen 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
8. Felten A, Grandry B, Lagrange PH, 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
9. Tokue Y, Shoji S, Satoh K, Watanabe A, Motomiya M (1992) Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 36:6–9
10. Van der Mee-Marquet N, Domelier AS, Girard N, Quentin R; The bloodstream infections study group of the Relais d'Hygiène du Centre (2004) Epidemiology and typing of *Staphylococcus aureus* strains isolated from bloodstream infections. J Clin Microbiol 42:5650–5657