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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

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Very-low-level methicillin-resistant *Staphylococcus aureus* (MRSA), or class 1 MRSA, is often misdiagnosed as methicillin-susceptible *S. aureus* (MSSA). We evaluated the performances of three methods for detection of low-level methicillin resistance: the disk diffusion method using the cephamycin antibiotics cefoxitin and moxalactam, the Vitek 2 system (bioMérieux), and the MRSA-screen test (Denka). Detection of the *mecA* gene by PCR was considered to be the "gold standard." We also determined the sensitivity of the oxacillin disk diffusion method with 5- and 1-μg disks and that of the Oxascreen agar assay with 6 mg of oxacillin liter^{-1} for detection of MRSA. We compared the distributions of MICs of oxacillin and cefoxitin by the E-test (AB Biodisk), and those of moxalactam by dilutions in agar, for MRSA and MSSA isolates. The 152 clinical isolates of *S. aureus* studied were divided into 69 MSSA (*mecA*-negative) and 83 MRSA (*mecA*-positive) isolates, including 63 heterogeneous isolates and 26 class 1 isolates (low-level resistance). The cefoxitin and moxalactam disk diffusion tests detected 100% of all the MRSA classes: cefoxitin inhibition zone diameters were <27 mm, and moxalactam inhibition zone diameters were <24 mm. The Vitek 2 system and the MRSA-screen test detected 94 and 97.6% of all MRSA isolates, respectively. The sensitivities of the 5- and 1-μg oxacillin disks were 95.2 and 96.4%, respectively, whereas that of the Oxascreen agar screen assay was 94%. All of the tests except the 1-μg oxacillin disk test were 100% specific. For the class 1 MRSA isolates, the sensitivity of the Vitek 2 test was 92.3%, whereas those of the MRSA-screen test and the disk diffusion method with cefoxitin and moxalactam were 100%. Therefore, the cefoxitin and moxalactam disk diffusion methods were the best-performing tests for routine detection of all classes of MRSA.

Staphylococcus aureus causes serious community-acquired and nosocomial infections. The introduction of benzylpenicillin had a dramatic effect on mortality rates due to invasive *S. aureus*. The increasing prevalence of benzylpenicillin-resistant *S. aureus* was initially overcome by the introduction of semisynthetic penicillins. Since that time, methicillin-resistant *S. aureus* (MRSA) has rapidly emerged and become a major clinical problem. In 1996, 57% of *S. aureus* isolates from French patients with nosocomial infections were methicillin resistant (1). Epidemiological studies on high-level MRSA, which is resistant to numerous antibiotics and antiseptics, revealed nosocomial outbreaks with clones disseminating nationally and internationally (2). Infections caused by very-low-level MRSA were first reported in Japan, where MRSA was more prevalent than elsewhere (14, 21). Subsequently, an increasing number of endemic and epidemic low-level MRSA strains that either were susceptible to most antistaphylococcal antibiotics, were susceptible to gentamicin, or did not produce β-lactamase were detected (4, 9, 23). The prevalence of community-acquired skin, soft-tissue, and disseminated infections caused by very-low-level MRSA in people without known risk factors increased recently in the United States and Europe (6, 10, 12). Four phenotypic classes of MRSA were identified by Tomasz et al. on the basis of population analyses of methicillin MICs in vitro. Three of these classes were heterogeneous (classes 1 to 3), and one was homogeneous (class 4); the methicillin MIC for class 4 was >800 mg liter^{-1}. For the major populations of class 1 to 3 isolates, methicillin MICs were 1.5 to 100 mg liter^{-1}, respectively, and for the minor populations, 10^{-8} to 10^{-2}, respectively, methicillin MICs were >100 mg liter^{-1} (27).

Nearly all MRSA isolates produce an additional penicillin-binding protein (PBP), named PBP2a. PBP2a binds β-lactams with a lower affinity than PBP2, the major physiological methicillin target. PBP2a is encoded by the *mecA* gene, a component of a larger DNA fragment designated the *mec* region (7, 14). The standard test used to identify MRSA is amplification of the *mecA* gene (22). The *mec* elements upstream and downstream of *mecA* are polymorphic. Nonetheless, two upstream genes, *mecR1* and *mecI*, are thought to regulate methicillin resistance (14, 15). Many other factors are involved in modulating the expression of methicillin resistance (7, 14).

Routine oxacillin tests often fail to detect very heterogeneous MRSA populations, which consequently are considered methicillin-susceptible *S. aureus* (MSSA) because of their usual susceptibility to most non-β-lactam antistaphylococcal antibiotics. Therefore, a number of parameters

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have been recommended to improve results: increasing the inoculum, growth at a low temperature, an oxacillin screen test with NaCl, or protracted incubation (17). The tests currently recommended by the NCCLS (National Committee for Clinical Laboratory Standards) are the oxacillin disk method, using 1 μg of oxacillin on a swab-inoculated Mueller-Hinton agar (MHA) plate supplemented with 2% NaCl and incubated at 35°C, and the oxacillin agar screen test using MHA supplemented with 4% NaCl. The Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM) recommends use of the oxacillin disk method, with 5 μg of oxacillin on an MHA plate, either supplemented with NaCl at 37°C or without NaCl at 30°C, flooded with the bacterial inoculum (24). More recent methods for detection of MRSA include the oxacillin E-test (AB BIODISK, Solna, Sweden) for determination of MICs, the automated Vitek 2 system (bioMérieux, La Balme les Grottes, France), and the MRSA-screen latex agglutination test (Denka, Seiken Co. Ltd., Tokyo, Japan), which detects PBP2a (3, 16, 18, 26, 27). Cephamycins were used extensively in Japan in the early 1980s, and as a result some MRSA and MSSA isolates became resistant to cefoxitin. Surprisingly, cefoxitin induced production of PBP2a in vitro in MSSA isolates for which cefoxitin MICs were high, and the disk diffusion assay with ceftizoxime (a cephamycin) proved to be a good assay for detection of low-level MRSA in Japan (19, 21).

We compared three methods for detection of MRSA, particularly the low-level class 1 MRSA: the cefoxitin and moxalactam (cephamycins) disk diffusion assays, the Vitek 2 system, and the MRSA-screen test. We subsequently measured the prevalence of clinical class 1 MRSA isolates among hospitalized patients and studied the distribution of *mecA*-regulatory genes in MRSA isolates.

MATERIALS AND METHODS

Bacterial strains. We studied 152 clinical *S. aureus* isolates from the St. Louis Hospital, Paris, France. These isolates formed three groups. The first comprised low-level MRSA index cases. These included eight isolates taken from seven patients and from the nose of a surgeon in the plastic surgery department in November and December 1998; three were class 1 MRSA and five were class 2 MRSA. The second group was a series of 95 consecutive clinical isolates collected between January and March 1999 from patients hospitalized in all departments except the intensive care unit and used to study the current prevalence of very-low-level MRSA. These isolates were collected from 90 patients, since 3 patients were found to harbor several *S. aureus* isolates with different resistance phenotypes. The third group comprised a selection of 49 clinical MRSA and MSSA isolates with different antibiotypes. Eight of these isolates were MSSA and were resistant to various antistaphylococcal antibiotics, and 41 were MRSA, including 13 class 1 isolates. Isolates were stored at −80°C. They were grown in air at 37°C on Columbia agar supplemented with 5% sheep blood before testing. *S. aureus* was identified by the mannitol fermentation test on Chapman medium, the coagulase-binding latex slide agglutination test (Fumouze Diagnostics, Levallois Perret, France), the tube coagulase test with oxalated rabbit serum and 18-h Staphylocoagulase broth (1 ml, incubated 4 h at 37°C; Bio-Rad, Marnes la Coquette, France), thermonuclease production on plates seeded with DNA (Bio-Rad) (100 μl of the 18-h Staphylocoagulase broth was heated for 5 min at 95°C, and DNase excretion was detected with 1 M HCl), and the API 32 Staph strip (bioMerieux) when necessary. Control strains used for all assays included the MSSA β-lactamase-negative strain ATCC 25923, the BORSA (β-lactamase-positive, borderline methicillin-resistant, *mecA*-negative *S. aureus*) strain CCUG 35302, the heterogeneous class 1 MRSA strain ATCC 43300^T, and the homogeneous MRSA strain CCUG 31966 from the Swedish Collection. *Micrococcus luteus* ATCC 4698 was used to test β-lactamase production.

Analysis of *mec* gene complex by amplification. DNA was extracted from three or four colonies suspended in 1.5 ml of water. After centrifugation, the pellet was resuspended in 200 μl of InstaGene Matrix (Bio-Rad). After 20 min at 56°C, the lysate was stirred and boiled for 8 min and then centrifuged to collect the DNA in the supernatant. PCR amplification was performed by using 1 U of *Taq* DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany). The reaction was carried out by using a Gene Amp PCR System 9600 (Perkin-Elmer). The *mecA* gene was amplified as described by Predari et al. (22). These conditions yielded a 528-bp PCR product corresponding to *mecA*. When *mecA* was present, the DNA was tested for the upstream regulatory genes *mecR1* and *mecI* with the primers and PCR conditions described by Kobayashi et al. (15). The 5′-end *mecRA* and 3′-end *mecRB* regions of the regulatory gene *mecR1* yielded PCR products of 310 and 236 bp, respectively. When *mecRB* was present, the DNA was tested for the presence of a *mecI* PCR product of 481 bp. For visualization of PCR products, 8-μl samples of the product were electrophoresed in 0.8% 1× TBE (8.9 M Tris, 8.9 M boric acid, 0.2 M EDTA) for 45 min at 100 V, stained with ethidium bromide, and photographed under UV illumination. The PCR products of the clinical isolates were visually compared with those of the reference strains.

Susceptibility testing methods. (i) Inocula for susceptibility testing. High-density inocula were made by diluting five colonies grown overnight on Columbia agar supplemented with 5% sheep blood (Bio-Rad) in 5 ml of Mueller-Hinton broth (Bio-Rad) or distilled water to prepare a suspension equivalent in density to 0.5 McFarland barium sulfate standard unit (average turbidity, 10^8 CFU ml^{-1}). Low-density inocula were made by diluting 200 μl of the high-density suspension in 20 ml of distilled water to a final concentration of approximately 10^6 CFU ml^{-1}.

(ii) Disk diffusion tests for resistance to oxacillin, cefoxitin, and moxalactam. The entire surface of the MHA plate (diameter, 90 mm) (Bio-Rad) was covered with the required inoculum, and the plate was air dried for 15 min before the disks were laid on the surface and incubation was performed for 18 h at the required temperature. Oxacillin resistance was determined with 1- and 5-μg disks according to the NCCLS and CASFM critical diameters, <13 and <20 mm, respectively (17, 24). In cases of heterogeneous growth, defined as the occurrence of small colonies in the circular growth inhibition area, the diameter of the inner limit of the small colonies' inhibition zone was taken into account.

(iii) Oxacillin agar screen. One hundred microliters of the high-density inocula was dropped onto MHA plates with 2% NaCl containing 6 μg of oxacillin ml^{-1}. If any growth occurred within 48 h, the isolate was considered to be oxacillin resistant.

(iv) Determination of oxacillin and cefoxitin MICs by the E-test and of moxalactam MICs by dilutions in agar. E-tests were performed according to the manufacturer's instructions on 150-mm-diameter MHA plates inoculated by swabbing in three directions. If heterogeneous growth occurred, the highest MIC (inner limit of the inhibition zone) was read. Moxalactam in the form of a dry powder (Chiomarin, Shionogi Co., Osaka, Japan) was diluted in MHA plates from 0.5 and 32 mg $liter^{-1}$. One microliter of the bacterial suspension (about 10^4 bacteria) was laid on the surface with an A400 multipoint inoculator (Dynex, Issy les Moulineaux, France). *S. aureus* ATCC 25923 was tested with each batch of medium. The MIC was defined as the lowest concentration that inhibited bacterial growth.

(v) Vitek 2. Susceptibility testing with the Vitek 2 system was performed according to the manufacturer's instructions. Readings were automatically taken every 15 min. The current NCCLS breakpoints for oxacillin susceptibility were used: MICs of ≤2 mg $liter^{-1}$ indicated susceptibility, and MICs of ≥4 mg $liter^{-1}$ indicated resistance.

(vi) Detection of PBP2a. The MRSA-screen test, which is based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a, was used according to the manufacturer's instructions.

(vii) β-Lactamase production. β-Lactamase production was measured by two methods. (i) Colonies from the edge of the inhibition zone of a 10-IU benzylpenicillin disk were streaked onto a nitocefin disk (bioMérieux), and β-lactamase production was characterized by the appearance of pink colonies within 15 min. (ii) If the first test was negative, Got's test was performed on an MHA plate containing approximately 10^8 CFU of *M. luteus* ml^{-1}. A benzylpenicillin disk was placed in the center of the agar plate, and a loopful of each isolate was streaked radially. After 24 h at 37°C, β-lactamase-producing *S. aureus* induced the growth of *M. luteus* in the vicinity of the streak.

Each set of tests was carried out by an investigator unaware of the individual oxacillin status of the isolates, and each was read by two independent observers.

(viii) Classification of MRSA isolates. We adopted the 72-h protracted incubation of the 5-μg oxacillin disk diffusion method to fit the classification system described by Tomasz et al. (25). Class 1 MRSA gave an inhibition zone larger

TABLE 1. Sensitivities of the recommended oxacillin methods and of the new methods for detection of 83 MRSA clinical isolates

Test	Sensitivity[a]		
	Overall	For class 1 MRSA	For other MRSA classes
Agar screen with 6 μg of oxacillin ml^{-1}	94	92.3	94.7
E-test for oxacillin MICs	91.6	73.1	100
Disk diffusion			
Oxacillin, 5 μg[b]	95.2	84.6	100
Oxacillin, 1 μg[b]	96.4	88.5	100
Cefoxitin, 30 μg[c]	100		
Moxalactam, 30 μg[c]	100		
Vitek 2	94	92.3	94.7
MRSA-screen	97.6	100	96.5

[a] Percentage of the 83 *mecA*-positive isolates for which positive test results were obtained.
[b] Inoculum, 10^8 CFU ml^{-1}. Incubation was carried out for 18 h at 37°C.
[c] Inoculum, 10^6 CFU ml^{-1}. Incubation was carried out for 18 h at 37°C.

than 20 mm within 18 h and a few colonies inside this zone within 36 to 72 h; classes 2 and 3 gave an inhibition zone larger than 20 mm within 18 h and a hazy growth of colonies inside this zone within 18 to 48 h; and class 4 showed either no inhibition zone or a zone of <20 mm within 18 h.

RESULTS

Performances of the different methods for the detection of MRSA. Among the 152 *S. aureus* isolates, 83 were MRSA, *mecA* positive, and 69 were MSSA, *mecA* negative.

Oxacillin 6-μg agar screen and oxacillin disk diffusion methods. The sensitivity of the agar screen test was 94% when the 31 MRSA isolates that gave hazy growth after 48 h were included (Table 1). The sensitivity of the 5-μg oxacillin disk method was 95.2% with the high-density inoculum at 37°C (Table 1), 41% with the low-density inoculum at 37°C, and 31.3% with the low-density inoculum at 30°C (data not shown). The 1-μg oxacillin disk diffusion test was 96.4% sensitive with the high-density inoculum at 37°C (Table 1). All of the methods except the 1-μg oxacillin disk test (specificity, 97.1%) were 100% specific.

Cefoxitin and moxalactam disk diffusion tests. The ranges of the inhibition zone diameters for the MRSA and the MSSA isolates were very distinct, except with the low-density inoculum at 30°C for one class 1 MRSA isolate (isolate 112) and one MSSA isolate (isolate 9), both of which gave a 26-mm cefoxitin inhibition zone diameter (Table 2). With the low-density inoculum at 37°C, all MRSA isolates showed cefoxitin inhibition zone diameters of <27 mm and moxalactam inhibition zone diameters of <24 mm, and all MSSA isolates showed larger diameters. With these critical diameters, cefoxitin and moxalactam disk diffusion tests were 100% sensitive and 100% specific (Table 1).

Oxacillin, cefoxitin, and moxalactam MICs. In cases of heterogeneous growth with the E-test, the recorded MIC corresponded to the highest limit of the inhibition of growth of the most resistant population. The E-test oxacillin MICs for the MSSA isolates were all <2 mg $liter^{-1}$, and those for the MRSA isolates were between 0.38 and 256 mg $liter^{-1}$ (>2 mg $liter^{-1}$ for 91.6% of MRSA isolates, and 0.38 to 1.5 mg $liter^{-1}$ for 8.4%—all class 1 isolates) (Fig. 1). For all MSSA isolates, E-test cefoxitin MICs were <4 mg $liter^{-1}$, whereas for all MRSA isolates, cefoxitin MICs were ≥4 mg $liter^{-1}$ (Fig. 1). The median cefoxitin MIC for MSSA isolates was 2 mg $liter^{-1}$, and the median cefoxitin MICs for class 1, classes 2 and 3, and class 4 MRSA isolates were 32, 48, and 256 mg $liter^{-1}$, respectively. For all MSSA isolates, moxalactam MICs were ≤8 mg $liter^{-1}$, and for all MRSA isolates except one (isolate 128, for which the moxalactam MIC was 4 mg $liter^{-1}$), moxalactam MICs were >8 mg $liter^{-1}$ (Fig. 1). The median moxalactam MIC for MSSA isolates was 8 mg $liter^{-1}$, and the median MICs for heterogeneous and homogeneous MRSA isolates were 32 and >32 mg $liter^{-1}$, respectively. The moxalactam MIC for *S. aureus* ATCC 25923 was 2 or 4 mg $liter^{-1}$, depending on the batch of medium.

Vitek 2. All of the MSSA isolates were Oxascreen negative, and oxacillin MICs for all MSSA isolates were ≤1 mg $liter^{-1}$. For 10 MRSA isolates, oxacillin MICs were <4 mg $liter^{-1}$. Five of these isolates, all of which belonged to classes 1 to 3, did not grow on the Oxascreen either, and were misclassified as oxacillin susceptible (Table 3).

MRSA-screen test. Two *mecA*-positive isolates gave false-negative results repeatedly on the MRSA-screen. Neither of these isolates belonged to class 1 (Table 3).

All the above assays gave satisfactory results with the reference strains.

Classification of isolates according to MRSA class, *mecA* status, and *mecA*-regulatory gene status. (i) *mecA* status and MRSA class. Sixty-nine of the 152 clinical *S. aureus* isolates studied were *mecA* negative and oxacillin susceptible. Of the 83 *mecA*-positive isolates, 26 belonged to class 1, 37 belonged to class 2 or 3, and 20 belonged to class 4.

(ii) *mecA*-regulatory genes. Seventy nine MRSA isolates harbored a *mecR1* gene; of these, 2 harbored the complete

TABLE 2. Inhibition zone diameters of cefoxitin and moxalactam disk diffusion tests for 83 *mecA*-positive (MRSA) and 69 *mecA*-negative (MSSA) *S. aureus* isolates

Cephamycin[a] disk	Inhibition zone diam (mm) at the following inoculum and incubation temp:					
	10^8 CFU ml^{-1}, 30°C		10^6 CFU ml^{-1}, 30°C		10^6 CFU ml^{-1}, 37°C	
	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
CTT	6–19	24–35	6–18 and 26[b]	26[c] and 27–33	6–20 and 23[b]	27–34
MOX	ND[d]	ND	6–17 and 23[b]	25–31	6–17 and 23[b]	24–30

[a] CTT, cefoxitin; MOX, moxalactam.
[b] Inhibition zone diameter for MRSA isolate 112. CTT and MOX critical diameters were <27 and <24 mm, respectively.
[c] CTT diameter for MSSA isolate 9.
[d] ND, not done.

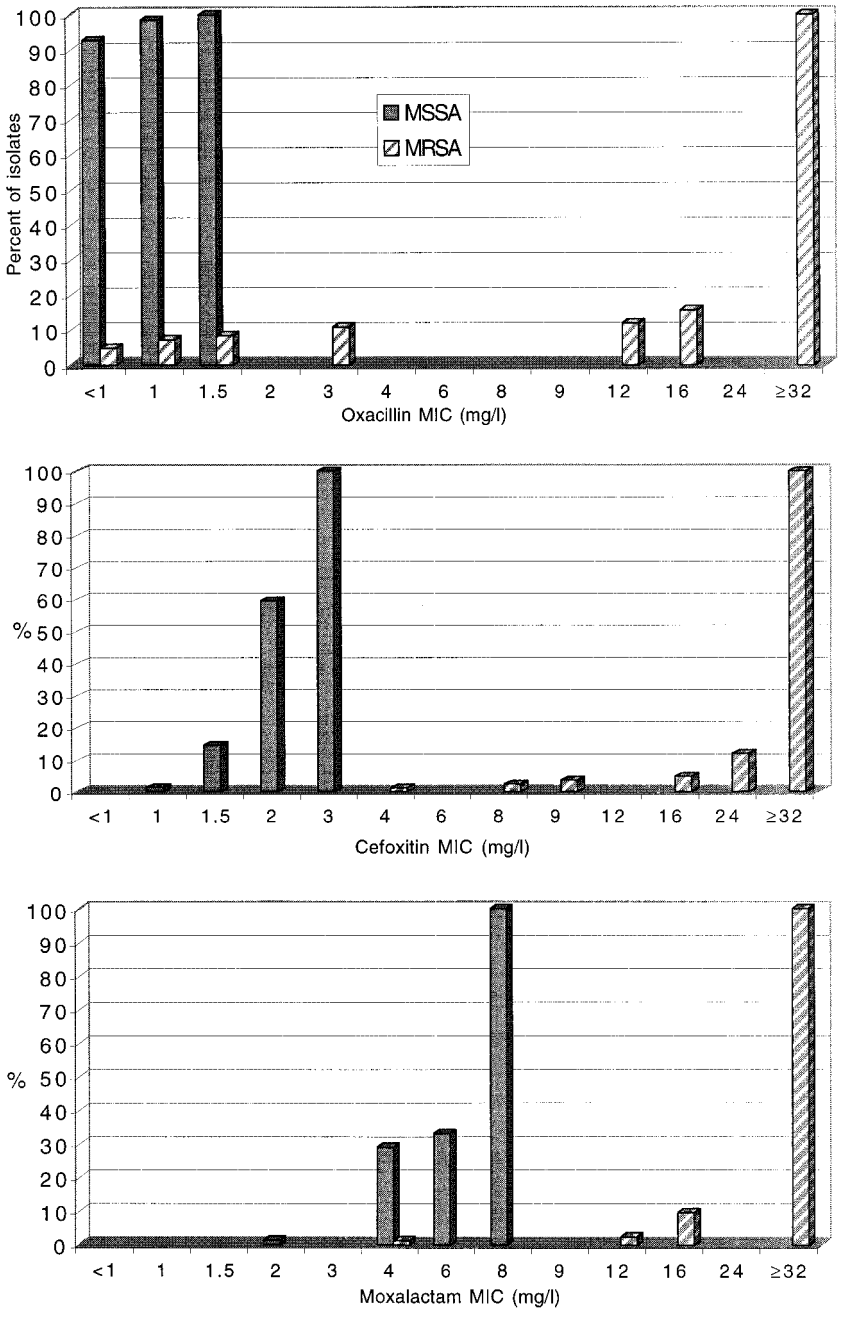

FIG. 1. Distribution of oxacillin, cefoxitin, and moxalactam cumulative MICs in *mecA*-negative (MSSA) and *mecA*-positive (MRSA) *S. aureus* isolates.

mecRA-mecRB (pattern of isolate 88) and 77 harbored the truncated *mecRA* only (pattern of isolate 112) (Fig. 2). Four MRSA isolates harbored no *mecR1* gene (pattern of isolate 105) (Fig. 2). *mecI* was found in the heterogeneous class 1 strain ATCC 43300^T but in no clinical isolate, unlike most class 1 Japanese isolates (14, 15). Neither MRSA class nor test performance was related to *mec*-regulatory genes: all of the 26 class 1 MRSA isolates, and all of the 7 MRSA isolates that were misclassified either by the Vitek 2 or by the MRSA-screen test, were of the predominant genotype.

DISCUSSION

The cefoxitin and moxalactam disk diffusion tests were found 100% sensitive and specific for MRSA under all conditions tested, except for one isolate under test conditions of a low-density inoculum and incubation at 30°C. This implies that the cephamycin disk test (cefoxitin or moxalactam) is an available alternative to the oxacillin disk method for routine antibiotic susceptibility testing at 37°C (24). According to our results, an *S. aureus* isolate that gives a cefoxitin diameter of <27

TABLE 3. Characteristics of the seven MRSA isolates that were misclassified by the Vitek 2 or the MRSA-screen test

Isolate designation	Result[a] with the following test:									
	Agar screen OXA-6[b]	E-test OXA-MIC	Disk diffusion[c]				Vitek 2[d]			MRSA-screen
			OXA-5	OXA-1	CTT-30	MOX-30	Oxascreen	MIC	Sum	
112	R	0.38	R	R	R	R	S	0.5	S	R
122	S	1	S	S	R	R	S	1	S	R
144	R	32	R	R	R	R	R	0.5	R	S
106	R	48	R	R	R	R	S	0.5	S	R
28	R	96	R	R	R	R	S	0.5	S	R
128	R	256	R	R	R	R	S	1	S	R
17	R	256	R	R	R	R	R	8	R	S

[a] R, resistant; S, susceptible. OXA-MIC, oxacillin MIC (>2 μg/ml indicates resistance).
[b] OXA-6, 6 μg of oxacillin/ml.
[c] Incubation was carried out for 18 h at 37°C. OXA-5 and OXA-1, 5- and 1-μg oxacillin disks, respectively (inoculum, 10^8 CFU ml^{-1}). CTT-30 and MOX-30, 30-μg cefoxitin and moxalactam disks, respectively (inoculum, 10^6 CFU ml^{-1}) (for critical diameters, see Table 2, footnote b).
[d] The result summarizes those of Oxascreen and MIC; R, growth on Oxascreen and/or a MIC of >2 μg/ml.

mm or a moxalactam diameter of <24 mm can be identified as MRSA. These diameters are higher than those corresponding to the respective MICs indicating resistance according to CASFM or NCCLS disk diffusion values (24). The strong correlation between cephamycin diameters and oxacillin resistance is mediated by still unknown mechanisms (14). This strong correlation between cephamycin MICs (cefoxitin MIC of ≥4 mg $liter^{-1}$, moxalactam MIC of >8 mg $liter^{-1}$) and methicillin resistance may be due to the interaction between PBP2a and various PBPs by still unknown mechanisms (14). Compared to cephalosporins, cephamycins have a high affinity for *S. aureus* PBP4, a protein which is involved in cell wall cross-linking (13, 20). Previous experiments showed a relationship between PBP2, PBP4, and methicillin resistance. The cephamycin MIC for an MRSA strain was 100 mg $liter^{-1}$, whereas that for its isogenic mutant, which was defective in PBP2, was 3 mg $liter^{-1}$ (20). In vitro induction of overexpression of PBP4 in an MSSA strain resulted in methicillin resistance (11). Ceftriaxone, an expanded-spectrum cephalosporin, has also recently been advocated as a presumptive test for MRSA; its performance (95% sensitivity and 97% specificity) (5) was lower than those of cephamycins.

The Vitek 2 test was 94% sensitive and 100% specific. The isolates which were misclassified were heterogeneous MRSA isolates. The sensitivity of the 6-μg/ml oxacillin agar screen method was also 94%. Previous trials showed that detection sensitivities were 95.3% with Vitek on heterogeneous French MRSA isolates and 97% with Vitek GPS 106 on multinational bloodstream isolates (3, 27). These trials did not detail results for very-low-level MRSA isolates. The Vitek 2 test produces rapid results: oxacillin susceptibility results within 5 h, identification to the species level and complete antimicrobial susceptibility results within 8 h.

The MRSA-screen test was 97.6% sensitive and did not misclassify any class 1 MRSA isolate as MSSA; the two misclassified isolates belonged to different clones (Centre National de Référence pour les Staphylocoques, Institut Pasteur, Paris, France). In previous assays, the MRSA-screen test was found to be 97 to 100% sensitive and 100% specific (5, 16, 18, 27). The MRSA-screen test is easy to perform, and the results are available within 15 min. Thus, it was the best commercially available test for distinguishing very-low-level class 1 MRSA from MSSA.

The detection sensitivities of the reference methods, oxacillin MICs of >2 mg $liter^{-1}$, the oxacillin agar screen, and disk diffusion tests with 5- or 1-μg oxacillin disks, were 91.6, 94, 95.2, and 96.4%, respectively. The oxacillin E-test failed to detect seven isolates correctly (MICs, 0.38 to 1.5 mg $liter^{-1}$; median MIC, 0.5 mg $liter^{-1}$), all of which were class 1 MRSA isolates. Surprisingly, the sensitivity of the oxacillin agar screen on MHA plates with 2% NaCl was lower than that of the 1-μg oxacillin disk diffusion method (with a high inoculum). In contrast, national quality con-

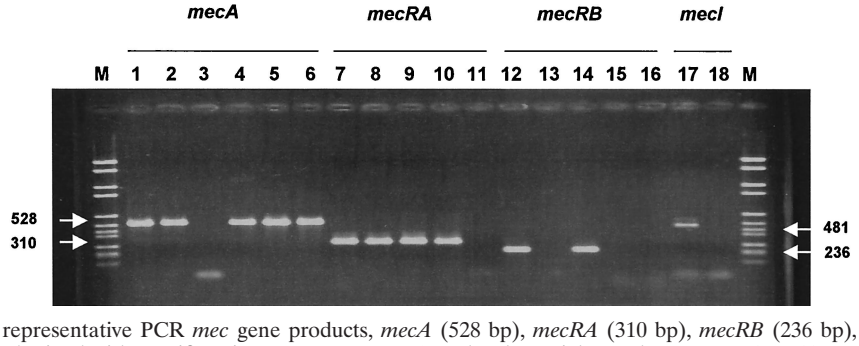

FIG. 2. Gel image of representative PCR *mec* gene products, *mecA* (528 bp), *mecRA* (310 bp), *mecRB* (236 bp), and *mecI* (481 bp), from different MRSA isolates, obtained with specific primers. Lane M, DNA molecular weight markers. Lanes 1, 7, 12, and 17, MRSA strain ATCC 44300; lanes 2, 8, and 13, MRSA strain CCUG 31966; lane 3, BORSA strain CCUG 35302; lanes 4, 9, 14, and 18, MRSA isolate 88; lanes 5, 10, and 15, MRSA isolate 112; lanes 6, 11, and 16, MRSA isolate 105.

trol of class 1 MRSA testing performed by the Laboratory Proficiency Testing Program of Ontario showed that 21% of laboratories using the standard disk test and 1% of those using an oxacillin agar screen reported an incorrect MSSA result (17). In the present study, all of the tests were 100% specific, except for the disk diffusion tests with 1 μg of oxacillin (97.1%) and 30 μg of cefoxitin at 30°C (98.6%). Specificities averaging 80% have been reported with the 1-μg oxacillin disk (7).

In this assay, the prevalence of MRSA was approximately 36%, and more than 10% of all *S. aureus* isolates were class 1 MRSA, as in most French hospitals. Nevertheless, class 1 MRSA may be misdiagnosed as MSSA with the usual tests, and the prevalence of their susceptibility to other antibiotics (benzylpenicillin [20% in the consecutive isolates], fosfomycin, rifampin, gentamicin, and erythromycin) is misleading. Community-acquired MRSA isolates from skin and soft tissue infections are usually susceptible to various antibiotics (10, 12). Many antibiotics, and even penicillins with good affinity for PBP2a, such as amoxicillin and benzylpenicillin, may be effective against β-lactamase-negative class 1 MRSA isolates (8). Regardless of the susceptibilities of these isolates to many classes of antibiotics, four recent cases of pediatric invasive community-acquired sepsis with very-low-level MRSA were fatal (6). In our institution, two-thirds of the *S. aureus* isolates originated from skin specimens, and 13% of these were class 1 MRSA. MSSA isolates from skin lesions probably acquired the *mecA* gene by horizontal transfer from other skin staphylococcal species before becoming class 1 to 4 in turn (14, 25). The index cases of heterogeneous MRSA formed a cluster of nosocomial skin infections in the plastic surgery department following reconstructive surgery after carcinoma removal. They belonged to three clones, which differed according to their MRSA classes (1, 2, and 3, respectively), their phage types, their antibiotypes, and their DNA profiles as determined by pulsed-field gel electrophoresis (data not shown).

In conclusion, the cefoxitin and moxalactam disk diffusion method was very suitable for detection of MRSA, particularly class 1 isolates. The MRSA-screen test identified all class 1 isolates. The Vitek 2 test (Oxascreen and oxacillin MIC) presented no benefit over the oxacillin MIC test alone. With some adaptation, these tests would also improve the detection of methicillin-resistant, coagulase-negative staphylococci [A. Felten, B. Grandry, P. H. Lagrange, and I. Casin, abstract from the 11th European Congress of Clinical Microbiology and Infectious Diseases 2001, Clin. Microbiol. Infect. **7**(Suppl. 1)**:**13, abstr. O-100, 2001].

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