Evaluation of the BBL® Crystal™ MRSA ID System for Rapid Detection of Methicillin Resistance in *Staphylococcus aureus*

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Twenty-four clinical isolates of *Staphylococcus aureus* collected from various geographic areas and four reference strains were studied by (i) agar diffusion with disks impregnated with 5 µg oxacillin and reading after incubation at 30°C for 24 hours, (ii) Southern hybridization with a probe specific for the *mecA* gene, and (iii) the BBL® Crystal™ MRSA ID system. There was perfect correlation between the three methods: the BBL® Crystal™ MRSA ID system detected methicillin resistance in the fifteen strains hybridizing with the *mecA* probe and classified as resistant by the oxacillin disk diffusion test; the thirteen remaining strains were susceptible by agar diffusion and by the BBL® test and did not hybridize with the *mecA* probe. The BBL® Crystal™ MRSA ID System, therefore, appears to be an accurate method for rapid detection of *Staphylococcus aureus* exhibiting homogeneous resistance to methicillin.

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) constitute a major problem in outbreaks of nosocomial infections worldwide (1). Rapid identification of these organisms is necessary to minimize their spread in hospitals. It is also important for adequate therapy to reliably identify such strains since they are resistant to all classes of β-lactam antibiotics, including cephalosporins (1).

Conventional methods for detection of MRSA consist in either disk diffusion or agar dilution. Unfortunately, these tests require a 24-hour incubation period and can fail to detect resistance (2) since phenotypic expression is often heterogeneous (3). The majority of MRSA carries the *mecA* gene which encodes production of the low-affinity penicillin-binding protein PBP2a responsible for methicillin resistance (4). Detection of the *mecA* gene by DNA-DNA hybridization (5) or by the polymerase chain reaction (6) has been successfully used. However, these methods require specialized staff and equipment and are not applicable in routine medical microbiology laboratories.

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The aim of the present study was to evaluate the sensitivity and specificity of the BBL® Crystal™ MRSA ID system designed to allow detection of MRSA from culture within four hours. The results obtained with clinical isolates and reference strains were compared with those of oxacillin disk-agar diffusion and DNA-DNA hybridization with a *mecA* specific probe.

Materials and Methods

Twenty four clinical isolates of *Staphylococcus aureus* (Table 1) were collected from three French hospitals in 1993. The organisms were cultured from various body sites including blood, wounds and urine. Isolates were identified using the Rapidec Staph method (bioMérieux, France). Four reference *Staphylococcus aureus* (Table 1, Ref. 7): FG30 and FG31 which express heterogeneous methicillin resistance, FG34, a β-lactamase overproducer, and FG35 which is methicillin-susceptible were also included.

Antibiotic susceptibility testing was performed by agar diffusion on Mueller-Hinton medium (Sanofi Diagnotics Pasteur, France) with disks (Sanofi Diagnotics Pasteur) impregnated with oxacillin (5 µg) or penicillin G (6 µg). Plates were incubated at 30°C and inhibition zones were measured after 24 hours.

For hybridization studies, a *Xba*I-*Pst*I 1070-bp fragment internal to the *mecA* gene was purified from plasmid pBBB62 (8) and labelled with [α-^{32}P] dCTP by nick translation (9). Total DNA (10) was digested

Table 1 Results of disk-agar diffusion test, DNA-DNA hybridization and BBL® Crystal™ MRSA ID System for detection of methicillin resistance in clinical isolates and reference strains of *Staphylococcus aureus.*

Strain	*Source or Reference*	*Inhibition zone diameter (mm) (clinical categorization)*		*DNA-DNA hybridization with the* mecA *probe*	*BBL® Crystal™ MRSA ID System*
		oxacillin	*penicillin*		
clinical isolates					
1	A	≤6 (R)	≤6 (R)	+	+
2	A	≤6 (R)	≤6 (R)	+	+
3	A	≤6 (R)	≤6 (R)	+	+
4	A	≤6 (R)	≤6 (R)	+	+
5	B	≤6 (R)	≤6 (R)	+	+
6	B	≤6 (R)	≤6 (R)	+	+
7	A	≤6 (R)	≤6 (R)	+	+
8	B	≤6 (R)	≤6 (R)	+	+
9	B	≤6 (R)	08 (R)	+	+
10	B	≤6 (R)	≤6 (R)	+	+
11	C	≤6 (R)	08 (R)	+	+
12	A	≤6 (R)	≤6 (R)	+	+
13	A	≤6 (R)	≤6 (R)	+	+
14	A	27 (S)	≤6 (R)	–	–
15	A	23 (S)	≤6 (R)	–	–
16	A	21 (S)	≤6 (R)	–	–
17	A	21 (S)	≤6 (R)	–	–
18	A	21 (S)	≤6 (R)	–	–
19	B	25 (S)	≤6 (R)	–	–
20	B	26 (S)	≤6 (R)	–	–
21	A	27 (S)	14 (R)	–	–
22	C	24 (S)	12 (R)	–	–
23	C	25 (S)	23 (R)	–	–
24	C	39 (S)	50 (S)	–	–
Reference strains					
FG30	7	≤6 (H)	≤6 (R)	+	+
FG31	7	14 (H)	09 (R)	+	+
FG34	7	25 (S)	13 (R)	–	–
FG35	7	26 (S)	29 (S)	–	–

A, hôpital Henri Mondor, Créteil, France; B, hôpital St Michel, Paris, France; C, hôpital Begin, St. Mandé, France.
R, methicillin-resistant; S, methicillin-susceptible ; H, methicillin-heterogeneous.
*+, methicillin-resistant; –, methicillin-susceptible.

with *Hin*dIII, resolved by electrophoresis in 1% agarose, transferred onto a Nytran membrane (Schleicher & Schuell, Germany) and hybridized under stringent conditions (10) with the *mecA* probe. Hybridization was detected by autoradiography.

The BBL® Crystal™ MRSA ID System (Becton Dickinson Microbiology Systems, USA) test was performed according to the instructions provided by the manufacturer. Briefly, a 0.5 McFarland equivalent bacterial suspension was prepared in BBL® saline. Dilutions were made to give a final concentration of 2×10^7 CFU/ml. For each bacterial sample, 150 µl of broth suspension were introduced into three separate wells of the microtiter test tray. The first well was used as a positive growth control, the second contained a final oxacillin concentration of 4 µg/ml, and the third containing 16 µg/ml vancomycin was used as a negative growth control. The tray was incubated at 35°C for 4 hours and fluorescence was monitored visually using a hand-held long wave UV lamp.

Results and Discussion

The 24 clinical isolates were first classified as resistant or susceptible to oxacillin and penicillin by the disk agar diffusion test according to the criteria of the Comité de l'Antibiogramme of the Société Française de Microbiologie (11). Strains with inhibition zones around the oxacillin disk of less than 20 mm were considered methicillin-resistant, those with zones >20 mm were considered methicillin-susceptible. Heterogenous expression of methicillin resistance was

identified by "squatter" colonies within the inhibition zone (3). Isolates with inhibition zones around the penicillin disk <29 mm were considered penicillin-resistant, although such strains may occasionally give a larger inhibition zone. Three phenotypic classes were defined among the clinical isolates (Table 1). Strains 1 to 13 were resistant to oxacillin and penicillin suggesting the presence of the *mecA* gene. Isolates 14 to 23 were resistant to penicillin only. Strain 24 was susceptible to both oxacillin and penicillin. The diffusion method has been recommended for detection of methicillin resistance in *Staphylococcus aureus* (1). However, several investigators (12, 13) have tested various conditions (medium supplemented with NaCl, different temperatures or periods of incubation) to optimize detection of resistance which is often phenotypically heterogeneous. Using our conditions, no heterogeneous expression of methicillin resistance among the 24 clinical isolates was observed. Heterogenous expression of methicillin resistance of reference strains FG30 and FG31 (7) was confirmed by the disk diffusion method (Table 1). Strains FG34 and FG35 were classified as methicillin-susceptible.

Because of the invariable presence of *mecA* in MRSA, detection of this gene provides an accurate method for identification of these strains, irrespective of environmental conditions that may affect the phenotypic expression of resistance (3, 5). Total DNA of all the strains (Table 1) studied was hybridized with a *mecA* specific probe and part of the results is presented in Figure 1. The *mecA* probe hybridized with a 4.2-kb *Hin*dIII fragment of total DNA from clinical isolates 1 to 13 and reference strains FG30 and FG31. We did not observe hybridization with DNA from the remaining strains studied. As expected, homology with the *mecA* probe was detected only in MRSA. A perfect correlation between DNA-DNA hybridization and disk diffusion results was observed.

Methicillin resistance was then evaluated in the 24 clinical isolates and the four reference strains with the BBL® Crystal™ MRSA ID System in a single blind study. This test uses a customized broth to enhance the growth of resistant strains and a fluorescent indicator placed in the bottom of the microtiter wells that is sensitive to the concentration of oxygen present in the broth. When oxygen is present, emissions from the indicator are quenched and no fluorescence is detected. When the oxygen is removed, fluorescence is easily detected. An actively respiring microorganism will consume oxygen and fluorescence will be observed. A microorganism which is not actively respiring will not consume the oxygen dissolved in the broth and will, therefore, quench the fluorescent emissions. In the presence of an antimicrobial agent,

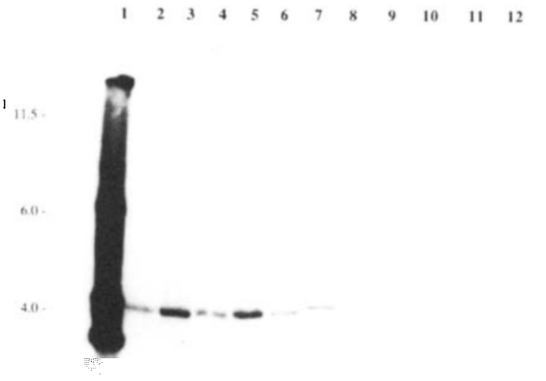

Figure 1 Southern hybridization of plasmid pBBB62 and *Hin*dIII-digested total DNA with the *mecA*-specific probe. Lane 1, plasmid pBBB62; lanes 2–6, clinical isolates of *Staphylococcus aureus* 1–5; lane 7, *Staphylococcus aureus* FG30; lanes 8–11, clinical isolates of *Staphylococcus aureus* 14–17; lane 12, *Staphylococcus aureus* FG34. Molecular sizes (in kilobases) are indicated.

the ability to observe fluorescence depends upon whether the bacteria are susceptible or resistant to that particular agent. For all strains positive control wells were fluorescent and negative control wells remained dark after 4 hours of incubation at 35°C. For strains 1 to 13 (Table 1) as well as for reference strains FG30 and FG31 fluorescence was detectable in the oxacillin well after four hours. These strains were classified as methicillin-resistant. Strains 14 to 24 and reference strains FG34 and FG35 were considered susceptible since they failed to fluoresce in the presence of oxacillin after four hours of incubation. A 100% correlation was thus observed for the 24 clinical isolates and the four reference strains between results with the BBL® Crystal™ MRSA ID System and those with the oxacillin disk diffusion or DNA-DNA hybridization methods. The BBL® Crystal™ MRSA ID System correctly identified heterogeneous methicillin-resistant strains FG30 and FG31. No intermediate amount of fluorescence was observed.

This study confirms the suitability of the BBL® Crystal™ MRSA ID System to detect methicillin resistance in clinical isolates of *Staphylococcus aureus*. The ability of the rapid fluorescent method to provide accurate results after only 4 hours of incubation is a major advantage. This could hasten the patient isolation process practised in many hospitals and allow rapid institution of alternate therapy. In addition, this test is easy to perform and to read and does not require expensive equipment.

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