

## Technical report

# Evaluation of the BBL Crystal MRSA ID System for detection of oxacillin resistance in *Staphylococcus aureus*

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### Abstract

**Aims**—To evaluate the BBL® Crystal™ MRSA ID System for detection of oxacillin resistance in *Staphylococcus aureus*.

**Methods**—52 methicillin resistant *S aureus* (MRSA) from five different countries and 85 methicillin susceptible *S aureus* (MSSA) were included. The species was confirmed by tube coagulation and detection of the clumping factor using the Staphaurex Plus. Clonal non-identity of the MRSA isolates was shown by pulsed field gel electrophoresis. MIC values (oxacillin) were determined using the Etest. Polymerase chain reaction was carried out to detect the *mecA* gene. The BBL Crystal MRSA ID System was carried out according to the manufacturer's instructions.

**Results**—The BBL Crystal MRSA ID System showed fluorescence in 45 of 52 MRSA (sensitivity 86.5%; negative predictive value 92.2%), and the specificity was 97.6% (positive predictive value 95.7%). Two of seven MRSA that failed to show fluorescence had MIC values (oxacillin) of 4 mg/litre.

**Conclusions**—The BBL Crystal MRSA ID System is a valuable test for detecting oxacillin resistance in *S aureus*. Its major advantage is the short time (4–5 hours) required to perform the test when organisms are grown on tryptic soy agar or sheep blood agar. Difficulties may arise in borderline resistant isolates.

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Keywords: oxacillin resistance; *Staphylococcus aureus*; screening; BBL Crystal MRSA ID system

Methicillin resistant *Staphylococcus aureus* (MRSA) continues to be a prominent problem in hospitals in the USA<sup>1,2</sup> and Europe.<sup>3</sup> Rapid identification is essential to limit the spread within a hospital.<sup>4</sup> Conventional methods to detect resistance to oxacillin such as disc diffusion or agar dilution require up to 24 hours of incubation, depending on the guideline. In addition, they may fail to detect oxacillin resistance when phenotypic expression of an isolate is heterogeneous.<sup>5</sup> Detection of the

*mecA* gene by polymerase chain reaction (PCR) is regarded as the reference method,<sup>6</sup> but it requires special equipment which is not yet available in most routine medical microbiology laboratories. The BBL® Crystal™ MRSA ID System has been designed to allow detection of oxacillin resistance in *S aureus* within four to five hours when organisms are cultured on tryptic soy agar or sheep blood agar,<sup>7</sup> allowing earlier treatment and more rapid implementation of infection control measures. In this study we evaluated the system using 52 non-identical MRSA isolates from five countries and 85 methicillin susceptible *S aureus* (MSSA) isolates.

### Methods

#### BACTERIAL ISOLATES

Seventy one MRSA isolates—obtained from from Argentina (n = 5), Belgium (n = 8), Canada (n = 24), Germany (n = 29), and Switzerland (n = 5)—were initially included in the study. Four isolates from Canada and two from Belgium were found to have pulsed field gel electrophoresis (PFGE) patterns that were identical to those of other isolates and they were therefore excluded from further analyses. Fifty four of the remaining 65 isolates represent distinct clones as shown by PFGE, while seven isolates from Canada and four from Belgium were classified as epidemiologically related isolates.<sup>8</sup> In addition, 80 MSSA isolated from clinical material were included in the study.

All isolates were stored in tryptic soy broth with 10% glycerol at –80°C. For the experiments, they were recultivated on sheep blood agar plates.

#### IDENTIFICATION BY AGGLUTINATION ASSAY AND PLASMA COAGULASE TESTING

Before the study the species *S aureus* was reconfirmed for each isolate by agglutination testing and tube coagulation. The Staphaurex Plus (Murex Diagnostika) was used as the agglutination test, according to the manufacturer's instructions, because of its high sensitivity for detecting *S aureus*.<sup>6,9</sup> Plasma coagulation was investigated with rabbit plasma (bio-Mérieux) and read after 2, 4, and 24 hours of incubation at 36±1°C, according to the criteria

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Table 1 BBL Crystal MRSA ID System with methicillin resistant (MRSA) and susceptible (MSSA) *S aureus* isolates; one of 137 isolates failed to show fluorescence in the positive control growth well

BBL Crystal MRSA ID System	MRSA (n = 52)	MSSA (n = 84)
Positive fluorescence	45	2
No fluorescence	7	82

of Sperber and Tatini.<sup>10</sup> Any isolate with a positive agglutination reaction and a coagulation reaction  $\geq 1$  was regarded as *S aureus*. Six isolates without agglutination or visible plasma coagulation were excluded from further analyses.

#### POLYMERASE CHAIN REACTION FOR DETECTION OF THE *mecA* GENE

All isolates were investigated for the presence of the *mecA* gene (PCR product, 533 bp) by PCR. The amplification was carried out with the following primers which are highly specific<sup>11</sup>:

*mecA*1: 5'-AAA ATC GAT GGT AAA GGT TGG C;

*mecA*2: 5'-AGT TCT GCA GTA CCG GAT TTG C.

The cycle for *mecA* gene determination was 94°C for one minute; 60°C for 30 seconds; 72°C for 30 seconds; 29 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds; 94°C for 30 seconds; 60°C for 30 seconds; and 72°C for four minutes. The amplification products were stained with ethidium bromide after separation on gel electrophoresis (agarose 2%), illuminated under ultraviolet light, and photographed.

#### E-TEST FOR DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

Minimum inhibitory concentration (MIC) was determined using the E-test, carried out according to the manufacturer's instructions. Mueller-Hinton agar supplemented with 2% NaCl was used.<sup>12</sup>

#### THE BBL CRYSTAL MRSA ID SYSTEM

The BBL Crystal MRSA ID System was employed according to the manufacturer's instructions (Becton Dickinson, Heidelberg, Germany). All trays with little fluorescence after four hours were incubated for another hour and reassessed. Fluorescence in the oxacillin containing well was regarded as evidence for oxacillin resistance when the positive growth control showed fluorescence and the negative growth control did not.

Table 2 Comparison of the sensitivities of the BBL® Crystal™ MRSA ID System on oxacillin resistant *Staphylococcus aureus* isolates from five different countries

Country	Oxacillin resistant <i>Staphylococcus aureus</i> (n)	BBL Crystal MRSA ID System positive (n)	Sensitivity
Argentina	5	5	100%
Belgium	6	6	100%
Canada	17	13	76.5%
Germany	19	17	89.5%
Switzerland	5	4	80%
All countries	52	45	83%

MRSA, methicillin resistant *Staphylococcus aureus*.

#### STATISTICAL ANALYSIS

The sensitivity, specificity, positive predictive value, and negative predictive value were calculated.<sup>13</sup>

#### Results

All 139 *S aureus* isolates were classified as resistant or susceptible to oxacillin by the oxacillin MIC values according to the National Committee for Clinical Laboratory Standards (NCCLS). Fifty two isolates were oxacillin resistant (MIC value  $\geq 4$  mg/litre; 51 *mecA* positive), 85 isolates were oxacillin susceptible (MIC value  $\leq 2$  mg/litre; 83 *mecA* negative). Two isolates were found to have MIC values of 3 mg/litre (both *mecA* positive) and were excluded from further analysis. There were 136 isolates that achieved sufficient growth in the control well within five hours (99.3%).

Of the MRSA, 86.5% were correctly identified as oxacillin resistant, with a negative predictive value of 92.2% (table 1). Four of the seven MRSA which failed to show fluorescence came from Canada (oxacillin MIC values: 4, 12, 48, and 256 mg/litre; all *mecA* positive), two came from Germany (oxacillin MIC values: 4 and 256 mg/litre; one *mecA* positive), and one came from Switzerland (oxacillin MIC value: 24 mg/litre; *mecA* positive; table 2).

Of the MSSA, 97.6% were found to be oxacillin susceptible by the BBL Crystal MRSA ID System, with a positive predictive value of 95.7%.

#### Discussion

Rapid detection of resistance to oxacillin in *S aureus* is very important for the clinical management of an infection and to implement appropriate infection control measures. It is claimed that the BBL Crystal MRSA ID System detects resistance to oxacillin within four to five hours when organisms are cultured on tryptic soy agar or sheep blood agar.<sup>7</sup> The use of screening cultures such as mannitol salt agar cancels out any speed advantage intrinsic to the kit because subcultures on tryptic soy agar or sheep blood agar would be necessary. The kit uses a fluorescent indicator which is sensitive to the presence of oxygen dissolved in the broth. When oxygen is present no fluorescence can be detected; when oxygen is removed (for example, by respiration of the bacteria in the presence of oxacillin) fluorescence is easily detected. In our study we found that the BBL Crystal MRSA ID System had a low sensitivity (86.5%) but a high specificity (97.6%). Other investigators have also found the system to be highly specific, but our sensitivity rate is the lowest ever reported. A sensitivity of 100% was reported for 142 MRSA isolates from a hospital in Saudi Arabia,<sup>14</sup> while Knapp *et al* reported 95% sensitivity in 69 *mecA* positive isolates.<sup>15</sup> A sensitivity of 92.9% among 170 *mecA* positive isolates was described in a European multicentre study,<sup>16</sup> and a sensitivity of 92.6% was reported from France among 27 *mecA* positive *S aureus* isolates.<sup>17</sup> Dutka-Malen *et al* found a sensitivity and a specificity of 100% among 13 MRSA and 11 MSSA isolates.<sup>18</sup> Willey *et al* found an accurate classi-

fication by the BBL Crystal MRSA ID System in 25 *mecA* positive and 18 *mecA* negative *S aureus* isolates.<sup>19</sup> In another report, difficulties with the test system were only observed in borderline resistant strains.<sup>20</sup>

Apart from our own study, no other investigator has proved clonal non-identity of the isolates. Retesting of copy strains cannot therefore be excluded. In our selection of MRSA isolates from five different countries, four of the seven MRSA without fluorescence in the test kit came from Canada, two from Germany, and one from Switzerland. An explanation for this could be the lack of oxacillin phenotypic resistance despite the presence of the *mecA* gene. Up to 16.7% of all oxacillin susceptible *S aureus* isolates may carry the *mecA* gene.<sup>21</sup> Of the seven isolates that failed to show fluorescence in the BBL Crystal MRSA ID System, two were found with MIC values of 4 mg/litre<sup>12</sup> and the remaining five isolates had MIC values  $\geq 12$  mg/litre. It has been shown previously that borderline resistant isolates are difficult to identify accurately.<sup>20</sup> It remains unclear why some highly resistant isolates apparently do not utilise oxygen in the presence of 2 mg/litre oxacillin. This observation might be most important for microbiological laboratories in Canada—the origin of the isolates giving the lowest test sensitivity in our study.

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