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European Multicentre Evaluation of a Commercial System for Identification of Methicillin-Resistant *Staphylococcus aureus*

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A commercial system for the rapid detection of methicillin-resistant *Staphylococcus aureus*, the BBL Crystal MRSA test (C-MRSA ID; Becton Dickinson, USA), was evaluated prospectively and compared with a polymerase chain reaction test for the presence of the *mecA* gene. Ten European centres tested a total of 676 isolates of *Staphylococcus aureus* from blood cultures. The system correctly identified 661 (97.8%) isolates within 4 h. All but three *mecA* gene-negative isolates (99.4% specificity) yielded a negative C-MRSA ID reaction, and 158 of 170 *mecA* gene-positive isolates were accurately detected (92.9% sensitivity). After repeated testing of discrepant results, sensitivity and specificity increased to 99% and 100%, respectively.

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Table 1: Agreement between the BBL Crystal MRSA ID (C-MRSA ID) system and the *mecA* detection test, and sensitivity, specificity, and predictive values of the C-MRSA ID, based on results obtained after 4h of incubation.

Site	No. of isolates	Agreement (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Barcelona	97	97	82	100	100	96
Bonn	54	100	NA	100	NA	100
Brussels	44	98	94	100	100	97
Dublin	26	92	100	85	75	100
Genova	49	96	71	100	100	95
Genoa	99	98	96	100	100	96
London	55	96	91	98	91	98
Lyon	62	97	82	100	100	96
Paris	96	100	100	100	100	100
Vienna	95	99	96	100	100	99
Total or mean	676	97.8	93	99.4	98.1	95.9

NA, not applicable; NPV, negative predictive value; PPV, positive predictive value.

The importance of methicillin-resistant *Staphylococcus aureus* (MRSA) as a nosocomial pathogen is well established. The accurate identification of these strains is of considerable clinical importance and remains a problem, especially for heteroresistant or borderline-resistant isolates (1). A rapid assessment of methicillin resistance is needed, especially in severe infections, since this property limits the choice of effective antibacterial drugs.

The purpose of this study was to evaluate a new susceptibility test system, the BBL Crystal MRSA ID test (C-MRSA ID; Becton Dickinson, USA), that is capable of detecting MRSA within 4 h. The system is based on the ability of MRSA to grow in the presence of 4 mg/l oxacillin and 2% sodium chloride, which results in a decrease of oxygen in the medium, leading to emission of fluorescence. The accuracy of the C-MRSA ID test was assessed by comparing its results with those of a polymerase chain reaction (PCR) test for the presence of the *mecA* gene (2), which encodes production of the low-affinity penicillin-binding protein PBP2a responsible for methicillin resistance (3).

Materials and Methods. Six hundred seventy-six strains of *Staphylococcus aureus* isolated from blood cultures in 1994 were studied. These included 170 methicillin-resistant *Staphylococcus aureus* and 506 methicillin-susceptible *Staphylococcus aureus*. The isolates originated from Barcelona (Spain), Bonn (Germany), Brussels (Belgium), Dublin (Ireland), Geneva (Switzerland), Genoa (Italy), London (UK), Lyon and Paris (France), and Vienna (Austria). All isolates were identified by the tube coagulase test and classified as methicillin-resistant on the basis of the presence of the *mecA* gene.

The C-MRSA ID system is based on the detection of staphylococcal growth by an oxygen-sensitive fluorescence indicator that detects oxygen consumption due to bacterial metabolism. The test consists of a sequence of three wells, including two control wells, one without antibiotic that allows growth (positive control) and another containing 16 mg/l vancomycin that inhibits growth (negative control). The test well contains 4 mg/l oxacillin and permits identification of methicillin resistance by fluorescence-indicated growth of the test strain.

All experiments were performed in each centre according to the manufacturer's recommendations. Fresh saline suspensions of each test isolate were adjusted to a turbidity of 0.5 McFarland standard. In order to obtain a final inoculum of 10^7 cfu/ml, a 0.5 ml aliquot of the standard suspension was transferred to a tube containing Mueller-Hinton broth with 2% NaCl. Four drops of the test broth suspension were added to each well. The panel was incubated in a 35°C incubator in air for 4 h. Bacterial growth was then visualized under ultraviolet illumination at 365 nm. The test well was read and compared with the positive and negative control wells.

The presence of the *mecA* gene was assessed using DNA extraction and PCR amplification methods as described previously (4). An intragenic fragment of the *mec* gene and the gyrase gene (*gyrA*) were coamplified simultaneously. The *gyrA* gene acts as an internal control to establish unequivocally the quality of the DNA extraction and amplification for each strain. Amplified

products were detected by agarose gel electrophoresis.

Results and Discussion. This prospective study confirmed the high incidence of septicaemia caused by MRSA in most parts of Europe. Methicillin resistance occurred in 0 to 50% of the *Staphylococcus aureus* strains isolated from blood cultures in each of the centres, with an overall proportion of 25% (170/676).

The agreement between the C-MRSA ID test and the reference test to detect the *mecA* gene, together with the sensitivity, specificity, and positive and negative predictive values of the C-MRSA ID, are presented in Table 1. A 97.8% correlation was observed between the results of both tests. The specificity, percentage agreement, positive predictive value, and negative predictive value were very satisfactory (> 95%). The sensitivity (93%) was also satisfactory but probably could be improved by an additional 1 h period of incubation, as suggested previously (5). Upon repeated testing, correct results were obtained for 13 of the 15 isolates from which results were initially discrepant.

However, two MRSA strains failed to produce a positive result, even after 5 h of incubation. One *mecA* gene-positive isolate was sensitive to the sodium chloride content of the Mueller-Hinton medium (agar or broth). The minimum inhibitory concentration of oxacillin for this isolate was > 4 mg/l or 0.06 mg/l, respectively, depending on the absence or presence of 4% sodium chloride. The other *mecA* gene-positive isolate demonstrated heterogeneous resistance to oxacillin detectable only with Mueller-Hinton agar containing 5% NaCl.

Since infection with MRSA can be extremely difficult to treat, the rapid identification of MRSA by the C-MRSA ID system may aid in the rational management of these infections in hospitals and in the prevention of MRSA spread. Technically, this test is easy to perform and to integrate into routine diagnostic workflow. It represents a useful alternative to other rapid antimicrobial susceptibility test systems (6–8) and molecular methods, especially since it does not require expensive equipment for interpretation.

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Comparison of Immunofluorescence with Enzyme Immunoassay for Detection of Q Fever

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To evaluate enzyme immunoassay (EIA) as an alternative to indirect immunofluorescence assay (IFA) to screen for Q fever in humans, 157 serum samples from patients suspected of having the disease were tested for immunoglobulin G antibodies

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