Comparative Evaluation of Two PCR-Based Methods for Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA): Xpert MRSA Gen 3 and BD-Max MRSA XT

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We compared two walk-away molecular diagnostic assays, the GeneXpert MRSA Gen 3 assay and the BD-Max MRSA XT assay. A total of 119 prospective swabs and 36 culture-positive samples were tested. Xpert MRSA Gen 3 had sensitivity of 95.7% and specificity of 100% versus 87.5% and 97.1% for BD-Max. The difference in agreement with the enriched culture results was significantly in favor of the Xpert assay ($P < 0.02$, McNemar nonparametric test).

The transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) from patient to patient in health care settings is a major concern due to a poorer prognosis associated with severe MRSA infections, such as bacteremia (1, 2). The rise in the incidence of methicillin-resistant *Staphylococcus aureus* infections was initially restricted to a few health care-associated clones and confined to a limited set of patients in high-risk groups. The molecular detection of MRSA directly from clinical samples is complicated by the frequent association of methicillin-susceptible *S. aureus* with methicillin-resistant coagulase-negative staphylococci. Distinguishing between this association and true MRSA requires the specific detection of the junction of the staphylococcal cassette chromosome in the *orfX* locus as well as positive detection of the *mec* gene encoding methicillin resistance (3). With the onset of community-acquired MRSA (4), outpatients and emergency room patients became at risk for MRSA carriage, further increasing the need for determination of carriage of MRSA. More recently, livestock-associated MRSA carrying variant mecC genes further complicated the epidemiology of MRSA by increasing its molecular diversity (5). Microbiology laboratories usually define MRSA phenotypically, but the underlying genetic structures responsible for this phenotype are constantly changing (6–8), and the molecular assays designed several years back are not necessarily relevant to the epidemiology observed today (9–11). A recent comparison of culture and a commercial molecular assay performed in a high-prevalence environment showed the molecular assay to be slightly less sensitive than enriched culture using ChromID MRSA agar but also showed that the results were not significantly different, although the molecular assay yielded results in hours instead of days (12).

Two fully automated walk-away platforms offer molecular detection of MRSA in nasal swabs: the BD-Max (Becton Dickinson, Le Pont de Claix, France) and the GeneXpert (Cepheid, Maurens Scopont, France) platforms. Both tests rely on the same principle (3) and detect the presence of the *mecA* gene and the *mecC* gene (*mecA/C*) as well as the presence of the junction between the staphylococcal cassette chromosome *mec* element (SCCmec) and the chromosome. However, the sequences of the primers and probes in the two assays are likely distinct, and the extraction processes rely on different principles. The performances of the assays of the previous generations have recently been found to be similar (13), but the release of updated versions warrants a renewed comparison. Moreover, the MRSA clones isolated in Europe and the United States are epidemiologically different and assay performances could be different (14, 15).

We evaluated the performances of early-release versions of Xpert MRSA Gen3 and BD-Max MRSA XT in Hospital Raymond Poincaré, Garches, France, a teaching hospital in the Paris public hospital system (Assistance Publique–Hôpitaux de Paris) affiliated with the Université de Versailles St. Quentin. At the time of the study, Xpert MRSA Gen3 was designated “research use only” (RUO) whereas BD-Max MRSA XT was CE-In Vitro Diagnostics (CE-IVD) marked. Patients in high-prevalence wards (spinal cord rehabilitation, intensive care unit, and bone and joint infection service) are routinely monitored for MRSA carriage, with the anterior nares swabbed on admission and weekly screening thereafter.

A prospective study that included 119 screened patients was performed in the course of 45 days using Copan Transys system Liquid Stuart single swabs to sample both anterior nares. The testing of leftover samples taken in the course of normal care is institutional review board (IRB) exempt according to French law. In order to ensure that all techniques were performed with identical starting materials, each swab was discharged in 500 µl of isotonic saline solution in a CryoFreeze vial (Nalgene). A 100-µl volume of the solution was used for selective culture on ChromID MRSA agar (bioMérieux, Marcy l’Etoile, France) and for overnight enrichment in tryptic soy broth (Bio-Rad, Marnes-La-Coquette, France) followed by overnight incubation.
France) at 36°C. A 100-µl volume of the broth was then plated on MRSA selective agar, with review of the plates performed after 24 h and 48 h. A 100-µl volume (each) was used for molecular detection on the BD-Max and Xpert platforms according to each manufacturer’s instructions. This process was adapted with minor modifications from the method used by Dalpke et al. (16) and validated using a quality control panel of organisms, including mecC isolates (Methicillin Resistant S. aureus 2013 EQA Programme; Qnostics [Glasgow, United Kingdom]); both assays successfully categorized all 12 samples. This divergence from the procedure recommended by the manufacturers could prove to lead to biased results, although we decided to favor identical starting materials for the two methods rather than the use of different swabs with potentially different starting materials.

Twelve of the 119 swabs prospectively tested were MRSA positive by culture (10%), 11 samples providing an actionable result within 24 h (sensitivity of 91.67%) and 1 requiring broth enrichment. This sample was detected by both the Xpert and BD-Max assays, suggesting that a low inoculum load is not a difficulty for molecular assays. The positive predictive values (PPV) for the Xpert and BD-Max assays were, respectively, 100% and 72.7%, and the negative predictive values (NPV) were 99.0% and 96.1%. One direct-culture-positive swab was detected by neither Xpert nor BD-Max. An isolated MRSA colony was resuspended in saline solution, and 10⁷ CFU was added to the elution reagent and an identical amount to the sample buffer. Both assays were positive for the amplification of the internal control and the meca/C gene targets but negative for SCC and mec right extremity junction (MREJ), hinting at incomplete primer and probe repertoires for this target. Three samples negative by culture and Xpert MRSA Gen 3 yielded positive MRSA detection by BD-Max MRSA XT. Results of further examination of the real-time fluorescence curves were suggestive of false positives of the detection algorithm as they were undistinguishable from the curves of the negative samples that were run simultaneously. This “false-positive” call is easily detected by review of the graphs as long as a positive sample is included in the run (Fig. 1), but detection of such a call can be challenging in the absence of positive samples because of the lack of fluorescence units and the autoscaling of the graphs.

To increase the number of positive samples tested on the molecular platforms, the swabs taken thereafter were similarly discharged in saline solution and the suspension was stored at 4°C for 18 to 24 h until the culture results were known. For 36 direct-culture-positive specimens, the molecular assays were then performed as previously described. The results are displayed in the flow chart (Fig. 2), and the performance of the assays is quantified in Table 1. Overall, the agreement of the Xpert MRSA Gen 3 results with culture results proved to be significantly superior to that of the BD-Max results as determined by the McNemar test (P = 0.023), with better sensitivity and specificity and a lower (0.6% versus 2.6%) and yet not statistically significant number of unresolved samples. Considering the whole study set, the PPV of Xpert MRSA remains at 100% whereas the PPV of BD-Max MRSA XT improves to reach 93.3%. However, the assays were not used strictly according to the manufacturer’s recommendation, and only 20% of the total inoculum was tested using each technique. Three of the four samples detected by Xpert MRSA Gen3 had SCC threshold cycle (Cₜ) values greater than 33, suggesting that the sample could possibly have been detected by both platforms using the entire swab. The fourth sample had a Cₜ value of 19.1, sugges-

![Representative MREJ fluorescence curves generated by the BD-Max MRSA XT in the same amplification and detection run.](http://jcm.asm.org/)

(A) “Backgrounded” PCR without true positive. (B) “Color-compensated” PCR without true positive. (C) “Backgrounded” PCR with true positive. (D) “Color-compensated” PCR with true positive. A10, MRSA true-negative sample; B3, MRSA true-positive sample; B9, MRSA false-positive sample. The software calls a positive MRSA result in the absence of amplification in the MREJ channel as determined by naked-eye interpretation of the curves. (A) The absence of fluorescence units on the graph and the autoscaling make the call difficult in the backgrounded PCR view in the absence of a positive result in the run. (C and D) In the presence of a true positive (sample B3), the lack of amplification of MREJ in the false positive (sample B9) is readily apparent.

FIG 1

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tive of an incomplete primer-probe repertoire in BD-Max MRSA XT. It is also noteworthy that only half of the nucleic acids extracted from the sample for BD-Max are used for the PCR, the other half being available for storage or further processing.

These results show that the performance of molecular assays for detection of methicillin resistance in *S. aureus* needs to be reevaluated periodically to ensure that they continue to match the current and local epidemiology of the organisms. Both of the tests reviewed here have been updated in terms of *mecC* detection as evidenced by the good performance seen with the *mecC* quality control isolate. A few isolates that still thwart these assays will be further analyzed in terms of SCC type and genetic makeup of the SCC.

Previous studies have addressed the laboratory efficiency of both platforms using earlier assay versions and have found discrepancies in the turnaround times depending on the number of assays processed and the maximum capacity of the instrument (13). Overall, the hands-on time requirements per sample with the two methods were found to be comparable in our experience. The turnaround time for the Xpert MRSA cartridge is 58 min once it has been loaded, and the throughput is dependent on the number of units installed. The instrument allows each of the slots to be used independently and provides a random access architecture allowing “on demand” testing by personnel with low to medium technical qualifications. The total turnaround time of the BD-Max assay is longer (around 120 min) and varies depending on the number of samples processed simultaneously, since a four-channel robotic arm performs all the pipetting. The instrument is not fully random access and is best suited to work with series of up to 24 samples. The BD-Max offers the interesting possibility of porting laboratory-developed assays to an automated walk-away molecular assay platform.

Overall, the RUO Xpert MRSA Gen 3 assay showed significantly better analytical performance than the BD MAX MRSA XT assay in testing a cohort of hospitalized patients in the setting of a French tertiary care center.

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**REFERENCES**


