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Evaluation of molecular assays for rapid detection of methicillin-resistant *Staphylococcus aureus*

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Abbreviations: Methicillin resistant *Staphylococcus aureus*, MRSA; Limit of detection, LoD; methicillin-resistant coagulase-negative staphylococci, MRCoNS; methicillin-sensitive coagulase-negative staphylococci, MSCoNS; colony forming units, cfu.

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ABSTRACT

The diagnostic sensitivity of BD-GeneOhm and Cepheid-Xpert was compared with culture on log-serial dilutions of well-characterized methicillin-resistant *Staphylococcus aureus* (MRSA) and non-MRSA isolates, and on nasal and groin swabs from patients with prior history of MRSA carriage. Sensitivities of GeneOhm and Xpert were high at 10^3 cfu/ml MRSA concentrations (92.3% and 96.3%, respectively) although decreased considerably (< 35%) at a log-lower concentration. Unexpectedly, both assays also detected select coagulase-negative staphylococci, which requires further evaluation.

TEXT

Effective and rapid laboratory diagnosis is critical for treating, managing, and preventing methicillin-resistant *Staphylococcus aureus* (MRSA) infections. PCR-based MRSA detection assays offer certain benefits over conventional culture techniques such as lower detection limits, high-throughput screening, and importantly, shorter time to detection. Currently, two of the most promising commercially available PCR-based assays for MRSA detection are GeneOhm MRSA (BD Diagnostics, Erembodegem, Belgium) and Xpert MRSA (Cepheid, Bouwel, Belgium) (reviewed in ref. 10). Both target the junction of the mobile element *SCCmec* (Staphylococcal cassette chromosome *mec*) carrying the *mecA* methicillin resistance gene in *S. aureus* (6).

We first evaluated and compared the diagnostic sensitivities of BD GeneOhm and Cepheid Xpert MRSA assays on patient screening samples compared to culture—both direct and after overnight-enrichment—on conventional/chromogenic media (BBL-CHROMagar, BD Diagnostics), followed by confirmatory testing, as previously described (9,21). Fifty-two nose and groin samples were prospectively collected in 1.5 ml brain heart infusion broth and 15% glycerol from 26 previously identified MRSA carriers at the University of Geneva Hospitals. Following manufacturers' recommendations, samples were tested on GeneOhm and Xpert that showed a similar sensitivity for MRSA detection (96% versus 93%, respectively), compared to direct culture that detected 28 samples as MRSA-positive (Table 1). Consistent with recent reports (1,21), an overnight enrichment protocol drastically increased the MRSA true positive status of the patient screening samples compared to direct-culture (42/52 versus 28/52). Of the 14 samples that did not show any MRSA colony-forming units (cfu) on direct-culture, Xpert

successfully detected 2 and GeneOhm 7 samples, suggesting an increased sensitivity of these PCR-based assays over direct cultures. However, taking preenriched-culture results as gold-standard, GeneOhm and Xpert showed significantly reduced sensitivities of 81% (McNemar test, *P*=0.039) and 66.7% (*P*=0.001), respectively (Table 1). However, sensitivities of GeneOhm and Xpert were not significantly different from each other with an overall concordance of 80.8% (n =42, Cohen's kappa=0.60); or 76.9% (n=20, kappa=0.54) and 84.6% (n=22, kappa=0.65) for nasal and groin samples, respectively. These data on previously identified MRSA carriers are similar to recent hospital-based studies showing comparable, high sensitivities of GeneOhm and Xpert on patient screening samples from the nose/groin or throat, compared to direct-culture, but a reduced performance compared to results of enriched culture (7,24). Only three samples with MRSA load of 100 cfu/ml or more were not detected by these assays. These constituted two groin samples for Xpert; and a nasal sample for GeneOhm from a patient that only carried MRSA in nose. Because certain *SCCmec* IV variants are reported not to be detected by these assays possibly due to an altered *SCCmec* element, we performed *SCCmec* genotyping as described (5). *SCCmec* I was the predominant clone identified in all but two isolates that harbored one each of *SCCmec* II and IV. Interestingly, the nasal sample that GeneOhm failed to identify carried *SCCmec* IV MRSA.

To identify the actual limits of detection (LoD) of GeneOhm and Xpert on divergent MRSA clones as well as to overcome the inherently low epidemiological diversity observed among clinical samples collected from a single hospital, we analyzed 27 distinct MRSA strains at defined concentrations. These strains harbored distinct *SCCmec* subtypes and comprised some of the most prevalent, well-characterized clonal lineages that have disseminated worldwide in hospitals and community, including animal-associated MRSA that are carried and cause

disease in humans (2,22) (Supplementary Table 1). MRSA strains were tested in serial dilutions on these assays from 10^0 through 10^5 cfu/ml (1,10,10^2,10^3,10^4,10^5) until a positive result was obtained. Both assays showed high sensitivities for detection of pure MRSA strains at concentrations of 10^3 cfu/ml with the average LoDs for GeneOhm (430 cfu/swab or 4300 cfu/ml) and Xpert (250 cfu/swab or 3300 cfu/ml) corroborating with previous data (16,17) (GeneOhm-MRSA-package insert) (Table 2). Nonetheless, the steep drop in sensitivity at 10^2 cfu/ml questions the ability of these assays to accurately detect MRSA carriage at lower concentrations including carriers that have completed topical decolonization treatment, but in whom complete eradication has not been achieved (14,23). Moreover, 3 MRSA strains could not be detected at 10^3 cfu/ml but at a log higher concentration in two independent experiments. These comprised MRSA harboring *SCCmec* III/ST239 (GeneOhm, human MRSA strain#9, Supplementary Table 1), *SCCmec* IV/ST398 (GeneOhm, animal MRSA#19), or *SCCmec* V/ST 398 (Xpert, animal MRSA# 20). The reduced sensitivities of detection observed for these MRSA corroborate previous reports of detection failures with human and animal MRSA harboring *SCCmec* types III, IV, and V on these assays (8,15,19,20). While the precise reason for this is unknown, sequence variations in the targeted *orfX-SCCmec* junction region, which are especially common in animal MRSA (13), are the most likely reason for the poor performance of the molecular assays with specific MRSA strains. Hence, from a clinical use perspective, iterative modifications of the molecular assays based on epidemiological changes will be necessary to sustain optimal sensitivities.

Lastly, we also studied cross-reactions to non-MRSA on mixtures of select MRSA and non-MRSA strains including various methicillin-resistant and -sensitive coagulase-negative staphylococci (MRCoNS and MSCoNS) (n = 25; Supplementary Table 1, strains #28 through

#52). Twenty-one mixtures of non-MRSA/MRSA were prepared as described in Supplementary information, and assayed on serial dilutions from 10^0 to 10^5 cfu/ml MRSA concentrations. Interestingly, an increased sensitivity (and lower LoD) was observed for MRSA in mixtures spiked with non-MRSA when compared to pure MRSA strains at similar concentrations (Table 2, lower panel). To study whether this increased sensitivity was due to cross-reactivity to non-MRSA strains, we tested all 25 pure non-MRSA strains individually as well as 8 mixtures comprising only non-MRSA at a single high concentration of 10^5–10^6 cfu/ml MRSA. Those showing false-positive results on either molecular assay were confirmed on log-serial dilutions. A false-positive detection of pure non-MRSA strains and their mixtures was observed sporadically with GeneOhm (all 5 MRCoNS and 1 of 3 MSCoNS tested) and Xpert (3 MRCoNS and 2 MSCoNS) (see Supplementary Table 2 for C_T values obtained for these strain dilutions). In a previous analytical study by Huletsky and colleagues, approximately 250 MRCoNS and MSCoNS were tested that did not show any false-positive detection on an in-house real-time PCR targeting *orfX-SCCmec* junction (6). GeneOhm and Xpert are also based on the same principle, although the primer targets might differ from Huletsky *et al.* (6). A US-based study tested 44 strains of MRCoNS and MSCoNS on Xpert and did not find any cross-reactivity (24), although the species and *SCCmec* types present in these strains were not described in the study. In yet another analytical study, Francois and colleagues showed false positive results on GeneOhm with MSSA, but MRCoNS were not tested (4). Some other clinical studies on large numbers of human screening samples have also shown false positive results, however, the underlying cross-reactive organisms could not be completely elucidated (3,7). Interestingly, similar to *S. aureus*, the vicinity of the *orfX* gene is a preferred site for insertion of *SCCmec* cassettes in other staphylococci, and frequent

exchange of parts or of entire *SCCmec* elements or even of non-*mecA*-containing *SCC* elements is also common in these organisms (11,12). Preliminary sequencing of the *orfX-SCCmec* junction region in select falsely positive MRCoNS has shown high homology to MRSA (Malhotra-Kumar et al, unpublished results). Thus, in addition to the well described cross-reactivity with MSSA (18), our study shows that presence of select MRCoNS in human screening samples could also impact the specificity of *orfX-SCCmec* targeting assays.

CONFLICT OF INTEREST STATEMENT

SM-K has received a speaker's honorarium from BD Diagnostics. We declare no conflict of interest.

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The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing the report.

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LEGENDS

Table 1. Sensitivities of GeneOhm and Xpert for MRSA detection from patient screening samples in comparison to direct and pre-enriched culture results.

Table 2. Sensitivities and limits of detection (LoD) of the two assays tested on pure strains and their defined mixtures at various concentrations.

Table 1

Assay	Samples	Sensitivity (95%CI) (Proportion of true positive samples)	
		Direct-culture	Preenriched-culture
GeneOhm	Nasal	90.9% (62.5–98.4) (10/11)	71.4% (50.0–86.2) (15/21)
	Groin	100% (81.6–100) (17/17)	90.5% (71.1–97.4) (19/21)
	All	96.4% (82.3–99.4) (27/28)	81.0% (66.7–90.0) (34/42)
Xpert	Nasal	100% (74.1–100) (11/11)	57.1% (36.6–75.5) (12/21)
	Groin	88.2% (65.7–96.7) (15/17)	76.1% (54.9–89.4) (16/21)
	All	92.9% (77.4–98.0) (26/28)	66.7% (51.6–79.0) (28/42)

Table 2

Sample	Assay	Limits of Detection (LoD)			Sensitivity at MRSA concentration		
		Range (cfu/ml)	Average LoD (cfu/ml) (95% CI)	Average LoD (cfu/swab)* (95% CI)	10^2 cfu/ml	10^3cfu/ml	10^4cfu/ml
MRSA isolates (n = 27)	GeneOhm	$1.4x10^2$–$4.1x10^4$	$4.3x10^3$ ($1.7x10^2$–$3.2x10^4$)	$4.3x10^2$ ($1.7x10^1$–$3.2x10^3$)	33.3% (n=9)	92.3% (n=25)	100% (n=27)
	Xpert	$1.4x10^2$–$2.0x10^4$	$3.3x10^3$ ($1.6x10^2$–$1.1x10^4$)	$2.5x10^2$ ($1.2x10^1$–$8.4x10^2$)	14.8% (n=4)	96.3% (n=26)	100% (n=27)
MRSA/Non-MRSA mixtures (n = 21)	GeneOhm	$5.4x10^0$–$5.1x10^3$	$2.0x10^3$ ($4.5x10^1$–$4.9x10^3$)	$2.0x10^2$ ($4.5x10^0$–$4.9x10^2$)	42.9% (n=9)	100% (n=21)	- #
	Xpert	$2.7x10^1$–$5.1x10^3$	$2.4x10^3$ ($3.7x10^1$–$5.0x10^3$)	$1.8x10^2$ ($2.7x10^0$–$3.7x10^2$)	38.1% (n=8)	100% (n=21)	-

*Calculated for a 100 µl and 75 µl sample input for GeneOhm and Xpert, respectively

Not determined as all MRSA-positive mixtures were detectable at the preceding lower concentration.