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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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1 **ABSTRACT**

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

9 **TEXT**

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

19 We first evaluated and compared the diagnostic sensitivities of BD GeneOhm and Cepheid
20 Xpert MRSA assays on patient screening samples compared to culture—both direct and after
21 overnight-enrichment—on conventional/chromogenic media (BBL-CHROMagar, BD
22 Diagnostics), followed by confirmatory testing, as previously described (9,21). Fifty-two nose
23 and groin samples were prospectively collected in 1.5 ml brain heart infusion broth and 15%
24 glycerol from 26 previously identified MRSA carriers at the University of Geneva Hospitals.
25 Following manufacturers' recommendations, samples were tested on GeneOhm and Xpert that
26 showed a similar sensitivity for MRSA detection (96% versus 93%, respectively), compared
27 to direct culture that detected 28 samples as MRSA-positive (Table 1). Consistent with recent
28 reports (1,21), an overnight enrichment protocol drastically increased the MRSA true positive
29 status of the patient screening samples compared to direct-culture (42/52 versus 28/52). Of the
30 14 samples that did not show any MRSA colony-forming units (cfu) on direct-culture, Xpert

31 successfully detected 2 and GeneOhm 7 samples, suggesting an increased sensitivity of these
32 PCR-based assays over direct cultures. However, taking preenriched-culture results as gold-
33 standard, GeneOhm and Xpert showed significantly reduced sensitivities of 81% (McNemar
34 test, $P=0.039$) and 66.7% ($P=0.001$), respectively (Table 1). However, sensitivities of
35 GeneOhm and Xpert were not significantly different from each other with an overall
36 concordance of 80.8% ($n=42$, Cohen's kappa=0.60); or 76.9% ($n=20$, kappa=0.54) and 84.6%
37 ($n=22$, kappa=0.65) for nasal and groin samples, respectively. These data on previously
38 identified MRSA carriers are similar to recent hospital-based studies showing comparable,
39 high sensitivities of GeneOhm and Xpert on patient screening samples from the nose/groin or
40 throat, compared to direct-culture, but a reduced performance compared to results of enriched
41 culture (7,24). Only three samples with MRSA load of 100 cfu/ml or more were not detected
42 by these assays. These constituted two groin samples for Xpert; and a nasal sample for
43 GeneOhm from a patient that only carried MRSA in nose. Because certain *SCCmec* IV
44 variants are reported not to be detected by these assays possibly due to an altered *SCCmec*
45 element, we performed *SCCmec* genotyping as described (5). *SCCmec* I was the predominant
46 clone identified in all but two isolates that harbored one each of *SCCmec* II and IV.
47 Interestingly, the nasal sample that GeneOhm failed to identify carried *SCCmec* IV MRSA.
48 To identify the actual limits of detection (LoD) of GeneOhm and Xpert on divergent MRSA
49 clones as well as to overcome the inherently low epidemiological diversity observed among
50 clinical samples collected from a single hospital, we analyzed 27 distinct MRSA strains at
51 defined concentrations. These strains harbored distinct *SCCmec* subtypes and comprised some
52 of the most prevalent, well-characterized clonal lineages that have disseminated worldwide in
53 hospitals and community, including animal-associated MRSA that are carried and cause

54 disease in humans (2,22) (Supplementary Table 1). MRSA strains were tested in serial
55 dilutions on these assays from 10^0 through 10^5 cfu/ml (1,10,10²,10³,10⁴,10⁵) until a positive
56 result was obtained. Both assays showed high sensitivities for detection of pure MRSA strains
57 at concentrations of 10^3 cfu/ml with the average LoDs for GeneOhm (430 cfu/swab or 4300
58 cfu/ml) and Xpert (250 cfu/swab or 3300 cfu/ml) corroborating with previous data (16,17)
59 (GeneOhm-MRSA-package insert) (Table 2). Nonetheless, the steep drop in sensitivity at 10^2
60 cfu/ml questions the ability of these assays to accurately detect MRSA carriage at lower
61 concentrations including carriers that have completed topical decolonization treatment, but in
62 whom complete eradication has not been achieved (14,23). Moreover, 3 MRSA strains could
63 not be detected at 10^3 cfu/ml but at a log higher concentration in two independent experiments.
64 These comprised MRSA harboring *SCCmec* III/ST239 (GeneOhm, human MRSA strain#9,
65 Supplementary Table 1), *SCCmec* IV/ST398 (GeneOhm, animal MRSA#19), or *SCCmec*
66 V/ST 398 (Xpert, animal MRSA# 20). The reduced sensitivities of detection observed for
67 these MRSA corroborate previous reports of detection failures with human and animal MRSA
68 harboring *SCCmec* types III, IV, and V on these assays (8,15,19,20). While the precise reason
69 for this is unknown, sequence variations in the targeted *orfX-SCCmec* junction region, which
70 are especially common in animal MRSA (13), are the most likely reason for the poor
71 performance of the molecular assays with specific MRSA strains. Hence, from a clinical use
72 perspective, iterative modifications of the molecular assays based on epidemiological changes
73 will be necessary to sustain optimal sensitivities.

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

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

100 exchange of parts or of entire *SCCmec* elements or even of non-*mecA*-containing *SCC*
101 elements is also common in these organisms (11,12). Preliminary sequencing of the *orfX*-
102 *SCCmec* junction region in select falsely positive MRCoNS has shown high homology to
103 MRSA (Malhotra-Kumar et al, unpublished results). Thus, in addition to the well described
104 cross-reactivity with MSSA (18), our study shows that presence of select MRCoNS in human
105 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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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 ³ cfu/ml	10 ⁴ cfu/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.