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ARTICLE

MRSA screening by the Xpert MRSA PCR assay: pooling samples of the nose, throat, and groin increases the sensitivity of detection without increasing the laboratory costs

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Abstract The performance of the Xpert MRSA polymerase chain reaction (PCR) assay on pooled nose, groin, and throat swabs (three nylon flocked eSwabs into one tube) was compared to culture by analyzing 5,546 samples. The sensitivity [0.78, 95 % confidence interval (CI) 0.73–0.82] and specificity (0.99, 95 % CI 0.98–0.99) were similar to the results from published studies on separated nose or other specimens. Thus, the performance of the Xpert MRSA assay was not affected by pooling the three specimens into one assay, allowing a higher detection rate without increasing laboratory costs, as compared to nose samples alone.

Introduction

The rapid and accurate detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers helps to reduce the risk of transmission to other patients. Rapid polymerase chain reaction (PCR)-based methods enable to confirm or refute MRSA carriage in patients within 2 h. Most of them were evaluated with nose specimens, whereas studies showed that multiple site sampling increases the sensitivity of MRSA detection [1–6].

The high price of commercially available rapid PCR tests for MRSA screening leads some laboratories to pool specimens of the same patient into one single assay [7], whereas others consider that these tests are not cost-effective [8]. Some studies addressed the effect of pooling nose and groin samples on tests' performances [9, 10]. As both throat and groin are additional important sites for MRSA detection [1, 5, 6], validation of the Xpert MRSA assay (Cepheid, Sunnyvale, CA, USA) carried out on these three swabs was required. Most of these studies were done using the Cepheid collection device (Venturi Transystem; Copan, Brescia, Italy). The new eSwab device (Copan) is increasingly used because it is suitable for the automated inoculation of agar plates, and is more sensitive to recovering bacteria by culture, including MRSA screening [11–13]. Therefore, we aimed to assess the performance [sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPP)] of the Xpert MRSA assay on pooled nose, throat, and groin specimens using eSwabs and culture as the gold standard.

Materials and methods

Screening samples (nose, groin, and throat) were performed using the eSwab MRSA system (Copan). This collecting device is composed of a screw-cap tube filled with 1 ml of Amies liquid and three swabs with flocked nylon fiber tips. Xpert MRSA tests were performed according to the manufacturer's instructions, except that 100 μL of the Amies liquid were used to perform the analysis. For culture, about 250 μl of the Amies liquid were inoculated into m-Staphylococcus broth (Difco, Basel, Switzerland) and incubated overnight at 35 °C. The broth was then inoculated onto chromogenic MRSA-Select agar (Bio-Rad, Marnes-la-Coquette, France) and incubated overnight at 35 °C [14]. The DNase and agglutination (Slidex Staph Plus; bioMérieux, Marcy-l'Etoile, France) tests were performed to confirm the identification of *S. aureus*. Susceptibility to oxacillin was determined by the cefoxitin disk diffusion

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method according to the Clinical and Laboratory Standards Institute (CLSI) recommendations [15]. The sensitivity, specificity, PPV, and NPV of the Xpert MRSA assay, and their 95 % confidence intervals (CIs), were calculated using the online calculator at http://faculty.vassar.edu/lowry/clin1.html.

Results

To assess the effect of pooling samples on rapid MRSA detection, we performed a preliminary investigation. Nose, groin, and throat sites of 50 known MRSA carriers were first swabbed with separate eSwab devices (three swabs in three separated tubes) and then with the eSwab MRSA system (three swabs in one tube). Each specimen (tube) was analyzed by the Xpert MRSA assay and culture. In addition, 150 μl of nose, groin, and throat separate samples were pooled at the laboratory before analysis. With the Xpert MRSA test, separate analyses of the nose, throat, and groin yielded a total of 38 (76 %) positive patients (Table 1), whereas when specimens were pooled, either by the nurses or at the laboratory, 34 (68 %) and 35 (70 %) patients were positive, respectively. Similar results were obtained with culture (Table 1). Thus, a loss in sensitivity of only 6 % could be attributed to pooling. This reduction is largely compensated by the benefit of adding throat and groin samples, which, overall, increases the detection from 52 % and 54 % to 76 % and 78 % for the Xpert MRSA test and culture, respectively (Table 1). These results are similar to the data reported in a larger study [1]. The sensitivities of the Xpert MRSA assay compared to culture as the gold standard were not significantly different regarding the site of sampling or the pooling protocol (harvested in one tube by nurses or pooled at the laboratory) (Table 1) and were similar to the 86 % sensitivity (95 % CI 0.81–0.91) reported by the manufacturer. Thus, we chose the eSwab MRSA system (three swabs in one tube) for a larger evaluation.

From July 2011 to May 2012, 5,555 pooled samples (nose, groin, and throat) from 3,774 patients were analyzed by both the Xpert MRSA assay and culture. Only nine samples (0.16 %) showed invalid results after being tested twice by the Xpert MRSA assay, and were excluded from the analysis. Among the 5,546 remaining samples, 297 (5.4 %) were positive by the Xpert MRSA test and 294 (5.3 %) by culture. Considering culture as the gold standard, we observed a total of 65 false-negative and 68 false-positive results. Thus, the sensitivity of the Xpert MRSA assay was 0.78 (95 % CI 0.72–0.82), the specificity 0.99 (95 % CI 0.98–0.99), the PPV 0.77 (95 % CI 0.72–0.82), and the NPV 0.99 (95 % CI 0.98–0.99). These results are similar to previously reported studies on the Xpert MRSA assay [7, 10, 16–21].

Discussion

Whether the Xpert MRSA test is adequate for detecting MRSA carriers is an important question. Among the 335 new MRSA carriers identified during this period, 37 (11 %) would have been missed if culture would not have been performed, a ratio similar to a previous report [22]. There are several reasons for that. Some studies report the failure of the Xpert MRSA assay to detect strains harboring SCC*mec* variants [20, 23] or the newly described *mec*C [24]. In our study, among the 65 false-negative results, 45 isolates were tested by the Xpert MRSA/SA nasal test (PCRs for *mec*A and SCC*mec*—chromosome junction) and were positive (data not shown). Moreover, at least 50 (77 %) were due to MRSA strains belonging to four predominant clones in our area (data not shown), which are usually correctly identified by the Xpert MRSA assay (ST45-IV, ST5-II, ST228-I, and ST8-VI; [25]). This indicates that the majority of false-negative results were not due to SCC*mec* variants. Another explanation could be the lower performance of the Xpert MRSA assay compared to culture. This hypothesis is supported by a study reporting

Table 1 Number of positive results and sensitivity of the Xpert MRSA assay compared to culture on pooled or nonpooled samples of the nose, groin, and throat among 50 known methicillin-resistant *Staphylococcus aureus* (MRSA) carriers

	No. of positives by Xpert MRSA	No. of positives by culture	Sensitivity (95 % CI)
Nose	26 (52 %)	27 (54 %)	0.89 (0.70–0.97)
Throat	21 (42 %)	27 (54 %)	0.78 (0.57–0.90)
Groin	31 (62 %)	34 (68 %)	0.88 (0.72–0.96)
Pooled results from separated analysis of the three sites[a]	38 (76 %)	39 (78 %)	0.92 (0.78–0.98)
Pooled from three separated eSwabs by lab technicians	35 (70 %)	36 (72 %)	0.86 (0.70–0.95)
Swabs pooled within one eSwab tube by nurses	34 (68 %)	36 (72 %)	0.86 (0.70–0.95)

[a] If one or more sites were positive, the pooled result was considered to be positive. It was considered to be negative only when the three sites were all negative

that the limit of detection of enrichment culture was about 15 times lower (40 CFU/ml) than the Xpert MRSA PCR (610 CFU/ml) [18].

In this work, we also observed 68 false-positive results. Among these, 33 (49 %) were due to the presence of methicillin-sensitive *S. aureus* strains that did not possess the *mec*A gene, but still possessed part of the SCC*mec* gene and the chromosome targeted by the Xpert MRSA test (detected according to a previously described protocol [22]). False-positive results could also be due to the presence of dead MRSA cells in former carriers.

In conclusion, the high NPV (99 %) of the Xpert MRSA assay that we observed when pooling nose, throat, and groin samples supports the use of this procedure to detect MRSA and to rapidly stop or avoid unnecessary preemptive isolation measures. By pooling these samples, we increased the efficiency of MRSA screening without increasing the laboratory costs. Moreover, by using the eSwab system, automated inoculation is possible.

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Conflict of interest All authors declare no conflicts of interest.

References

1. Senn L, Basset P, Nahimana I, Zanetti G, Blanc DS (2012) Which anatomical sites should be sampled for screening of methicillin-resistant *Staphylococcus aureus* carriage by culture or by rapid PCR test? Clin Microbiol Infect 18(2):E31–E33
2. Batra R, Eziefula AC, Wyncoll D, Edgeworth J (2008) Throat and rectal swabs may have an important role in MRSA screening of critically ill patients. Intensive Care Med 34(9):1703–1706
3. Bishop EJ, Grabsch EA, Ballard SA, Mayall B, Xie S, Martin R, Grayson ML (2006) Concurrent analysis of nose and groin swab specimens by the IDI-MRSA PCR assay is comparable to analysis by individual-specimen PCR and routine culture assays for detection of colonization by methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 44(8):2904–2908
4. Eveillard M, de Lassence A, Lancien E, Barnaud G, Ricard JD, Joly-Guillou ML (2006) Evaluation of a strategy of screening multiple anatomical sites for methicillin-resistant *Staphylococcus aureus* at admission to a teaching hospital. Infect Control Hosp Epidemiol 27(2):181–184
5. Mertz D, Frei R, Jaussi B, Tietz A, Stebler C, Flückiger U, Widmer AF (2007) Throat swabs are necessary to reliably detect carriers of *Staphylococcus aureus*. Clin Infect Dis 45(4):475–477
6. Matheson A, Christie P, Stari T, Kavanagh K, Gould IM, Masterton R, Reilly JS (2012) Nasal swab screening for methicillin-resistant *Staphylococcus aureus*—how well does it perform? A cross-sectional study. Infect Control Hosp Epidemiol 33(8):803–808
7. Nulens E, Descheemaeker P, Deurenberg RH, Stobberingh EE, Gordts B (2010) Contribution of two molecular assays as compared to selective culture for MRSA screening in a low MRSA prevalence population. Infection 38(2):98–101
8. Wassenberg MW, Kluytmans JA, Bosboom RW, Buiting AG, van Elzakker EP, Melchers WJ, Thijsen SF, Troelstra A, Vandenbroucke-Grauls CM, Visser CE, Voss A, Wolffs PF, Wulf MW, van Zwet AA, de Wit GA, Bonten MJ (2011) Rapid diagnostic testing of methicillin-resistant *Staphylococcus aureus* carriage at different anatomical sites: costs and benefits of less extensive screening regimens. Clin Microbiol Infect 17(11):1704–1710
9. Jeyaratnam D, Gottlieb A, Ajoku U, French GL (2008) Validation of the IDI-MRSA system for use on pooled nose, axilla, and groin swabs and single swabs from other screening sites. Diagn Microbiol Infect Dis 61(1):1–5
10. Kelley PG, Grabsch EA, Howden BP, Gao W, Grayson ML (2009) Comparison of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay, BD GeneOhm MRSA assay, and culture for detection of nasal and cutaneous groin colonization by MRSA. J Clin Microbiol 47(11):3769–3772
11. Verhoeven P, Grattard F, Carricajo A, Pozzetto B, Berthelot P (2010) Better detection of *Staphylococcus aureus* nasal carriage by use of nylon flocked swabs. J Clin Microbiol 48(11):4242–4244
12. Smismans A, Verhaegen J, Schuermans A, Frans J (2009) Evaluation of the Copan ESwab transport system for the detection of methicillin-resistant *Staphylococcus aureus*: a laboratory and clinical study. Diagn Microbiol Infect Dis 65(2):108–111
13. Saegeman V, Flamaing J, Muller J, Peetermans WE, Stuyck J, Verhaegen J (2011) Clinical evaluation of the Copan ESwab for methicillin-resistant *Staphylococcus aureus* detection and culture of wounds. Eur J Clin Microbiol Infect Dis 30(8):943–949
14. Nahimana I, Francioli P, Blanc DS (2006) Evaluation of three chromogenic media (MRSA-ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 12(12):1168–1174
15. Clinical and Laboratory Standards Institute (CLSI) (2009) Performance standards for antimicrobial susceptibility testing. Approved standard MS-100-S19. CLSI, Wayne, PA
16. Marlowe EM, Miller JM, LaForga E, Amos M, Novak-Weekley SM (2009) eSwab nares specimens cultured with BioRad MRSASelect™ media are comparable to the Cepheid Xpert™ MRSA PCR for use in MRSA Surveillance. In: Proceedings of the 109th General Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania, May 2009. American Society for Microbiology, Washington, DC
17. Martens KD, De Beenhouwer H, Frans J, Van den Abeele A, Cartuyvels R, Coppens G (2009) Evaluation of eSwab for surveillance of MRSA by Xpert MRSA and culture on pooled samples. In: Proceedings of the 19th European Congress of Clinical Microbiology and Infectious Diseases, Helsinki, Finland, 15(S4): S199, P797
18. Rossney AS, Herra CM, Brennan GI, Morgan PM, O'Connell B (2008) Evaluation of the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the GeneXpert real-time PCR platform for rapid detection of MRSA from screening specimens. J Clin Microbiol 46(10):3285–3290
19. Hombach M, Pfyffer GE, Roos M, Lucke K (2010) Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in specimens from various body sites: performance characteristics of the BD GeneOhm MRSA assay, the Xpert MRSA assay, and broth-enriched culture in an area with a low prevalence of MRSA infections. J Clin Microbiol 48(11):3882–3887
20. Laurent C, Bogaerts P, Schoevaerdts D, Denis O, Deplano A, Swine C, Struelens MJ, Glupczynski Y (2010) Evaluation of the Xpert MRSA assay for rapid detection of methicillin-resistant *Staphylococcus aureus* from nares swabs of geriatric hospitalized patients and failure to detect a specific *SCC*mec type IV variant. Eur J Clin Microbiol Infect Dis 29(8):995–1002

21. Wolk DM, Picton E, Johnson D, Davis T, Pancholi P, Ginocchio CC, Finegold S, Welch DF, de Boer M, Fuller D, Solomon MC, Rogers B, Mehta MS, Peterson LR (2009) Multicenter evaluation of the Cepheid Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) test as a rapid screening method for detection of MRSA in nares. J Clin Microbiol 47(3):758–764
22. Blanc DS, Basset P, Nahimana-Tessemo I, Jaton K, Greub G, Zanetti G (2011) High proportion of wrongly identified methicillin-resistant *Staphylococcus aureus* carriers by use of a rapid commercial PCR assay due to presence of staphylococcal cassette chromosome element lacking the *mecA* gene. J Clin Microbiol 49(2):722–724
23. Roisin S, Laurent C, Nonhoff C, Deplano A, Hallin M, Byl B, Struelens MJ, Denis O (2012) Positive predictive value of the Xpert MRSA assay diagnostic for universal patient screening at hospital admission: influence of the local ecology. Eur J Clin Microbiol Infect Dis 31(5):873–880
24. García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD, Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK, Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA (2011) Meticillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11(8):595–603
25. Basset P, Senn L, Prod'hom G, Bille J, Francioli P, Zanetti G, Blanc DS (2010) Usefulness of double locus sequence typing (DLST) for regional and international epidemiological surveillance of methicilin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 16(8):1289–1296