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

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Running title: Pooled nose throat and groin samples with Xpert-MRSA

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Abstract

The performance of the Xpert-MRSA PCR assay on pooled nose, groin, and throat swabs (3 nylon flocked eSwabs into one tube) was compared to culture by analyzing 5546 samples. Sensitivity (0.78, 95%CI 0.73-0.82) and specificity (0. 99, 95%CI 0.98-0.99) were similar to results from published studies on separated nose or other specimens. Thus, the performance of Xpert-MRSA 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

Rapid and accurate detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers helps reducing the risk of transmission to other patients. Rapid PCR-based methods enable to confirm or refute MRSA carriage in patients within two hours. Most of them were evaluated with nose specimens, whereas studies showed that multiple site sampling increase 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 test's performances [9, 10]. As both throat and groin are additional important site for MRSA detection [1, 5, 6], validation of Xpert-MRSA (Cepheid, Sunnyvale, USA) done 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 automated inoculation of agar plates, and is more sensitive to recover bacteria by culture, including MRSA screening [11-13]. Therefore, we aimed at assessing the performance (sensitivity, specificity, positive [PPV] and negative [NPP] predictive values) of Xpert-MRSA on pooled nose, throat, and groin specimens using eSwabs and culture as the gold standard.

Material 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 1ml of Amies liquid and three swabs with flocked nylon fiber tip. Xpert-MRSA tests were performed according to manufacturer 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), incubated overnight at 35°C. The broth was then inoculated onto chromogenic MRSA-Select agar (Biorad, Marne-la-Coquette,

France), incubated overnight at 35°C [14]. Sensitivity, specificity, PPV and NPV of Xpert-MRSA, and their 95% confidence intervals, were calculated using the online calculator (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 swabbed first with separate eSwab devices (3 swabs in 3 separated tubes) and then with the eSwab MRSA system (3 swabs in one tube). Each specimen (tube) was analyzed by Xpert-MRSA 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 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 increase the detection from 52% and 54% to 76% and 78% for Xpert-MRSA and culture, respectively (Table 1). These results are similar to data reported in a larger study [1]. Sensitivities of Xpert-MRSA compared to culture as 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 choosed the eSwab MRSA system (3 swabs in one tube) for a larger evaluation.

From July 2011 to May 2012, 5555 pooled samples (nose, groin and throat) were analyzed both by Xpert-MRSA and culture. Only 9 samples (0.16%) showed invalid results after being tested twice by Xpert-MRSA, and were excluded from the analysis. Considering culture as the gold standard, we observed among the 5546 remaining samples a total of 65 false negative

and 68 false positive results. Thus, the sensitivity of Xpert-MRSA 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 study on Xpert-MRSA [7, 10, 15-20].

Discussion

Whether the Xpert-MRSA test is adequate to detect 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 [21]. There are several reasons for that. Some studies report the failure of Xpert-MRSA to detect strains harboring SCC*mec* variants [19, 22] or the newly described *mec*C [23]. In our study, among 65 false negatives, at least 55 (85%) were due to MRSA strains belonging to four predominant clones in our area (data not shown), which are usually correctly identified by Xpert-MRSA (ST45-IV, ST5-II, ST228-I, and ST8-VI; [24]). This indicates that the majority of false negatives were not due to SCC*mec* variants. Another explanation could be the lower performance of Xpert-MRSA compared to culture. This hypothesis is supported by a study reporting that the limit of detection of enrichment culture was about 15 time lower (40 CFU/ml) than Xpert-MRSA PCR (610 CFU/ml) [17].

In this work, we also observed 68 false positives. Among these, 33 (49%) were due to the presence of methicillin-sensitive *S.aureus* strains that did not possess the *mec*A, but still possess part of the SCC*mec* and the chromosome targeted by Xpert-MRSA (detected according to a previously described protocol [21]). False positives could also be due to the presence of dead MRSA cells in former carriers.

In conclusion, the high NPV (99%) of Xpert-MRSA 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.

Transparency Declaration

All authors declare no conflicts of interest

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Table 1. Number of positive results and sensitivity of Xpert MRSA compared to culture on pooled or non pooled samples of nose groin and throat among 50 known 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 3 sites*	38 (76%)	39 (78%)	0.92 (0.78-0.98)
Pooled from 3 separated eSwabs by lab technicians	35 (70%)	36 (72%)	0.86 (0.70-0.95)
Swabs pooled within one eSwab tube by the nurses	34 (68%)	36 (72%)	0.86 (0.70-0.95)

*, if one or more sites were positive, the pooled result was considered positive. It was considered negative only when the 3 sites were negative.