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

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

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24 **Abstract**

25 The performance of the Xpert-MRSA PCR assay on pooled nose, groin, and throat swabs (3
26 nylon flocked eSwabs into one tube) was compared to culture by analyzing 5546 samples.

27 Sensitivity (0.78, 95% CI 0.73-0.82) and specificity (0.99, 95% CI 0.98-0.99) were similar to
28 results from published studies on separated nose or other specimens. Thus, the performance of
29 Xpert-MRSA was not affected by pooling the three specimens into one assay, allowing a
30 higher detection rate without increasing laboratory costs, as compared to nose samples alone.

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33 **Introduction**

34 Rapid and accurate detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers
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2 35 helps reducing the risk of transmission to other patients. Rapid PCR-based methods enable to
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4 36 confirm or refute MRSA carriage in patients within two hours. Most of them were evaluated
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6 37 with nose specimens, whereas studies showed that multiple site sampling increase the
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9 38 sensitivity of MRSA detection [1-6].

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12 39 The high price of commercially available rapid PCR tests for MRSA screening leads some
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14 40 laboratories to pool specimens of the same patient into one single assay [7], whereas others
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17 41 consider that these tests are not cost-effective [8]. Some studies addressed the effect of
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19 42 pooling nose and groin samples on test's performances [9, 10]. As both throat and groin are
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22 43 additional important site for MRSA detection [1, 5, 6], validation of Xpert-MRSA (Cepheid,
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24 44 Sunnyvale, USA) done on these three swabs was required. Most of these studies were done
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26
27 45 using the Cepheid collection device (Venturi Transystem; Copan, Brescia, Italy). The new
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29 46 eSwab device (Copan) is increasingly used because it is suitable for automated inoculation of
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32 47 agar plates, and is more sensitive to recover bacteria by culture, including MRSA screening
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34 48 [11-13]. Therefore, we aimed at assessing the performance (sensitivity, specificity, positive
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37 49 [PPV] and negative [NPP] predictive values) of Xpert-MRSA on pooled nose, throat, and
38
39 50 groin specimens using eSwabs and culture as the gold standard.

42 51 **Material and Methods**

43 52 Screening samples (nose, groin and throat) were performed using the eSwab MRSA system
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46 53 (Copan). This collecting device is composed of a screw-cap tube filled with 1ml of Amies
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49 54 liquid and three swabs with flocked nylon fiber tip. Xpert-MRSA tests were performed
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52 55 according to manufacturer instructions, except that 100 μ L of the Amies liquid were used to
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54 56 perform the analysis. For culture, about 250 μ l of the Amies liquid were inoculated into m-
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57 57 *Staphylococcus* broth (Difco, Basel, Switzerland), incubated overnight at 35°C. The broth
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59 58 was then inoculated onto chromogenic MRSA-Select agar (Biorad, Marne-la-Coquette,
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59 France), incubated overnight at 35°C [14]. Sensitivity, specificity, PPV and NPV of Xpert-
60 MRSA, and their 95% confidence intervals, were calculated using the online calculator
61 (<http://faculty.vassar.edu/lowry/clin1.html>).

62 **Results**

63 To assess the effect of pooling samples on rapid MRSA detection, we performed a
64 preliminary investigation. Nose, groin and throat sites of 50 known MRSA carriers were
65 swabbed first with separate eSwab devices (3 swabs in 3 separated tubes) and then with the
66 eSwab MRSA system (3 swabs in one tube). Each specimen (tube) was analyzed by Xpert-
67 MRSA and culture. In addition, 150 µl of nose, groin and throat separate samples were pooled
68 at the laboratory before analysis. With the Xpert-MRSA test, separate analyses of nose, throat
69 and groin yielded a total of 38 (76%) positive patients (Table 1), whereas when specimens
70 were pooled, either by the nurses or at the laboratory, 34 (68%) and 35 (70%) patients were
71 positive, respectively. Similar results were obtained with culture (Table 1). Thus, a loss in
72 sensitivity of only 6% could be attributed to pooling. This reduction is largely compensated
73 by the benefit of adding throat and groin samples, which overall increase the detection from
74 52% and 54% to 76% and 78% for Xpert-MRSA and culture, respectively (Table 1). These
75 results are similar to data reported in a larger study [1]. Sensitivities of Xpert-MRSA
76 compared to culture as gold standard were not significantly different regarding the site of
77 sampling or the pooling protocol (harvested in one tube by nurses or pooled at the laboratory)
78 (Table 1) and were similar to the 86% sensitivity (95%CI 0.81-0.91) reported by the
79 manufacturer. Thus, we choosed the eSwab MRSA system (3 swabs in one tube) for a larger
80 evaluation.

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

85 and 68 false positive results. Thus, the sensitivity of Xpert-MRSA was 0.78 (95%CI 0.72-
86 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
87 0.99 (95%CI 0.98-0.99). These results are similar to previously reported study on Xpert-
88 MRSA [7, 10, 15-20].

89 Discussion

90 Whether the Xpert-MRSA test is adequate to detect MRSA carriers is an important question.
91 Among the 335 new MRSA carriers identified during this period, 37 (11%) would have been
92 missed if culture would not have been performed, a ratio similar to a previous report [21].
93 There are several reasons for that. Some studies report the failure of Xpert-MRSA to detect
94 strains harboring SCC*mec* variants [19, 22] or the newly described *mecC* [23]. In our study,
95 among 65 false negatives, at least 55 (85%) were due to MRSA strains belonging to four
96 predominant clones in our area (data not shown), which are usually correctly identified by
97 Xpert-MRSA (ST45-IV, ST5-II, ST228-I, and ST8-VI; [24]). This indicates that the majority
98 of false negatives were not due to SCC*mec* variants. Another explanation could be the lower
99 performance of Xpert-MRSA compared to culture. This hypothesis is supported by a study
100 reporting that the limit of detection of enrichment culture was about 15 time lower (40
101 CFU/ml) than Xpert-MRSA PCR (610 CFU/ml) [17].

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

107 In conclusion, the high NPV (99%) of Xpert-MRSA that we observed when pooling nose,
108 throat and groin samples supports the use of this procedure to detect MRSA and to rapidly
109 stop or avoid unnecessary preemptive isolation measures. By pooling these samples we

110 increased the efficiency of MRSA screening without increasing the laboratory costs.

111 Moreover, by using the eSwab system, automated inoculation is possible.

112

113 **Transparency Declaration**

114 All authors declare no conflicts of interest

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- 5 119 [1] Senn L, Basset P, Nahimana I, Zanetti G, Blanc DS (2012) Which anatomical sites
6 120 should be sampled for screening of methicillin-resistant *Staphylococcus aureus* carriage by
7 121 culture or by rapid PCR test? *Clin Microbiol Infect* 18 (2):E31-33
- 8 122 [2] Batra R, Eziefula AC, Wyncoll D, Edgeworth J (2008) Throat and rectal swabs may
9 123 have an important role in MRSA screening of critically ill patients. *Intensive Care Med* 34
10 124 (9):1703-1706
- 11 125 [3] Bishop EJ, Grabsch EA, Ballard SA, Mayall B, Xie S, Martin R, Grayson ML (2006)
12 126 Concurrent analysis of nose and groin swab specimens by the IDI-MRSA PCR assay is
13 127 comparable to analysis by individual-specimen PCR and routine culture assays for detection
14 128 of colonization by methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 44 (8):2904-
15 129 2908
- 16 130 [4] Eveillard M, de Lassence A, Lancien E, Barnaud G, Ricard JD, Joly-Guillou ML
17 131 (2006) Evaluation of a strategy of screening multiple anatomical sites for methicillin-resistant
18 132 *Staphylococcus aureus* at admission to a teaching hospital. *Infect Control Hosp Epidemiol* 27
19 133 (2):181-184
- 20 134 [5] Mertz D, Frei R, Jaussi B, Tietz A, Stebler C, Fluckiger U, Widmer AF (2007/8/15)
21 135 Throat swabs are necessary to reliably detect carriers of *Staphylococcus aureus*. *Clin Infect*
22 136 *Dis* 45 (4):475-477
- 23 137 [6] Matheson A, Christie P, Stari T, Kavanagh K, Gould IM, Masterton R, Reilly JS
24 138 (2012) Nasal Swab Screening for Methicillin-Resistant *Staphylococcus aureus*-How Well
25 139 Does It Perform? A Cross-Sectional Study. *Infect Control Hosp Epidemiol* 33 (8):803-808
- 26 140 [7] Nulens E, Descheemaeker P, Deurenberg RH, Stobberingh EE, Gordts B (2010)
27 141 Contribution of two molecular assays as compared to selective culture for MRSA screening in
28 142 a low MRSA prevalence population. *Infection* 38 (2):98-101
- 29 143 [8] Wassenberg MW, Kluytmans JA, Bosboom RW, Buiting AG, van Elzakker EP,
30 144 Melchers WJ, Thijsen SF, Troelstra A, Vandenbroucke-Grauls CM, Visser CE, Voss A,
31 145 Wolffs PF, Wulf MW, van Zwet AA, de Wit GA, Bonten MJ (2011) Rapid diagnostic testing
32 146 of methicillin-resistant *Staphylococcus aureus* carriage at different anatomical sites: costs and
33 147 benefits of less extensive screening regimens. *Clin Microbiol Infect* 17 (11):1704-1710
- 34 148 [9] Jeyaratnam D, Gottlieb A, Ajoku U, French GL (2008) Validation of the IDI-MRSA
35 149 system for use on pooled nose, axilla, and groin swabs and single swabs from other screening
36 150 sites. *Diagn Microbiol Infect Dis* 61 (1):1-5
- 37 151 [10] Kelley PG, Grabsch EA, Howden BP, Gao W, Grayson ML (2009) Comparison of the
38 152 Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay, BD GeneOhm MRSA
39 153 assay, and culture for detection of nasal and cutaneous groin colonization by MRSA. *J Clin*
40 154 *Microbiol* 47 (11):3769-3772
- 41 155 [11] Verhoeven P, Grattard F, Carricajo A, Pozzetto B, Berthelot P (2010) Better detection
42 156 of *Staphylococcus aureus* nasal carriage by use of nylon flocked swabs. *J Clin Microbiol* 48
43 157 (11):4242-4244
- 44 158 [12] Smismans A, Verhaegen J, Schuermans A, Frans J (2009) Evaluation of the Copan
45 159 ESwab transport system for the detection of methicillin-resistant *Staphylococcus aureus*: a
46 160 laboratory and clinical study. *Diagn Microbiol Infect Dis* 65 (2):108-111
- 47 161 [13] Saegeman V, Flamaing J, Muller J, Peetermans WE, Stuyck J, Verhaegen J (2011)
48 162 Clinical evaluation of the Copan ESwab for methicillin-resistant *Staphylococcus aureus*
49 163 detection and culture of wounds. *Eur J Clin Microbiol Infect Dis* 30 (8):943-949
- 50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 164 [14] Nahimana I, Francioli P, Blanc DS (2006) Evaluation of three chromogenic media
 165 (MRSA-ID, MRSA-Select and CHROMagar MRSA) and ORSAB for surveillance cultures of
 166 methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 12 (12):1168-1174
- 167 [15] Marlowe EMM, J. M.; LaForga, E.; Amos, M.; Novak-Weekley, S. M. (2009) eSwab
 168 nares specimens cultured with BioRad MRSASelect™ media are comparable to the Cepheid
 169 Xpert™ MRSA PCR for use in MRSA Surveillance. 109th Gen Meet Am Soc Microbiol
 170 American Society for Microbiology, Washington, DC
- 171 [16] Martens KDB, H.; Frans, J.; Van den Abeele, A.; Cartuyvels, R.; Coppens, G. (2009)
 172 Evaluation of eSwab for surveillance of MRSA by Xpert MRSA and culture on pooled
 173 samples. Clinical Microbiology and Infection 15 (S4):S199, P797
- 174 [17] Rossney AS, Herra CM, Brennan GI, Morgan PM, O'Connell B (2008) Evaluation of
 175 the Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) assay using the GeneXpert
 176 real-time PCR platform for rapid detection of MRSA from screening specimens. J Clin
 177 Microbiol 46 (10):3285-3290
- 178 [18] Hombach M, Pfyffer GE, Roos M, Lucke K (2010) Detection of methicillin-resistant
 179 *Staphylococcus aureus* (MRSA) in specimens from various body sites: performance
 180 characteristics of the BD GeneOhm MRSA assay, the Xpert MRSA assay, and broth-enriched
 181 culture in an area with a low prevalence of MRSA infections. J Clin Microbiol 48 (11):3882-
 182 3887
- 183 [19] Laurent C, Bogaerts P, Schoevaerdts D, Denis O, Deplano A, Swine C, Struelens MJ,
 184 Glupczynski Y (2010) Evaluation of the Xpert MRSA assay for rapid detection of methicillin-
 185 resistant *Staphylococcus aureus* from nares swabs of geriatric hospitalized patients and failure
 186 to detect a specific SCCmec type IV variant. Eur J Clin Microbiol Infect Dis 29 (8):995-1002
- 187 [20] Wolk DM, Picton E, Johnson D, Davis T, Pancholi P, Ginocchio CC, Finegold S,
 188 Welch DF, de Boer M, Fuller D, Solomon MC, Rogers B, Mehta MS, Peterson LR (2009)
 189 Multicenter evaluation of the Cepheid Xpert methicillin-resistant *Staphylococcus aureus*
 190 (MRSA) test as a rapid screening method for detection of MRSA in nares. J Clin Microbiol
 191 47 (3):758-764
- 192 [21] Blanc DS, Basset P, Nahimana-Tessema I, Jatton K, Greub G, Zanetti G (2011) High
 193 Proportion of Wrongly Identified Methicillin-Resistant *Staphylococcus aureus* Carriers by
 194 Use of a Rapid Commercial PCR Assay Due to Presence of Staphylococcal Cassette
 195 Chromosome Element Lacking the mecA Gene. Journal of Clinical Microbiology 49
 196 (2):722-724
- 197 [22] Roisin S, Laurent C, Nonhoff C, Deplano A, Hallin M, Byl B, Struelens MJ, Denis O
 198 (2011) Positive predictive value of the Xpert MRSA assay diagnostic for universal patient
 199 screening at hospital admission: influence of the local ecology. Eur J Clin Microbiol Infect
 200 Dis
- 201 [23] Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD,
 202 Walpole E, Brooks K, Pickard DJ, Teale C, Parkhill J, Bentley SD, Edwards GF, Girvan EK,
 203 Kearns AM, Pichon B, Hill RL, Larsen AR, Skov RL, Peacock SJ, Maskell DJ, Holmes MA
 204 (2011/8) Methicillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human
 205 and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11
 206 (8):595-603
- 207 [24] Basset P, Senn L, Prod'homme G, Bille J, Francioli P, Zanetti G, Blanc DS (2010)
 208 Usefulness of double locus sequence typing (DLST) for regional and international
 209 epidemiological surveillance of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol
 210 Infect 16 (8):1289-1296

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.