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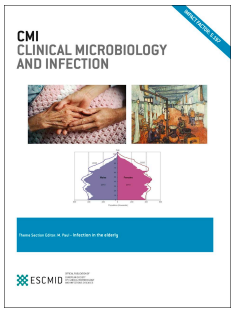

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Evaluation of three consecutive versions of a commercial rapid PCR test to screen for methicilin-resistant *Staphylococcus aureus*

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Abstract

Objectives. Screening for Methicillin-resistant *Staphylococcus aureus* (MRSA) is part of many recommendations to control MRSA. Several rapid PCR tests are available commercially and updated versions are constantly released. We aimed to evaluate the performance of three consecutive versions (G3, Gen3 and NxG) of the XpertMRSA test.

Methods. Routine samples for MRSA screening were simultaneously tested by culture and rapid PCR. The three versions of XpertMRSA were used successively and compared to culture.

Results. A total of 3512, 2794 and 3288 samples were analyzed by culture and by the G3, Gen3 and NxG XpertMRSA versions, respectively. The rates of positive by culture in the three groups were 5.0%, 4.7% and 4.3%, respectively. The sensitivity improved over time (71.4 [95%CI, 64.0 – 77.9], 82.3 [95%CI, 74.4 – 88.2] and 84.3% [95%CI, 77.0-89.7], respectively), but non-significantly. The specificity (98.4 [95%CI, 97.9 – 98.8], 96.8 [95%CI, 96.0 – 97.4] and 99.1 [95%CI, 98.7-99.4], respectively) and the positive likelihood ratios (45.7 [95%CI, 34.4 – 60.8], 25.6 [95%CI, 20.5 – 32.0], 97.1 [95%CI, 66.3 – 142.4]) were significantly lower in the Gen3 version ($p<0.00001$).

Conclusions. These significant differences in performance shows the importance to evaluate each new version of a commercial test.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major cause of hospital-acquired infection with increasing morbidity, mortality and associated costs. Many countries have implemented recommendations and guidelines to prevent

MRSA spread (1, 2). Among these, screening at risk patients in order to manage positive carriers with additional control measures is advocated. Culture of screening samples remains the traditional and cheapest way to detect such microorganism, unfortunately with a turnaround time (TAT) to results of at least one or two days. With rapid commercial tests, a TAT of few hours can be achieved and this might be beneficial to the health care institution in several situations, such as (i) reduced time of pre-emptive isolation of the patient, (ii) an earlier control of the spread of the pathogen and (iii) a better management of the patient's flow. For these reasons, the XpertMRSA screening test (Cepheid, Sunnyvale, CA) has been introduced in our hospital since 2009 and evaluated for a pool sample of nose, groin and throat swab (3).

The XpertMRSA test is based on the amplification of the junction of the staphylococcal chromosomal cassette mec (SCC*mec*) and the chromosome. The earlier versions (up to G3) were designed based on the sequences of SCC*mec* I to IV. With the discovery of the last SCC*mec* XI and its specific *mecC* gene, it became a requisite to update the test to all SCC*mec* types. This was done in version Gen3 with the added amplification of the *mecA* or *mecC* genes, whereas version NxG benefited from a new design of all primers and an optimization to render the test more robust.

In the present study, we prospectively evaluated the performance of three consecutive versions (G3, Gen3 and NxG) of the rapid PCR-based Xpert MRSA test, using culture methods and antimicrobial susceptibility testing as the reference standards for comparison. We also aimed to investigate discordances between culture and PCR test with a special focus on so-called "false positives".

Materiel and Methods

Setting: The University Hospital of Lausanne is a 1'100-bed tertiary care hospital. Active surveillance cultures are part of its MRSA control program.

Microbiological methods: Screening samples (nose, groin, and throat) were performed using the eSwab MRSA system (Copan, Italy) (4). This collecting device is composed of a screw-cap tube filled with 1 ml of Amies liquid and three swabs with flocked nylon fibber tips. The XpertMRSA test and culture were performed on all samples in parallel as previously described (3). Culture was considered as the gold standard and consisted in incubation of the sample into an enrichment broth followed by plating onto chromogenic agar which was incubated for 28 hours. In case of invalid or error result with the XpertMRSA test, a second assay was performed. In the G3 version, Xpert MRSA yield positive results if only the SCCmec-chromosome junction is detected, whereas in the Gen3 and NxG versions both this junction and the *mecA* gene must be detected. Based on these results, the performances of the consecutive versions of the XpertMRSA were evaluated between March 2014 and March 2015 for the version G3, between March 2015 and February 2016 for Gen3, and between June 2016 and May 2017 for NxG. These performance indicators were the sensitivity, specificity, positive and negative predictive values (PV), the positive and negative likelihood ratio (LR).

In cases of discordance between the results of rapid test and culture, additional analyses were performed prospectively. In case of false positive (rapid test positive, culture negative), the initial enrichment broth, which was kept at 4℃, was inoculated onto one *S. aureus* chromogenic plate (SAID, bioMérieux, Marcy l'Etoile, France) and one M-select plate (Bio-Rad, Marnes-la-Coquette, France). Growth of characteristic colonies on SAID and not on M-select was suspect of a *S.aureus* with a "SCCmec-

like" element (5). Antimicrobial susceptibility testing and an XpertMRSA test (done directly on a colony) were performed to confirm or not this hypothesis. To investigate if the culture conditions were responsible for the false positive result, the initial broth was re-incubated for 24 hours and the stored sample was inoculated in a brain heart infusion (BHI) broth with 24h and 48h of incubation. All broths were inoculated onto M-select and SAID plates. In case of false negative, the XpertMRSA test was performed on one colony of the MRSA isolate which was kept frozen for further analysis.

Patients' data: Patient files of all discordance were retrospectively investigated for the search of risk factors for MRSA (MRSA history, infection with MRSA or MSSA, hospitalisation during the year before the discordant results, hospitalisation during the epidemic period 2008-2012 (6), transfer from an abroad hospital or from a nursing home, antibiotic treatment during the previous month, urinary or intra-vascular catheter, wounds, surgery and dialysis).

Statistical methods: The performance indicators were presented with their 95% confidence intervals according the Wilson score method (7, 8) proposal for positive and negative LR. These were calculated using the online calculator at http://vassarstats.net/clin1.html Comparison of proportion of false positives of the 3 versions of XpertMRSA tests was based on the chi2 test.

This study was approved by the local ethics commission (Commission Cantonale d'Ethique de la Recherche sur l'Etre Humain, Lausanne, Switzerland) under the number 2016-01045. Only patients giving a general authorisation to use their data and samples were included in the study.

Results

Clinical performance characteristics

A total of 9594 samples processed by rapid test and culture were included in the study. For 63 samples, the XpertMRSA results were invalid even after the second assay (Suppl. Materials, Table S1). Among the remaining 9531 samples, 445 were positive by culture and 514 by XpertMRSA (Suppl. Materials, Table S2). For each version of the test, the numbers of samples, periods of use, prevalence (percentage of culture positive), sensitivity, specificity, positive and negative predictive values and likelihood ratios (PLR and NLR) are shown in Table 1. The specificity and the PLR were significantly lower in the Gen3 version ($p<0.00001$ for Gen3 versus G3 or NxG). Sensitivity improved, but non-significantly, between the G3 and the NxG versions (Table 1).

Discordances, false positives

Among the 514 positive samples by PCR, 164 (32%) were culture-negative (Table 2). A significant reduction of discordance was observed in the last NxG generation of the test (G3 vs Gen3 : $p< 0.0001$; G3 vs NxG : $p=0.011$; Gen3 vs NxG $p< 0.000001$). The version Gen3 had the higher rate of false positive, most of them remaining negative after supplementary cultures. The positive likelihood ratio was the best performer for NxG; the NxG test was nearly 100 times more likely to be positive when the culture was too (Table 1).

The subculture of the enrichment broth onto a *S. aureus* chromogenic agar (SAID) allowed us to identify 63 MSSA isolates, which were phenotypically susceptible to methicillin and positive to the XpertMRSA test. This is highly suggestive for the presence of a SCCmec-like element (9). A significant decreased of such MSSA was observed between the version G3 or Gen3 and NxG ($p= 0.107$ and 0.0091).

Following additional cultures, a total of 18 MRSA were found. Five were obtained after a new subculture of the initial broth onto M-select agar, 2 by increasing the incubation of the enrichment broth to 48h, and 9 by inoculating the sample into a BHI broth. Two additional MRSA requiring specific condition were recovered (one needed an enriched CO_2 atmosphere and the second grew only on the SAID agar). The last version NxG showed the lower rate of false positive for which additional cultures revealed the presence of MRSA (G3 vs NxG : p=0.014).

The patient charts with false positive results and definitive negative culture were reviewed in order to find risk factors for MRSA infection/colonization (Suppl. Materials, Table S3). Among the 79 patients, 19/79 (24.2%) had at least one other sample positive for MRSA (5 developed an infection with MRSA) and 39/79 (49%) had an antibiotic treatment at the time of sampling or the month before. Most interesting, among the 12 false positive with the NxG version, 6 (50%) had at least one other sample positive for MRSA.

Discordances, false negatives

Among the 9017 negative samples by PCR, 95 were found positive by culture (Suppl. Materials, Table S2). Molecular typing of these isolates showed that 74 (78%) possessed a SCCmec type I, II, IV V and VI which are recognized by all the version of the XpertMRSA test (Suppl. Materials, Table S4). Moreover, the 22 isolates recovered during the use of version NxG were tested with the XpertMRSA assay and were all positive. For both Gen3 and NxG, negative likelihood ratio (LR-) were considered good (<0.2).

Discussion

The aim of our study was to define the clinical performances of different versions of the rapid XpertMRSA test using pooled samples of nose, throat and groin. Significant differences were observed in the specificity and the positive predictive value between the different versions. The specificity was significantly better in the G3 (98.4%) and NxG (99.1%) versions than in the Gen3 version (96.8%). This is in agreement with a recent study showing the specificity of NxG to be better than Gen3 (10). Similarly, the PPV was also found to be better in the G3 version (70.6%) and NxG (81.4%) than in Gen3 (55.7%). A change in the incidence of MRSA in the population could explain these differences. However, this incidence remained stable over the period of the study (2014-2017) in our hospital and in the area (data from www.anresis.ch). The significant decrease of the specificity and the PPV in the version Gen3 was due to a higher number of false positives which could not be explained by further testing of the sample (Table 2).

While not significant, the sensitivity and the NPV were both improved during the successive versions G3, Gen3 and NxG; whereas Jacquim et al. (10) showed the higher sensitivity with the Gen3 version.

There are several reasons to explain discordances between results of XpertMRSA and culture. The first explanation is the presence of MSSA strain harboring a SCCmec-like element. The presence of such isolates in samples explained 38.4% of the false positives in our study and 25% in another study done in 2011 in France (11). We have previously shown that half of these isolates have the upstream sequence from the insertion site of the SCCmec highly similar to the SCC sequence (9). Others were due to isolates that have a SCC-like element (naturally without the *mecA* gene), and only a minority are former MRSA, which lost their *mecA* gene. The

amplification of the *mecA* or *mecC* gene was included in the last two versions of the XpertMRSA assay, Gen3 and NxG, in order to decipher such cases. Our results showed a significant improvement as only 13 (0.27 % of all XpertMRSA assay) such isolates were recorded in the NxG version compared to 24 (0.68 %) and 26 (0.93 %) in the G3 and Gen3 versions. We did not explain why the rate did not decrease with the version Gen3 which also include the *mecA* and *mecC* PCR. The addition of the *mecA* and *mecC* PCR in the assay did not resolved all cases. In our study, using the version NxG, we found the concomitant presence in the sample of MSSA with SCCmec-like and methicilin-resistant coagulase-negative staphylococci (data not shown), which led to a positive XpertMRSA result.

The low bacterial charge of the sample and the culture condition might also explain these discordances. In our study, among the 85 false positive, 5 were found positive after a second culture and showed either a low number of colonies or the growth of other colonies, which may have hidden the MRSA. By increasing the incubation time of the enrichment broth, using a second broth (BHI) and a different agar plate, MRSA could be grown from 13 initially negative samples. Interestingly, we fortuitously isolated one MRSA that needed a CO_2 enriched atmosphere to grow. The addition of such growth condition during the NxG version period did not revealed other similar strains (data not shown). Finally, discrepancies between rapid test and culture results could also be explained by the non-homogeneity of the sample despite the use of flocked swabs.

The question of flagging patients as MRSA carrier based only on XpertMRSA results is raised. Considering the current version of XpertMRSA, 27/145 (18.6%) were false positive. Among them, 13 could be explained by the presence of MSSA with SCCmec-like element (which can be identified by routine culture of positive samples).

This leaves only 14 (9.7%) false positive among which two showed the presence of MRSA after additional culture, and for the remaining 12 samples, 6 had at least one other sample positive for MRSA (Table S3). Thus, if MSSA with SCCmec-like are identified by the laboratory, the probability that a positive XpertMRSA test reflect the past, present or future status of MRSA carrier of the patient is high. For these reasons, we advise to i) detect SCCmec-like MSSA and ii) flag the patient as MRSA carrier based on a positive Xpert MRSA result.

False negative XpertMRSA results might be explained by inadequate or insufficient coverage of the diversity of SCC*mec* elements. The lower sensitivity (71.4 %) was observed with the G3 version, which was originally developed to target SCCmec I to IV. With the inclusion of all other SCCmec types known up to date, the sensitivity increased to over 80% in the Gen3 and NxG versions. The better coverage of the Nx3 XpertMRSA assay has already been assessed on a wide collection of diverse MRSA isolates by Becker et al. (12). Nevertheless, MRSA isolates recovered from all false negative samples using the NxG version were positive when tested with this assay. This highlights the sufficient coverage of the SCC*mec* type by the XpertMRSA assay in our epidemiological setting. The limit of detection (LOD) of XpertMRSA might also explain false negative specimens with a low charge of MRSA. An experimental assai showed that, following our laboratory protocols, at the limit of detection NxG XpertMRSA need an inoculum 100x higher than for culture to be positive (data not shown). Experimental errors could also be the reason of false negative. However, we did not retested these samples with XpertMRSA to investigate this hypothesis. The non homogeneity of the sample or the genetic diversity within SCC*mec* types (13) might be other hypothesis to explain these false negative.

One limitation of our study is that it was conducted in one center and consecutively. The advantage of a monocentric study is that standard laboratory procedures were used all over the study period. Due to logistic and financial resources, testing the three versions in parallel on the same samples was not feasible. However, we believe the possible effects of the consecutive study were limited due to a stable local epidemiology of MRSA (stable and low incidence, no recorded outbreaks, no predominant clone).

In conclusion, significant differences in performance were observed between the different versions of the PCR Xpert® MRSA test. This was unexpected and shows the importance to evaluate new versions of commercial test. Fortunately, the worst version was used only for a year and was replaced by a version showing much better performances.

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Transparency declaration

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Table 2. Additional analysis on the 164 false positive samples (Xpert® MRSA positive, culture negative).

	G3	Gen3	NxG	p-values
No of false positive	52	85	27	G3 vs Gen3 : p< 0.0001 G3 vs NxG : p=0.01 Gen3 vs NxG p< 0.000001
MSSA with SCC-like element	24	26	13	G3 vs Gen3 : p=0.272 G3 vs NxG : p= 0.107; Gen3 vs NxG : p= 0.0091
MRSA found after supplementary cultures	8	8	2	G3 vs Gen3 : p=0.85 G3 vs NxG : p=0.014 Gen3 vs NxG p=0.36
Negatives	20	51	12	G3 vs Gen3 : p < 0.00001 G3 vs NxG : p=0.218 Gen3 vs NxG : p < 0.000001

Table 1. Data and performances of the different XpertMRSA versions compared to culture*.

	G3	Gen3	NxG
Period	04.2014-03.2015	03.2015-02.2016	06.2016-05.2017
No analysis	3503	2776	3252
Prevalence (95%CI)**	5.0% (4.3-5.8)	4.7% (4.0-5.5)	4.3% (3.7-5.1)
Sensitivity % (95%CI)	71.4 (64.0 – 77.9)	82.3 (74.4 – 88.2)	84.3 (77.0-89.7)
Specificity % (95%CI)	98.4 (97.9 – 98.8)	96.8 (96.0 – 97.4)	99.1 (98.7-99.4)
PPV % (95%CI)	70.6 (63.2 – 77.1)	55.7 (48.4 – 62.8)	81.4 (73.9-87.2)
NPV % (95%CI)	98.5 (98.0 – 98.9)	99.1 (98.6 – 99.4)	99.3 (98.9-99.5)
PLR (95%CI)	45.7 (34.4 – 60.8)	25.6 (20.5 – 32.0)	97.1 (66.3 – 142.4)
NLR (95%CI)	0.29 (0.23 – 0.37)	0.18 (0.11 – 0.26)	0.16 -0.11 – 0.23
Accuracy (%)	97.1	95.5	97.4

*. Based on results before additional analysis on discrepant results.
**. Based on positives by culture.