Rapid Detection of *Staphylococcus aureus* in Lower Respiratory Tract Secretions from Patients with Suspected Ventilator-Associated Pneumonia: Evaluation of the Cepheid Xpert MRSA/SA SSTI Assay

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A preclinical evaluation was conducted to evaluate the performance of the Cepheid Xpert assay on 135 lower respiratory tract secretions for detection of methicillin-resistant *Staphylococcus aureus* and *S. aureus*. Compared with the quantitative culture, the sensitivity, specificity, and positive and negative predictive values were 99.0%, 72.2%, 90.7%, and 96.3%, respectively.

Ventilator-associated pneumonia (VAP) is associated with high morbidity and mortality rates (15, 18), particularly in those patients who receive inadequate antimicrobial treatment (8, 19). Microbiologic information in patients with suspicion of VAP relies mainly on the quantitative culture of lower respiratory tract (LRT) secretions, and conventional bacterial isolation, identification, and antimicrobial susceptibility testing takes no less than 48 to 72 h. *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) are important etiologic agents of VAP and account for variable proportions that range from 19.5% to 34.4% of all episodes in different regions (3, 4, 9). Empirical treatment of VAP in many institutions requires the administration of potentially toxic or costly anti-MRSA antibiotics, such as vancomycin or linezolid.

Rapid molecular diagnostic tests for VAP are an unmet medical need recently acknowledged by the Infectious Diseases Society of America (IDSA) (1). In past years, the automated Cepheid Xpert MRSA/SA SSTI real-time PCR assay (Cepheid, Sunnyvale, CA) has been FDA and CE (European Community) approved for the simultaneous detection of methicillin-susceptible *S. aureus* (MSSA) and MRSA in skin and soft tissue infection swabs and blood cultures (21). This test has also been used directly on peri-operative bone and joint samples for the diagnosis of osteoarticular infections (7). The use of the assay for the direct detection of MSSA and MRSA in LRT secretions in the diagnosis of VAP is an off-label use not endorsed by the manufacturer, and to the best of our knowledge, this assay has not yet been evaluated in this setting.

We conducted a preclinical comparison of Xpert MRSA/SA assay to the gold standard, the quantitative culture method, in order to evaluate the performance of this assay directly on LRT secretions from patients with suspicion of VAP. Over a period of 15 months, all LRT samples (endotracheal aspirates) received in the microbiology laboratory from patients admitted to the intensive care units (ICUs) of our hospital and suspected of having VAP were examined by Gram stain and routinely cultured onto sheep blood, chocolate, and McConkey agar plates by the standard quantitative culture technique, which was considered the gold standard method. Plates were incubated for 18 to 24 h at $35 \pm 2^\circ\text{C}$ aerobically and in 5% CO_2 (chocolate agar) (22). Colony counts of $\geq 10^4$ CFU/ml of either MRSA or MSSA were considered significant and a positive test result (quantitative culture), whereas counts below 10^4 CFU/ml of either MRSA or MSSA were considered a negative test result (qualitative culture) (2). The

absence of growth, or any colony counts of other microorganisms, was also considered negative. Identification and susceptibility testing were performed using the MicroScan automated microdilution method (Siemens, Sacramento, CA) by following the manufacturer's recommendations. Breakpoints were determined by the Clinical and Laboratory Standards Institute (6). The Xpert assay testing was conducted in endotracheal aspirates showing Gram-positive cocci in clusters. Samples were adsorbed onto a cotton swab which was directly introduced in the extraction buffer vial of the Xpert assay, transferred into the cartridge, treated according to the manufacturer's instructions, and placed into the GeneXpert instrument according to the program. The overall process was completed in 58 min. The assay is based on the simultaneous amplification of three targets (*spa*, *mecA*, and staphylococcal cassette chromosome *mec* element [SCC*mec*]) and on fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The random-access Xpert performs extraction, amplification, and detection of the targets in a single-use cartridge. Results obtained by Xpert were compared with those obtained by the quantitative and qualitative cultures.

SCC*mec* types and subtypes were determined by PCR as previously described (12, 14, 23). Sensitivity, specificity, and positive and negative predictive values, with their 95% confidence intervals, were calculated. Invalid kit results and mechanical errors were discarded for further analysis.

Among 143 consecutive endotracheal aspirates showing Gram-positive cocci in clusters, 105 were positive for *S. aureus* by the quantitative culture (58 MSSA and 47 MRSA specimens). In 7 additional samples, the culture yielded *S. aureus* at $<10^4$ CFU/ml (4 MSSA and 3 MRSA specimens). Eight specimens yielded either invalid Xpert kit results or mechanical errors (3 of them from the same patient with MRSA, 3 with MSSA, and 2 with absence of growth in the culture). We finally evaluated 135 endotracheal aspirates being 99 positive, $\geq 10^4$ CFU/ml of MSSA ($n = 55$) or MRSA ($n = 44$), and 36 negative. The performance of the Xpert

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TABLE 1 Performance of the Xpert MRSA/SA SSTI assay in 135 endotracheal aspirates compared to quantitative and qualitative cultures

Type of culture	No. of positive samples/total no. of samples, % (95% confidence interval) ^a			
	Sensitivity	Specificity	PPV	NPV
Quantitative	98/99, 99.0 (96.5–100.0)	26/36, 72.2 (56.2–88.2)	98/108, 90.7 (84.8–96.7)	26/27, 96.3 (87.3–100.0)
Qualitative	105/106, 99.1 (96.7–100.0)	26/29, 89.7 (76.9–100.0)	105/108, 97.2 (93.7–100.0)	26/27, 96.3 (87.3–100.0)

^a PPV, positive predictive value; NPV, negative predictive value.

assay is summarized in Tables 1 and 2. The Xpert assay correctly detected all 55 MSSA isolates, and 43 out of the 44 MRSA isolates. In the kit-negative specimen, the *mecA* gene was detected, but the detection of the *SCCmec* showed up in cycle number 37, above the cutoff value given by the manufacturers, and the system interpreted the assay as negative for MRSA. Further PCR analysis of the *SCCmec* type showed that this isolate presented an *SCCmec* type IVc cassette. Additional *SCCmec* typing of 10 out of the 44 MRSA isolates recovered in this study showed that 7 of them carried *SCCmec* type IVa and 3 carried *SCCmec* type IVc, and all were detected by the Xpert assay. The Xpert assay correctly detected the 7 additional samples with *S. aureus* at <10⁴ CFU/ml (qualitative culture). In the remaining 29 culture-negative samples, the Xpert assay was negative in 26 but gave 3 additional positive results (one MRSA and two MSSA). These samples corresponded to patients with isolation of *S. aureus* in different samples taken 1 to 10 days before the performance of the Xpert assay in the endotracheal aspirate. Compared with the quantitative culture, the sensitivity, specificity, and positive and negative predictive values of the Xpert assay were 99.0%, 72.2%, 90.7%, and 96.3%, respectively. Compared with cultures showing any amount of *S. aureus* (qualitative culture), the sensitivity, specificity, and positive and negative predictive values of the Xpert assay were 99.1%, 89.7%, 97.2%, and 96.3%, respectively.

VAP represents a great challenge to clinicians, and the best diagnostic approach is still under debate and controversy. From the microbiological point of view, rapid tests that allow detection of the microorganisms most frequently implicated as a cause of VAP are necessary. As shown by our results, the Xpert MRSA/SA SSTI assay has proved to be in our hands a user-friendly, robust, and rapid method for the detection of MRSA and MSSA in a complex type of sample, such as LRT samples from patients with suspicion of VAP. In our study, performance of the real-time PCR Xpert assay on these samples after Gram stain allowed direct and

rapid detection of MRSA and MSSA with a high sensitivity (99%) and a negative predictive value of 96.3%. A short reporting time is crucial to administrate adequate antibiotic therapy, and results were obtained in less than 1 h.

We obtained seven positive Xpert results in which microbiological culture had counts of <10⁴ CFU/ml of *S. aureus*. Recently, it has been recommended that endotracheal aspirates with non-quantitative cultures should be used as the initial diagnostic strategy in patients with suspicion of VAP (13). Consequently, the Xpert-positive/quantitative culture-negative results should not be considered false-positive results. We also obtained three additional Xpert-positive samples in which the culture did not show growth of *S. aureus*. In two of them, *S. aureus* was isolated in previous cultures several days before the performance of the PCR assay, and the patients were under antibiotic treatment. In the remaining case, the organism was isolated from a nasal swab the same day. It is known that the culture has a lower sensitivity in comparison with the PCR assay (lower limit of detection from 48 to 109.4 CFU/test) (20, 21) and that prior antibiotic therapy can lead to a negative culture. In addition, a positive test result does not necessarily indicate the presence of viable organisms but rather the presence of amplifiable DNA from dead bacteria in treated patients, as it happened in two of our cases. This fact could explain the discrepancies in this study, since bacterial DNA can be detected in clinical samples for more than 10 days after successful treatment (17). The Xpert failed to detect one MRSA isolate grown in culture. Although the assay detected the *mecA* gene, it failed to detect *SCCmec*. It has been described that this assay detects MRSA strains belonging to *SCCmec* types I, II, III, IV, IVa, IVb, IVc, IVd, V, and VI, but one limitation of the Xpert is that the presence of mutations or polymorphisms in primer and probe binding regions may affect detection of new or unknown MRSA variants, resulting in a false-negative result (5, 11, 16, 17). In our study, further analysis showed that the MRSA isolate presented an *SCCmec* type IVc. In order to investigate if the system had limitations for the detection of this type of cassette, we analyzed the *SCCmec* type in 10 additional MRSA isolates detected in this study. Three of them also presented this type of cassette, were detected by the system, and were interpreted as positive.

In our study, 5 samples (3.5%) were not valuable due to invalid kit results. It has been suggested that prewarming of the reagents for 15 to 30 min at 35°C avoids this inconvenience (10), but we did not repeat testing on our samples under these circumstances. In addition, in three samples, the system gave mechanical errors (2.09%) due to probe check failure. These figures are comparable to those found with samples from various body sites in other studies (10, 16).

The Infectious Diseases Society of America (IDSA) does not believe that regulatory clearance of a new diagnostic molecular test for VAP by the FDA should require that the test validates the presence of pneumonia or another variant of respiratory infec-

TABLE 2 Detection of MRSA and MSSA in 135 endotracheal aspirates by culture and by Xpert

Type of culture	Organism identified by standard culture (no. of samples)	No. of samples in which the indicated organism was detected by Xpert		
		MRSA	MSSA	No <i>S. aureus</i>
Quantitative	MRSA (44)	43	1	0
	MSSA (55)	0	55	0
	No <i>S. aureus</i> or <10 ⁴ UFC/ml (36)	3	4	29
Qualitative	MRSA (47)	46	1	0
	MSSA (59)	0	59	0
	No <i>S. aureus</i> (29)	1	2	26

tion. Instead, the focus should be the accuracy of the new molecular method in the detection of a given bacterial or viral organism that will reduce antibiotic overuse (1), and in our study the Xpert assay proved to be useful for the detection of MRSA and MSSA in this context.

In summary, the Xpert MRSA/SA SSTI assay is a rapid and sensitive method for the detection of MRSA and MSSA in LRT secretions from patients with suspicion of VAP. This assay could be useful for the diagnosis of MRSA/MSSA pneumonia in ventilated patients. The assay may be best used, with a higher specificity, to test LRT secretions from patients with a high clinical suspicion of MRSA/MSSA pneumonia but without previous evidence of positive cultures of different samples. Finally, the clinical impact of this test should be assessed in future studies.

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The authors declare that they have no conflicts of interest.

REFERENCES

1. Anonymous. 2011. An unmet medical need: rapid molecular diagnostics tests for respiratory tract infections. *Clin. Infect. Dis.* 52(Suppl 4):S384–S395.
2. Aucar JA, Bongera M, Phillips JO, Kamath R, Metzler MH. 2003. Quantitative tracheal lavage versus bronchoscopic protected specimen brush for the diagnosis of nosocomial pneumonia in mechanically ventilated patients. *Am. J. Surg.* 186:591–596.
3. Bouza E, et al. 2012. Ventilator-associated pneumonia due to methicillin-resistant *Staphylococcus aureus*: risk factors and outcome in a large general hospital. *J. Hosp. Infect.* 80:150–155.
- 3a. Cercenado E, et al. 2009. Rapid detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in lower respiratory tract secretions from patients with ventilator-associated pneumonia: evaluation of the Cepheid Xpert assay, poster D-775. 49th Intersci. Conf. Antimicrob. Agents Chemother., San Francisco, CA, 12 to 15 September 2009.
4. Chastre J, Fagon JY. 2002. Ventilator-associated pneumonia. *Am. J. Respir. Crit. Care Med.* 165:867–903.
5. Chongtrakool P, et al. 2006. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. *Antimicrob. Agents Chemother.* 50:1001–1012.
6. Clinical and Laboratory Standards Institute. 2012. Performance standards for antimicrobial susceptibility testing, 22nd informational supplement. M100-S22, vol 32, no 3. Clinical and Laboratory Standards Institute, Wayne, PA.
7. Dubois-Bourandy A, et al. 2011. Direct detection of *Staphylococcus osteoarticular* infections by use of Xpert MRSA/SA SSTI real-time PCR. *J. Clin. Microbiol.* 49:4225–4230.
8. Iregui M, Ward S, Sherman G, Fraser VJ, Kollef MH. 2002. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. *Chest* 122:262–268.
9. Jones RN. 2010. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. *Clin. Infect. Dis.* 51(Suppl 1):S81–S87.
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.* 47:3769–3772.
11. Laurent C, et al. 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.* 29:995–1002.
12. Milheirico C, Oliveira DC, de Lencastre H. 2007. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: ‘SCC*mec* IV multiplex.’ *J. Antimicrob. Chemother.* 60:42–48.
13. Muscedere J, et al. 2008. Comprehensive evidence-based clinical practice guidelines for ventilator-associated pneumonia: diagnosis and treatment. *J. Crit. Care* 23:138–147.
14. Oliveira DC, de Lencastre H. 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 46:2155–2161.
15. Melsen WG, Rovers MM, Bonten MJ. 2009. Ventilator-associated pneumonia and mortality: a systematic review of observational studies. *Crit. Care Med.* 37:2709–2718.
16. 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.* 46:3285–3290.
17. Rupp J, Fenner I, Solbach W, Gieffers J. 2006. Be aware of the possibility of false-positive results in single-locus PCR assays for methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* 44:2317.
18. Timsit JF, Zahar JR, Chevret S. 2011. Attributable mortality of ventilator-associated pneumonia. *Curr. Opin. Crit. Care* 17:464–471.
19. Torres A, Ferrer M, Badia JR. 2010. Treatment guidelines and outcomes of hospital-acquired and ventilator-associated pneumonia. *Clin. Infect. Dis.* 51(Suppl 1):S48–S53.
20. Wolk DM, et al. 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.* 47:758–764.
21. Wolk DM, et al. 2009. Rapid detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays. *J. Clin. Microbiol.* 47:823–826.
22. York MK, Gilligan P. 2004. Lower respiratory tract cultures, p 3.11.2.1–3.11.2.15. In Isenberg HD (ed), *Clinical microbiology procedures handbook*, 2nd ed, vol 1. ASM Press, Washington, DC.
23. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* 43:5026–5033.