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Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) and PCR-Based Rapid Diagnosis of *Staphylococcus aureus* Bacteremia.

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Key words: MALDI-TOF MS, GeneXpert MRSA, *Staphyloccocus aureus* bacteremia, antibiotic resistance, antibiotic susceptibility testing

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Effective empirical treatment is of paramount importance to improve the outcome of patients with *Staphylococcus aureus* bacteremia. We aimed to evaluate a PCR-based rapid diagnosis of methicillin resistance (GeneXpert MRSA) after early detection of *S. aureus* bacteremia using MALDI-TOF MS. Patients with a first episode of *S. aureus* bacteremia identified using MALDI-TOF MS were randomized in a prospective interventional open study between October 2010 and August 2012. In the control group, antibiotic susceptibility testing was performed after MALDI-TOF MS identification on blood culture pellets. In the intervention group, a GeneXpert MRSA was performed after *S. aureus* identification. The primary outcome was the performance of GeneXpert MRSA performed directly on blood cultures. We then assessed the impact of early diagnosis of methicillin resistance on the empirical treatment. There were 197 episodes of *S. aureus* bacteremia included in the study, of which 106 were included in the intervention group. Median time from MALDI-TOF MS identification to GeneXpert MRSA result was 97 minutes (range 25-250). Detection of methicillin resistance using GeneXpert MRSA had a sensitivity of 99% and a specificity of 100%. There was less unnecessary coverage of methicillin-resistant *S. aureus* (MRSA) in the intervention group (17.1% vs. 29.2%, $P = 0.09$). GeneXpert MRSA was highly reliable in diagnosing methicillin resistance when performed directly on positive blood cultures. This could promote sparing unnecessary prescriptions of anti-MRSA agents and introducing earlier adequate coverage in unsuspected cases.

Introduction

Staphylococcus aureus is a major cause of community-acquired and nosocomial bacteremia.[1,2] The associated mortality, which ranges from 15 to 60%, is higher in cases of methicillin-resistant *S. aureus* (MRSA).[3,4] Early introduction of appropriate empirical antibiotic therapy improves the outcome of patients with *S. aureus* bacteremia.[5,6]

Although the Gram stain result of positive blood cultures detected by automated blood culture systems has a high impact on empiric antibiotic therapy,[7] definitive identification of the etiological agent and determination of its susceptibility to antibiotics may imply a 24 to 48-hours delay. Empirical antibiotic therapy is based on clinical assessment and the epidemiological setting. In the case of positive blood cultures with Gram-positive cocci in clusters, empirical coverage of MRSA may be automatic in high-prevalence settings. Wider use of vancomycin or new anti-Gram-positive antibiotics may cause unnecessary costs and toxicity, and favour the development of resistance.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was prospectively assessed for the rapid diagnostic of bloodstream infections.[8-12] Our experience of bacterial identification by MALDI-TOF MS on positive blood culture pellets after an ammonium chloride erythrocyte-lysing procedure led to the correct identification at the species level of 79% of the pellets tested, and more specifically all the 25 episodes of *S. aureus* bacteremia were correctly identified with a score ≥ 1.7, theoretically indicating an identification reliable at the genus level only.[9] Others groups also confirmed high performances of *S. aureus* identification in blood cultures using MALDI-TOF MS.[10-12] Whilst the early identification of *S. aureus* is possible with MALDI-TOF MS, the time to obtaining antibiotic susceptibility results (AST) remains unchanged. The identification of methicillin resistance using MALDI-TOF MS remains experimental without clinical validation to date.[13,14]

PCR-based tests targeting the mecA gene for the identification of MRSA were used at first for the rapid diagnosis of MRSA carriage with the aim of preventing its nosocomial

transmission.[15] This technique applied to positive blood cultures with Gram-positive cocci in clusters showed a high sensitivity of 96 to 100% for the detection of MRSA.[16-18] As a routine procedure, it would generate high costs due to the frequency of coagulase-negative staphylococci in blood cultures. In our center, 1020 bottles of blood cultures returned positive for coagulase-negative staphylococci during the study period.

In this pilot study, we aimed to test the combination of early detection of *S. aureus* using MALDI-TOF MS with a PCR-based detection of methicillin resistance in blood cultures. We tested the accuracy of this method to detect the resistance to methicillin based on subsequent antibiotic susceptibility results in the setting of an innovative combined diagnostic of *S. aureus* bacteremia. We also wanted to assess the consequences of early PCR-based identification of methicillin resistance regarding empirical antibiotic therapy and particularly the prescription of unnecessary MRSA coverage.

Materials and methods

Design and case definition:

Patients with a first episode of *S. aureus* bacteremia identified using MALDI-TOF MS were included in a prospective, randomized open study between October 2010 and August 2012 in Lausanne University Hospital Center, an 850-bed primary and tertiary care hospital in western Switzerland where *S. aureus* resistance to methicillin was 23% in 2010 and indications for MRSA screening were extended after a hospital-wide outbreak in 2008. We excluded cases with known antibiotic susceptibility results (e.g. transfers from others hospitals) and patients who were dead at time of diagnosis of bacteremia. Randomization occurred using a unique number assigned to each hospitalized patient in our center, which was not linked to patient characteristics. In the control group (odd number), standard AST was performed after MALDI-TOF MS identification (time to result of about 24-48 hours for standard antibiotic susceptibility results). In the intervention group (even number), MALDI-TOF MS identification was coupled to a direct rapid PCR-based test (GeneXpert MRSA, Cepheid, Sunnyvale, CA) performed directly on the positive blood culture pellets, in addition to standard AST.

Mode of randomization was concealed from the clinicians. We planned to include around 100 cases in the intervention group to assess the performances of GeneXpert in the setting of this combined diagnostic of *S. aureus* bacteremia. For each case, a standardized case report form (CRF) including epidemiological, clinical and biological data, as well as the sequence of empirical antibiotic therapy, was filled in.

Procedures

Positive blood cultures were detected using the BACTEC 9240 automated blood culture system (Becton Dickinson, Sparks, MD). Direct MALDI-TOF MS has been performed routinely in our center on all positive blood cultures since September 2009. Mass spectra were acquired on a Microflex LT MALDI-TOF MS (Bruker Daltronics, Bremen, Germany) after a standardized ammonium chloride erythrocyte-lysing procedure as described by Prod'hom *et al.*[9] Spectral analysis and comparison with the database were done using MALDI BioTyper 2.0 software. Identification of *S. aureus* on positive blood cultures with Gram-positive cocci in clusters was considered adequate when the score value was ≥ 1.7.[9] In the control group, Gram stain and MALDI-TOF MS results were sequentially reported orally to clinicians during working hours (08h00 to 17h00) as well as electronically using the laboratory information system. Clinicians were contacted the following day for blood cultures turning positive out of hours. AST was started concomitantly. In the intervention group, the same procedure was followed; in addition, a PCR-based rapid test targeting spa, mecA and SCC genes (GeneXpert MRSA) was immediately launched on the blood culture pellets. Clinicians were told of this ongoing test when informed of the *S. aureus* bacteremia. The result of GeneXpert MRSA was then provided orally during daytime hours as well as electronically using a standardized recall of previously issued test result data.[16,18] An infectious diseases consultation was recommended in all cases of *S. aureus* bacteremia.

Study definitions and outcomes

Adequacy of empirical antibiotic therapy was defined as the prescription of an active antibiotic, as confirmed by AST performed on subcultures. *Adequacy of empirical*

antibiotic therapy ("MALDI-TOF-directed treatment") was assessed similarly for both groups after informing the clinician of the *S. aureus* bacteremia, secondarily to MALDI-TOF MS identification, as this corresponded to the time of intervention and allowed us to test the impact of the immediate GeneXpert availability on the choice of empirical treatment. Glycopeptides and daptomycin were considered appropriate for MRSA bacteremia. Drugs with activity against methicillin-susceptible S. aureus (MSSA), adequate dosing for bacteremia and intravenous administration were considered appropriate treatments for MSSA bacteremia. *Immunosuppression* included neutropenia, HIV infection and immunosuppressive drugs. Bacteremia cases were considered *nosocomial* if blood cultures were taken ≥ 48h after hospitalization, and *complicated* if local or metastatic complications occurred during follow-up. *Penicillin allergy* was defined based on the medical record. *Severe sepsis*/*septic shock* were defined according to standard definitions.[19] *Renal insufficiency* was defined as an increase from baseline creatinine value of more than 50% and was evaluated at day 3, day 7 and day 14. The first outcome was the accuracy of GeneXpert MRSA to detect the resistance to methicillin based on subsequent AST. We secondarily wanted to assess the consequences regarding MRSA coverage.

Statistics

Categorical variables were compared using the chi-squared or Fisher's exact tests when appropriate; continuous variables were compared using the Mann-Whitney U test. Analyses were conducted using the GraphPad Prism software version 5.03 (GraphPad software, San Diego, California). A p value of ≤ 0.05 was considered significant.

Results

There were 224 episodes of *S. aureus* bacteremia during the study period (figure 1). During this period, 5622 bottles of blood cultures returned positive. Twelve episodes were excluded as failures of MALDI-TOF MS identification, of which 9 were polymicrobial bacteremia. False identifications of *S. aureus* (identification score ≥ 1.7) did not occur during the study period. Four additional episodes were excluded as they

occurred during the weekend initially. Altogether, 208/220 *S. aureus* bacteremia (94.5% of all tested) were correctly identified by MALDI-TOF MS (identification score ≥ 1.7) and 197 were included in the study. Using the numbers assigned to each patient at hospital admission, 106 patients were assigned to the intervention group and 91 to the control group.

Patient characteristics

Although randomization led to unequal numbers, the groups were well balanced in their epidemiological and clinical characteristics (table 1). In particular, 94% of all empirical antibiotic therapies were considered adequate in both groups. Of 197 episodes of bacteremia, 43 (21.8%) were due to MRSA. In both groups, the most frequent sources of bacteremia were intravascular catheters and skin/soft tissue infections. An unknown origin of infection was more frequent in the control group ($p = 0.04$).

Performance of GeneXpert MRSA and MALDI-TOF MS

In the intervention group, a correct identification of methicillin susceptibility was possible in 105/106 cases. There were 24 episodes of MRSA bacteremia and 82 episodes of MSSA bacteremia. For the only case of MSSA bacteremia with an undetermined result, repetition of GeneXpert led to a correct result. There was no false-positive detection of resistance to methicillin. Considering the indeterminate result initially obtained as a false-negative, a sensitivity of 99% and a specificity of 100% were thus measured for the detection of methicillin resistance on positive blood culture pellets with Gram-positive cocci in clusters identified as *S. aureus* with MALDI-TOF MS.

Identification of *S. aureus* with MALDI-TOF MS as evaluated on 220 episodes had a sensitivity of 94.5% and a specificity of 100%, as no specimen was wrongly identified as *S. aureus* by MALDI-TOF MS during the study period. The 5.5% of *S. aureus* not identified by MALDI-TOF MS all exhibited scores < 1.7; thus, no false identification occurred when the score was ≥ 1.7. No *S. aureus* was wrongly identified as another species during the study period.

Time to result

There were 65/106 episodes (61.3%) which led to direct telephone transmission of MALDI-TOF MS identification of *S. aureus* and/or GeneXpert result to the clinician in the intervention group. Other cases were transmitted only by the laboratory information system, as they occurred out of hours. The median time from MALDI-TOF MS identification to GeneXpert result (56 evaluable episodes) was 97 minutes (range 25-250). Median time from blood culture positivity to GeneXpert result (57 evaluable episodes) was 201 minutes (range 100-430).

Impact on empirical antibiotic therapy (table 2)

As shown in table 1, both groups had similarly high degrees of initial adequacy of empirical antibiotic therapy (94%) as confirmed by AST. Among the 43 patients with MRSA bacteremia, 34 (79.1%) were previously known MRSA carriers, and thus, more than 90% of patients with MRSA bacteremia received an empirical coverage of MRSA in both groups. There was less unnecessary use of glycopeptides for MSSA in the intervention group, although this difference did not reach statistical significance (17.1% vs. 29.2%, $p = 0.09$). When excluding patients with penicillin allergy, who were overrepresented in the intervention group (10/106 – 9.4% vs. 5/91 – 5.5%, table 1), the rate of unnecessary use of glycopeptides for MSSA decreased from 26.1% (18/69) in the control group to only 8.1% (6/74) in the intervention group ($p < 0.01$). For the two patients in the intervention group that did not receive an early coverage of MRSA, rapid detection of MRSA with GeneXpert allowed an early coverage of MRSA prior to the AST. For five out of 14 patients (35.7%) receiving unnecessary use of glycopeptides for MSSA in the intervention group, the rapid detection of MSSA with GeneXpert allowed the introduction of a betalactam treatment prior to the availability of a routine AST that needed an overnight incubation.

Toxicity

No skin rashes were documented in both groups, although 95% of patients with empirical MRSA coverage in the control group and 89% in the intervention group were

treated with vancomycin. Regarding renal insufficiency, there was no statistically significant difference in rates of renal insufficiency between the intervention group and the control group at day 3 (0% vs. 3.2%), day 7 (9.4% vs. 4.4%) or day 14 (7.5% vs. 6.6%).

Discussion

In this prospective, randomized interventional study including 197 patients with *S. aureus* bacteremia, we documented a very high reliability of GeneXpert performed directly in blood cultures in diagnosing methicillin resistance, as 99% of cases were correctly diagnosed hours prior to the routine AST. Contrary to data from our own center established in a prospective study of *S. aureus* carriage, where 12.9% of patients were wrongly identified as MRSA carriers using GeneXpert MRSA performed on screening swabs,[20] we did not document false-positive tests with this assay when applied to blood cultures. High performances have already been published by groups performing this molecular diagnosis directly on positive blood culture pellets with Gram-positive cocci in clusters.[16-18] As the excellent performances of MALDI-TOF MS for the identification of *S. aureus* in blood cultures were also confirmed in a real-life setting, we think that the combination of both techniques would help to spare the unnecessary use of the expensive molecular diagnosis in cases of coagulase-negative staphylococci cases, which are often contaminants.

Appropriate empirical antibiotic therapy remains the most critical determinant of prognosis for patients with bloodstream infections.[6, 21] In severely diseased patients with sepsis, as was the case in 10% of our patients, time to initiation of an effective antibiotic therapy was shown to predict the risk of death, as each hour of delay was associated with a significant decrease in survival.[22] In the setting of *S. aureus* bacteremia, delay until effective treatment (e.g. absence of MRSA coverage when indicated) was associated with increased mortality and longer hospital stay.[23] Thus, reducing the time to result is critical in clinical microbiology. As the standard of blood culture management,[24] Gram stain reporting showed a major impact on empirical antibiotic

therapy and improvement of its turnaround time was associated with an improvement of patient prognosis.[25] More recently, routine application of MALDI-TOF MS on blood cultures was associated with an 11% increase in the appropriateness of empirical antibiotic regimen, although this study was not designed to assess mortality.[26]

Data on the clinical impact of PCR-based diagnosis of methicillin resistance are scarce to date. Bauer *et al*. demonstrated in a single-center study performed in a high-incidence environment that molecular diagnosis made a possible decrease to the mean length of stay by more than 6 days and thus decreased hospital costs.[27] In another setting of 20% of resistance to methicillin among *S. aureus*, the use of GeneXpert was associated with 54% of earlier appropriate vancomycin prescription in patients with MRSA bacteremia and 25% of avoidance of unnecessary use of vancomycin.[28] As most patients with MRSA bacteremia in our study were previously known carriers, an adequate coverage was already provided to most of them before the GeneXpert result. The use of GeneXpert allowed us to spare some unnecessary prescriptions of anti-MRSA agents especially in the subgroup of patients with penicillin allergy, although other confounding factors might exist, and to introduce an earlier adequate coverage in the few unsuspected cases. This may be of greater value in settings of higher incidence of community-associated MRSA, which remains very low in Switzerland. Furthermore, this highly specific test allowed us to adequately prescribe betalactams in the few MRSA carriers with MSSA bacteremia. This may be useful given the higher efficacy of betalactams,[29-30] although the cost-effectiveness of such a strategy should be evaluated.

In conclusion, we have documented high performance of GeneXpert for the diagnosis of methicillin resistance of *S. aureus* in blood culture pellets. Our work thus emphasizes the usefulness of coupling rapid PCR on blood culture to MALDI-TOF MS identification in clinical practice. These results should now be confirmed in larger studies also investigating clinical outcomes and including a cost-effectiveness analysis as well as in settings of higher MRSA incidence, where the combination of MALDI-TOF MS with

GeneXpert MRSA should be of significant help to improve the management of *S. aureus* bacteremia.

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Conflicts of interest

None. The producer of GenXpert had no implication in the study design.

Figure 1: Study flow chart showing the exclusion criteria and randomization

Abbreviation: SAB = *S. aureus* bacteremia. AST = Antibiotic susceptibility testing

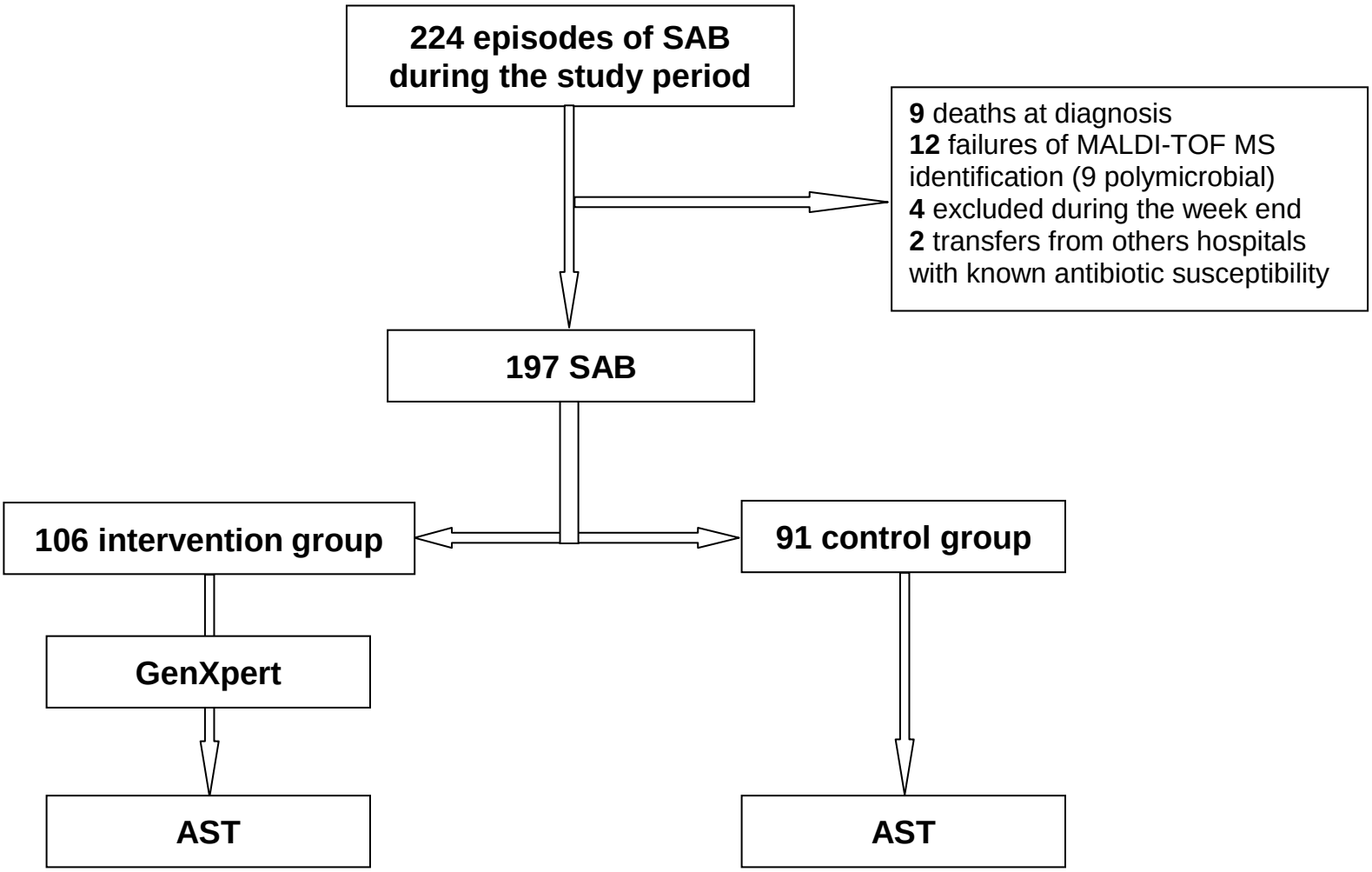

Table 1: Patient characteristics according to intervention

Characteristics	**Intervention** ***N = 106***	**Control** ***N = 91***	***P* value**
Sex (female)	32 (30.2)	27 (29.7)	1.0
Age (median/range-years)	66/0-94	67/0-93	0.4
ICU stay before bacteremia	16 (15.1)	12 (13.2)	0.68
Nosocomial bacteremia	50 (47.2)	49 (53.8)	0.39
Source of bacteremia			
Intravascular catheter	37 (34.9)	32 (35.2)	1.0
Skin and soft tissue infection	28 (26.4)	27 (29.7)	0.22
Others	16 (15.1)	21 (23.1)	0.2
Unknown	25 (23.6)	11 (12.1)	**0.04**
Severe sepsis/septic shock	11 (10.4)	9 (9.9)	0.82
Known MRSA carrier	19 (17.9)	17 (18.7)	1.0
Immunosuppression	25 (23.6)	21 (23.1)	1.0
Diabetes	31 (29.2)	28 (30.8)	0.88
IDU	4 (3.8)	5 (5.5)	0.74
Dialysis	9 (8.5)	4 (4.4)	0.39
Penicillin allergy	10 (9.4)	5 (5.5)	0.42
Bacteremias due to MRSA	24 (22.6)	19 (20.9)	0.86
ID consultation	97 (91.5)	84 (92.3)	1.0
Adequate empirical therapy	100 (94.3)	86 (94.5)	1.0
Complicated bacteremia	32 (30.2)	29 (31.9)	0.88

Data are n (%), unless stated otherwise.
Abbreviations: ICU = intensive care unit. IDU = injecting drug user. ID = infectious diseases.

Table 2: Impact of the GeneXpert MRSA performed directly on positive blood cultures on the empirical antibiotic therapy

	Intervention ***N = 106***		**Control** ***N = 91***	
	MRSA	*MSSA*	*MRSA*	*MSSA*
Empirical anti-MRSA tx, all	22/24 (91.7%)	14/82 (17.1%)*	18/19 (94.7%)	21/72 (29.2%)*
Empirical anti-MRSA tx, without PA	20/22 (90.9%)	6/74 (8.1%)#	16/17 (94.1%)	18/69 (26.1%)#

Data are n (%). Tx = treatment, PA = penicillin allergy
*: p = 0.09, #: p = 0.01 (Fisher's exact test)
Empirical anti-MRSA treatment = prescription of glycopeptides or daptomycin

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