Matrix-assisted laser desorption ionization time-of-flight mass spectrometry and PCR-based rapid diagnosis of *Staphylococcus aureus* bacteraemia

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Abstract

Effective empirical treatment is of paramount importance to improve the outcome of patients with *Staphylococcus aureus* bacteraemia. We aimed to evaluate a PCR-based rapid diagnosis of methicillin resistance (GeneXpert MRSA) after early detection of *S. aureus* bacteraemia using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Patients with a first episode of *S. aureus* bacteraemia 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 directly on blood cultures. We then assessed the impact of early diagnosis of methicillin resistance on the empirical treatment. In all, 197 episodes of *S. aureus* bacteraemia were 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 min (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 MRSA in the intervention group (17.1% versus 29.2%, p 0.09). GeneXpert MRSA was highly reliable in diagnosing methicillin resistance when performed directly on positive blood cultures. This could help to avoid unnecessary prescriptions of anti-MRSA agents and promote the introduction of earlier adequate coverage in unsuspected cases.

Keywords: Antibiotic resistance, antibiotic susceptibility testing, GeneXpert MRSA, matrix-assisted laser desorption ionization time-of-flight, *Staphyloccocus aureus* bacteraemia
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Introduction

Staphylococcus aureus is a major cause of community-acquired and nosocomial bacteraemia [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* bacteraemia [5,6].

Although Gram staining of positive blood cultures has a high impact on empirical antibiotic therapy [7], definitive identification of the aetiological agent and determination of its antibiotic susceptibility may imply a delay of 24–48 h. Empirical antibiotic therapy is based on clinical assessment and 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 diagnosis 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 species level of 79% of tested pellets and, more specifically, all 25 episodes of *S. aureus* bacteraemia were correctly identified with a score ≥1.7, theoretically indicating an identification that is reliable at genus level only [9]. Other groups also confirmed high performances of *S. aureus* identification in blood cultures using MALDI-TOF MS [10–12]. Although 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].

Tests based on PCR and 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 96–100% for the detection of MRSA [16–18]. As a routine procedure, it would generate high costs because of the frequency of coagulase-negative staphylococci in blood cultures. In our centre, 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* bacteraemia. 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* bacteraemia identified using MALDI-TOF MS were included in a prospective, randomized open study between October 2010 and August 2012 in Lausanne University Hospital Centre, 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 other hospitals) and patients who were dead at time of diagnosis of bacteraemia. Randomization was performed using a unique number assigned to each hospitalized patient in our centre, which was not linked to patient characteristics. In the control group (odd number), standard AST was performed after MALDI-TOF MS identification (time to antibiotic susceptibility result of about 24–48 h). In the intervention group (even number), MALDI-TOF MS identification was coupled to a GeneXpert MRSA test (Cepheid, Sunnyvale, CA, USA) performed directly on 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* bacteraemia. For each case, a standardized case report form 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, USA). Direct MALDI-TOF MS has been performed routinely in our centre on all positive blood cultures since September 2009. Mass spectra were acquired on a Microflex LT MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) after a standardized ammonium chloride erythrocyte-lysing procedure [9]. Spectral analysis and comparison with the database were carried out 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 (0800–1700 h) as well as electronically using the laboratory information system. Clinicians were contacted the following day for blood cultures that turned 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* bacteraemia. 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* bacteraemia.