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The prevalence, population structure and screening test specificity of penicillin-susceptible *Staphylococcus aureus* bacteremia isolates in Malmö, Sweden

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Summary *Objectives:* The objectives of this study were to examine the prevalence of penicillin-susceptible bacteremic *Staphylococcus aureus* in the Malmö area in 2014, to re-evaluate the phenotypic methods of penicillinase detection on these isolates, and to investigate the clonal distribution of penicillin-susceptible isolates.
Methods: All non-redundant *S. aureus* from blood in the Malmö catchment area in southern Sweden 2014 were screened for penicillin susceptibility using PcG 1U disk diffusion, E-test PcG and the nitrocefin test. All isolates screened as likely susceptible were subjected to PCR for detection of penicillinase (*blaZ*) and *spa*-typing.
Results: Almost one out of three bacteremic isolates (80/257; 31.1%) were susceptible to penicillin. All screening methods except for the nitrocefin test alone had a low proportion of isolates falsely tested as susceptible, but no method used in the study had perfect specificity compared with PCR. Penicillin-susceptible isolates had a distinct phylogenetic distribution, and two clonal complexes (CC5 and CC45) constituted half of the isolates.
Conclusion: Almost one third of *S. aureus* isolated from blood in southern Sweden in 2014 was susceptible to penicillin. Considering that intravenous penicillin has theoretical advantages compared with the standard treatment in the study area, we argue that routine testing of penicillin susceptibility should be reconsidered.

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Introduction

Despite many advances in the treatment of bacterial infections, bacteremic *Staphylococcus aureus* infections still pose a major challenge for infectious diseases physicians. Bacteremia caused by *S. aureus* is common and associated with frequent complications, such as endocarditis and osteomyelitis[1,2] as well as with significant mortality.[3] Penicillin G remains the theoretical first choice for treatment of penicillin-susceptible *S. aureus* (PSSA) bacteremia. For penicillin-susceptible isolates, given standard doses, intravenous penicillin generally provides higher fT > MIC (proportion of time of free plasma concentrations above the minimum inhibitory concentration) than cloxacillin.[4] It has in one study been shown to have equivalent or even superior clinical effect compared to other tested betalactams,[5] even though the latter requires confirmation in prospective studies. In contrast, vancomycin treatment has been associated with higher rates of recurrence and higher mortality than betalactam treatment in methicillin-susceptible *S. aureus* (MSSA) infections.[6,7]

A rapid selection of penicillinase-producing *S. aureus*, as well as a pandemic of penicillin-resistant strains occurred in the 1940's and 50's following wide-scale introduction of penicillin.[8] Epidemiological studies suggest that penicillin-susceptible isolates still circulate, but it is generally stated that they are very rare (<5%).[9] Another review suggests a difference in rates of penicillin-susceptible isolates between community-acquired *S. aureus* (75–85% penicillin-resistant) and hospital-acquired *S. aureus* (90–95% penicillin-resistant).[5] In a global perspective, the pandemic spread of methicillin-resistant *S. aureus* (MRSA) has marginalized penicillin as a treatment option even further.[10] In Sweden, a country where rates of MRSA among invasive *S. aureus* infections remain very low (1%), routine testing for penicillin susceptibility was discontinued decades ago, attributed to the fact that susceptible isolates were considered very rare and that alternative, safe treatment options existed. Scattered epidemiological reports from recent years have suggested that the prevalence of PSSA in some regions may be greater than 5%. Numbers from the US have suggested rates at 15–30%,[11,12] while studies from Denmark and Canada have suggested levels at or above 20%.[5,13]

A major concern when testing for penicillin susceptibility in *S. aureus* is that traditional screening methods have not been considered reliable, since the penicillinase causing resistance is not constitutionally expressed. Different studies have questioned the reliability of phenotypic testing.[14,15] The EUCAST standard testing of PcG 1U disk diffusion testing seems to predict true susceptibility well,[16] but includes a step of subjective determination of zone edge appearance,[17] which potentially makes it less reproducible.

The purpose of the current study was three-fold. The first two objectives were to examine the prevalence of PSSA from blood cultures in our catchment area and to re-evaluate the phenotypic methods of penicillinase detection on these isolates. Thirdly, we performed *spa*-typing of our penicillin-susceptible isolates to investigate the clonal distribution, as well as to validate that the phenotypic methods were tested on a range of non-clonal isolates.

Methods

Study setting

The study was performed in Malmö, Sweden, at a laboratory of clinical microbiology serving an area with a population of approximately 500,000 patients. In the laboratory, all bacterial strains isolated from blood are normally saved. All available non-redundant *S. aureus* isolates saved in the laboratory were included in the study. In order to exclude repeat isolates, individual patients were indexed by number by the database provider. No patient-level data was used in the study.

Culture conditions and DNA preparation

All included *S. aureus* were streaked on blood agar plates and incubated overnight at 37 °C in an aerobic environment. Isolates were confirmed as *S. aureus* using standard microbiology techniques including slide agglutination with Pastorex® (Bio-Rad Laboratories, Hercules, CA) and MALDI-TOF (Biotyper®; Bruker, Solna, Sweden). In order to release bacterial DNA, 5 bacterial colonies from each isolate were heated in sterile distilled water at 98 °C for 10 min and centrifuged at 10,000 g for 3 min. Supernatants were collected and stored at −20 °C.

Antimicrobial susceptibility testing

Disk diffusion was used for routine antimicrobial susceptibility testing.[18] All clinical isolates had been tested for resistance to cefoxitin, gentamicin, vancomycin, clindamycin, ciprofloxacin, fusidic acid, trimethoprim–sulfamethoxazole, lincomycin and rifampicin. Antimicrobial susceptibility was interpreted according to nordicAST breakpoints of the study period.[19]

All available isolates were tested for penicillinase production using three phenotypic screening tests: nitrocefin testing (Céfinase discs; Biomerieux, Marcy l'Etoile, France), E-tests for penicillin G (Biomerieux) on Mueller-Hinton agar plates and finally disk diffusion testing using PcG 1U disks on Mueller-Hinton agar plates. Interpretation of susceptibility was based upon nordicAST/EUCAST guidelines.[19] The only exception to this rule was the zone edge appearance, which was not monitored. Interpretations were based upon zone diameter alone.

Polymerase chain reaction (PCR) for detection of the staphylococcal penicillinase

A flow chart of our procedure for testing of *S. aureus* is outlined in Fig. 1. All available *S. aureus* isolates that were tested negative in the nitrocefin β-lactamase test and were penicillin-susceptible according to either PcG (1U) E-test or disk diffusion (or both) were subjected to PCR to detect the penicillinase gene (*blaZ*) using primers: 5′-TTCAACACCTGCTGCTTTCGG-3′ and 5′-CCTTCATTACACTCTTGGCGGTTTC-3′. PCR products were separated by electrophoresis on a 1% agarose gel. All PCRs included an internal positive control. A subset of isolates tested as

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