Staphylococcus aureus from public hospitals in KwaZulu-Natal, South Africa – infection detection and strain-typing

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The objectives of the study were: 1) to determine the range of infections caused by Staphylococcus aureus, 2) to establish one/more rapid, reliable, reproducible and cost-effective methods for the detection of methicillin-resistant S. aureus (MRSA) in resource-constrained healthcare settings, and 3) to compare South African strains with those found globally using multi-locus strain typing (MLST). A total of 241 S. aureus isolates collected from 16 hospitals in KwaZulu-Natal were subjected to antibiotic susceptibility testing using the CLSI disc sensitivity testing method. A random selection of 24 putative MRSA and a matched control group of methicillin-sensitive S. aureus isolates were subjected to agar-based methods, the Etest and screen latex agglutination methods for MRSA detection using polymerase chain reaction as the gold standard. Strain typing was undertaken by MLST. S. aureus was implicated in a range of infections. Sensitivity, specificity, positive predictive values, negative predictive values and costs of the various detection tests showed that the use of oxacillin disc agar diffusion and oxacillin screening salt agar was appropriate for routine laboratory testing for the identification of MRSA. MLST identified the ST8-SCCmec type IV (67%), ST239-SCCmec type III (13%), ST8-SCCmec type II (8%), ST5-SCCmec type IV (8%) and ST45-SCCmec type IV (1%) clones confirming the mobility of SCCmec type IV (79%). Infections by S. aureus are a public health concern in South Africa as they are worldwide. Antibiotic prescribing trends may account for the de novo evolution of strains identified globally. Routine surveillance for MRSA is thus advocated.

Introduction

Staphylococcus aureus is implicated in a variety of diseases ranging from superficial skin infections to severe life-threatening infections and is amongst the leading causes of nosocomial infections. Its adaptive capacity is epitomised by its response to the use of antibiotics. Penicillin was rendered ineffective within 10 years of its introduction in the early 1940s; methicillin-resistant S. aureus (MRSA) emerged in 1960, within two years of the clinical use of isoxazolyl penicillins; epidemic, multidrug-resistant MRSA clones spread globally by the 1980s and glycopeptide resistance emerged in the early 2000s. MRSA are frequently resistant to a wide-range of antimicrobials including the β-lactams, tetracyclines, macrolides, lincosamides, aminoglycosides, quinolones, disinfectants, heavy metals and antiseptics, and, MRSA infections are associated with worse clinical outcomes, substantial morbidity and mortality and increased healthcare costs. The need to monitor MRSA rapidly and cost-effectively especially within under-resourced public health systems is thus particularly important and a necessary step towards its containment.

The major mechanism of resistance in MRSA is the production of modified penicillin-binding proteins (PBPs), and the production of a supplemental PBP2a which is encoded by a chromosomal meca gene and which is insensitive to inactivation by β-lactam antibiotics. The meca gene is carried on the staphylococcal cassette chromosome mec (SCCmec) which is a mobile, exogenous genetic element. The type of recombinase genes (crr) and the class of mec complex carried determines six major types of SCCmec differing in size and content. Multi-locus sequence typing (MLST) of seven housekeeping genes in combination with SCCmec typing applied to a substantial international S. aureus strain collection concluded that MRSA evolved on at least 20 occasions from methicillin-sensitive S. aureus (MSSA) clones resulting in a small number of pandemic MRSA clones.

The epidemiology of MRSA outbreaks is critical to the understanding of its dissemination, the identification of the MRSA strains circulating globally, and their relatedness to each other and to susceptible isolates. MLST is a highly discriminatory method of bacterial isolate characterisation allowing sequence data to be readily compared between laboratories.

This study describes the range of infections and susceptibility patterns attributable to S. aureus, particularly MRSA; it compares the sensitivity, specificity, positive predictive value, negative predictive value and cost of the different detection methods to determine a rapid and cost-effective method for the detection of MRSA in resource constrained settings in developing countries and it compares South African strains with those found globally.

Materials and methods

Bacterial strains

Microbiology laboratories at 16 hospitals in KwaZulu-Natal, South Africa, participated in a multi-centre surveillance study by submitting a maximum of 100 consecutive, non-repetitive isolates during the
period January 2001 to December 2002 (University of KwaZulu-Natal Ethical Clearance No.:0092A). Of the 1,270 isolates collected, 277 (21.8%) were *S. aureus* and 241 (87% of the total *S. aureus* isolated) were ultimately used in this study (36 isolates were excluded as a result of contamination or loss of viability upon storage). Speciation was verified on the basis of positive mannitol fermentation, catalase and coagulase tests. *S. aureus* ATCC 27952 and ATCC 25923 served as the positive MRSA and negative MRSA controls, respectively. The latter also served as the control for susceptibility testing.

**Antibiotics**

The antibiotic test panel consisted of benzylpenicillin, ampicillin, amoxicillin-clavulanate, oxacillin, cefalothin, cefepime, amikacin, chloramphenicol, cotrimoxazole, erythromycin and vancomycin, particularly chosen because of their availability within the public healthcare system.

**Susceptibility testing**

Susceptibility testing was performed by means of the Kirby Bauer agar diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines. Discs were obtained from Mast Diagnostics, Merseyside, UK.

**MRSA detection**

The comparison of MRSA detection methods took the form of a nested case-control study where a random selection of 24 putative MRSA and a matched control (by hospital) of MSSA isolates formed the sample. Isolates were regarded as putative MRSA based on growth on oxacillin screening plates (6 µg/ml of oxacillin + 4% NaCl in Mueller Hinton agar), used for MRSA detection in public hospitals. Although the sample number was determined for convenience as commercially available latex agglutination tests were available in pack sizes of 24, the number of MRSA used in this study nevertheless constituted 43% of the putative MRSA total.

Isolates were subjected to polymerase chain reaction (PCR) detection of the **meca** gene, oxacillin screening test (6 µg/ml of oxacillin + 4% NaCl on Mueller Hinton agar), disc diffusion test on Mueller Hinton agar, and disc diffusion test with 2% sodium chloride in Mueller Hinton agar as described previously. The oxacillin Etest (AB Biodisk, Solna, Sweden) with 2% sodium chloride in Mueller Hinton agar served as the positive MRSA and negative MRSA controls, respectively. The latter also served as the control for susceptibility testing.

**Results and discussion**

*S. aureus* has long been recognised as a common pathogen in human illness with several studies having documented the *S. aureus* as the causative microorganism in diverse disease states. A 28% prevalence was shown in skin, soft tissue, bone and joint infections in hospitalised patients from 134 institutions in Washington over a one year period, a median prevalence of 39% was evidenced in blood culture isolates processed by laboratories servicing 62 hospitals in the southern and eastern Mediterranean over a two-year period and a 36% prevalence was found in a three-month carrier study in a neonatal unit in Benin, to name a few. Skin and soft tissue infections accounted for some 73% of the diagnoses in this study, otitis media for 7.5%, surgical site infections and septicemia for 2.5% each and a range of miscellaneous diagnoses accounted for the rest. Twenty-seven isolates were from community-acquired infections and three of the 27 were putative MRSA.

Table 1 shows the percentage resistance of all isolates, MRSA and MSSA to the antibiotics tested. Nine (3.7%) of the isolates were susceptible to all antibiotics, three (1.2%) were resistant to a single antibiotic (erythromycin) while the remaining 229 (95.1%) were multi-resistant, evidencing resistance to two to 11 of the antibiotics tested. Resistance to penicillin occurred concomitantly with resistance to ampicillin in all isolates with clavulanate restoring sensitivity to penicillins in 109 (45%) of the isolates, indicating the expected expression of penicillinases. The lack of total resistance to amoxicillin-clavulanate, cefalothin and cefepime amongst the confirmed MRSA isolates and the unexpected resistance to oxacillin in the confirmed MSSA isolates depicted in Table 1 may be attributed to the discordant MRSA detection test results discussed below.

It must be remembered that the 48 isolates subjected to MRSA detection tests were putative MRSA and MSSA based on growth on oxacillin screening plates [6 µg/ml of oxacillin + 4% NaCl in Mueller Hinton agar]. Vancomycin retained its efficacy.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance (%)</th>
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<tbody>
<tr>
<td></td>
<td>All isolates N=241</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>95</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>95</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>50</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>23</td>
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<tr>
<td>Cefalothin</td>
<td>20</td>
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<tr>
<td>Cefepime</td>
<td>10</td>
</tr>
<tr>
<td>Amikacin</td>
<td>11</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>10</td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole</td>
<td>22</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>40</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>24</td>
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<tr>
<td>Vancomycin</td>
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</tr>
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The literature contains numerous studies investigating optimal methods for phenotypic detection of MRSA. Oxacillin-salt screening plates containing 6 µg/ml oxacillin plus 4% NaCl, broth microdilution tests with 2% NaCl, agar dilution tests with 2% NaCl and disc diffusion tests are often cited, but the expression of resistance is reported to be markedly affected by test conditions, including, but not limited to, the agent tested (oxacillin vs methicillin with the former reported as the more stable), culture medium (Columbia and Mueller Hinton agars yielding better discrimination than IsoSensitest agar), NaCl concentration (2% as discriminatory as 5% which is not tolerated by some salt-sensitive organisms), and incubation temperatures.
Optimal conditions for phenotypic susceptibility testing are reported to vary between strains and no single phenotypic test is likely to detect all resistant strains. Sensitivity, specificity, positive predictive values, negative predictive values and costs of the various detection tests to which the 24 MRSA and 24 MSSA isolates were subjected showed that the oxacillin screening plates and disc diffusion methods with 0% and 2% NaCl supplementation showed greater sensitivity and specificity as compared than the Etest, and latex agglutination methods for MRSA detection. Combining phenotypic tests did not significantly improve any of the parameters (p values ranged from 0.25 to 0.9) contrary to the study by Baddour, Abuelkheir and Fatani (2007).

Eight isolates showed discordant results; four were identified as MRSA by all phenotypic tests but not by PCR; one was MRSA-positive on PCR but negative on all the phenotypic tests; two were detected as MRSA by the disc sensitivity tests in Mueller Hinton Agar with 0% and 2% sodium chloride, but not by the other tests; one was detected as MRSA by the disc sensitivity tests in Mueller Hinton agar with 2% sodium chloride while two were missed by the Etest and the latex agglutination test although positive on PCR. The oxacillin screening plates and disc diffusion methods with 0% and 2% NaCl supplementation were thus considered appropriate for routine identification of MRSA in resource-constrained healthcare settings.

MLST results (Table 3) showed that 67% (16/24) MRSA belonged to the ST8-SCCmec type IV/EMRSA-2 and -6 clones found in Finland, France, Germany, Ireland, Netherlands, UK and USA; 13% (3) belonged to the ST239-SCCmec type III/EMRSA-1, -4, -11, Por/Bra, Vienna clone found in Finland, Germany, Greece, Ireland, Netherlands, Poland, Portugal, Slovenia, Sweden, UK and USA; 8% (2) belonged to the ST8-SCCmec type II/Irish-I clone found in Ireland, UK and USA, 8% (2) belonged to the ST5-SCCmec type IV/paediatric clone found in France, Portugal, UK, and USA and 4% (1) belonged to the ST45-SCCmec type IV/Berlin clone found in Belgium, Finland, and USA.
Germany, and Sweden (http://www.mlst.net). This study confirmed the enhanced mobility of SCCmec type IV (19/24-79%) attributed to it being the smallest structural SCCmec type.2

ESH37 was one of the three putative community-acquired MRSA randomly selected for nested cased-control study and it was found to belong to the ST8-SCCmec type IV/EMRSA-2 and -6 clone. It evidenced the SCCmec type IV, a trait shared by community-acquired MRSA globally15 and belonged to ST8, the most prevalent community-acquired MRSA clone in the US.16 It was resistant to all the β-lactams on the antibiotic panel but retained sensitivity to the other antibiotics, a characteristic of community-acquired MRSA reported in other studies.16,17

Two MSSA isolates (ADD69 and GJC7) had the same allelic profile as ST8-SCCmec type IV, one (RKK8) had the same allelic profile as ST239-SCCmec type III, two (ADD69 and GJC7) had the same allelic profile as ST8-SCCmec type II, four (EDD95, EGU42, PS61 and RKK4) had the same allelic profile as ST5-SCCmec type IV and one (RKK49) had the same allelic profile as ST45-SCCmec type IV. While this provides some evidence for the potential evolution of MRSA from MSSA, it is more likely that antibiotic prescribing patterns may account for the de novo evolution of strains identified globally because all except one of the MSSA were isolated at hospitals different from those in which the MRSA were collected. To our knowledge, this is the first report of MLST of S. aureus from South Africa.

Routine surveillance, prudent antibiotic use and optimised infection control practices11 are some of the strategies advocated for the containment of MRSA in a South African healthcare system experiencing an ever-increasing incidence and prevalence of infections as a result of HIV and AIDS, yet ill-equipped to deal with the clinical and pharaco-economic implications of resistance.

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References