Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi, Kenya

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## S U M M A R Y

**Background:** Skin and soft tissue infections (SSTIs) are among the most common infectious diseases and a frequent cause of hospital visits. In this study we sought to assess the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and antibiotic susceptibility patterns in SSTIs in patients attending hospitals in Kenya.

**Methods:** Eighty-two *S. aureus* isolates recovered from SSTIs from both inpatients and outpatients were screened for antibiotic susceptibility, possession of staphylococcal cassette chromosome mec (SCCmec) gene type, and the Panton-Valentine leukocidin (PVL) toxin gene. The prevalence of MRSA was investigated in relation to the type of patient and infection type, as well as the type of health care facility.

**Results:** Of 60 boil cultures, 39 (65%) grew *S. aureus*, of out of which 34 (87.2%) were MRSA. Of the 60 abscess cultures, 14 (23.3%) grew *S. aureus*, of which 10 (71.4%) were MRSA. Of 34 cellulitis cultures, 18 (52.9%) grew *S. aureus*, of which 16 (88.8%) were MRSA. Of 25 ulcer cultures, 11 (44%) grew *S. aureus*, of which nine (81.8%) were MRSA. Sixty-nine of 82 *S. aureus* (84.1%) were MRSA, with 52 (75.4%) possessing SCCmec II type and 14 (20.3%) being positive for the PVL gene. Based on hospitals, it was noted that most MRSA were isolated at publicly funded health care facilities serving an economically disadvantaged segment of Nairobi’s population, such as those living in urban informal settlements. All 82 *S. aureus* were susceptible to vancomycin and resistant in high numbers to macrolides, aminoglycosides, and quinolones. Bacterial isolates were mostly susceptible to vancomycin, ciprofloxacin and co-trimoxazole, and none was resistant to vancomycin. However, most organisms showed decreased susceptibility to erythromycin and clindamycin.

**Conclusions:** These findings suggest that SCCmec II MRSA and a PVL strain of MRSA are significant pathogens in patients with SSTIs presenting to hospitals in Kenya, and that MRSA cases are prevalent at publicly funded health care facilities.

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## 1. Introduction

*Staphylococcus aureus* is a major pathogen responsible for various infections including bacteremia, pneumonia, skin and soft tissue infections (SSTIs), and osteomyelitis. ¹ ² Over the past 50 years *S. aureus* has acquired resistance to previously effective antimicrobials including the penicillinase-resistant ones like methicillin.³ Today, methicillin-resistant *S. aureus* (MRSA) poses a serious therapeutic problem worldwide.⁴ ⁵ The percentage of hospitals isolating MRSA in the developed countries increased from 2% in the 1970s to 30% in the 1990s.⁶ ⁷ Although MRSA is a serious global pathogen, studies are largely skewed to affluent regions and as such there is very little information regarding the frequency and characteristics of MRSA in developing countries, especially in Africa.

Carriage of MRSA by healthcare workers has been studied extensively with respect to the role that this may play in spreading MRSA to patients. A study published in 2009 found the prevalence of MRSA in doctors and nurses working in the intensive care units and surgical units in six hospitals in Tripoli, Libya to be 36.8%. In Africa, data on MRSA, particularly antibiotic susceptibilities, are extremely limited and the magnitude of the problem is yet to be quantified.⁸ ⁹ ¹⁰ A study aimed at determining the prevalence and antibiotic susceptibility patterns of MRSA in African countries found relatively high prevalence rates in Nigeria, Kenya, and Cameroon (21–30%), and below 10% in Tunisia, Malta, and Algeria.⁹ Surveillance of staphylococcal colonization and cross-infection in hospitals located in Cape Verde showed an *S. aureus* carriage rate of
41%, demonstrating extensive colonization of both inpatients and outpatients. This observation emphasizes the need to maintain surveillance and control of MRSA infections in Africa.

This study was an attempt to assess the prevalence of MRSA and antibiotic susceptibility patterns in SSTIs in patients attending hospitals in Kenya.

2. Materials and methods

2.1. Study design

This was a prospective study involving a convenience sample of patients (inpatients and outpatients) who presented with SSTIs to five different health facilities in Nairobi, Kenya, during 2005–2007. The health facilities were categorized as: facility 1: a public tertiary research center; facilities 2 and 3: private hospitals in Nairobi; facility 4: a public district hospital; and facility 5: a public tertiary referral hospital. Written informed consent to participate in this study was required, and all patients agreed to participate by signing an informed consent form.

2.2. Inclusion and exclusion criteria

Patients were screened by trained nursing staff or a treating physician for inclusion/exclusion in the study either immediately after admission, or during the outpatient visits. Inclusion criteria for patients were: admission to an inpatient/outpatient service, presence of an active SSTI as determined by the treating physician, at least 18 years of age, ability to understand risks/benefits, and consent to participation in the study. Patients were excluded if they had an uncomplicated skin or superficial skin structure infection such as simple skin abrasion. Patients with necrotizing fasciitis, gas gangrene, and osteomyelitis were excluded from the study. Children and those patients who refused to sign the informed consent form were excluded. SSTIs were defined as cellulitis, skin abscess, infected skin ulcer, infected surgical incision, infected traumatic wound, diabetic foot ulcer, decubitus ulcer, ischemic ulcer, or infected bite. For the latter four diagnoses, the infection had to involve the soft tissues only, without concomitant osteomyelitis.

2.3. Bacterial isolates and culture conditions

Culture swabs from SSTIs were immediately streaked onto mannitol salt and nutrient agar. Plates were incubated at 35 °C for 48 h in aerobic conditions. Colonies demonstrating S. aureus colony morphology were subcultured onto trypticase soy agar with 5% defibrinated sheep blood (Nissui Pharmaceutical Co. Ltd) and incubated overnight at 35 °C. Identification of S. aureus was based on colony morphology on trypticase soy agar supplemented with 5% sheep blood, growth on mannitol salt agar, Gram stain, and a positive tube coagulase test using rabbit plasma (Oxoid Ltd, Hampshire, UK).

2.4. Antimicrobial susceptibility testing

Confirmed S. aureus were screened for antibiotic sensitivity with nine antibiotics by disk diffusion method, as standardized by the Clinical and Laboratory Standards Institute (CLSI). Antibiotics included methicillin (10 μg), oxacillin (1 μg), cefoxitin (30 μg), erythromycin (15 μg), gentamicin (10 μg), ciprofloxacin (5 μg), clindamycin (10 μg), co-trimoxazole (25 μg), and vancomycin (30 μg). All tests were performed on Mueller–Hinton agar (Oxoid Ltd, Hampshire, UK) and interpreted after incubation at 37 °C overnight.

2.5. Oxacillin, cefoxitin, and inducible clindamycin resistance, and penicillin binding protein 2a (PBP2a) screening

In addition, all isolates were subjected to oxacillin resistance screening agar, Mueller–Hinton agar (supplemented with 4% NaCl containing 6 μg of oxacillin per ml), and cefoxitin disk diffusion test using 30 μg cefoxitin disks on Mueller–Hinton agar, as recommended by CLSI guidelines. The zone diameters measured around each disk were interpreted as described previously. The double-disc diffusion D-test with a 2 μg clindamycin disk and a 15 μg erythromycin disk on the Mueller–Hinton agar plate was used to test for inducible clindamycin resistance. Isolates were further screened using a PBP2a latex agglutination test kit (SlideX MRSA detection; Denka Seiken). All MRSA and isolates for which the PBP2a result was discrepant with the phenotypic result had their MRSA phenotypes confirmed by testing for meca, as previously described. S. aureus ATCC 29213 (susceptible to methicillin) and S. aureus ATCC 43300 (resistant to methicillin) were used as controls.

2.6. DNA extraction

Total DNA from MRSA isolates was prepared by growing the strain on 5% sheep blood agar plates for 24 h at 37 °C. One to five bacterial colonies were suspended in 50 μL of sterile distilled water and heated at 99 °C for 10 min. The bacterial suspension was centrifuged at 30 000 × g for 1 min, and 2-μl aliquots of the supernatant containing total DNA were used for the PCR assays.

2.7. SCCmec typing and PVL gene detection

The nucleotide sequences of meca primers and their respective amplified products have been described previously. The primer sets were designed to anneal to unique regions and to generate amplicons that would allow identification of the meca gene based on the molecular mass of its PCR product. As an internal positive control, S. aureus ATCC 43300 was used. A multiplex PCR containing six pairs of primers including specific primers for SCCmec types I, II, III, IV, and V, and the primer specific for the mecA gene (Table 1), was used to determine SCCmec type I–V and meca for all MRSA isolates, using methods published previously. The presence of the lukS-PV and lukF-PV genes encoding PVL components was determined by a PCR-based method with the primer pair and thermocycler conditions as reported by Lina et al.

2.8. PCR conditions and assays

All PCR assays were performed directly from genomic DNA extracted from cultures grown on agar plates as described previously. Target genes, size, and primers are shown in Table 1. The SCCmec multiplex PCR typing assay contained six pairs of primers including the specific primers for SCCmec types I, II, III, IV, and V, and the primers for the meca gene. A 10-μl aliquot of the PCR product was analyzed in 1.5% agarose gel, and the PCR products were observed under a UV transilluminator after staining with 0.5 μg/ml ethidium bromide.

3. Results

3.1. Overall prevalence of bacterial isolates

One hundred and seventy-nine specimens were collected from 100 patients at the five different health care facilities. Sixty-nine specimens grew MRSA, 13 grew methicillin-susceptible S. aureus (MSSA), and 46 grew other bacteria including coagulase-negative...
Table 1
Primer sets used in the study. The primer sets were designed to anneal to unique regions and to generate amplicons that would allow identification of mecA, SCCmec, or PVL genes based on the molecular mass of their PCR product.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5′–3′)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCmec I</td>
<td>Type I-F</td>
<td>GCTTAAGAGCTGCTTACAGG</td>
<td>613</td>
<td>11</td>
</tr>
<tr>
<td>SCCmec II</td>
<td>Type I-R</td>
<td>GCTTACTGACTAGACTGCT</td>
<td>398</td>
<td>11</td>
</tr>
<tr>
<td>SCCmec III</td>
<td>Type II-F</td>
<td>CGTGAAGATGATGACGGC</td>
<td>280</td>
<td>11</td>
</tr>
<tr>
<td>SCCmec IV</td>
<td>Type III-F</td>
<td>CATTATATGCATGAGCG</td>
<td>325</td>
<td>11</td>
</tr>
<tr>
<td>SCCmec V</td>
<td>Type V-F</td>
<td>ATCATTAGGTAAAATGTCTGCATGATCCA</td>
<td>533</td>
<td>11</td>
</tr>
<tr>
<td>luk-PV-1</td>
<td>Type V-R</td>
<td>AGTTGTGCTCTGAAAGATACG</td>
<td>432</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2
Bacterial strains by health care facility and patient type

<table>
<thead>
<tr>
<th>Facility*</th>
<th>Bacterial isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>MSSA</td>
<td></td>
</tr>
<tr>
<td>Inpatient</td>
<td>Outpatient</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>F2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>F3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>F4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>F5</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>17</td>
</tr>
</tbody>
</table>

MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.
* F1, public tertiary research center; F2 and F3, private hospital in Nairobi; F4, public district hospital; and F5, public tertiary referral hospital.

Staphylococcus, corynebacteria, Streptococcus, and Micrococcus, which were more likely to represent colonization/contamination than infection. Fifty-one cultures grew yeasts and were not considered for further screening.

The prevalence of *S. aureus* at each of the facilities is shown in Table 2. The prevalence of *S. aureus* strains in relation to the type of infection is shown in Figure 1. Most swab cultures were positive for *S. aureus*. Of the 60 boil cultures, 39 (65%) grew *S. aureus*, of which 34 (87.2%) were MRSA. Of the 60 abscess cultures, 14 (23.3%) grew *S. aureus*, of which 10 (71.4%) were MRSA. Of 34 cellulitis cultures, 18 (52.9%) grew *S. aureus*, of which 16 (88.8%) were MRSA. Of 25 ulcer cultures, 11 (44%) grew *S. aureus*, of which nine (81.8%) were MRSA.

3.2. Antimicrobial sensitivity patterns

Antimicrobial susceptibility data are summarized in Table 3 and Figure 2. Most isolates were susceptible to vancomycin, ciprofloxacin, and co-trimoxazole, and none was resistant to vancomycin. However, most organisms showed decreased sensitivity to gentamicin and erythromycin. Out of 128 isolates, 82 (64.1%) were *S. aureus*; 69 (53.9%) were MRSA and 13 (10.2%) were MSSA. Fifty-one (62.2%) of the *S. aureus* isolates were erythromycin-resistant but clindamycin susceptible, out of which 19 (37.2%) isolates showed inducible clindamycin resistance; 31 (60.8%) showed constitutive resistance and one (2.0%) isolate was negative indicating Macrolide-streptogramin type/MS type (Table 4).

3.3. Analysis of mec and PVL genes

All *S. aureus* isolates were screened for the mecA gene and those that were positive were further screened for SCCmec type. All

Figure 1. Prevalence of MRSA and MSSA isolation based on clinical presentation.
(Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PVL+ MRSA, Panton-Valentine leukocidin toxin-positive MRSA; PVL- MRSA, Panton-Valentine leukocidin toxin-negative MRSA.)

Table 3
Disk diffusion test for all *Staphylococcus aureus* isolates to nine antibiotics, on Mueller–Hinton agar with 4% NaCl, as recommended in the Clinical and Laboratory Standards Institute guidelines10

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Sensitive, n (%)</th>
<th>Intermediate, n (%)</th>
<th>Resistant, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>82 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Methicillin</td>
<td>13 (15.9)</td>
<td>0 (0)</td>
<td>69 (84.1)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>21 (25.6)</td>
<td>6 (7.3)</td>
<td>55 (67.1)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>13 (15.9)</td>
<td>0 (0)</td>
<td>69 (84.1)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>13 (15.9)</td>
<td>0 (0)</td>
<td>69 (84.1)</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>20 (24.4)</td>
<td>11 (13.4)</td>
<td>51 (62.2)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13 (15.9)</td>
<td>0 (0)</td>
<td>69 (84.1)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 (18.3)</td>
<td>11 (13.4)</td>
<td>56 (68.3)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>51 (62.2)</td>
<td>0 (0)</td>
<td>31 (37.8)</td>
</tr>
</tbody>
</table>
methyl-Ç, cefoxitin-, oxacillin-, and gentamicin-resistant phenotypes carried mecA. Five isolates (7.2%) had SCCmec I, 52 (75.4%) had SCCmec II, one (1.4%) had SCCmec I and II, and two (2.9%) had SCCmec IV. No SCCmec III or V was detected, and nine (13.1%) strains were non-typeable. None of those with the MSSA phenotype were positive for mecA. The PVL gene was detected in 14 (20.3%) MRSA isolates and was represented in all clinical presentations.

4. Discussion

To our knowledge, this is the first study to report the prevalence of MRSA among patients presenting with SSTIs in Kenya. Previous studies in the region have been limited to specific departments10,13 and therefore information on the general prevalence of MRSA in patients has been lacking. Sixty-nine of 82 S. aureus (84.1%) recovered from SSTIs were MRSA, which is higher than previous findings in the region,6,8 suggesting an increase in MRSA prevalence over the years. This is a wake-up call for health stakeholders in the region to put into place measures to mitigate the spread of MRSA. Of the total MRSA, 14 (20.3%) were PVL-positive and 75.4% possessed SCCmec type II, suggesting that SCCmec II MRSA and a PVL strain of MRSA are significant pathogens in patients with SSTIs presenting to hospitals in Kenya. Publicly funded health care facilities had a higher MRSA isolation rate compared to private hospitals, a finding consistent with a previous study in Kenya.14 The prevalence of MRSA in proven staphylococcal infections in inpatients and outpatients were 75% and 25%, respectively. These findings emphasize the need for healthcare workers to take necessary precautions to avert nosocomial infections in inpatient departments, as well as the potential source of MRSA infection in the community. SSTIs are among the most common infectious diseases and a frequent cause of visits to healthcare providers. The majority of cultures were positive for S. aureus, with 87.2% of those from boils, 71.4% from abscesses, and 88.8% from cellulitis being positive for MRSA. These findings suggest that MRSA is a significant pathogen in these types of infection.

Antimicrobial susceptibility patterns of the MRSA isolates in this study are typical of MRSA from other recent reports.4,6,8,15,16 Although some strains were susceptible to a number of other non-Ç-lactam antibiotics, including ciprofloxacin, erythromycin, and clindamycin, this study demonstrated a reduction in the choice of antibiotic in the case of MRSA infections. Fortunately all MRSA isolates in the present study were susceptible to vancomycin. Since vancomycin is the reserved drug for treating MRSA infections, these data provide useful information for the healthcare personnel.

Most community-associated MRSA (CA-MRSA) strains carry SCCmec IV or SCCmec V and are susceptible to most non-Ç-lactam antimicrobials, while hospital-associated MRSA (HA-MRSA) strains often carry SCCmec I, II, or III.15 However, some studies have shown that nosocomial MRSA can carry SCCmec IV.17-19 Two MRSA isolates in this study were SCCmec IV-positive. The genotypes of MRSA clone isolates are distinct in different geographic locations and therefore the genotypes of MRSA clones in Kenya in relation to other global MRSA clones may not be the same. We suggest the need for a study to screen for CA-MRSA in Kenya to provide information on the importance of these strains in hospitals.

This study clearly demonstrates that MRSA is a significant pathogen in SSTIs and an important cause of wound infections among the populations at the health facilities covered by this study. These data provide an insight into the current epidemiology of MRSA in Kenya and may be important in setting guidelines for preventing Staphylococcus-related SSTIs in health care facilities. They are also necessary for drawing up effective therapeutic guidelines and for S. aureus antibiogram surveillance.

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Ethical approval: Permission to carry out this study was granted by the Institutional Review Board (IRB) of the Kenya Medical Research Institute.

Conflict of interest: The authors would like to declare no potential conflict of interest.

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