Prevalence of clindamycin inducible resistance among methicillin-resistant 
Staphylococcus aureus at Bugando Medical Centre, Mwanza, Tanzania

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Abstract: Methicillin-resistant Staphylococcus aureus (MRSA) has been recognized world wide as an important causative agent of nosocomial and community acquired infections. Clindamycin has been considered as an alternative drug for the treatment of such strains. However, the possibility of clindamycin inducible resistance complicates the choice of treatment. The aim of this study was to determine the prevalence of clindamycin inducible resistance of MRSA at Bugando Medical Centre (BMC) in Mwanza Tanzania. A total of 600 clinical specimens of pus, wound swabs and aspirates from patients admitted at BMC surgical wards were processed over a period of 4 months. Of these, 160 of S. aureus clinical isolates were analysed. MRSA was identified using cefoxitin disc, oxacillin disc and oxacillin agar. Inducible clindamycin resistance was detected using erythromycin (15µg) and clindamycin (2µg) discs placed 15mm apart on Muller Hinton agar. Of the 160 isolates, 26 (16.3%) were found to be MRSA. Overall prevalence of inducible clindamycin resistance (iMLS\(_g\)) was 28.8% (46/160), with 22% (30/134) of methicillin-susceptible S. aureus (MSSA) and 61% (16/26) of MRSA exhibiting inducible clindamycin resistance (\(P=0.0001\)). Constitutive resistance (cMLS\(_g\)) was found in 1 (3.7%) of the MRSA isolates and was not detected among MSSA. MS\(_g\) phenotype was detected in 1 (3.8%) of MRSA isolates and 2 (1.5%) of MSSA. Eight (29.6%) of the MRSA isolates were sensitive to both clindamycin and erythromycin. In conclusion, a high prevalence of inducible clindamycin resistance was observed among S. aureus with significant association between MRSA and inducible clindamycin resistance. It is important that susceptibility test of staphylococci is routinely done to facilitate early detection of clindamycin inducible resistance in the country.

Key words: prevalence, clindamycin, methicillin-resistant, Staphylococcus aureus, Tanzania

Introduction

Staphylococcus aureus has been recognized world wide as an important causative agent of nosocomial and community acquired infections (Jan et al., 2002). The increase of methicillin resistant S. aureus (MRSA) among staphylococci is an increasing problem and clindamycin is considered to be one of the potent alternative agents available to address this problem (Fokas et al., 2005). In the USA, Canada and Europe, available statistics indicate that MRSA accounts for up to 40% of nosocomial S. aureus infections in large hospitals and 25%-30% of such infections in smaller hospitals (Jan et al., 2002). Epidemiological data on MRSA and inducible clindamycin resistance are scarce in Africa. The prevalence of MRSA in eight countries from 1996 to1997 was reported to be relatively high (21-30%) in Nigeria, Kenya and Cameroon and low (10%) in Tunisia and Algeria (Kesah et al., 2003). Few reports of the antimicrobial susceptibility of S. aureus in Tanzania are available. In a study at the Muhimbili National Hospital in Dar es Salaam, Tanzania, Urassa et al. (1999) observed a 0.4% prevalence of methicillin resistant S. aureus. Recently, at the same hospital, 12% of S. aureus were found to be resistant to cloxacillin (Bloomberg et al., 2007).

Macrolide resistance is increasing worldwide. For instance, inducible resistance has been found to be more than 50% in MSSA and constitutive phenotype resistance more than 80% in MRSA (Lina et al., 1999). Inducibleclindamycin resistance was reported in 10.8% in MSSA and 82% in MRSA in South Africa (Adebayo et al., 2006). Emergence of resistance to clindamycin in staphylococci shortly after therapeutic use of erythromycin has been reported (Gopal Rao, 2000). Resistance to macrolides, lincosamides and streptogramin B (MLS antibiotics) is mainly due to acquisition of erythromycin resistant methylase (erm) gene, which encodes enzyme that methylate the 23rRNA (Fokas et al., 2005). Constitutive resistance phenotype is resistant to macrolides, lincosamides and streptogramin-B but inducible resistance is expressed in the presence of methylase synthesis inducer like erythromycin (14 rings) and azithromycin (15 rings) macrolides (Gopal Rao, 2000). Data on inducible clindamycin resistance among MRSA in Tanzania is not available. This

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study was therefore carried out to determine the 
prevalence of MRSA and inducible clindamycin 
resistance among *S. aureus* isolates from 
patients admitted at Bugando Medical Centre 
in Mwanza, Tanzania.

**Material and Methods**

**Study area**
The study was done at Bugando Medical Centre 
(BMC) in Mwanza, in north-western Tanzania. 
It is situated along the shores of Lake Victoria 
and has 900 bed capacity. BMC is a consultant 
and teaching hospital for the Lake and Western 
zones of the Tanzania. It serves as a referral 
centre for tertiary specialist care for a catchment 
population of approximately 13 million people 
(http://www.bugandomedicalcentre.go.tz) 
from Mwanza, Mara, Kagera, Shinyanga, Tabora 
and Kigoma.

**Laboratory protocol**
A total of 600 clinical specimens (pus, wound 
swabs, aspirates) from patients admitted at BMC 
surgical wards were processed over a period of 
4 months (April-July 2008). All specimens were 
inoculated on sheep blood agar, MacConkey 
agar (OXOID UK) and incubated at 37°C 
aerobically for 24h. Identification of *S. aureus* 
was first done using colony morphology on 
5% sheep blood agar. Cream to golden yellow 
colonies with or without haemolysis were 
further identified using Gram stain and catalase 
test; followed by staphylase and DNAse tests 
(Murray *et al.*, 1995). Staphylase test (OXOID 
UK) was done and interpreted as recommended 
by the manufacturer. DNAse test was done by 
pouring 1N HCl to the overnight colonies on 
DNAse test agar (OXOID, UK). Clearance of 
precipitated DNA around the colonies was 
reported as positive (Murray *et al.*, 1995).

MRSA detection was done using cefoxitin, 
oxacillin discs (OXOID UK) and oxacillin 
screen agar (5% NaCl, 6µg/ml oxacillin) as 
described previously (Murray *et al.*, 1995; Saxena *et al.*, 2003). Bacterial colonies were suspended in 
saline to a turbidity of 0.5 McFarland standards 
and inoculated on a Muller Hinton agar plate 
(OXOID UK). Plates were incubated at 37°C for 
cefoxitin disc and at 33°C in the case of oxacil-
lin disc and oxacillin agar. All isolates resistant 
to cefoxitin and oxacillin were considered 
as MRSA and its correlation to the presence of 
mecA gene is more than 95% (Felten *et al.*, 2002; 

Clindamycin inducible resistance was 
detected as described previously. Erythromycin 
(15µg) and clindamycin (2µg) (OXOID UK) 
discs were placed 15-20mm apart edge to 
edge (Adebayo *et al.*, 2006; Delialioglu *et al.*, 
2005; Ravisekhar *et al.*, 2006). Appearance 
of flattened clindamycin zone between 
clindamycin and erythromycin forming D 
shape with erythromycin resistance was 
considered as positive clindamycin inducible 
resistant (iMLS). Resistance to both discs was 
recorded as constitutive resistance (cMLS) 
and resistance to erythromycin alone was taken as 
MS phenotype. Interpretation of the diameters 
of zone inhibition was as follows: Erythromycin 
(E)-sensitive (S) = ≥ 23mm, E-Intermediate (I) =14 
to 22mm, E-resistance = ≤ 13mm; Clindamycin 
(DA)–S= ≥21mm, DA-I=15-20 mm, DA-R = ≤14mm (Fokas *et al.*, 2005; CLSI 2000).

Disc susceptibility testing for penicillin 
(10IU), oxacillin (1µg), cefoxitin (30µg), 
vancomycin (30µg), ciprofloxacin (5µg), 
chloramphenicol (30µg), tetracycline (30µg) 
and sulfamethaxazole/trimethoprim (25µg) (UK 
OXOID) was done using standard procedures 
(CLSI, 2000). For the quality control of media, 
incubation conditions and discs and *Escherichia 
coli* strain ATCC 25922 from Mwanza Medical 
Research Centre of the National Institute of 
Medical Research was used.

**Results**

Among 600 non-repetitive clinical specimens 
160 (26.6%) *S. aureus* were isolated. Methicillin- 
resistant *S. aureus* were identified in 26 (16.3%) 
of all *S. aureus* isolates. Almost all 155/160 
(97%) of *S. aureus* isolates were resistant to 
penicillin and all the *S. aureus* isolates were 
sensitive to vancomycin. Rates of resistance to 
chloramphenicol, ciprofloxacin, tetracycline 
and sulfamethaxazole/trimethoprim among 
MRSA were 10 (39%), 14 (54%), 18 (69%) and 24 
(92%), respectively (Table 1).
Table 1: Susceptibility of MRSA isolates to common antibiotics

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Overall prevalence of inducible clindamycin resistance (iMLS\(_B\)) was 46(28.8%), with 30/134 (22%) of methicillin-susceptible \(S. aureus\) (MSSA) and 16/26 (61.5%) of MRSA exhibiting inducible clindamycin resistance (Table 2). There was significant association between MRSA and inducible clindamycin resistance (iMLS\(_B\)) among staphylococci isolates (\(X^2 = 14.75, P=0.0001\) (Table 2).

Constitutive resistance (cMLS\(_B\)) was found in 1 (3.7%) among MRSA isolates and was not detected among MSSA. Among MRSA isolates resistance to erythromycin alone with negative D test (MS\(_B\) phenotype) was detected in 3.8% of the isolates and 1.5% of MSSA isolates (Figure 1). Eight (29.6%) of MRSA isolates were found to be sensitive to both clindamycin and erythromycin. All MRSA isolates were preserved for future molecular analysis.

Table 2: D test among methicillin-sensitive \(S. aureus\) (MSSA) and methicillin-resistant \(S. aureus\) (MRSA)

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<th>Negative D test N (%)</th>
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<td>MSSA</td>
<td>30 (22%)</td>
<td>104 (78%)</td>
<td>134 (83%)</td>
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<td>MRSA</td>
<td>16 (61.5%)</td>
<td>10 (38.5%)</td>
<td>26 (17%)</td>
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<tr>
<td>Total</td>
<td>46 (28.8%)</td>
<td>114 (71.2%)</td>
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\(\chi^2=14.76, P=0.0001\)
Discussion

In the present study *S. aureus* was commonly isolated from surgical patients (26.6%). Similar observations have been reported elsewhere that *S. aureus* is the cause of most wound infections among hospitalized patients (Jan et al., 2002; Bloomberg et al., 2007). Only 3.1% of the isolates were sensitive to penicillin, possibly due to production of β-lactamases enzyme which hydrolyzes penicillin. Similar findings have been reported recently at the Muhimbili National Hospital in Dar es Salaam where as high as 100% of *S. aureus* isolates were reported to be resistant to penicillin (Bloomberg et al., 2007). In our study using disc diffusion method the resistance to oxacillin and cefoxitin which signifies MRSA was observed in 16.2% of isolates. Disc diffusion especially oxacillin disc has been used in most developing countries to detect MRSA (Felten et al., 2002). In developed countries MIC methods has been replaced by molecular methods that detect meca gene (Brown et al., 2001). Use of cefoxitin disc have been found to have high correlation (>95%) with the presence of meca gene (Felten et al., 2002; Swenson et al., 2005). In few cases where the low resistance to oxacillin and cefoxitin does not correlate to the presence of meca gene disc variation and hyperproduction of penicillinase can explain the discrepancy (Chambers et al., 1990; Yassin et al., 1997). Our findings are similar to those reported from the Muhimbili National Hospital study where 12% of hospital isolated *S. aureus* were resistant to cloxacillin (Bloomberg et al., 2007). This is yet, relatively lower prevalence compared to the findings (24-74%) elsewhere (Jan et al., 2002; Adebayo et al., 2006).

Clindamycin is a useful drug in the treatment of both methicillin susceptible and resistant staphylococcal infections. In our study clindamycin inducible resistance (iMLS\(_B\)) was observed in 61% and 22% of MRSA and MSSA, respectively. Similarly, a study in Kwa-Zulu Natal in South Africa has indicated that 10.8% of MSSA and 82% of MRSA had inducible clindamycin resistance. Other studies conducted elsewhere have reported high prevalence of clindamycin inducible resistance among MSSA (Ravisekhar et al., 2006; Patel et al., 2006). A significant association between MRSA and inducible clindamycin resistance and the presence of MS phenotype in both MSSA and MRSA was observed in our study. Recent studies in Turkey and Nepal have detected MS phenotype only in MSSA (Fokas et al., 2005; Azap et al., 2005).

In the present study, the constitutive clindamycin resistance was identified in one MRSA isolate and was absent in all MSSA. This trend is in contrast with other studies in Korea where the majority of MRSA had constitutive resistance (cMLS\(_B\)) (Kim et al., 2004). This indicates that the incidence of constitutive and inducible resistance in staphylococcal isolates is likely to vary by regions. Moreover, the low constitutive clindamycin resistance in our study may also be due to the fact that the drug is not commonly used, and hence there is less selection of resistant strains. The development of resistance to antibiotics is related to the wide-spread use of the respective antibiotic (Yajarayma et al., 2005). To the best our knowledge this is the first study describing clindamycin inducible resistance among MRSA and MSSA in Tanzania.

Our findings indicate that the majority of MRSA isolates were resistant to sulfamethoxazole/trimethoprim. Similar observations have been reported in South Africa (Adebayo et al., 2006). As in other studies all MRSA isolates were sensitive to vancomycin and chloramphenicol. Chloramphenicol can be used in our setting for non serious MRSA infection and in case of bacteraemia vancomycin is recommended (Jan et al., 2002; Bloomberg et al., 2007). One-third of MRSA isolates and three quarters of MSSA were sensitive to both...
erythromycin and clindamycin. The drugs therefore, can still be used for non-serious staphylococcal infections in the study area. However, it is important that this is guided by culture results.

In conclusion, high prevalence of inducible clindamycin resistance was observed among *S. aureus* isolates, with significant association between MRSA and inducible clindamycin resistance. It should be noted that the results of this study represent *S. aureus* from a single hospital and may not be a representative of the rest of the country. Since it is likely that the prevalence of iMLS$_B$ and MRSA clones differs from region to region, it is recommended that clinical microbiology laboratories should routinely include disc diffusion induction tests to facilitate detection of clindamycin inducible resistance (iMLS$_B$) in the country. The need to establish antimicrobial resistance surveillance in the tertiary care facilities in Tanzania to track the emergence of drug resistant bacterial agents is also strongly recommended.

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