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Vol. 11(18), pp. 705-711, 14 May, 2017
DOI: 10.5897/AJMR2017.8481
Article Number: DD1C0DC64225
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antimicrobial susceptibility patterns of *Staphylococcus aureus* and coagulase negative staphylococci isolated from humans in Nairobi, Kenya

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Received 9 February, 2017; Accepted 26 April, 2017

Nosocomial infections due to multidrug resistant *Staphylococcus aureus* are an important health problem worldwide. Antimicrobial resistance prolongs the duration of hospitalization, thereby increasing the cost of patient care. For a long time, methicillin was considered as drug of choice for treatment of penicillin-resistant staphylococcal infections. Emergence of methicillin resistance reduced the available options for treatment of nosocomial and community acquired *S. aureus*. Normally, such strains are only sensitive to glycopeptides such as vancomycin and teicoplanin. Recent reports show that methicillin resistant *S. aureus* (MRSA) have become multiply resistant to other drugs such as fluoroquinolones, trimethoprim-sulfamethoxazole (SXT), clindamycin or erythromicin and there are reports of vancomycin resistant strains from different parts of the world. The aim of this study was to determine the susceptibility patterns of *Staphylococcus* isolates from humans. Drug susceptibility testing of isolates was determined using the disk diffusion method. A total of 110 *S. aureus* (SA) and 23 coagulase negative staphylococcus (CoNS) isolates from human sources were studied. Both SA and CoNS isolates were completely sensitive to vancomycin. On one hand, there was a comparable high resistance for both SA and CoNS to penicillin G, augmentin and tetracycline. On the other hand, there was significantly high resistance to erythromycin (69.6%), SXT (69.6%), oxacillin (82.6%), ciprofloxacin (52.2%) and clindamycin (39.1%) among CoNS when compared with SA isolates (erythromycin 38.2%, SXT 38.2, oxacillin (33.6%), ciprofloxacin (26.4%), clindamycin 18.2%) with p values 0.0090, 0.0099, 0.0001, 0.0239 and 0.0483, respectively. These high levels of resistance, calls for continuous surveillance studies to monitor for *S. aureus* infections in the community and hospital settings and the emergence of vancomycin resistant isolates.

Key words: Methicillin resistant, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), antibiotic susceptibility, vancomycin, coagulase negative staphylococci.

INTRODUCTION

Staphylococcus aureus is a common cause of both community and hospital-acquired infections. Clinical

syndromes associated with severe disease include bacteraemia, pneumonia, endocarditis, septic arthritis, osteomyelitis and deep abscess formation (Enright et al., 2000; Loir et al., 2003, Abdulgader et al., 2015).

Mortality from invasive *S. aureus* disease was high during the pre-antibiotic era. However, the introduction of penicillin in the 1930s had a dramatic impact on the treatment of *S. aureus* infections. The semisynthetic penicillin methicillin was introduced in 1959 to overcome the problems that arose from the increasing prevalence of penicillinase-producing isolates of *S. aureus* resistant to penicillin G and penicillin V. However, methicillin-resistant *S. aureus* (MRSA) strains rapidly emerged and became a major clinical problem within hospitals during the 1960s in Europe and the 1970s in the United States and elsewhere (Enright et al., 2000). MRSA was first reported in England in 1961, shortly after its introduction (Mulvey et al., 2001). Worldwide reports show that MRSA strains are resistant to most other classes of antimicrobial agents and are susceptible only to glycopeptides and a few new investigational drugs (Enright et al., 2000; Lee, 2003).

MRSA is common also in the African region (Falagas et al., 2013). From an African multicentre study by Kesah et al. (2003), methicillin resistance was detected in 213 (15%) of the 1440 isolates tested. In another study, the rate of MRSA was relatively high in Nigeria, Kenya and Cameroon (21 to 30%) and below 10% in Tunisia, Malta and Algeria. More than 60% of MRSA were multidrug resistant, with relatively high resistance to erythromycin, gentamicin and oxacillin. Fusidic acid, co-trimoxazole, rifampicin and ciprofloxacin exhibited moderate efficacy. All MRSA isolates were sensitive to vancomycin (Kesah et al., 2003). In a 1995 study conducted in Nairobi, it was found that about 90% of patients admitted in burn units were infected with MRSA and thereby significantly increasing duration of stay in hospital and treatment cost (Muthotho et al., 1995).

The determination of antimicrobial susceptibility of a clinical isolate is often crucial for the optimal antimicrobial therapy of infected patients. This requirement is crucial especially at a time when reports indicate that resistance is increasing and there is emergence of multidrug resistant microorganisms. Standard procedures and breakpoints have been defined to predict therapeutic outcome both in time and at different geographic locations (Fluit et al., 2001).

Monitoring of multidrug resistant methicillin resistant *S. aureus* is therefore an important public health aspect in Kenya, where MRSA may increase the duration of stay in hospital and treatment costs. In addition, drugs used to treat MRSA are not readily available and affordable in this country.

The aim of this study was to determine the proportion of MRSA and investigate the antimicrobial susceptibility patterns of both coagulase positive and coagulase negative staphylococcal strains isolated from humans in Nairobi, to commonly used antibiotics and vancomycin.

MATERIALS AND METHODS

This was a descriptive and cross sectional study of staphylococci isolates obtained from studies on wounds and blood borne infections and stored at the Centre for Microbiology Research-Kenya Medical Research Institute.

Identification of *Staphylococcus* species

Samples were subcultured aerobically and growing colonies were Gram stained. Gram-positive cocci clusters were tested for production of catalase and coagulase enzymes and confirmed by API Staph Identification system (BioMerieux Inc., Durham, USA). Confirmed isolates were stored in Tryptic soy broth containing 15% glycerol and frozen at -80°C until further processing.

β-Haemolysis test

Isolates confirmed as *S. aureus* were inoculated onto blood agar and incubated for 18 to 24 h. Isolated colonies were picked and re-suspended in sterile normal saline (0.85%) to give a final inoculums equivalent 0.5 McFarland turbidity standard before inoculating them on Mueller Hinton agar (Oxoid, Hampshire, UK) plates each containing 20 ml of the medium to attain a uniform depth of 4 mm.

Antimicrobial susceptibility testing

All isolates were tested for susceptibility to a panel of 12 antibiotics (penicillin 10 units, ciprofloxacin 5 µg, chloramphenicol 30 µg, clindamycin 2 µg, gentamicin 10 µg, erythromycin 15 µg, oxacillin 1 µg, amoxycillin/clavulanic acid (Augmentin) 10/20 µg, tetracycline 30 µg, sulphamethoxazole-trimethoprim 25 µg, methicillin 5 µg and vancomycin 30 µg) by using the disk diffusion technique following guidelines of Clinical and Laboratory Standards Institute (CLSI, 2002).

The antibiotic disks were placed on each agar plate at equal distance from each other and plates were incubated aerobically at 35°C for 16 - 18 h, except for vancomycin, methicillin and oxacillin which were incubated for 24 h. *Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 35218 were used to control for growth of bacteria and efficacy of antibiotic disks. The size of zones of inhibition were recorded and interpreted according to CLSI standards (CLSI, 2015).

RESULTS

Identification of *Staphylococcus* species

A total of 133 staphylococcal isolates were studied. Out

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Table 1. β Haemolysis patterns of the staphylococci isolates.

Isolate	Beta (β) haemolytic N (%)	Non-haemolytic N (%)	Total
Staphylococcus aureus	98 (89.1)	12 (10.9)	110
CoNS	19 (82.7)	4 (17.3)	23
Total	117 (88.0)	16 (12.0)	133

CoNS = Coagulase-negative staphylococci.

Table 2. Antimicrobial susceptibility of 133 *Staphylococcus* species to 12 antibiotics.

Antibiotic	Resistant N (%)	Intermediate N (%)	Sensitive N (%)
Penicillin	122(91.7)	0(0.0)	11(8.3)
Augmentin	100(75.2)	0(0.0)	33(24.8)
Tetracycline	62(46.6)	2(1.5)	69(51.9)
Erythromycin	58(43.6)	13(9.8)	62(46.6)
SXT	58(43.6)	9(6.8)	66(49.6)
Oxacillin	56(42.1)	0(0.0)	77(57.9)
Methicillin	46(34.6)	7(5.3)	80(60.2)
Ciprofloxacin	41(30.8)	4(3.0)	88(66.2)
Chloramphenicol	40(30.1)	5(3.8)	88(66.2)
Gentamicin	38(28.6)	2(1.5)	93(69.3)
Clindamycin	29(21.8)	9(6.8)	95(71.4)
Vancomycin	0(0.0)	0(0.0)	133(100.0)

SXT= Sulfamethoxazole-Trimethoprim.

of these, 110 (82.7%) were *S. aureus* (SA) and 23 (17.3%) coagulase-negative staphylococci (CoNS).

β-Haemolysis in *Staphylococcus* species studied

In total, 88% of all isolates were β haemolytic and the rest were non hemolytic. *S aureus* isolates were significantly more β hemolytic in comparison with the CoNS isolates (Table 1).

Antimicrobial susceptibility of Staphylococcus species

All isolates were highly sensitive to vancomycin (100%) but highly resistant to penicillin G (91.7%). The order of increasing resistance was penicillin G > augmentin > tetracycline > erythromycin = SXT > oxacillin > methicillin > ciprofloxacin > chloramphenicol > gentamicin > clindamycin > vancomycin (Table 2). Isolates were relatively more sensitive to gentamicin and clindamycin showing resistance in only less than 30% of all isolates.

Comparison of antibiotic susceptibility of *S. aureus* and coagulase negative *Staphylococcus* species

Both *S. aureus* and coagulase-negative staphylococci isolates were completely sensitive to vancomycin. As indicated in Table 3, *S. aureus* were more resistant to most drugs except oxacillin, when compared with coagulase-negative staphylococci (Table 3). There was significantly high resistance among coagulase-negative staphylococci isolates from human sources to erythromycin (69.6%), SXT (69.6%), oxacillin (86.2%), ciprofloxacin (52.2%) and clindamycin (39.1%) as compared to *S. aureus* isolates from human sources (erythromycin 38.2%, SXT 38.2, clindamycin 18.2%) with P values 0.0090, 0.0099, 0.0001, 0.0239 and 0.0483, respectively) (Table 5).

There was significantly higher resistance to oxacillin in coagulase-negative staphylococci than in *S. aureus.* The percentage resistance of *S. aureus* and coagulase-negative staphylococci to oxacillin was 29.2 and 55.7% (P value = 0.0003), respectively. Overall resistance to penicillin and augmentin was significantly higher for *S. aureus* than coagulase-negative staphylococci (P values = 0.0001 for both).

Table 3. Comparison of overall resistance of *S. aureus* and coagulase- negative staphylococci.

Antibiotic	*Staphylococcus aureus* resistant N (%)	CoNS resistant N (%)	P value[a]
Penicillin	101(91.8)	21(91.3)	1.0000
Augmentin	82(74.5)	18(78.3)	0.7967
Tetracycline	50(45.5)	12(52.2)	0.6479
Erythromycin	42(38.2)	16(69.6)	0.0099
SXT	42(38.2)	16(69.6)	0.0099
Oxacillin	37(33.6)	19(82.6)	0.0001
Methicillin	35(31.8)	11(47.8)	0.1550
Ciprofloxacin	29(26.4)	12(52.2)	0.0239
Chloramphenicol	27(24.5)	13(56.5)	0.0768
Gentamicin	30(27.3)	8(34.8)	0.4583
Clindamycin	20(18.2)	9(39.1)	0.0481
Vancomycin	0(0.0)	0(0.0)	-
Total	110(100)	23(100)	

[a]Fisher's exact test; SXT = sulfamethoxazole-trimethoprim; CoNS=coagulase-negative staphylococci.

Table 4. Proportion of isolates resistant to one or more of the eleven antibiotics tested.

Isolate	Total isolates	Resistant to Number of antibiotics											
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Staphylococcus aureus	110	8	13	25	15	7	5	3	5	5	7	9	8
CoNS	23	1	0	2	1	3	1	2	1	4	3	2	3
Total (%)	133 (100)	9 (6.8)	13 (9.7)	27 (20.3)	16 (12.0)	10 (7.5)	6 (4.5)	5 (3.8)	6 (4.5)	9 (6.8)	10 (7.5)	11 (8.3)	11 (8.3)

CoNS =Coagulase-negative staphylococci.

Table 5. Summary of multidrug resistance for all *S. aureus* and CoNS isolates.

Isolate	Sensitive N (%)	Resistant to 1 or 2 N (%)	MDR N (%)	Total no. of isolates
Staphylococcus aureus	8 (7)	38 (35)	64 (58)	110
CoNS	1 (4)	2 (9)	20 (87)	23
Overall total	9 (6.8)	40 (30.0)	84 (63.2)	133

[a]Fisher's exact test; CoNS =coagulase-negative staphylococci.

MRSA were detected by determining the susceptibility to oxacillin or methicillin. As shown in Table 3, SA isolates were more resistant to oxacillin than methicillin (33.6 and 31.8% respectively). In contrast, CoNS showed a different trend with oxacillin and methicillin isolates at the proportion of 82.6 and 47.8%, respectively. Out of 12 drugs that were tested, only vancomycin completely inhibited the growth of all isolates tested.

Only 7% of all isolates were completely sensitive to all antibiotics (Tables 4 and 5). Sixty three percent of all isolates were multidrug resistant (those resistant to three or more drugs). The remaining 30% of the isolates were resistant to one or two drugs (Table 5). Approximately 8% of all isolates were resistant to all the tested drugs except vancomycin. Other isolates were resistant to one or two drugs (9.7 and 20.3%, respectively). Isolates resistant to between three and ten drugs varied between 4.5 and 12% of all isolates (Table 5). Considered individually, the proportion of multidrug resistant (MDR) isolates in *S. aureus* and coagulase negative was 58 and 87%,

respectively.

DISCUSSION

This study shows that the commonly available drugs such as penicillin, augmentin, tetracycline, erythromycin and SXT are no longer reliable in treating nosocomial and community acquired staphylococcal infections. For instance, out of all isolates tested, 11 were resistant to all antibiotics except vancomycin. Some reports indicate increase of vancomycin intermediate/resistant *S. aureus* (VISA/VRSA) isolates worldwide (Lowy, 2003; Monaco et al., 2016). Vancomycin resistance became of more concern since the demonstration of successful transfer of the *vanA* gene from enterococci to *S. aureus* under laboratory conditions (Noble et al., 1992) and after reports of staphylococcal isolates resistant to vancomycin in some countries (Hiramatsu et al., 1997a, b; Sieradzki et al., 1999; Chang et al., 2003; Whitener et al., 2004; Palazzo et al., 2005; Lee et al., 2015).

Because isolates in this study did not show resistance to vancomycin, it is advisable to limit the use of glycopeptides to treat only MRSA infections so as to reduce the selective pressure and likely emergence of resistance to this class of antibiotics. Decreased staphylococcal susceptibility to vancomycin is not due to transfer of *vanR* genes from vancomycin-resistant enterococci (VRE) or to small colony variants, as noted in staphylococci for other antimicrobial agents (Mitsuyama et al., 1997) but appears to be a gradual selection process due to treatment pressure. Glycopeptide - resistant mutants of *S. aureus* have been experimentally selected by increasing the levels of vancomycin present during *in vitro* growth (Daum et al., 1992; Sieradzki and Tomasz, 1997). The use of avoparcin, another member of the glycopeptide class of antibiotics, as a growth-promoting agent in the production of food animals is often cited as playing a role in the spread of glycopeptide-resistant microorganisms (Aarestrup et al., 1996; Van den Bogaard et al., 1997; Witte, 1997, Economou and Gousia, 2015). Although, it is well recognized that vancomycin resistance is more prevalent in the United States than in Europe, it has not been explained why avoparcin usage fails to correlate with the different epidemiologies of resistance between the two continents; avoparcin was never approved for use in animals in the United States, in contrast to its broad use as a growth-promoting agent in Europe (Donnelly et al., 1996; Leclercq and Courvalin, 1997). There is no explanation yet, to this continental variation and more theories are required to explain the difference in the glycopeptide resistance. Based on the outcome of this study and available literature, the use of avoparcin should also be controlled to avoid spread of resistance. In order to avoid the emergence of glycopeptide resistance in currently susceptible staphylococci isolates, the use of avoparcin in Kenya should also be highly controlled. The fact that the use of glycopeptides should be controlled is supported by a number of reports. According to a study by Tenover et al. (1998) glycopeptide-intermediate *S. aureus* (GISA) isolates represented mutants selected *in vivo* with increased resistance to glycopeptides as a result of prolonged exposure of the organisms to constant levels of vancomycin in an opportune environment.

In the current study, all isolates had vancomycin zones greater than 14 mm. According to CLSI guidelines, any staphylococcal isolates with zones diameters of 14 or less should be tested by an MIC method. When it is determined that isolates have elevated MIC to vancomycin (>= 4µg/ml), these should be sent to a reference laboratory (CLSI, 2015). According to a study by Tenover and et al. (1998), the disk diffusion testing, which is widely used around the world does not differentiate strains with reduced susceptibility to vancomycin from susceptible strains (MIC range, 0.5 to 2 µg/ml). Accordingly, the disk diffusion test should not be used alone for testing staphylococci resistance with vancomycin. The same study suggests that disk diffusion tests with another glycopeptide, teicoplanin may be of value for identifying isolates with reduced susceptibility to glycopeptides. They also recommend the use of vancomycin agar screening test as method for testing staphylococci MICs and detect isolates with reduced glycopeptide susceptibility (Tenover et al., 1998).

The isolates showed a decreasing trend of resistance in the order, penicillin G > augmentin > tetracycline > erythromycin > SXT > oxacillin > methicillin > ciprofloxacin > chloramphenicol> gentamicin > clindamycin > vancomycin. Similar trends have been observed in South Africa (Marais et al., 2009). This indicates that the antibiotic class of penicillins has higher chances of treatment failure and should not be recommended for treatment of staphylococcal infections. Ciprofloxacin, chloramphenicol, gentamicin and clindamycin should be sought as alternative treatment for MRSA infections. Vancomycin is the most effective drug of all the tested antibiotics when β lactams are ineffective (Shakibaie et al., 2002; Casey et al., 2007; Gad et al., 2010). However, due to poor tissue diffusion and moderate bactericidal activity, vancomycin can be combined with rifampicin for deep-seated infections (Aubry-Damon et al., 1998).

According to the overall proportions of resistant isolates in this study, the authors arbitrary classified the antibiotics with respect to these isolates as highly resistant if percentage resistance is between 50 and 100% as was the case with penicillin and augmentin. Tetracycline, erythromycin, SXT, oxacillin, methicillin, ciprofloxacin, chloramphenicol and gentamicin were moderately resistant (resistance between 25 and 49%). Clindamycin was the only drug to which less than 25% of the isolates were resistant. Interestingly, all isolates were 100% susceptible to the antibiotic vancomycin.

A notable phenomenon with coagulase-negative

staphylococci is that they are significantly more resistant to most antibiotics and particularly to oxacillin as compared to *S. aureus*. This is consistent with previous studies by Reynolds et al. (2004) where 76% of coagulase-negative staphylococci isolates were oxacillin resistant when compared with 42% of *S. aureus*. In another study, rates of oxacillin resistance among *S. aureus* and CoNS isolates were 59.3 and 78.5%, respectively (Johnson et al., 2003). The *mec* gene responsible for *S. aureus* resistance is postulated to have originated from a different species of staphylococci. Although, many methicillin-resistant strains appear to be descendants of a limited number of clones, some appear to be multi-clonal in origin, suggesting the horizontal transfer of *mec* DNA (Archer and Niemeyer, 1994; Abdulgader et al., 2015). Hence it is possible that the higher resistance to oxacillin in these coagulase negative isolates could be a source to transfer *mec* gene to other staphylococci isolates. This was previously confirmed in studies by Wielders et al. (2002).

MRSA isolates often are multiply resistant to commonly used antimicrobial agents, including erythromycin, clindamycin and tetracycline, a situation that was evident in this study. MRSA isolates in this study were not only resistant to β-lactam antibiotics but also to chloramphenicol, clindamycin, ciprofloxacin, erythromycin, gentamicin and sulphamethoxazole-trimethoprim.

Both *S. aureus* and coagulase-negative staphylococci isolates from humans were highly resistant to penicillin and augmentin. However, about 9% of these isolates were sensitive penicillin. Treatment with these drugs should only be prescribed after sensitivity testing to penicillin.

Indiscriminate use and sale of antimicrobials, sale of antibiotics without prescription, sale of under dose preparations, brand substitution and self-medication can enhance the development of drug resistance (Indalo, 1997; Shakibaie et al., 2002, Roess et al., 2013). Therefore, to control the spread of MRSA, it is advised that both antibiotic use regulation and contact preventions be strictly observed. Similarly, culture surveillance is an important control measure (Farr, 2004; Drees et al., 2016).

Future work is required to determine the clonal relationship among MRSA isolates from a wider area of study, which can be used as epidemiological reference tool during MRSA outbreaks in hospitals and also aid in management and control of these infections. Using molecular tools, the relatedness between *S. aureus* and coagulase negative staphylococci can possibly indicate the ease with which staphylococci can transfer resistance genes among different genus.

Conclusion

Although, the study isolates were multidrug resistant, there was no isolate which was vancomycin resistant. The fact that 11 (8.3%) of all isolates were resistant to 11 out of 12 tested antibiotics is an alarming situation. Vancomycin should therefore be controlled in hospitals to avoid quick emergence of resistance to this life saving drug. There is a relatively high proportion of oxacillin resistance isolates among coagulase negative staphylococci, these may be responsible for the transfer of *MecA* gene responsible for oxacillin resistance to the more pathogenic *S. aureus* strains. Therefore, medical institutions should regularly perform vancomycin agar screening to determine any emergence of glycopeptide and other antibiotics resistance among staphylococci isolates.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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