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PREVALENCE OF METHICILLIN RESISTANT *Staphylococcus aureus* AMONG HEALTHY UNIVERSITY STUDENTS

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is among the currently ravaging human pathogens responsible for most of health-care associated and community acquired infections around the world. The aim of this study was to determine the antibiotic profiles and prevalence of MRSA isolates among healthy undergraduate students. A total of 300 urine, stool and nasal swab samples from various anatomical sites of the study participants were cultured on mannitol salt agar and identified as S. aureus using catalase, coagulase and DNAse tests. Antibiotic susceptibility determination was performed using the disk diffusion susceptibility method whilst MRSA were identified by cefoxitin disk diffusion method as per CLSI guidelines. Beta-lactamase production was carried out by combination of the acidimetric and iodometric tube methods. The result obtained showed the presence of S. aureus with 41.3% prevalence. The distribution of the isolates was as follows: 65 isolates (43.3%) from urine, 17 (34.0%) from stool and 42 (42.0%) from nasal swab. There was however no association between prevalence and sample type (p value=0.74). In the assessment of drug susceptibility profile for S. aureus isolates, it was observed that 30.8% of the isolates from urine samples were resistant to gentamicin, 52.9% of the isolates from stool also resistant to gentamicin whilst S. aureus isolates from nasal swab exhibited 14.3% resistance to gentamicin. All the S. aureus isolates showed 100% resistance to augmentin and ceftazidime. Out of the 124 S. aureus isolates recovered, a total of 45 (36.3%) were MRSA. However, there was no association between prevalence and sample type (p value=0.82). Majority of the MRSA isolates were susceptible to gentamicin, and ofloxacin while highest resistance was recorded for augmentin and ceftazidime. Among the 124 S. aureus isolates, a total of 94, (75.8 %) were beta-lactamase producers. This study has demonstrated high prevalence of MRSA isolates from apparently healthy individuals and underlines the need for periodic surveillance studies of this type.

KEYWORDS: *Antimicrobial resistance, MRSA, Antibiotic susceptibility profile, Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus till date remains the foremost pathogen responsible for the majority of health-care associated infections globally (Cercenado *et al*., 2008). Infections resulting from this bacteria manifest in diverse clinical syndromes including necrotizing pneumonia, skin and soft tissue infections as well as other complicated specific forms like endocarditis, toxic shock syndrome and scalded skin syndrome (Nnachi *et al.,* 2014). These organisms cause infections in most parts of the human body but the skin has been reported to be most affected (Daum, 2007). The organism is a colonizer of the nasal passage with a carrier rate ranging from 15 - 35% amongst clinically healthy individuals (File, 2008). Susceptibility to colonization by this organism has been described to be peculiar to some groups of individuals notably health-care workers, nursing home inhabitants, prison inmates, military recruits and children (Ben-David *et al*., 2008; Ho *et al*., 2008). A proven channel of transmission of this pathogen is through exposure or contact of patients with healthcare workers who are carriers (Joshi *et al.,* 2013).

Treatment of this infection had earlier relied on the use of Penicillin antibiotics. However, over the course of time an apparent display of resistance to this group of antibiotics ushered in strains of this bacterium known worldwide as methicillin resistant *Staphylococcus aureus* (MRSA). These new strains have since become notorious in causing nosocomial infections (Alamin *et al.,* 2013). The possibility of transmission of health-care associated MRSA (HA-MRSA) to the community became almost unavoidable because of contact of patients with healthcare workers and their families.

MRSA infections presents with a mirage of challenges ranging from increased length of admission in the hospitals as well as increased morbidity and mortality which all together hampers infection control and treatment strategies (Macedo-Vinas *et al*., 2013; Kim *et al*., 2014). The burden of MRSA infections has further been heightened by the emergence of community-acquired MRSA (CA-MRSA) and livestock-acquired MRSA (LA-MRSA). The transmission of HA-MRSA into the community will amount to a major source of endogenous infection with accompanying human to human transmission which could go on to become a national menace owing to the poor healthcare status of many developing nations (David and Daum, 2010; Sakwinska *et al.,* 2011).

Reports of studies on prevalence of community-associated MRSA (CA-MRSA) are also emerging in Nigeria; 10.8% in apparently healthy school children in Okada, Edo State (Okwu *et al*., 2012), 41% in apparently healthy University students in Ekpoma, also of Edo State (Eke *et al*., 2012), 60.7% in healthy inhabitants of Uturu communities in Abia State (Ibe *et al*., 2013), 71.7% in healthy women volunteers in the Abuja Capital Territory (Onanuga *et al*., 2005).

The CA-MRSA strains have a great potential to cause an epidemic in the community, which can spread to the hospital (Kluytmans-VandenBergh and Kluytmans, 2006). This hence

necessitates the accumulation of adequate data regarding the prevalence rate of infection caused by this organism which is still adjudged as the biggest concern of healthcare related pathogens.

Studies have also shown 33% of healthy persons carry *Staphylococcus aureus* in their nose, usually without any illness but these could be constant sources of infection to other people. The importance of detecting MRSA strains is mainly attributed to its colonization in patients and health care workers, which serves as a constant source of infection and for transmission.

Generally, the prevalence rates of MRSA across some countries in the African continent have been shown to range from 25 - 50% (Falagas *et al.,* 2013). Studies conducted in South Africa, Nigeria, Kenya, and Cameroon found the prevalence ranging from 21 to 33.3% (Bouchillon *et al.,* 2004; Kesah *et al.,* 2003). A report from Kenya found the MRSA prevalence rate of 84.1% among skin and soft tissue infections (SSTIs) which was higher than previous findings in the region (Maina *et al.,* 2013). These observations seem to suggest a trend of increasing MRSA cases over the years and hence the need for continued monitoring and control of MRSA infections.

METHODS

Collection of Samples

A total of 300 urine, stool and nasal swab samples were obtained from apparently healthy students who consented to take part in the research.

Isolation of Organisms

The samples were inoculated onto mannitol salt agar using the streak plate method of inoculation. Inoculated plates were incubated at 37°C for 24hr. Upon establishment of growth, bacterial colonies showing typical characteristics of *S. aureus* (golden yellow pigmentation on mannitol salt agar) resulting from fermentation of mannitol were sub cultured onto freshly prepared nutrient agar plates. The bacterial isolates were identified as *S. aureus* based on their morphology, Gram-staining, catalase properties, coagulase and DNase tests according to the CLSI Guidelines, (2013). The resulting pure colonies were stored in agar slants for antimicrobial assays.

Antimicrobial Susceptibility Testing

The identified *S. aureus* isolates were tested for susceptibility to 8 different antimicrobial agents by the disc diffusion method on Mueller Hinton agar. The antimicrobial agents tested were: augmentin, 30µg (AUG), cloxacillin, 25µg (CXC), erythromycin, 15µg (ERY), Ceftazidime, 30µg (CAZ), Ofloxacin, 10µg (OFL), gentamicin, 10µg (GEN), Ceftriaxone, 30µg (CRO), and Cefotaxime 30µg (CTR). Discrete colonies from an 18hr nutrient agar plate of each isolate was suspended into sterile nutrient broth in a tube to achieve a bacterial suspension equivalent to 0.5 McFarland turbidity Standard**.** A cotton swab was dipped into the bacterial suspension and the swab pressed on the side of the tube to drain excess fluid. The entire surface of the agar plate was then inoculated with the same swab of inoculum, rotating the plate to ensure confluent growth of the bacteria. The antibiotics discs were placed on Mueller Hinton agar plates already seeded with the isolates. The plates were incubated at 35^{o}C for 24hr and observed for zones

of inhibition, measured using a ruler and recorded. The zones of inhibition produced by the antibiotics against the isolate was used to categorize them as either susceptible, resistant and intermediate status after comparing the zone of inhibition produced by the antibiotics against the isolate with that of a reference guide provided by the CLSI.

Detection of MRSA

A suspension of each isolate was prepared from the colonies from an overnight growth on nutrient agar plate. A suspension of the overnight growth was prepared with sterile saline and the turbidity was adjusted to 0.5 McFarland's standard. A sterile swab was dipped into this suspension and the excess of inoculum was removed by pressing it against the sides of the tube. The swab was then inoculated on Mueller-Hinton agar plate to create a lawn of the organisms. Cefoxitin disks, which are used for methicillin testing, were placed on each inoculated Mueller-Hinton plates. The plates were incubated for 24hrs at 37°C. The diameter of the zone around the disc was measured and the results were interpreted according to the CLSI guidelines. The isolates with a zone of inhibition less than 22mm were reported as MRSA strains. *Staphylococcus aureus* ATCC 25923 was used as the control strain.

Beta-lactamase Production Test

All *S. aureus* strains were screened for beta-lactamase production by employing combination of the acidimetric and iodometric tube methods as described in Livermore and Derek (2005).

Statistical Analysis

Comparative resistant rates of *S. aureus* strains from the different clinical specimens were statistically analysed by T-test and results were considered significant at 95% confidence level.

RESULTS

A total of 124 (41.3%) of isolates obtained from the different clinical samples were identified as *S. aureus* on the basis of their colonial morphology and biochemical characteristics. The distribution of the isolates was as follows: 65 isolates (43.3%) from urine, 42 (42.0%) from nasal swab and 17 (34.0%) from stool (Table 1).

A greater percentage of the isolates were resistant to ceftazidime, augmentin and cloxacillin whilst susceptibility to ofloxacin and gentamicin were observed (Figures 1 and 2). Of the total 124 isolates, 36.3% were found to express methicillin resistance (Table 2) while 75.8% were beta-lactamase producers (Table 3). There was no statistically significant association ($\chi 2 = 0.5943$; $p = 0.74$) between the prevalence of *S. aureus* and sample type, there was no association ($\chi 2 = 0.392$; $p = 0.82$) between the prevalence of MRSA and sample type. In the same vein there was no significant association between beta-lactamase production and sample type ($\chi 2 = 0.082378$; $p=0.96$).

Table 1: Isolation rate of *S. aureus* from urine, stool and nasal swabs

Sample	No. of sample Collected	No. (%) of *S. aureus* isolated	p-Value
Urine	150	65 (43.3)	0.74
Nasal swab	100	42 (42.0)	
Stool	50	17 (34.0)	
Total	300	124 (41.3)	

DF = 2; X^2 = 0.59432

Table 2: Prevalence of MRSA producing *Staphylococcus aureus*

Sample Type	Total Number of isolates	No. (%) positive of MRSA	p-Value
Urine	65	25 (38.5)	0.82
Nasal swab	42	13 (31.0)	
Stool	17	7 (41.2)	
Total	124	45 (36.3)	

DF = 2; X^2 = 0.39202

Table 3: Prevalence of Beta-lactamase producing *Staphylococcus aureus*

Sample Type	Total Number of isolates	No.(%) positive to β-lactamase	p-Value
Urine	65	51 (78.5)	0.96
Nasal swab	42	31 (73.8)	
Stool	17	12 (70.6)	
Total	124	94 (75.8)	

DF = 2; X^2 = 0.082378

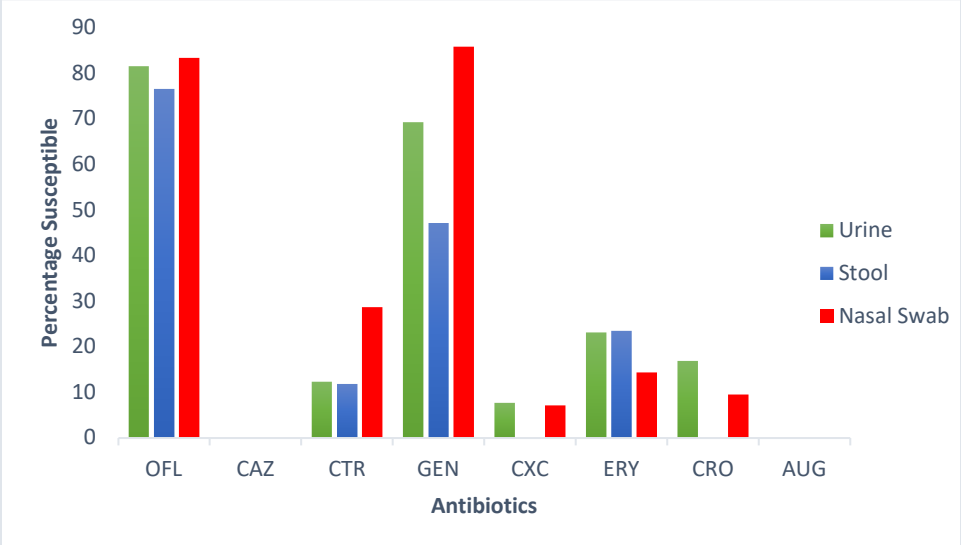

Fig 1: Susceptibility pattern of the *S. aureus* isolates from urine, stool and nasal swabs

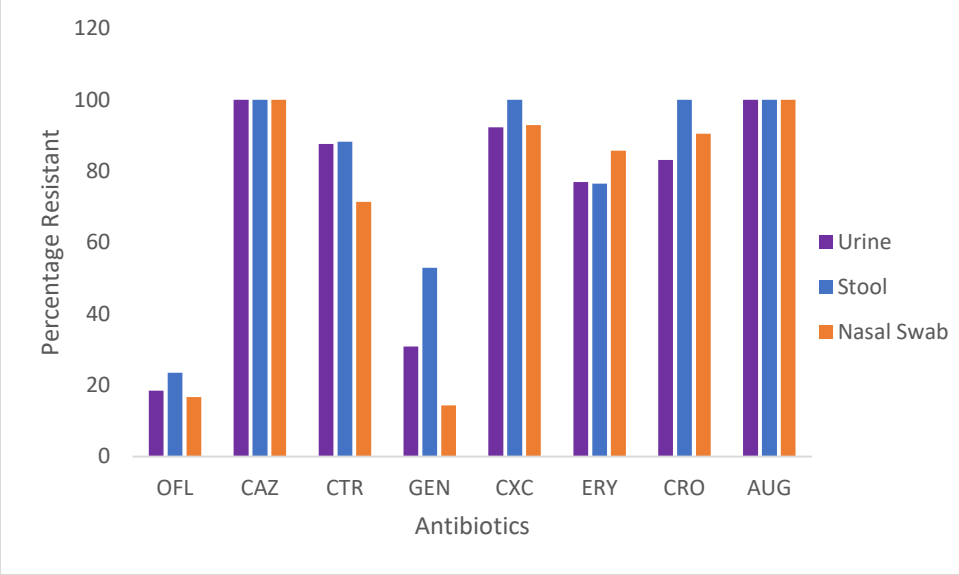

Fig. 2: Resistance Profile of the *S. aureus* from urine, stool and nasal swabs

Table 4: Drug Profile of the Methicillin Resistant *S. aureus*

Antibiotics	No. (%) Susceptible	No. (%) Intermediate	No. (%) Resistant
Ceftriaxone	21 (46.7)	6 (13.3)	18 (40.0)
Gentamicin	34 (75.6)	3 (6.7)	8 (17.8)
Ofloxacin	39 (86.7)	0 (0.0)	6 (13.3)
Ceftazidime	3 (6.7)	5 (11.1)	37 (82.2)
Erythromycin	16 (35.5)	8 (17.8)	21 (46.7)
Cloxacillin	5 (11.1)	0 (0.0)	40 (88.8)
Augmentin	2 (4.4)	3 (6.7)	40 (88.8)
Cefotaxime	14 (31.1)	4 (8.8)	27 (60.0)

DISCUSSION

Staphylococcus aureus is a very common cause of nosocomial infections. In this study, the isolation rate of *S. aureus* was 41.3% with the highest frequency occurring among urine and nasal samples. It is of note that this organism when isolated in specific patient population can be an important primary urinary pathogen. The present work occurred among apparently healthy students and therefore may be regarded as colonization as isolation of *S. aureus* from urine samples in the absence of bacteremia is often considered as colonization. However, it is quite difficult to define the role of *S. aureus* as a cause of symptomatic urinary tract infection as opposed to colonization (Muder *et al*., 2006). The overall prevalence of 41.3% of *S. aureus* is comparable to the 56.4% reported by Finney *et al*. (2013) for *S. aureus* and 50% for CoNS among normal healthy student. Similarly, the study by Anueyiagu *et al* (2020) also showed an overall frequency of isolation of *S. aureus* of 50.8% among healthy veterinary students.

In this study, a prevalence rate of 36.3% MRSA was recorded. This is lower than those of Onanuga *et al.* (2005) and Olowe *et al.* (2007), where 69.0% and 47.8% were recorded respectively but were slightly higher than that of Nwankwo *et al.* (2010), which revealed prevalence rate of 28.6%. The high prevalence of MRSA bacteria obtained in this study (36.3%) could be attributed to the irrational use of antibiotics (particularly beta-lactam drugs) as young students are known to often resort to self-medication as a way of minimizing health care costs. This encourages pathogenic bacteria (including *S. aureus*) to develop resistance to these drugs over time.

Different studies have depicted variations in the prevalence rates of MRSA in different countries. Over 50% prevalence rate of MRSA was reported in Portugal and Italy; 25% in England, Greece and France; 2% in the Netherlands and Switzerland (Fluit *et al.,* 2001). Prevalence of MRSA ranged from 23.6% in Australia to over 61 % in Taiwan and Singapore, and more than 70% in Japan and Hong Kong (Diekema *et al.,* 2001). Differences in the length of study period, number of study sites, sample size, sample type may be factors that could contribute to variations in the prevalence rate of MRSA.

In the assessment of drug susceptibility profile for the *S. aureus* isolates, it was observed that 30.8% of the isolates from urine samples presented resistance to Gentamicin, 52.9% of the isolates from stool also resistant to Gentamicin whilst *S. aureus* isolates from nasal swab exhibited 14.3% resistance to gentamicin. None of the isolates from the respective samples were however susceptible to Augmentin and ceftazidime showing 100% resistance Cloxacillin and Augmentin (82.4%). The results also showed that the MRSA isolates had high resistance to cloxacillin 88.8%, augmentin 88.8% and ceftazidime 85.7% respectively for isolate from urine, stool and nasal swab which support earlier findings, that MRSA strains are equally resistant to all β-lactam antibiotics (Onanuga *et al*., 2005). Majority of the MRSA isolates were susceptible to gentamicin and ofloxacin (Table 4). This may suggest that these antibiotics remain important in the management of *S. aureus* in this community. The sensitivity profile of the isolates to the flouroquinolones and gentamicin is in line with a previously published work (Ajoke *et al.,* 2012). The trends of drug susceptibility as observed in this investigation is in context with previous report on a similar study by Arumugam *et al.* (2016). These results reflect heavy antibiotic use and confirm, as the general trend, that MRSA strains have a highly multi-antibiotic resistant state.

In the present study all *S. aureus* isolates were tested for beta-lactamase production. It was shown that 94/124 (75.8%) were beta-lactamase producers. Beta-lactamase producing strains of *S. aureus* in the present study were much higher than that has been reported by previous study conducted in Ethiopia (Dilnessa and Adane, 2016) but more or less the same as reported in by Efuntoye and Amuzat, (2007). The high percentage values observed for the β-lactamse positive isolates explains the increased resistance to some of the antibiotics evaluated in this study.

CONCLUSION

The resistance displayed by *Staphylococcus aureus* to many groups of antimicrobial agents used represents a serious concern in therapeutic option available to the clinician in managing such infections. Methicillin resistance - the marker of multi-drug resistance showed a moderate MRSA prevalence (36.3%) amongst isolates of *Staphylococcus aureus*. Reducing this burden by good infection control practices such as strict hand washing, identifying MRSA carriers and treating them as well as prudent use of antimicrobial agents is recommended. Adherence to infection-control measures is further advocated to forestall transmission and spread of these infections caused by MRSA.

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