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Research article

Prevalence and antimicrobial susceptibility profiles of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates among healthy individuals in Okada, South-South, Nigeria.

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Abstract

Staphylococcus aureus and community-acquired *S. aureus* (CA-MRSA) nasal carriage rates have been investigated in various populations of the world. The values vary in different communities. The objectives of this study were to determine the prevalence of *S. aureus* and CA-MRSA colonization in nasal carriers among healthy individuals in Okada, South-South, Nigeria and their resistance profiles to commonly used antibiotics in the community. A total of 360 nasal swab samples were collected from healthy participants in the region and screened for *S. aureus* using standard microbiological procedures. Kirby-Bauer disc diffusion technique was applied to determine their antimicrobial susceptibility profiles and MRSA isolates. A total of 50 (13.9%) *S. aureus* and 25 (6.9%) CA-MRSA isolates were obtained. Of these, 30 (16.2%) *S. aureus* and 15 (8.1%) CA-MRSA isolates were obtained from the female participants while 20 (11.4%) *S. aureus* and 10 (5.7%) CA-MRSA isolates from the males. The highest prevalence values of 27 (18.6%) *S. aureus* and 15 (10.3%) CA-MRSA were obtained from the age range 9-14 years;

followed by the age range 3-8 years with values of 10 (17.2%) *S. aureus* and 6 (10.3%) CA-MRSA. The age ranges 25 years and above showed no nasal carriage of *S. aureus* isolates. There was no statistical significant in age and sex. The isolates were resistant to: ampicillin (100%), cloxacillin (100%), penicillin (100%), tetracycline (84%), chloramphenicol (66%), gentamicin (66%), erythromycin (62%), streptomycin (58%), and methicillin (50%). All the CA-MRSA isolates obtained in this study showed multi-drug resistant to at least five antibiotics tested and 24% were resistant to all the antibiotics. In conclusion, there is a need to reassess policies on antibiotics use both within and outside the hospital environment.

Key words: Community-acquired methicillin-resistant *S. aureus* (CA-MRSA), nasal carriage, prevalence, antimicrobial susceptibility profiles, healthy individuals.

Introduction

Staphylococcus aureus, a common and important human pathogen causing nosocomial and community-acquired infections can colonize the anterior nares and skin in both adults and children (1, 2, 3).The bacterium is transmitted to the nares by contaminated hands and from surfaces where it can survive for months. However, transmission occurs mainly through person-to-person contact (4).

S. aureus infections are assumed to arise from nasal carriage because the anterior nares are the most frequent carriage site (2). The infections of the bacterium cause significant morbidity and mortality in both the community and hospital settings (5). The infections range from superficial skin and soft tissue infections to bacteremia, endocarditis, osteomyelitis (6, 7).

The prevalence of nasal carriage of *S. aureus* varies widely between different populations. Also, the prevalence of *S. aureus* infections is higher in carriers than in non-carriers and the carrier prevalence ranges from 20-65% in both patients and healthy populations (3).

S. aureus nasal colonization can be an indicator of high risk for subsequent infection as methicillin-resistant *S. aureus* (MRSA) (8, 9). MRSA is a multi-drug resistant strain of *S. aureus* which is characterized by antibiotic resistance to penicillins, clindamycin, tetracycline, macrolides, aminoglycosides, fluoroquinolones etc (10, 11, 12, 13, 14). MRSA is a major health problem and nasal carriers of MRSA including community-acquired MRSA (CA-MRSA) are prone to septicemia, wound infections, skin and soft tissue infections, toxic shock syndrome to life threatening conditions (15, 16, 17, 18, 3, 19). Low rates of CA-MRSA carriage have led to widespread outbreaks of CA-MRSA diseases in athletic and paediatrics populations (20, 21).

This study was carried out to determine the prevalence and antimicrobial susceptibility profiles of *S. aureus* and CA-MRSA isolated from nasal swab samples of healthy individuals in Okada community, South-South, Nigeria.

Materials and Methods

Media and Antibiotics

The media used were: Mannitol salt agar (Chapman medium USP. Eur. Pharm), Mueller-Hinton agar, Nutrient broth and agar used were from Maharashtra, India. The antibiotics discs used were: methicillin (5µg) (Oxoid, UK), erythromycin (5µg), ampicillin (10µg), tetracycline (10µg), streptomycin (10µg), penicillin (10U), cloxacillin (5µg), gentamicin (10µg) and chloramphenicol (10µg) from Abtek Biological Limited.

Sample collection

Using sterile cotton wool swabs pre-wetted with sterile saline, one nasal swab sample from both anterior nares was collected from each participant. Collection of nasal swab samples was by inserting the swab into the nares and gently rotating it three times (22). Samples were collected randomly from 360 apparently healthy individuals in a period of April- June, 2011. An informed consent was obtained from the volunteers. They were not on any antibiotics and had not been hospitalized in the last one year at the period of sampling. The samples were labeled, packaged, transported to the laboratory and cultured within 1-3 hours of collection.

Identification procedures

Each nasal swab sample was inoculated (in duplicates) into mannitol salt agar and 5% blood agar plates. The plates were incubated aerobically at 37^0C for 24- 48 hours. A control strain *S. aureus* NCIB 8588 was also included. The characteristic isolates obtained were identified using standard microbiological methods which included colonial morphology, Gram's staining reaction and biochemical tests (23). Isolates that were Gram-positive cocci in clusters, catalase, coagulase, deoxyribonuclease and mannitol fermentation positive were considered as *S. aureus* in this study.

Test for MRSA strains

S. aureus isolates were tested for methicillin susceptibility by using modified Kirby-Bauer disc diffusion technique (23). Few colonies of each *S. aureus* isolate were adjusted at 0.5% McFarland standard and each streaked uniformly with sterile cotton wool swab in Mueller-Hinton agar plates containing 4% w/v sodium chloride (NaCl) to obtain confluent growth. Methicillin (5µg) discs were placed in inoculated plates and then incubated aerobically at 35^0C for 24-48 hours (22). Inhibition zone diameters of the isolates were measured in millimeters with a ruler. Isolates were classified as resistant (≤ 10mm), intermediate (11-12mm) or sensitive (≥ 13mm) based on the interpretative chart according to Clinical and Laboratory Standards Institute (24).

Antimicrobial susceptibility testing

The antimicrobial susceptibility profile of each *S. aureus* isolate was determined using eight antibiotics that are commonly used locally in the treatment of *S. aureus* infections. Modified Kirby-Bauer's disc diffusion technique was used (23). Mueller-Hinton agar was used and the plates were incubated at 37^0C for 18- 24 hours. Zones of inhibition were measured in millimeters and recorded.

Statistical analysis

Frequencies were obtained and percentages were calculated for the study variables. Chi-square was used to calculate significance as described by Jipa and Amariei (25).

Results and Discussion

A total of 50 (13.9%) *S. aureus* and 25 (6.9%) CA-MRSA isolates were obtained from 360 nasal swab samples screened. The prevalence values of *S. aureus* and CA-MRSA isolates among apparently healthy individuals in Okada community were thus 13.9% and 6.9% respectively. Among the *S. aureus* carriers, 20 (11.4%) were males and 30 (16.2%) females. For CA-MRSA carriers, 10 (5.7%) were males and 15 (8.1%) female subjects (table 1).

S. aureus carriers were highest in the age group of 9-14 years with 18.6%, followed by 3-8 years with 17.2%, then15-19 years with 10.6%. Age groups 25-29 years and above showed no carriers probably because of the small sample size or *S. aureus* carriers were absent in these age groups. CA-MRSA carriers were highest in the age groups of 3-8 and 9-14 years with 10.3%, followed by 20-24 years with 4.2%, then 15-19 years with 2.9% (table 2).

There was no significant difference ($p > 0.05$) in the prevalence values of *S. aureus* and CA-MRSA isolates between male and female subjects as well as between the age groups in this study.

The antimicrobial susceptibility test showed that the *S. aureus* isolates were resistant to ampicillin, cloxacillin and penicillin with 100% while tetracycline with 84%, chloramphenicol and gentamicin 66%, erythromycin 62%, streptomycin 58% and methicillin 50% (table 3).

The prevalence of multi-drug resistant CA-MRSA isolates is shown on table 4. Multi-drug resistance is defined in this study as resistance to four or more of the antibiotics tested. Thus, 25 (100%) of the CA-MRSA isolates were multi-drug resistant to the antibiotics tested in this study.

S. aureus, a world-wide pathogen whose natural reservoir is man has been reported to cause nosocomial as well as severe community-associated infections of skin and soft tissues. It is increasingly developing resistance to many antibiotics (19). Results of the nasal swab cultures of this study indicated that 13.9% of the apparently healthy individuals in Okada community carried *S. aureus* in their anterior nares. This is lower than the 17.1% reported by Rosina and Estifanos (26) and 20-65% by Citak, *et al*., (3). The prevalence of CA-MRSA carriers was 6.9% in this study which is lower than the 11.1% reported by Ankur, *et al*., (22). The variation in values is in agreement with the reports of Ankur, *et al*., (22) and Okwu, *et al*., (19) who stated that the carriage rates vary in different communities. In this study, the females were more carriers than the males and the age groups 3-8 and 9-14 years than the others. However, there was no statistical significant difference in staphylococcal carriage by age or sex. This is in agreement with the reports of Ankur, *et al*., (22); Khanna, *et al*., (27) and Okwu, *et al*., (19).

The highest level of antimicrobial resistance showed by *S. aureus* isolates in this study was observed in ampicillin, cloxacillin and penicillin which are in agreement with the reports of Okwu, *et al*., (19). Most of the isolates were resistant to the other antibiotics used and their resistance profiles are similar to the profiles reported by Okwu, *et al*., (19). This is probably because the strains of *S. aureus* isolated in this study might be similar to the strains isolated by Okwu, *et al*., (2012). The similarity of strains was however not investigated in this study. The old agent chloramphenicol that was reported by Peng, *et al*., (28) as still highly active against MRSA and may therefore be used in the treatment of MRSA infections was poorly active in this study. However, this is in conformity with previous observations that most isolates of *S. aureus* were resistant to a large number of commonly prescribed antibiotics (29). The resistance to methicillin may be due to the expression of mecA gene or as a result of the thickening of the cell wall of the organisms (30, 31). Resistance to the other antibacterial agents may be driven by the acquisition of discrete genetic accessory elements comprising plasmids, transposable genetic elements (insertion sequences and transposons) and genomic islands (32).

The level of multi-drug resistance shown by CA-MRSA isolates in this study is of great concern. They were resistant from five to nine antibacterial agents and the resistance rate is higher than the values reported in different studies (19). The increased resistance could be due to self-medication, inappropriate prescription and indiscriminate use of antibiotics (33).

Conclusion

The present study has lent a voice to the already existing body of knowledge that *S. aureus* and CA-MRSA are increasingly becoming resistant to majority of the available antibiotics in use and subsequently have developed into a challenging public health problem (34). Therefore, there is a need to reassess policies on antibiotics use within and outside the hospital environments. Hence, local sensitivity profiles of the organisms should be known and the same reviewed periodically (7). Also, community medical personnel should effectively enlighten the general public to discourage the indiscriminate use of antimicrobial agents and promote their rational use.

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Table1: Prevalence of *S. aureus* and CA-MRSA in male and female subjects

Sex	Number (%) sampled	Frequency (%) of *S. aureus*	Frequency (%) of CA-MRSA
Male	175 (48.6%)	20 (11.4%)	10 (5.7%)
Female	185 (51.4%)	30 (16.2%)	15 (8.1%)
Total	360 (100%)	50 (13.9%)	25 (6.9%)

Table 2: Prevalence of *S. aureus* and CA-MRSA in different age ranges

Age range	Number sampled	Frequency (%) of *S. aureus*	Frequency (%) of CA-MRSA
3-8	58 (16.1%)	10 (17.2%)	6 (10.3%)
9-14	145 (40.3%)	27 (18.6%)	15 (10.3%)
15-19	104 (28.9%)	11 (10.6%)	3 (2.9%)
20-24	24 (6.7%)	2 (8.3%)	1 (4.2%)
25-29	12 (3.3%)	0	0
30-34	9 (2.5%)	0	0
35-above	8 (2.2%)	0	0
Total	360 (100%)	50 (13.9%)	25 (6.9%)

Table 3: Antibiotic susceptibility profiles of the 50 *S. aureus* isolates

Antibiotic	Resistant no. (%)	Intermediate no. (%)	Susceptible no. (%)
Chloramphenicol (10µg)	33 (66%)	5 (10%)	12 (24%)
Ampicillin (5µg)	50 (100%)	0 (0)	0 (0)
Cloxacillin (5µg)	50 (100%)	0 (0)	0 (0)
Penicillin (10U)	50 (100%)	0 (0)	0 (0)
Gentamicin (10µg)	33 (66%)	0 (0)	17 (34%)
Streptomycin (10µg)	29 (58%)	12 (24%)	9 (18%)
Tetracycline (10µg)	42 (84%)	5 (10%)	3 (6%)
Erythromycin (5µg)	31 (62%)	14 (28%)	5 (10%)
Methicillin (5µg)	25 (50%)	1 (2%)	24 (48%)

Table 4: Prevalence of multi-drug resistant CA-MRSA isolates

Parameter	Frequency of multi-drug resistant isolates	
	Number	Percentage (%)
Fully sensitive	0	0
Resistant to 1 agent	0	0
Resistant to 2 agents	0	0
Resistant to 3 agents	0	0
Resistant to 4 agents	0	0
Resistant to 5 agents	4	16%
Resistant to 6 agents	6	24%
Resistant to 7 agents	5	20%
Resistant to 8 agents	4	16%
Resistant to 9 agents	6	24%