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Prevalence of Nasal Carriage of Community-associated Methicillin-resistant *Staphylococcus aureus*(CA-MRSA) among Healthy Primary School Children in Okada, Nigeria

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Abstract
Methicillin- resistant *Staphylococcus aureus* (MRSA) infections are very difficult to cure because MRSA strains are resistant against almost all clinically available antibiotics. The objectives of this study were to determine the prevalence of MRSA colonization in nasal carriers among healthy school children in Okada community and their resistance patterns to nine commonly used antibiotics. A total of 120 nasal swab samples were collected from healthy school children and screened for *S. aureus* using standard microbiological procedures. Disc diffusion technique was applied to determine their antibiotic susceptibility profiles. A total of 22 (18.3%) *S. aureus* and 13 (10.8%) MRSA isolates were obtained. Of these, 12 (20%) *S. aureus* and 7 (11.7%) MRSA were obtained from females while 10 (16.7%) *S. aureus* and 6 (10%) MRSA were from males. Also, 12 (19.4%) S. *aureus* and 7 (11.3%) MRSA were from the age range 9-14years while 10 (17.3%) S. *aureus* and 6 (10.3%) MRSA were from the age range 3-8years. There was no statistical significant in age and sex. The isolates were resistant to ampicillin(100%), cloxacillin(100%), penicillin(100%), tetracycline(82%), chloramphenicol(73%), erythromycin(68%), gentamicin(64%), streptomycin(56%) and oxacillin(55%). All the MRSA isolates (13) obtained showed multi-drug resistance to at least five antibiotics tested.
Key words: Community-associated methicillin resistant *Staphylococcus aureus (CA-MRSA),* healthy school children, nasal carriers, prevalence.

1. Introduction

Staphylococcus aureus is one of the most successful and adaptable human pathogen. It is also a common colonizer of the skin and nose, carried by 30-50% of the total population. The bacterium can be carried asymtomatically for weeks or months on mucous membranes but only transiently on intact skin (Archer, 1998; Rosina and Estifanos, 2007).

Methicillin- resistant *S. aureus* (MRSA) is recognized as one of the major causes of infections in humans, occurring in both the community and the hospital. It causes skin infections, osteoarthritis and respiratory tract infections in the community (Didier *et al*., 2004). Nasal carriers of MRSA are also prone to septicemia, wound infections and occasionally toxic shock syndrome (Memon, 2006; Ikeagwu *et al.,* 2008 and Mohammad *et al.,* 2009).

The development of antibiotic resistance in *S. aureus* has contributed to its emergence as an important pathogen in a variety of settings (Daini and Akano, 2009). Drug resistant *S. aureus* is the major cause of infections especially in hospital settings (Rosina and Estifanos, 2007; Mahmood *et al.,* 2010). Frequent administration of systemic antibiotics modified nasal *S. aureus* from methicillin-sensitive *S. aureus* (MSSA) to MRSA. This strain renders almost all antibiotics useless, including the most potent penicillinase-stable β-lactams (oxacillin, methicillin, nafcillin and cephalosporins). Strains resistant to vancomycin and ciprofloxacin are also emerging (Rosina and Estifanos, 2007).

The prevalence of drug resistant strains of *S. aureus* varies between different countries ranging from 7.5-25% (Ankur *et al.,* 2008). This study was carried out to determine the prevalence and antibiotics resistance of *S. aureus* isolated from nasal samples of primary school children in Okada, Edo State, Nigeria.

2. Materials and Methods

2.1 Antibiotics and media

Antibiotics discs used were: oxacillin (5µg) (Oxoid, UK), cloxacillin (5µg), erythromycin (5µg), gentamicin (10µg), penicillin (10U), streptomycin (10µg), tetracycline (10µg), ampicillin (10µg) and chloramphenicol (10µg) from Abtek Biological limited.

Mannitol salt agar (Chapman medium USP, Eur Pharm), Mueller-Hinton agar, nutrient broth and nutrient agar used were from Maharashtra, India.

2.2 Sample collection

Nasal swabs from the anterior nare of each nostril of each subject were collected using sterile cotton swabs. Collection was by inserting the swab and gently rotating it three times (Ankur *et al.,* 2008). Samples were collected randomly from 120 healthy primary school children in Okada community after an informed consent over a period of two weeks. All the volunteers were not on any antibiotic and had not been hospitalized in the last one year at the period of sampling. The samples were labeled, packaged and transported to the laboratory.

2.3 Identification procedures

Each nasal sample was inoculated (in duplicates) onto mannitol salt agar and blood agar and the plates were incubated aerobically at 37^{o}C for 24h. A control strain, *S. aureus* NCIBB 8588 was also included. The characteristic isolates obtained were identified using standard microbiological methods which included colonial morphology, Gram's stain reaction and biochemical tests (Cheesbrough, 2002). Isolates that were Gram-positive cocci in clusters, catalase, coagulase and mannitol fermentation positive were considered as *S. aureus* in this study.

2.4 Test for MRSA strains

S. aureus isolates were tested for methicillin resistance by using modified Kirby-Bauer disc diffusion technique (Cheesbrough, 2002). Inocula were adjusted at 0.5% McFarland standard and each streaked uniformly with swab sticks in Mueller-Hinton agar plates containing 4% sodium chloride (NaCl) to obtain confluent growth. Oxacillin (5µg) discs were placed in the plates which were then incubated aerobically at 35^{0}C for 24h (Ankur *et al.,* 2008). Zone diameters of the isolates were measured in millimetres with a ruler. Isolates were classified as resistant, intermediate or sensitive based on the interpretative chart updated according to the current NCCLS standards (NCCLS, 2002).

2.5 Antibiotic susceptibility testing

The antibiotic susceptibility pattern of all the *S. aureus* isolates was determined using nine antibiotics by the modified Kirby-Bauer diffusion technique (Cheesbrough, 2002). Mueller-Hinton agar (without 4% NaCl) medium was used and the plates were incubated at 37^{0}C for 18-24h. Zones of inhibition were also measured and recorded.

2.6 Statistical analysis

Frequencies were obtained and percentages were calculated for study variables. Chi-square was used to calculate significance as described by Jipa and Amariei (2012).

3. Results and Discussion

Nasal swabs from 120 subjects were examined and among them, 22 (18.3%) were found to be positive for *S. aureus* and 13 (10.8%) were oxacillin-resistant (referred to as MRSA isolates). The prevalence of *S. aureus* and CA-MRSA isolates among the primary school children in Okada was thus estimated to be 18.3% and 10.8% respectively. Among the staphylococcal carriers, 10 (16.7%) were from males and 12 (20.0%) were from females (table 1).

S. aureus and CA-MRSA carriers were higher in the age group of 9-14years (19.4% and 11.3%) than 3-8years (17.3% and 10.3%) respectively, as shown in table 2. There was no significant difference (X^2 cal was less than X^2 tab) in carriage rates of *S. aureus* and CA-MRSA isolates among male and female subjects as well as among the two age groups.

The antibiotic susceptibility test showed that the *S. aureus* isolates were highly resistant to ampicillin (100%), cloxacillin (100%), penicillin (100%), tetracycline (82%), chloramphenicol (73%), erythromycin (68%), gentamicin (64%), oxacillin (59%) and streptomycin (55%) as shown in table 3. The prevalence of multi-drug resistance in the CA-MRSA isolates is shown in Table 4. Multi-drug resistance was defined in this study as resistance to four or more of the antibiotics tested. Thus, 13 (100%) of the CA-MRSA isolates showed multi-drug resistance to the antibiotics used in this study.

S. aureus, a world-wide pathogen whose natural reservoir is humans, is reported to cause nosocomial as well as severe community-associated infections of skin and soft-tissues. It is increasingly developing resistance to many antibiotics (Lowy, 2003; Onanuga *et al.,* 2005 and Rosina and Estifanos, 2007). *S. aureus* nasal carriage rates have been investigated in various populations of the world but no documented information about healthy carriers of the organism in Okada community, Edo state, Nigeria is available. Nasal carriage of *S. aureus* varies in different communities (Rosina and Estifanos, 2007 and Ankur *et al.,* 2008). Results of the nasal cultures of this study indicate that 18.3% of the subjects examined carried *S. aureus* in the anterior nares. This is in agreement with the study of Rosina and Estifanos (2007) who reported 17.1%. The prevalence of MRSA in the apparently healthy school children was estimated to be 10.8% which was in line with the result of 11.1% reported by Ankur *et al.* (2008). There was no statistically significant difference observed in staphylococcal carriage by sex and age of study subjects. This is also in agreement with the reports of Rosina and Estifanos (2007) and Ankur *et al*., (2008).

The highest level of resistance was observed in ampicillin, cloxacillin and penicillin respectively, which is in agreement with the reports of Aravind *et al.* (2000), Onanuga *et al.* (2005), Rosina and Estifanos, (2007) and Nkwelang *et al.* (2009). Most of the *S. aureus* isolates in this study were resistant to other commonly used

antibacterial agents. This result is in line with similar studies from Nigeria as well as other countries (Beyene and Abdisa, 2005; Onanuga *et al.,* 2005 and Mahmood *et al.,* 2010). This is in conformity with previous observations that most isolates of *S. aureus* are resistant to a large number of commonly prescribed antibiotics (Onanuga *et al.,* 2005). The resistance to oxacillin (59%) has been widely reported internationally and even in our communities (Olayinka *et al.,* 2005). The resistance may be due to the expression of mecA gene or as a result of the thickening of the cell walls of the organisms (Dennis *et al.,* 2002 and Zer *et al.,* 2009). Nevertheless, the resistance rate in this study is higher than the values reports in different studies. The increased resistance could be due to self medication and indiscriminate use of antibiotics by parents to treat their children (Barana, 2003). Frequent administration of systemic antibiotics modified nasal *S. aureus* from MSSA to MRSA (Nielsan *et al.,* 1998). The level of multi-drug resistance shown by MRSA from the healthy children in this study is of great concern. About 46.2% of the MRSA isolates were resistant to six antibiotics; 23.1% were resistant to seven while 7.7% to eight antibiotics. None of the isolates were fully sensitive. These observations confirmed the postulation that healthy members of the community are the highest reservoir of antimicrobial resistant bacteria (Lamikanra *et al.,* 1996).

Emergence of multi-drug resistant bacteria is attributed to inappropriate prescription, self-medication and indiscriminate use of antibiotics. This is of immerse concern to health workers and officials and calls for effective measures including public enlightenment to promote rational use of antibiotics and to discourage their indiscriminate use.

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

Sex	Number sampled	Frequency(%) of *S. aureus*	Frequency of MRSA
Male	60	10(16.7%)	6(10.0%)
Female	60	12(20.0%)	7(11.7%)
Total	120	22(18.3%)	13(10.8%)

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

Age range	Number sampled	Frequency(%) of *S. aureus*	Frequency of MRSA
3-8	58(48.3%)	10(17.3%)	6(10.3%)
9-14	62(51.7%)	12(19.4%)	7(11.3%)
Total	120	22(18.3%)	13(10.8%)

Table 3. Antibiotic susceptibility pattern of the 22 *S. aureus* isolates

Antibiotics	Resistant No (%)	Intermediate No (%)	Susceptible No (%)
Ampicillin (5µ g)	22 (100)	0 (0)	0 (0)
Chloramphenicol (10µg)	16 (73)	3 (14)	3 (14)
Cloxacillin (5 µg)	22 (100)	0 (0)	0 (0)
Penicillin (10U)	22 (100)	0 (0)	0 (0)
Gentamicin (10µ g)	14 (64)	0 (0)	8(36)
Streptomycin (10µ g)	12 (55)	10 (45)	0 (0)
Tetracycline (10µ g)	18 (82)	4 (18)	0 (0)
Erythromycin (5µ g)	15 (68)	6 (27)	1 (5)
Oxacillin (5µ g)	13 (59)	0 (0)	9 (41)

Table 4. Prevalence of multi drug resistance among 13 CA-MRSA isolates

Parameter	Frequency of multi-drug resistance	
	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	3	23%
Resistant to 6 agents	6	46%
Resistant to 7 agents	3	23%
Resistant to 8 agents	1	8%
Resistant to 9 agents	0	0%