NJBAS
Available online at http://www.ajol.info/index.php/njbas/index
Nigerian Journal of Basic and Applied Science (June, 2019), 27(1): 114-121
DOI: http://dx.doi.org/10.4314/njbas.v27i1.15
ISSN 0794-5698

Full Length Research Article

Prevalence Of Methicillin-Resistant Staphylococci Among Apparently Healthy Students Attending A Tertiary Institution In Benin City, Nigeria

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ABSTRACT
This study was aimed at determining the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative staphylococci (MRCoNS) among apparently healthy students of a tertiary institution in Benin City, Nigeria. A total of 350 students were recruited for the study and nasal swabs were collected alongside demographic data. These swabs were processed microbiologically using standard techniques to recover staphylococci. Antimicrobial susceptibility and methicillin-resistance was determined using a phenotypic method (cefoxitin resistance). A total of 148 (42.3%) of 350 students were culture positive for *S. aureus*, while 72 (20.6%) were positive for CoNS. Students from Faculty of Dentistry showed the highest prevalence of nasal MRSA (40.0%) and MRCoNS (20.0%). Ofloxacin and gentamicin were the most active antibacterial agents against MRSA with 89.1% and 87.3% respectively been susceptible, while gentamicin was the most active antibiotic against MRCoNS (75.0%). Nasal colonization by MRSA and MRCoNS was unaffected by area of residence and gender (P > 0.05). The nasal carriage rate of MRSA and MRCoNS was 37.2% and 33.3% respectively. The study recommends periodic review of nasal colonization rates among apparently healthy subjects. Regulated use of antimicrobial agents is imperative in order to stem the tide of resistance.
Keywords: Methicillin-resistance, Staphylococci, Students, Antibiotics

INTRODUCTION
Since the first detection of methicillin-resistance in *Staphylococcus aureus* in 1961, the worldwide prevalence of MRSA being implicated in hospital and community-acquired infection has been on the rise (Olowe *et al.,* 2013). Coagulase-negative staphylococci (CoNS) belong to normal microbial flora of the skin and mucous membranes (Azih and Enabulele, 2013). These organisms have relatively low virulence but are increasingly recognized as agents of clinically significant infection of the bloodstream and other sites (Azih and Enabulele, 2013). The incidence of methicillin-resistant CoNS (MRCoNS) causing infection are now on the rise and have equally been implicated in clinical infections worldwide (Ibadin *et al.,* 2017).

The association between *S aureus* nasal carriage and staphylococcal disease was first reported in 1931 (Solberg, 1965; Sollid *et al.,* 2014). Due to an increase in staphylococcal infection, subsequent studies which investigated a causal relationship between nasal carriage of *S aureus* and infection was proven by the fact that the nasal *S aureus* strain and the infecting strain shared the same phage type or genotype (von Eiff *et al.,* 2001; Sollid *et al.,* 2014). CoNS have also increasingly drawn attention as nasal carriage has been shown in recent studies (Abadi *et al.,* 2015).

The specific mechanism of resistance to methicillin/oxacillin in staphylococci is due to expression of *mecA* gene (Ito *et al.,* 1999). *mecA* gene codes for penicillin binding protein 2a (PBP2a), a transpeptidase with low affinity for β-lactams which confers resistance to methicillin and other β-lactam antibiotics in staphylococci harboring the gene (Ito *et al.,* 1999). Methicillin-resistant staphylococci are therefore typically

difficult to treat owing to resistance that is shown to different antibacterial drugs.

Though molecular methods have emerged as the gold standard for detecting methicillin resistance in staphylococci, phenotypic methods have also proven reliable though differing sensitivities and specificities subsist in several studies (Olowe *et al.,* 2013; Ibadin *et al.,* 2017). The use of cefoxitin antibacterial as a surrogate marker for methicilin resistance is however very reliable when employed in agar dilution, broth micro-dilution or disc diffusion techniques (CLSI, 2013).

Methicillin-resistant staphylococci have been shown to be prevalent in health institutions, causing a wide range of clinical infections across Nigeria (Olowe *et al.,* 2013; Ibadin *et al.,* 2017). Efforts are now increasingly being made to explore the carrier status of healthcare workers (Rongpharpi et al., 2013), patients (von Eiff et al., 2001), apparently healthy individuals and companion animals in order to ascertain their role in transmission (Okodua et al., 2013). This study was however aimed at determining nasal carriage of MRSA and MRCoNS among apparently healthy students in a tertiary institution in Benin City, Nigeria.

MATERIALS AND METHODS

Study Population

A total of 350 apparently healthy students who had not taken antibiotics in the last one month were recruited for this study. These were students from various Faculties of the University of Benin, Benin City, Nigeria.

Sample Collection and Processing

Nasal swab was collected from all participants. Demographic data such as: Faculty of the student, gender, age, level, on-campus and off-campus residence. The nasal swabs from all participants were inoculated on 3% NaCl nutrient agar. The plates were incubated aerobically for 24 h at 37^0C.

Bacterial Isolates

Emergent bacterial isolates from culture plates were identified following Gram stain and appropriate biochemical tests namely catalase, coagulase (slide and/or tube), citrate utilization, indole, gelatin hydrolysis, Vogues-Proskauer, sucrose, maltose, lactose and glucose as described in standard Medical Microbiology laboratory manual (Cheesbrough, 2009). All *Staphylococcus* spp recovered were thereafter stored at 4°C on Mueller Hinton agar slants for further work.

Susceptibility Testing

Antimicrobial susceptibility testing was carried out following the recommendation of British Society for Antimicrobial Chemotherapy (BSAC) method (Andrew, 2009). The test colonies were emulsified in sterile distilled water and the turbidity matched with 0.5 McFarland. Once matched, a sterile cotton wool swab was dipped in the organism suspension and excess liquid was removed by turning the swab on side of the test tube. The entire surface of Mueller–Hinton agar plate was seeded by swabbing in three directions with the swab. The antibiotic discs were placed on the plate with the use of a sterile forceps. The antibiotics used include the following: ceftazidime (30µg), cefuroxime (30 µg), ceftriaxone (30 µg), Cloxacillin (5µg), amoxicillin-clavulanate (30µg), erythromycin (5µg), ofloxacin (5 µg), gentamicin (10 µg) (all from Abtek U.K).

Screening for methicillin-resistance

All *Staphylococcus* spp isolated were screened for methicillin-resistance by following CLSI guidelines using 30 µg cefoxitin discs (Abtek U.K) (CLSI, 2013). Plates were read after incubation at 35°C for 18 h. Zone diameter ≤ 21mm was deemed cefoxitin resistant.

Statistical Analysis

The frequency data were compared using the chi square (X^2) test. The statistical software INSTAT® was used for the analysis. A p value of < 0.05 was deemed statistically significant.

RESULTS

A total of 350 apparently healthy students were recruited for this study. Of this number, 148 (42.3%) of students were culture positive for *S. aureus*, while 72 (20.6%) were positive for CoNS. The prevalence of *S. aureus* and CoNS were significantly higher among students of the Faculties of Dentistry (p= 0.0118) and Medicine (p=0.0113) respectively, compared to students from other Faculties (Table 1).

The nasal carriage rate of MRSA and MRCoNS among apparently healthy students in this study was 37.2% and 33.3% respectively. The prevalence of MRSA and MRCoNS were not significantly different (p>0.05) and were not affected by students Faculty (p>0.05) (Table 2). Similarly, gender of students and location of their residence did not significantly (p>0.05) affect the prevalence of MRSA and MRCoNS (Table 3).

Gentamicin and ofloxacin were the most active antibacterial agents against MRSA, MSSA MRCoNS and MSCoNS (Tables 4 and 5) with MRSA isolates been significantly (p<0.0001) more susceptible to gentamicin and ofloxacin than MSSA isolates (Table 4). MSCoNS isolates were significantly (p=0.0312) more susceptible to cefuroxime than their MRCoNS counterparts (Table 5). Generally, the susceptibilities of all isolates to the other tested antibacterial agents were poor.

Table 1: Distribution of staphylococci according to different faculties

Faculty	Number of students tested	*Staphyhlococcus aureus*	CoNS
Basic medical sciences	100	58(58.0)	16 (16.0)
Management sciences	14	7 (50.0)	2 (14.3)
Art	34	8 (23.5)	3 (8.8)
Pharmacy	18	8 (44.4)	2 (11.1)
Agricultural sciences	24	6 (25.0)	9 (37.5)
Physical sciences	14	6 (42.9)	2 (14.3)
Engineering	11	2 (18.2)	1(9.1)
Dentistry	5	3 (60.0)	0
Medicine	26	10 (38.5)	12 (46.2)
Law	9	1 (11.1)	1 (11.1)
Education	24	11 (45.8)	4 (16.7)
Life sciences	36	14 (38.9)	10 (27.8)
Social sciences	35	14 (40.0)	10 (28.6)
TOTAL	350	148(42.3)	72 (20.6)

Staphylococcus aureus: p=0.0118; CoNS: p=0.0113, CoNS- Coagulase negative staphylococci, number in brackets = value in percentage.

Table 2: Distribution of methicillin-resistant staphycococci among students from different faculties.

Faculty	*Staphylococcus aureus*		CoNS		P value
	No. tested	MRSA	No. tested	MRCoNS	
Basic Medical Sciences	58	24 (43.6)	16	5 (31.3)	0.5691
Management Sciences	7	3 (42.9)	2	1 (50.0)	1
Art	8	2 (25.0)	3	1 (33.3)	1
Pharmacy	8	4 (50.0)	2	1 (50.0)	1
Agricultural sciences	6	2 (33.3)	9	3 (33.3)	1
Physical Sciences	6	3 (50.0)	2	1 (50.0)	1
Engineering	2	0	1	1(100.0)	1
Dentistry	3	2 (66.7)	0	0	ND
Medicine	10	4 (40.0)	12	5 (41.7)	1
Law	1	0	1	1 (100.0)	
Education	11	3(27.3)	4	1 (25.0)	1
Life Sciences	14	3 (21.4)	10	3 (30.0)	0.6653
Social Sciences	14	5 (35.7)	10	2 (20.0)	0.6529
TOTAL	148	55 (37.2)	72	24 (33.3)	0.685

MRSA vs Faculty: p=0.8926; MRCoNs vs Faculty: p=0.8595, MRCoNS-Methicillin resistant coagulase negative staphylococci, MRSA-Methicillin resistant *Staphylococcus aureus*, CoNS- Coagulase negative staphylococci, ND- Not done, number in brackets = value in percentage

Table 3: Prevalence of methicillin-resistant *Staphylococcus aureus* and Coagulase negative staphylococci among students in relation to area of residence and Gender

	Number of isolates tested	Methicillin-Resistant(%)	p value
Residence			
S. aureus			0.3956
On- Campus	100	40 (40.0)	
Off- Campus	48	15 (31.3)	
CoNS			0.7998
On-campus	42	15 (35.7)	
Off-campus	30	9 (30.0)	
Gender			0.1305
S. aureus			
Male	56	16 (28.6)	
Female	92	39 (42.4)	
CoNS			0.5591
Male	34	13 (38.2)	
Female	38	11 (28.9)	

CoNS-Coagulase negative staphylococci

Table 4: Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates recovered from apparently healthy students

Antibacterial drugs	MRSA (%) n = 55	MSSA (%) n = 93	p
Cloxacillin (5 µg)	1(1.8)	3 (3.2)	0.6098
Erythromicin (5 µg)	29 (52.7)	33 (35.5)	0.0598
Gentamicin (10 µg)	49 (89.1)	37 (39.8)	<0.0001
Amoxicillin-clavulanate (30 µg)	1 (1.8)	6 (6.45)	0.3775
Ofloxacin (5 µg)	48 (87.3)	40 (43.0)	<0.0001
Ceftazidime (30 µg)	4 (7.3)	11 (11.8)	0.5448
Cefuroxime (30 µg)	3 (5.5)	10 (10.8)	0.4238
Ceftriaxone (30 µg)	3 (5.5)	13 (14.0)	0.097

MRSA-Methicillin resistant *Staphylococcus aureus*, MSSA- Methicillin susceptible *S. aureus*, n = number of isolates tested

Table 5: Antimicrobial susceptibility of Coagulase negative *staphylococci*

Antibacterial drugs	MRCoNS (%) n = 24	MSCoNS (%) n = 48	p
Cloxacillin (5 µg)	0 (0)	9 (18.8)	0.0588
Erythromycin (5 µg)	8 (33.3)	20 (41.7)	0.6691
Gentamicin (10 µg)	18 (75.0)	23 (47.9)	0.0529
Amoxicillin-clavulanate (30 µg)	1 (4.2)	7 (14.6)	0.3534
Ofloxacin (5 µg)	14 (58.3)	22 (45.8)	0.4533
Ceftazidime (30 µg)	2 (8.3)	10 (20.8)	0.3134
Cefuroxime (30 µg)	1 (4.2)	14 (29.2)	0.0312
Ceftriaxone (30 µg)	3 (12.5)	11 (22.9)	0.4611

MRCoNS- Methicillin resistant coagulase negative staphylococci, MSCoNS- Methicillin susceptible coagulase negative staphylococci, n = number of isolates tested

DISCUSSION

The ecological niche for *S. aureus* has long been identified as the anterior nares in man (Sollid *et al.,* 2014). The carriage rate varies from one geographical location to another (14). Several studies have in recent times shown nasal carriage for CoNS (Abadi *et al.,* 2015).

In this study, the carriage rate of *S. aureus* and CoNS was 42.3 and 20.6% respectively among apparently healthy students. The carriage rate of *S. aureus* observed is slightly higher than a similar study in Ekpoma, Edo state which reported 35.4% among apparently healthy residents of the town (Okodua *et al.,* 2013). The carriage rate is equally higher than another study in Thailand which evaluated nasal swabs of medical students, where a colonization rate of 29.7%, 30.5% and 39.4% respectively was observed for *S. aureus* when screened thrice (Treesirichod *et al.,* 2014). The nasal colonzation rate of CoNS was comparatively lower than *S. aureus* in this study and approximately half the rate observed for *S. aureus.* This finding differs strikingly from an Iranian study among students where nasal colonization rate was reported as 71.1% (Abadi *et al*., 2015). A carriage rate of 6.25% had been earlier reported among hospital personnel and students in Ile-Ife (Shittu *et al.,* 2006). Our study therefore shows an increase in nasal colonization of CoNS in comparison with a previous study in Southern Nigeria.

Methicillin-resistance in staphylococci is strongly associated with resistance to beta-lactam antibiotics (Abadi *et al.,* 2015; Ibadin *et al.,* 2017). This includes an array of antibiotics namely penicillin, cephalosporins and carbapenems. The

nasal colonization rate of MRSA and MRCoNS among students in this study was 37.2% and 33.3% respectively. Previous studies have demonstrated a causal link between nasal carriage of staphylococci and subsequent infection (von Eiff *et al.,* 2001). Several researchers have also shown a relationship between nasal carriage of staphylococci among healthcare workers and an outbreak of MRSA in wards (Belani *et al.,* 1986). Our study however observed a comparatively higher carriage rate among students of medicine, dentistry and other health professions in comparison with other Faculties. Though reasons may not be very clear, these students usually have compulsory postings in the hospital, thereby increasing their risk of exposure to these resistant bacterial strains among patients, hospital items, and specimens. A recent study showed a high prevalence of MRSA and MRCoNS among clinical specimens in the teaching hospital of University of Benin (Ibadin *et al.,* 2017). A previous study in Thailand which evaluated carriage rate of *S. aureus* among students in preclinical classes by collecting nasal swabs prior to working in the hospital (the first), following the first rotation (the second) and at the end of the rotation schedule in the hospital (the last) observed an increasing carriage rate of 29.7%, 30.5% and 39.4%, respectively (Treesirichod *et al.,* 2014). This may explain the higher prevalence observed among medical and dental students in this study.

In this study, students who resided on-campus had higher prevalence of MRSA and MRCoNS. The difference was however statistically insignificant in comparison with students residing off-campus. The finding was not too surprising as both groups are community dwellers with similar living conditions. Similarly, the difference between the nasal colonization of MRSA and MRCoNS for males and females was not statistically significant. The finding agrees with several previous studies (Okodua *et al*., 2013; Abadi *et al.,* 2015; Ayepola *et al.,* 2018).

MRSA showed poor susceptibility to most antibacterial agents tested in this study. Gentamicin and ofloxacin were however the most active antibacterial agents against MRSA and showed statistical significance in comparison with MSSA. The susceptibility profile of MRSA to gentamicin in this study is similar to another in Ekpoma which observed 100% susceptibility of MRSA to gentamicin (Okodua *et al.,* 2013). In that study however, 100% susceptibility was observed for MSSA to gentamicin unlike this study where 39.8% was observed. MSSA were equally resistant to several other antibiotics tested. Antibiotic abuse is rife in our environment as has been previously stated (Ibadin *et al.,* 2017). Similarly, MRCoNS and MSCoNS showed poor acitivity to the antibacterial agents tested. Gentamicin was however the most active antibiotic with 75% of MRCoNS being susceptible while MSCoNS were poorly susceptible. The finding compares with an Iranian study which reported 100% efficacy for gentamicin against nasal CoNs from students in which several SCC*mec* types were detected (Abadi *et al.,* 2015). The observation that most MSCoNS were resistant to other antibacterial agents may imply that some other mechanism of resistance may be at play and poses potential health risk to carriers should such strain be implicated in opportunistic infection. Abuse of antibiotics can serve to create selective pressure, ensuring the survival of resistant bacterial strains (Ayepola *et al.,* 2018).

CONCLUSION

The prevalence of nasal MRSA and MRCoNS in this study was 37.2% and 33.3% respectively. Gentamicin was the most effective antibiotic against methicillin resistant staphylococci. Regulated use of antimicrobial agents is imperative in order to stem the tide of resistance.

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