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RELATIONSHIP BETWEEN SOME SELECTED SOCIO DEMOGRAPHIC PROFILES AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG APPARENTLY HEALTHY RESIDENTS IN EKPOMA, NIGERIA

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ABSTRACT

This study assesses the relationship between some selected demographic profiles and methicillin resistant *Staphylococcus aureus* (MRSA) amongst 384 apparently healthy residents in Ekpoma, Edo, Nigeria. Following standard protocol, nasal swab samples were subjected to bacteriological investigation and *Staphylococcus aureus* isolates were identified by mannitol fermentation and coagulase positivity. Antimicrobial susceptibility test was performed via Kirby-Bauer's disc diffusion method on Mueller-Hinton agar medium. Results showed that *Staphylococcus aureus* was present in 136 (35.4%) samples (male: 33.8% and female: 37.0%) among which 27.9% were methicillin-resistant (male; 27.3%; and female; 28.6%). The distribution of nasal colonisation of *Staphylococcus aureus* was significantly higher ($P < 0.05$) among those within the age group of 41–50 years (66.7%) and civil servants (52.9%). Also, MRSA was common among age group 31 – 50 years (50%) and amongst civil servants (33.3%). The MRSA isolated were resistant to penicillin, ampicillin, cloxacillin (92.1%), tetracycline (68.4%), chloramphenicol (18.4%), streptomycin (18.4%), erythromycin (18.4%) and gentamicin (0%). Judging by the results, MRSA is prevalent in the study area and the use of gentamicin may be recommended as the drug of choice for the treatment of multi-drug resistant MRSA.

Keywords: Demographic profile, Antibiotic resistant, *Staphylococcus aureus*, Ekpoma.

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INTRODUCTION

Staphylococcus aureus is a facultative anaerobic gram positive coccal bacterium. It is frequently found as part of normal skin flora on the skin and nasal passages (Kluytmans *et al*; 1997). The ability to up-regulate virulence factors under stressful stimuli (e.g. host immune response or circulating antibiotics) is a key factor in the enabling of *Staphylococcus aureus* to persist in the blood stream, to seed deep tissues, and to form secondary foci of infection. *Staphylococcus aureus* strains have effectively been able to adhere to and colonize the skin and mucosa of nares, to form protective biofilms and to develop resistance to several antibiotics. Consequently, despite the availability of many antibiotics with activity against wild strains type, *Staphylococcus aureus* has remained a highly successful and increasingly clinically important Gram positive pathogen (Weidenmaier *et al;* 2004).

Methicillin-Resistant *Staphylococcus aureus* (MRSA) are strains of *Staphylococcus aureus* which are resistant to methicillin and related penicillins and are particularly difficult to treat because they are also resistant to most other common antibiotics (Cheesbrough, 2000). It is well recognized that nasal carriage of MRSA represents a major risk factor for subsequent infection and transmission of this pathogen (Kluytmans *et al;* 1997; Wertheim *et al.,* 2005). Although several studies have reported the prevalence of MRSA nasal carriages in patients in health care settings (Warren *et al*., 2006; Munoz et *al.,* 2007); this subject has rarely been investigated in healthy individuals (Kuehnert *et al*., 2006).

Community–acquired MRSA did not evolve de novo in the community but represents a hybrid between MRSA that spread from the hospital environment, and strains that were once easily treatable in the community (Zautner, 2010). Since healthy carriers of MRSA are potential source for the spread of these organisms to other body sites as well as to other individuals, this study was therefore designed to determine the prevalence as well as the antibiotic susceptibility patterns of Methicillin–Resistant *Staphylococcus aureus* (MRSA) among apparently healthy residents in Ekpoma, Edo, Nigeria.

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MATERIALS AND METHODS

Study area: This project work was carried out in Ekpoma town located in Esan West Local Government Area, Edo State, Nigeria. Ekpoma lies between longitude 6.13°E and latitude 6.73°N and has a population of about 61,870 people (Population of Cities, 2007).

Sample size: A total of 384 nasal swab samples were collected randomly from apparently healthy male and female subjects. The sample size was calculated using the formula by Araoye (2004):

$$n = \frac{Z^2 pq}{d^2}$$

Where n = the desired sample size (when population is greater than 10,000). Z = the standard normal deviate, usually set at 1.96 (or more simply at 2.0), which corresponds to the 95 percent confidence level. p = the proportion in the target population estimated to have a particular characteristic. Since there was no reasonable estimate, p was taken as 50% (i.e. 0.50). q = 1.0 – p

d = degree of accuracy desired, usually set at 0.05 or occasionally at 0.02.

$$n = \frac{(1.96)^2 (0.50)(0.50)}{(0.05)^2} = 384$$

Sample population: This study involved 384 male and female residents of Ekpoma, Edo State, Nigeria.

Research design: This study was carried out within a period of two months. A total of 384 nasal swab samples from apparently healthy males and females were collected randomly and used for this project work.

Exclusion criteria: Subjects with respiratory infections such as catarrh, sore throat, asthma, common cold and other ailments as well as those who were on antibiotics or inhaler spray were excluded from this study.

Sample collection: Three hundred and eighty four (384) nasal swab samples used for the study were collected randomly from apparently healthy subjects in Ekpoma. Subject's age ranged between 16– 65 years.

The samples (nasal swabs) were collected in good light vision from subjects bending their heads backward to collect the specimens deep down the anterior passages using a sterile swab stick. Both right and left nostrils were swabbed. They were labeled alongside the data for sex, age and occupation. The swab sticks were carefully returned to their sterile containers, sealed with adhesive tape, and labelled as appropriate. The collected specimens were then taken to the laboratory where bacteriological analysis was carried out immediately.

Data analysis: Data were analyzed using Statistical Package for Social Sciences (SPSS) at a P value of 0.05 and 95% level of confidence and presented as mean±S.D (standard deviation) in suitable tables.

RESULTS

Of the 384 nasal swabs from the apparently healthy subjects examined, carriage rate of *Staphylococcus aureus* was found to be 136 (35.4%). In relation to sex, the nasal carriage of *Staphylococcus aureus* was 66 (33.8%) for males and 70 (37.0%) for females but the difference was not statistically significant ($P > 0.05$).

Out of 136 *Staphylococcus aureus* isolated, 38 (27.9%) were found to be methicillin-resistant. The prevalence of MRSA in males was 18 (27.3%) and was almost the same with those of females, 20 (28.6%); but the difference was not statistically significant ($P > 0.05$) (Table 1).

Table 2: shows the distribution of *Staphylococcus* species among the studied subjects by age groups. Nasal carriage of *Staphylococcus aureus* was found to be high within the age group (41 – 50) years and ≤ 20 years with prevalence of 6 (66.7%) and 27 (49.0%) respectively. The prevalence was generally low in the age group above 50 years. The difference in the nasal carriage rate of *Staphylococcus aureus* among the various age groups was statistically significant ($P < 0.05$).

The percentage of MRSA isolated from studied subjects was high within the age groups of 31 – 40 years and 41 – 50 years with a prevalence rate of 6 (50%) and 3 (50%) respectively, but less (11.1%) within the age group of ≤ 20 years. The prevalence was generally low in the age group above 50 years. The difference in the nasal carriage of *Staphylococcus aureus* among the various age groups was statistically significant ($P < 0.05$).

Table 1: Distribution of *Staphylococcus* Species among Studied Subjects by Sex

Sex	N	*S. aureus* (%)	MRSA (%)	MSSA (%)	*S. albus* (%)	No Growth (%)
Males	195	66 (33.8)	18 (27.3)	48 (72.7)	62 (31.8)	67 (34.4)
Females	189	70 (37.0)	20 (28.6)	50 (71.4)	34 (18.0)	85 (45.0)
Total	384	136 (35.4)	38 (27.9)	98 (72.1)	94 (25.0)	152 (39.6)
		$\chi^2 = 0.427$	$\chi^2 = 0.028$			
		P = 0.513	P = 0.867			

Key: N = number of isolates; MRSA = Methicillin-Resistant *Staphylococcus aureus;* MSSA = Methicillin-Sensitive *Staphylococcus aureus*

Table 2: Distribution of *Staphylococcus* Species among Studied Subjects by Age Groups

Age	N	*S. aureus* (%)	MRSA (%)	MSSA (%)	*S. albus* (%)	No Growth (%)
≤ 20	55	27 (49.0)	3 (11.1)	24 (88.9)	3 (5.5)	25 (45.5)
21 – 30	283	91 (32.0)	26 (28.6)	65 (71.4)	86 (30.0)	106 (38.0)
31 – 40	31	12 (38.7)	6 (50.0)	6 (50.0)	4 (12.9)	15 (48.4)
41 – 50	9	6 (66.7)	3 (50.0)	3 (50.0)	0 (0)	3 (33.3)
> 50	6	0 (0)	0 (0)	0 (0)	3 (50)	3 (50)
Total	384	136 (35.4)	38 (27.9)	98 (72.1)	96 (25.0)	152 (39.6)
		$\chi^2 = 13.092$	$\chi^2 = 8.167$			
		P = 0.011	P = 0.043			

Table 3: Distribution of *Staphylococcus* Species among Studied Subjects by Occupation

Occupation	N	*S. aureus* (%)	MRSA (%)	MSSA (%)	*S. albus* (%)	No Growth (%)
Students	305	106 (35.0)[a]	29 (27.4)	77 (72.6)	89 (29.0)	110 (36.0)
Civil servants	34	18 (52.9)[b]	6 (33.3)	12 (66.7)	4 (11.8)	12 (35.3)
Others*	45	12 (26.7)[c]	3 (25.0)	9 (75.0)	3 (6.6)	30 (66.7)
Total	384	136 (35.4)	38 (27.9)	98 (72.1)	96 (25.0)	152 (39.6)
		$\chi^2 = 6.130$	$\chi^2 = 0.329$			
		P = 0.047	P = 0.848			
		a vs b => $\chi^2 = 4.362$; P = 0.037				
		a vs c => $\chi^2 = 1.148$; P = 0.284				
		b vs c => $\chi^2 = 5.676$; P = 0.017				

Table 4: Antibiotic Susceptibility Patterns of the Two *Staphylococcus aureus* Strains Isolated

Antibiotic	MSSA (%), n = 98		MRSA (%), n = 38		χ^2 for Resistant Strains	P Value
	S	R	S	R		
Erythromycin	95 (96.9)	3 (3.1)	31 (81.6)	7 (18.4)	9.483	0.002
Gentamicin	98 (100)	0 (0)	38 (100)	0 (0)	120.941	0
Streptomycin	92 (93.9)	6 (6.1)	31 (81.6)	7 (18.4)	4.791	0.029
Tetracycline	33 (33.7)	65 (66.3)	12 (31.6)	26 (68.4)	0.054	0.816
Chloramphenicol	73 (74.5)	25 (25.5)	31 (81.6)	7 (18.4)	0.765	0.382
Cloxacillin	28 (28.6)	70 (71.4)	3 (7.9)	35 (92.1)	6.652	0.010
Penicillin	3 (3.1)	95 (96.9)	0 (0)	38 (100)	1.190	0.275
Ampicillin	6 (6.1)	92 (93.9)	0 (0)	38 (100)	2.434	0.119

Key: N = total number of isolates; S = Sensitive; R = Resistant; MRSA = Methicilin-Resistant *Staphylococcus aureus*; MSSA = Methicilin-Sensitive *Staphylococcus aureus*

Table 3: shows the distribution of *Staphylococcus* species among studied subjects by occupation. The nasal carriage of *Staphylococcus aureus* was high (18; 52.9%) among civil servants and less (106; 35.0%) among students and the difference was statistically significant ($P < 0.05$). Similarly, nasal carriage of *Staphylococcus aureus* was higher (106; 35.0%) in students than others (12; 26.7%) but the difference was not statistically significant ($P > 0.05$); while nasal carriage among civil servants was higher 18 (52.9%) than others 12 (26.7%) and was statistically significant ($P < 0.05$)

The prevalence of MRSA was high (6; 33.3%) in civil servants and less in students and others with prevalence of (29; 27.4%) and (3; 25%) respectively. The difference in the nasal carriage among the various types of occupation was not statistically significant ($P > 0.05$).

Table 4: shows the antibiotic susceptibility patterns of the two *Staphylococcus aureus* strains isolated. From the table, gentamicin had the highest percentage of sensitivity (100%) on MRSA strain. Cloxacillin had the least percentage sensitivity (7.9%) while the strain was generally resistant to penicillin and ampicillin. The percentage of erythromycin resistance to Methicillin-Sensitive *Staphylococcus aureus* (MSSA) strain was 3.1% but 18.4% for MRSA strain and the difference was statistically significant ($P < 0.05$). Similarly, the percentage of streptomycin resistance to MSSA strain was 6.1% but 18.4% for MRSA strain and the difference was statistically significant ($P < 0.05$). The percentage of cloxacillin resistance to MSSA strain was 71.4% but 92.1% for MRSA. The difference was also statistically significant ($P < 0.05$)

DISCUSSION

The observed nasal carriage rate of *Staphylococcus aureus* (35.4%), agrees with the findings of Mainous *et al* (2006) who found that the prevalence of nasal carriage of *Staphylococcus aureus* in US population was 32.40%. The agreement between these results might be due to the similarities in the method of isolation.

Similarly, our findings on the statistically non-significant but higher nasal carriage rate of *Staphylococcus aureus* in females (37.0%) than in males (33.8%), is at variance with the findings of Lamikanra *et al* (1985) that a significantly greater proportion of females (65.0%) than males (46.5%) were carriers of *Staphylococcus aureus* in 548 Nigerians screened. The noted variation might be due to differences in the areas of study as well as the sample size.

Also, the fact that the observed nasal carriage of methicillin-resistant *Staphylococcus aureus* was 27.9%, is similar to that reported in India, where out of 129 *Staphylococcus aureus* isolates, 31 (24%) isolates were oxacillin resistant and these are referred to as MRSA (Majumdar *et al.,* 2009). Although nasal carriage rate of MRSA in males (27.3%) was almost the same with those of females (28.6%), the difference was statistically significant. This finding is at variance with the study carried out by Shakya *et al* (2010) , who observed an MRSA prevalent rate of 5.6% and 8.5% in male and female respectively ($P > 0.05$). Comparatively, the hospital study-setting by Majumdar *et al.,* 2009, might account for the observed differences.

Furthermore, the comparative difference in the prevalence of *Staphylococcus aureus* amongst the subjects of varying ages, contradicts the findings reported by Shakya *et al* (2010). The difference in the two results could be due to the differences in the age range of studied subjects. In addition, the high prevalence of MRSA among the ages 31 – 40 (6; 50%) and 41 – 50 (3; 50%) years compared to those of ages ≤ 20 years and above 50 years indicates that age difference might be the determining factor.

As regards the occupation-based significant difference in carriage rate among the subjects, no clear bases for comparison exist since no similar study has been documented. Nevertheless, the observed prevalence among students is at variance with the study conducted among University students in Brazil by Prate et al. (2010), whereby the percentage of nasal carriage of *Staphylococcus aureus* was 40.8%. However, the inclusion of other participants apart from students, as well as the differences in sample size and study area, may account for this variation. On the other hand, the observed higher carriage rate of MRSA amongst civil servants, may possibly be due to antibiotic abuse, while the rare usage of gentamicin might account for its highest percentage sensitivity.

Finally, the results of this study have shown that the nasal carriage rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among apparently healthy residents in Ekpoma is 27.9%, although MRSA resistance to commonly used antibiotics was considered low when compared to values from some other studies. However, the application of molecular biology techniques to monitor the epidemiology of MRSA in healthy individuals is highly recommended. Also, gentamicin is recommended as the drug of choice for the treatment of multi-drug resistant MRSA infections.

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AUTHOR'S CONTRIBUTION

Okodua, M. and Ebhodaghe, E.E. were involved in the literature search and manuscript drafting. Ebhodaghe, E.E. and Adeleke, G. were involved in sample collection, while Ebhodaghe, E.E., Turay AA. and Ijiekhuamen, M were involved in sample analysis. All the authors were involved in the proof-reading of this manuscript.