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Research article

Epidemiology of methicillin-resistant *Staphylococcus aureus* among hospitalized patients and apparently healthy individuals in Ekiti and Ondo States, Nigeria

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ABSTRACT

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitalized patients and apparently healthy individuals was investigated in two western states of Nigeria using standard microbiological methods. One thousand and two hundred non-repeat isolates of *S. aureus* were recovered from the subjects. At varying degrees the isolates were resistant to cotrimoxazole (54.8%), augmentin (36.9%), pefloxacin (35.9%), gentamycin (28.3%), erythromycin (24.9%), vancomycin (10.3%), ofloxacin (5.2%) and ciprofloxacin (0.3%). One hundred and fifty six (13.0%) were resistant to methicillin out of which 4.8% and 8.2% were from healthy individuals and patients respectively. There was no correlation between prevalence of MRSA and age or sex ($p < 0.05$). There was no correlation between the antibiotic resistance pattern in MRSA from healthy volunteers and patients ($P < 0.05$). A total of 9.0%, 12.2% and 21.2% of the MRSA were resistant to 3, 4 and 5 antibiotics respectively. Antibacterial activities of five biocides examined using agar diffusion method showed that 38.5%, 53.2%, 59.6%, 61.5% and 71.8% of the MRSA were not inhibited by Izal®, Morigad®, Septol®, Dettol® and Purit® respectively, at concentrations two times higher than the in-use concentration. This finding points to the fact that MRSA occurs among patients and in the communities in the study areas, which calls for a public health concern and awareness.

KEYWORDS: Hospital-acquired MRSA; community acquired MRSA; biocides, epidemiology; multiple antibiotic resistance.

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen causing diseases and infections in both man and animal. It causes wide varieties of acute suppurative infections which include superficial, subcutaneous, sub-mucous infections[47,13,38,2,32,50] characterized by inflammation, necroses, and abscess formation[5,7,31].

Staphylococcus aureus is an extremely versatile and frequent pathogen of humans both in the community and in the hospitals[10,35,7,37]. Despite standardized hygienic measures, the high rate of tolerance, adaptation to new environments and

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increasing resistance to antibiotics are the major factors responsible for the recent upsurge in the rate of staphylococcal infections[27,10,50].

The acquisition of *mec*A gene by *S. aureus* has been confirmed to be responsible for its methicillin resistance[29]. Methicillin-resistant *S. aureus* (MRSA) was first isolated in England and was initially considered a nosocomial pathogen, however in recent years, increasing numbers of MRSA strains have been isolated worldwide from patients with community-acquired infections[45,42,43,21,6]. *Staphylococcus aureus* represents a major public health threat, as MRSA strains are the leading cause of nosocomially acquired infections. MRSA infections present a challenge to infection control and treatment strategies, resulting in increased morbidity, mortality, and length of hospitalization and health care costs[44,28,6,38].

Kesah *et al.*[26] reported 21–30% MRSA in 8 African countries including Nigeria. Shittu et al.[48] also reported emergence of a variant of epidemic strain MRSA in South Western Nigeria. However, this study was aimed at isolating and determining of methicillin resistance among *S. aureus* strains from Ekiti and Ondo States, Nigeria.

MATERIALS AND METHODS

COLLECTION AND PROCESSING OF SAMPLES

One thousand and two hundred non-repeat samples of *Staphylococcus aureus* from various clinical specimens and apparently healthy volunteers were collected in Ekiti and Ondo States of Nigeria. Informed consent was obtained from all patients before the samples were collected and all experiments were performed in accordance with the international ethical standards. Samples were inoculated on Mannitol Salt Agar (MSA) and incubated at 35°C for 24 h. Colonies with brilliant yellow appearance were sub-cultured to get pure isolates. Pure isolates were identified by standard methods described by Olutiola *et al.*[39] and Fawole and Oso[18] while the results were interpreted according to Holts and collegues[23].

ANTIBIOTIC SENSITIVITY TESTING

The isolates were grown at 37 °C in Mueller-Hilton broth (Oxoid) for 18 h and diluted to an optical density of 0.1 (0.5 McFarland Standard) and stored at 4 °C. The disc diffusion method was used for susceptibility testing as described by Clinical and Laboratory Standard Institute, CLSI[12]. The isolates were tested against the following commercial antibiotic with their concentrations (µg): amoxicillin (25), augmentin (30), ciprofloxacin (10), cotrimoxazole (25), erythromycin (5), gentamicin (10), ofloxacin (30), oxacillin (5), perfloxacin (5), and vancomycin (5). Oxford *Staphylococcus aureus* (NCTC 6571) was used as control strain. Multiple antibiotic resistance was determined for strains that are not susceptible to oxacillin, which is taken for methicillin resistance according to Onemu and Ophori[40]. Agar dilution method was used to determine susceptibility of isolates to commonly used biocides.

STATISTICAL ANALYSIS OF DATA

Statistical analysis was done using SPSS (version 17) to determine frequency distribution, mean, harmonic mean, standard deviation, analysis of variance (ANOVA), Duncan Multiple Range and Pearson correlation coefficient.

RESULTS

The distribution of the *S. aureus* strains recovered from subjects is represented in Table 1. A total of 498 (41.5%) and 702 (56.5%) was recovered from both male and female subjects respectively. The antibiotic susceptibility test of 1,200 *S. aureus* is shown in Table 2. . One hundred and fifty-six (13.0%) were resistant to methicillin antibiotic.

Table 1. The distribution of *S. aureus* recovered from the study areas.

State	Healthy (%)		Patients (%)		Total	
	Male	Female	Male	Female	Male	Female
Ekiti	154 (12.8)	284 (23.7)	114 (9.5)	48 (4.0)	268 (22.3)	332 (27.7)
Ondo	191 (15.9)	308 (25.7)	39 (3.3)	62 (5.2)	230 (19.2)	370 (30.8)
Total	345 (28.8)	592 (49.3)	153 (12.8)	110 (9.2)	498 (41.5)	702 (58.5)

Antibiotic susceptibility test showed that most of the MRSA isolates were resistant to all the antibiotics tested in varying degrees. Resistance to cotrimoxazole was highest (87.2%) whereas it was least against ciprofloxacin (1.3%). This was followed by ofloxacin (37.2% resistance) and pefloxacin (46.8% resistance). One hundred and thirty six (87.2%) strains being highest were resistant to cotrimoxazole, 130 (83.3%) to amoxicillin and 118 (75.6%) to vancomycin. There was no statistically significant difference between the antibiotic resistance pattern in MRSA from apparently healthy volunteers and patients *($p<0.05$)*.

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Table 2. Distribution of *Staphylococcus aureus* according to antibiotics susceptibility testing.

Antibiotic	Ekiti (n = 600)				Ondo (n = 600)			
	Healthy (%)		Patients (%)		Healthy (%)		Patients (%)	
	R	S	R	S	R	S	R	S
Aug	159(13.3)	279 (23.3)	91 (7.6)	71 (5.9)	113 (9.4)	386 (32.2)	80 (6.7)	21 (1.8)
Amox	78 (6.5)	360 (30.0)	57 (4.8)	105 (8.8)	50 (4.2)	449 (37.4)	44 (3.7)	57 (4.8)
Cip	1 (0.08)	437 (36.4)	1 (0.08)	161 (13.4)	1 (0.08)	498 (41.5)	0 (0)	101 (8.4)
Cotr	211 (17.6)	227 (18.9)	109 (9.1)	53 (4.4)	251 (20.9)	248 (20.7)	87 (7.3)	14 (1.2)
Ery	91 (7.6)	347 (28.9)	53 (4.4)	109 (9.1)	84 (7.0)	415 (34.6)	71 (5.9)	30 (2.5)
Gen	125 (10.4)	313 (26.1)	97 (8.1)	65 (5.4)	83 (6.9)	416 (34.7)	35 (2.9)	66 (5.5)
Oflo	30 (2.5)	408 (3.4)	24 (2.0)	138 (11.5)	7 (0.6)	492 (41.0)	1 (0.08)	100 (8.3)
Oxa	9 (0.8)	429 (35.8)	51 (4.3)	111 (9.3)	49 (4.1)	450 (37.5)	47 (3.9)	54 (4.5)
Pef	202 (16.8)	236 (19.7)	67 (5.6)	95 (7.9)	118 (9.8)	381 (31.8)	44 (3.7)	57 (4.8)
Van	8 (0.7)	430 (35.8)	33 (2.81)	129 (10.8)	42 (3.5)	457 (38.1)	41 (3.4)	60 (5.0)

AMX=amoxicillin, AUG=augmentin, CIP=ciprofloxacin, COT=cotrimoxazole, ERY=erythromycin, GEN=gentamicin, OFL=ofloxacin, OXA=oxacillin, PEF=perfloxacin, VAN=vancomycin, S=Susceptible, R=resistance

Multiple antibiotic resistance (MAR) of MRSA strains was investigated. All the organisms showed multiple resistance in varying degrees ranging. Forty two (26.9%), 28 (18.0%) and 6 (3.9%) were resistant to 6, 7 and 8 antibiotics among the MRSA strains respectively. None was resistant to all the 9 antibiotics tested (Table 3).

Table 3. Multiple Antibiotic Resistance (MAR) Strain among MRSA strains.

Number of Antibiotic	MRSA strain	Percentage
3	14	9.0
4	19	12.2
5	33	21.2
6	42	26.9
7	28	18.0
8	6	3.9

Most of the MRSA isolates tested against the various concentrations/dilutions of the commonly used disinfectants were resistant. Overall, 156 (100.0%) strains of MRSA from both Ekiti and Ondo States were resistant to all the disinfectants at the concentrations lower than the in-use concentrations recommended by manufacturers. At the recommended concentrations, 109 (69.9%) strains of MRSA from Ekiti and Ondo states were resistant to Izal® whereas 145 (92.9%), 145 (92.9%), 144 (92.3%) and 140 (89.7%) were resistant to Septol®, Dettol®, Purit® and Morigad® respectively.

At concentrations above the recommended in-use concentrations, the antimicrobial activity of the disinfectants revealed that 91 (58.3%) MRSA strains from or from Ekiti and Ondo were resistant to Izal®while 109 (69.9%), 126 (80.0%) and 130 (83.3%) resistant to Morigad®, Dettol® and Purit® respectively. Septol® was the least effective among all the disinfectants with only 16.0 % of the isolates susceptible to it.

DISCUSSION

This study summarizes the results of a two-year surveillance in the two selected states of Nigeria. Most of the health care facilities surveyed are tertiary care specialist hospitals, tertiary institutions in Ekiti and Ondo States, Nigeria. A total of 1,200 isolates of *S. aureus* from various non-repeat clinical samples was recovered in the participating hospitals and communities out of which 156 (13.0%) were identified as MRSA. This was below that of Onemu and Ophori[40] that reported 79% resistance to methicillin among the clinical *S. aureus* isolates. This incidence of MRSA among the patients observed in this study was lower than the range between 24. % and 34.1 % recorded by Akujobi *et al.*[5] among different cadres of healthcare workers in Nigeria. Onemu and Ophori[40] reported 79.0% while 34.1% recorded by

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Akujobi *et al.*[5] in Edo and Anambra States both in Nigeria. Fifty eight (37.2%), 23 (40.0%) and 98 (62.8%) MRSA strains were isolated from apparently healthy volunteers, apparently healthy hospital personnel/health care workers (HCWs) and patients respectively. The high incidence of MRSA among patients could be due to reasons ranging from being acquired from hospital workers who introduced the organism into hospital environment (as nosocomial infection) from the community, abuse/misuse of antibiotics and low immunity in patients as a result of disease conditions[17,7,25,40]. This agrees with earlier reports by Abraham and collegues[1]. There was no statistically significant difference between the prevalence of MRSA and age or sex ($p<0.05$).

The antibiotic sensitivity pattern of the isolates shows that ciprofloxacin was the most effective followed by ofloxacin, pefloxacin, gentamycin, erythromycin, augmentin, vancomycin, amoxycillin and cotrimoxazole in that order (Table 2). Statistically, there was no significant difference between the resistance to antibiotics among MRSA from healthy volunteers and patients ($p < 0.05$).The result of this study reveals the efficacy of members of floroquinolones over the other antibiotics. The study shows that the likes of cotrimoxazole, amoxycillin, vancomycin, augmentin and erythromycin, to a high degree, were an unreliable empirical choice of antibiotics for the treatment of infections with MRSA strains as the aetiological agents[17,46,49].

Considering the general overview of the antibiotic susceptibility profile of the strains isolated in this study, the susceptibility of the MRSA strains to ciprofloxacin (98.7 %) may be due in part to it non-availability and exorbitant cost unlike the commonly used antibiotics which are cheaper and could easily be afforded. The newer and expensive antibiotics have undoubtedly high antibacterial efficacy against MRSA in this study.

The antibacterial activity of five commonly used biocides on the 156 MRSA strains showed the efficacy of the biocides used Izal®, Morigad®, Septol®, Purit® and Dettol® in that order on MRSA strains isolated. The finding in this study agrees with reports that resistance to benzalkonium chloride, a biocide, has been closely linked to oxacillin resistance in *S. aureus*[33,24,16].

It was reported that resistance to both biocides and antibiotics can be plasmid-mediated. Plasmid-mediated resistance to biocides is a well-recognized phenomenon. Such resistance to quaternary ammonium chlorides and other biocides has been identified in nosocomial pathogens[51,14]. Resistance to antibiotics and biocides may be due to the common target site between the two antimicrobial agents which may bring about selection of mutants altered in such target by either agents or the emergence of cross-resistance[9,20,30]. It has been suggested that subtle differences in the biocide susceptibility of antibiotic-resistant strains might facilitate their selection and maintenance in the environment by low-sub-effective concentrations of the agents[34,35,41,15] owing to indiscriminate and on inappropriate use[11,19,22,16,24,41].

This study shows that there is a relatively high incidence of MRSA (13%) that are resistant to antibiotics and biocides in the study locations. A continuous surveillance is therefore recommended because of the public health importance of the organism and the high danger this may potent.

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