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Prevalence Of Methicillin- Resistant Staphylococcus Aureus And Coagulase-Negative Staphylococci Among Male Students In A Private Tertiary Institution And Their Enterotoxin -producing Potential

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ABSTRACT

In order to determine the prevalence and antimicrobial resistance among methicillin-resistant, methicillin-sensitive and coagulase-negative staphylococci in a tertiary institution, nasal swabs were collected from 100 male students. A total of 98 staphylococci were isolated, out of which 51 were coagulase positive. Methicillin resistance among the *Staphylococcus aureus* isolates was 25.5%. The carriage rate of methicillin-resistant coagulase-negative staphylococci among the subjects was 11%. *Bacillus* spp. was isolated in pure cultures from 5 subjects and of these isolates one was resistant to methicillin. Antimicrobial susceptibility of the methicillin- resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCONS) isolates to various antibiotics tested showed that, in most cases, the MRSA were less susceptible to the antibiotics than MRCONS. None of the MRSA was resistant to vancomycin. There were no identified risk factors in this study. Twenty-six strains of *Staphylococcus aureus* isolates produced staphylococcal enterotoxins. Staphylococcal enterotoxin A (SEA) was produced by 21 strains (8 from the 13 MRSA isolates). *Staphylococcus saprophyticus* produced SEA (11 strains), SEB (2 strains) and SEC (2 strains).

INTRODUCTION

Staphylococcus aureus infections have continued to rise in health care facilities in recent times, with increased emergence of strains resistant to methicillin called methicillin-resistant *S. aureus* (MRSA). Studies have also shown that these strains have become important in community-acquired infections [1-4]. A number of studies have investigated health care workers and individuals associated with them for nasal carriage of *S. aureus*. Weng-Tsung *et al*. [5] and Ciftci *et al*. [6], in their studies involving children in Turkey and Taiwan reported 13.2% and 0.3% community-acquired MRSA carriage in health workers' children respectively. In a similar study involving adult health care workers', Kalsoom *et al.* (7) found the prevalence of nasal carriage of *S. aureus*, coagulase-negative staphylococci (CONS) and MRSA to be 48%, 46% and 14% respectively. A 2007 report estimated the number of MRSA infections treated in hospitals to have doubled in the USA (8). Many clinical infections arise from spread from healthy carriers but most surveillance of *S. aureus* and MRSA have focused on individual with invasive infection rather than on an entire population (4,9). *S. aureus* nasal carriage, present in about 20% of the general population, has been identified as a key factor for the subsequent development of community-acquired and nosocomial staphylococcal infections (10, 11). Methicillin-resistant *S. aureus* carriage has been significantly correlated with the presence of skin lesions, antibiotic usage and prior hospitalization (12). There is paucity of data on the prevalence and antibiotic susceptibility patterns of MRSA in community settings such as a University far removed from a hospital setting. Most data on the prevalence and susceptibility patterns of *S. aureus* in Nigeria have been from clinical isolates. Nasal carriage of methicillin-resistant *S. aureus* has varied prevalence from region to region and from one country to another (13, 14). Proper knowledge of the prevalence and local antimicrobial resistance patterns of *S. aureus* is essential for understanding the epidemiology of *S. aureus* infections and prescription of adequate therapy and formulation of infection control policies. There are few reports in Nigeria on the enterotoxin production ability of isolates of *S. aureus* from nasal passage of healthy carriers. This study aims at documenting the prevalence, antibiotic susceptibility patterns and enterotoxigenicity potentials of staphylococci isolated from male students in a private University in southwestern Nigeria.

MATERIALS AND METHODS

Sample population, Specimen Collection & Processing

The study population involves male students of Redeemer's University, Mowe, Ogun State, Nigeria. Informed consent was obtained from the students before samples collection. The sampled population was about 12% of the total male students in the University at the time when this study was carried out. All participants were students who had not been exposed to the health care system or attended hospitals or used any antibiotic during the previous 6 months. Written questionnaires containing information such as practices like nose picking, smoking, handwashing, handshake, and closeness to or relationship with health workers were completed by the subjects examined. The specimens were collected as follows: a sterile cotton-tipped swab was moistened in sterile distilled water and swirled inside the anterior nares and rotated clockwise and anticlockwise four times and plated by streaking onto mannitol salt agar (MSA) (Oxoid), a selective medium for the isolation of S. *aureus.* The plates were incubated at 37°C for 24 hours and observed for growth. After growth, staphylococci were identified on the basis of colonial characteristics, Gram stain and biochemical reactions, namely, catalase test, coagulase test (both slide and tube methods), urease test, DNAase test with DNAse agar plate (Oxoid Ltd. , Basingstoke, U.K.), alkaline phosphatase test, β-galactosidase test and by fermentation of carbohydrates such as glucose, sucrose, lactose, mannitol, mannose and trehalos. Pure cultures of isolates were preserved on tryptone soy agar (Oxoid) slants for further analysis. *Bacillus* species were characterized by conventional microbiological methods using morphology of vegetative cells, shape and position of spores, nitrate reduction, degradation of starch, urea, casein, gelatin, acid production from glucose, mannitol, xylose, citrate utilization, lecithinase test, growth at 4°, 10°, 25°, 30°, 37°, 40°, 50°, 55°C, growth in nutrient broth with 6% NaCl [15].

Antimicrobial susceptibility testing

The agar diffusion method recommended by the CLSI [16] was employed to determine the susceptibility to antimicrobial agents. The following antibiotic discs were tested: ciprofloxacin, 5µg; penicilin G, 10g; gentamicin, 10µg; amoxycilin, 25µg; rifampicin, 5µg; tetracycline, 10µg; vancomycin, 30µg; erythromycin, 5µg; chloramphenicol, 30µg, augmentin, 30µg. Zone diameters were measured after incubation for 24 hours at 37°C and results were classified following CLSI [16] interpretive standards. For the determination of methicillin resistance, the cefoxitin disk test [16] was employed. The medium used was Mueller – Hinton agar (Oxoid) and the inoculum of isolates was standardized to 0.5 MacFarland turbidity.

Enterotoxin production test

The SET-RPLA kit made by Oxoid, UK was employed to determine the enterotoxin production of the isolates of *Staphylococcus aureus* and coagulase-negative staphylococci, according to manufacturer's instructions.

Statistical analysis

The data obtained by questionnaires and experiment were analysed using SPSS software, version 15.0 [17]

RESULTS

A total of 103 strains of bacteria were isolated from the 100 nasal samples collected. A total of 95 out of 100 students were found to be carriers of *Staphylococcus* species. Out of those positive for staphylococci 51 (53.7%) were carriers of *S. aureus*. Four of those that carried *S. aureus* also carried coagulase-negative staphylococci (3 with mixed culture of *S. epidermidis* and one with mixed culture of *S. saprophyticus*). The CONS isolated included *S. epidermidis* (11 strains), *S saprophyticus* (30 strains), *S. haemolyticus* (5 strains) and *S. saccharolyticus* (2 strains). The carriage rate of CONS among the subjects was 48%. The prevalence of MRSA among the *S. aureus* isolates was 25.5% (Table No.1). Five samples yielded *Bacillus* spp. as pure cultures, which included *Bacillus subtilis* (4 isolates) and *Bacillus cereus* (1 isolate).

The antimicrobial susceptibility of the MRSA and MRCONS is shown in Table No.2. The susceptibly of the S. aureus isolates showed that the methicillin-resistant coagulase-negative strains were more susceptible to the antibiotics tested when compared with the MRSA isolates. Only for chloramphenicol and vancomycin were higher resistance recorded for MRCONS than for MRSA. Majority of the MRSA isolates were resistant to penicillin (96.9%), amoxycillin (84.6%) and augmentin (76.9%).

Result of the enterotoxin production showed that 8 of the 13 MRSA produced staphylococcal enterotoxin A (SEA) while some of the other *S. aureus* strains produced SEA (16 strains) and SEC (5 strains). The CONS, particularly *S. saprophyticus* produced SEA (11 strains), SEB (2 strains) SEC (2 strains).

Table No.1: Number of isolates resistant and sensitive to methicillin

Organism	Methicillin resistant	Methicillin sensitive	Total
Staph. aureus	13	38	51
CONS	11	36	47
Bacillus spp.	1	4	5
Total	25	78	103

Table No. 2: Antibiotic resistance pattern of methicillin resistant *S. aureus* and coagulase- negative staphylococci.

Antibiotic	% resistant	
	Staphylococcus aureus	Coagulase-nagative staphylococci
Penicillin	92.3	81.8
Vancomycin	0	9.1
Ciprofloxacin	46.2	18.2
Rifampicin	23.1	18.2
Augmentin	76.9	63.6
Amoxycillin	84.6	81.8
Erythromycin	46.6	45.5
Gentamicin	53.8	27.3
Tetracycline	76.9	36.4
Chloramphenicol	15.4	36.4

DISCUSSION

Among the 100 students investigated, 51 (51%) were carriers of *S. aureus.* The carriage rate is higher compared with previous data in the United States [18] and among New York State prisoners [19].

It was found in this study that the ages of those who had nasal carriage of *S. aureus* fell mostly between ages 19 and 21 (58%). Twenty- five *S. aureus* were obtained from this group and of these 8 strains (32%) were MRSA strains. Prakash *et al.* [20] also reported 38% of *S. aureus* within the age group of 21-30 years with 21 isolates being methicillin resistant. However in investigations involving lower age groups in Taiwan and Turkey, a much lower percentages of MRSA were reported [6, 5]. Ogunzkaya-Artan *et al.* [21] reported 18% nasal carriage of *S. aureus* and 5.6% of MRSA among healthy preschool children. In this study 23.4% of the coagulase-negative staphylococci were resistant to methicillin, a result similar to that of Kalsoom *et al*.[7] and Guchi *et al*. [22] where 22% and 23.5% of CONS were resistant to methicillin respectively. The MRSA carriage rate 13/51 *S. aureus* isolates (25.5%) and among the students investigated (13%) were higher than those reported in previous data from studies from Italy [23], Trinidad and Tobago [24] and Canada [25].

The antibiotic susceptibility profiles observed in this study indicated that the MRSA were not only resistant to methicillin but to a range of other antibiotics tested.

For example resistance to penicillin was highest, 92.3% followed by amoxycillin, 84.6%, followed by augmentin and tetracycline, 76.9% each, ciprofloxacin and erythromycin (46.3% each). The least resistance was observed for chloramphenicol, 15.4%. The percentage resistance of MRSA to ciprofloxacin and erythromycin were higher than those reported by Prakash *et al*. [20] but lower for vancomycin. None of the MRSA in this study was resistance to vancomycin. In this study, it was observed that 16.7% of the health care workers children were carriers of MRSA. One practice of the student examined, i.e., cleaning of nostrils showed a close relationship to nasal carriage of MRSA with a P value of 0.069 (χ^2= 3.302, df= 1). Since there were no identified risk factors in this study it was difficult to link our community isolates of MRSA to any risk factor. Some studies [26, 27] have linked recent use of antibiotics to colonisation with community-acquired MRSA but our study could not validate this by reason of the subjects examined.

There had been reports by some authors of the production of enterotoxins by coagulase- negative *Staphylococcus* isolated from humans and animals [28, 29]. In this study, majority of the enterotoxin-producing *S. aureus* produced staphylococcal enterotoxin A. The production of enterotoxins by strains of *S. aureus* is of public health significance because of their role in diarrhoea, sinusitis, sepsis and osteomyelitis [30, 31]. SEA has been reported as the most common staphylococcal enterotoxin involved in food intoxication outbreak all over the world. Enterotoxin production by coagulase–negative staphylococci should also not be ignored as CONS are becoming increasingly important as pathogens.

The results of this investigation suggests that nasal carriage of *Staphylococcus aureus* continue to play a significant role in the pathogenesis of community-acquired infections and underscores the need for continued surveillance to control emerging infections associated with MRSA. Nasal carriage of MRSA and CONS producing enterotoxin could also add to the burden of staphylococcal infections like food poisoning, sinusitis, septic arthritis [29, 30, 32, 33].

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