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PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* WITH REDUCED SUSCEPTIBILITY TO VANCOMYCIN AMONG HEALTHY WOMEN IN TWO NIGERIAN METROPOLITAN CITIES.

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections, which had been uniformly susceptible to vancomycin, are increasingly reported to develop resistance to the same antibiotic worldwide and infections caused by *S. aureus* with reduced susceptibility to vancomycin are a new clinical and public health dilemma. This study investigated its prevalence in urine of healthy women in two cities of Nigeria and it resistance pattern to other antibiotics. "First catch" urine samples collected from healthy women volunteers in Abuja and Zaria cities in Nigeria were cultured and screened for *S. aureus* using standard microbiological procedures. The isolates were then subjected to antibiotic susceptibility testing using disc diffusion technique. A total of 114 *S. aureus* isolates and 57 coagulase-negative staphylococci (CoNS) were isolated from 300 samples of urine screened. Isolates from Abuja were made up of 60 (63.2%) *S. aureus* and 35 (36.8%) CoNS, while those from Zaria women were made up of 54 (71.7%) *S. aureus* and 22 (28.9%) CoNS. Of the *S. aureus* isolated, 46(76.7%) and 37(68.5%) were methicillin-resistant in Abuja and Zaria respectively while 37 (80.4%) and 33(89.2%) of the methicillin-resistant isolates in Abuja and Zaria respectively had reduced susceptibility to vancomycin. These strains had very low resistance to ofloxacin, ciprofloxacin, sparfloxacin and gentamicin but high resistance to cephalexin, ampicillin and clindamycin. The high prevalence of *S. aureus* with resistance to both methicillin and vancomycin in the urine of healthy women in these cities emphasizes the importance of the prudent use of antibiotics and the use of infection-control precautions to prevent their transmission.

***Keywords: Staphylococcus aureus*, prevalence, methicillin, vancomycin, resistance, urine, healthy women.**

INTRODUCTION

Staphylococcus aureus has been recognized as one of the major cause of infection in human beings, occurring in both community and the hospital. (1,2) *S. aureus* was uniformly sensitive to Penicillin when it was introduced in the late 1940s but resistant strains producing β-lactamase enzyme soon emerged (3,4). The introduction of the β-lactamase stable penicillins (methicillin) marked an initial victory over β-lactamase producing penicillin-resistant *S. aureus* infections until the 1980s when methicillin-resistant *S. aureus* (MRSA) became endemic in many hospitals, (5,6). This resistance was found to be as a result of the production of altered cell wall synthesis enzymes, the penicillin-binding proteins (PBPs) that are encoded on the *mecA* gene and function by preventing the binding of penicillins (4).

Glycopeptide antibiotics (vancomycin, teicoplanin) introduced into clinical practice in 1958 for the

treatment of Gram-positive bacteria, (6) were found to be effective in the treatment of MRSA infections by virtue of their unique mechanism of actions (1, 7). The emergence of Vancomycin -resistant strains of coagulase-negative staphylococci (CoNS) caused concern that such observation might presage similar developments in *S. aureus* (6, 8, 9). The threat of vancomycin resistant in *S. aureus* has been the topic of intense research and concern in the scientific world (10). Various levels of vancomycin-resistance have since been encountered in MRSA infections worldwide (10). Problems created by the increasing level of antimicrobial resistance have focused attention on measures for fighting antimicrobial resistance, foremost of which is susceptibility surveillance (11).

MRSA are often multi-drug resistant leading to failure of empirical therapy; therefore, the knowledge of the prevalence of such pathogens and their antimicrobial susceptibility pattern will be essential for infectious disease mangers in their routine work.

It is generally believed that effective antibiotic therapy in developing countries is being compromised by the large reservoir of antibiotic resistant bacteria that exists within their population. The healthy members of the community represent the largest reservoir of bacteria resistant to antimicrobial agents.

This study determined the prevalence of MRSA and MRSA that are resistant to vancomycin in urine samples from healthy women in Abuja and Zaria, Nigeria.

MATERIALS AND METHODS

Sample collection

First "clean catch" urine samples were collected randomly from 150 women each from Abuja and Zaria communities after informed consent had been obtained from each woman. Samples were collected into sterile bottles, kept in an iced-bag and transported to the laboratory.

Bacteriology

Each urine sample was immediately inoculated into Mannitol Salt agar (Lab M, International Diagnostics, UK) plates and incubated aerobically at 37^{O}C for 18hrs. The characteristic isolates were asceptically isolated and characterized using established microbiological methods, which included colonial morphology, Gram stain characteristics, ability to produce enzymes peroxidase and coagulase (12, 13).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility pattern of *Staphylococcus aureus* isolates to ampicillin 10μg (Medreich, India), cephalexin 30μg (Fidson, India), clindamycin 2μg (Pharmacia, Belgiun), gentamicin 10μg (Wuham, China), ofloxacin 5μg (Pathoteq, India), ciprofloxacin 5μg (Fidson, India), pefloxacin 5μg (Fidson, India), sparfloxacin 5μg (Pathoteq, India), vancomycin 30μg (Dumex, S. Denmark), and methicillin 10μg (Oxoid, UK) were determined by the modified Kirby –Bauer diffusion technique (12). Standardized overnight culture of each isolate (counting about 10^6 cfu/ml) was used to flood the surface of Mueller Hinton agar (MHA) plates, excess drained off and dried. The standard antibiotic discs were then asceptically placed at reasonable equidistance on the inoculated MHA plates and allowed to stand for 1hr. The plates (prepared in duplicates for each isolate) were then inoculated at 37^{O}C for 18hrs (14).

The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and isolates classified as "resistant" "intermediate" or 'sensitive" based on the standard interpretative chart updated according to the current NCCLS standard (12, 15).

Statistical Analysis

Frequencies were obtained and percentages were calculated for study variables. Chi-square and two-tailed Fisher's exact test were used to calculate probabilities and determine significance. A p-value of less than or equal to 0.05 is considered to be statistically significant ($p \leq 0.05$).

RESULTS

Bacteriology

A total of 114 *Staphylococcus aureus* isolates and 57 coagulase-negative staphylococci (CoNS) were isolated from 300 urine samples screened. Isolates from Abuja women were made up of 60 (63.2%) *Staphylococcus aureus* and 35 (36.8%) CoNS while those from Zaria women were made up of 54 (71.1%) *Staphylococcus aureus* and 22 28.9%) CoNS.

Antimicrobial Susceptibility Testing

The Performance Standards for Antimicrobial Disc Susceptibility Test Approved Standard was described by NCCLS(15). By this an isolate was considered methicillin resistant when diameter of zone of inhibition produced by methicillin $10\mu g$ disc was $\leq$17mm and Vancomycin resistant when the diameter of zone of inhibition produced by Vancomycin $30\mu g$ disc was $\leq$ 15mm. Table 1 shows the relative proportion of the *Staphylococcus aureus* isolates that are methicillin-resistant (MRSA) and Vancomycin-resistant (VRSA) in Abuja and Zaria. The resistance pattern of the MRSA and VRSA isolates from Zaria and Abuja to other antimicrobial agents is shown in Figures 1 and 2. The two-tailed fisher's exact test was conducted on the relative proportion of MRSA and VRSA. It showed that the observed difference between the two cities with respect to MRSA and VRSA isolates was not statistically significant ($p>0.05$) while the difference observed between VRSA and MRSA isolates in Abuja and in Zaria was significant ($p<0.05$). That is, there is a significant relationship between the isolates of VRSA and MRSA in each of the two localities.

Table 1: Distribution of Staphylococci, MRSA and VRSA among healthy women in the two cities

CITIES	TOTAL SAMPLE (URINE)	No of Staphylococci $n_A = 95$ $n_Z = 76$		MRSA No (%)	VRSA No (%)	MRSA/VRSA No (%)
		Co NS No (%)	**S.aureus** No (%)			
ABUJA	150	35 (36.8)	60(63.2)	46(76.7)	41(68.3)	37(61.7)
ZARIA	150	22(28.9)	54(71.1)	37(68.5)	36(66.7)	33(61.1)

Key: n_A = Number in Abuja n_Z = Number in Zaria

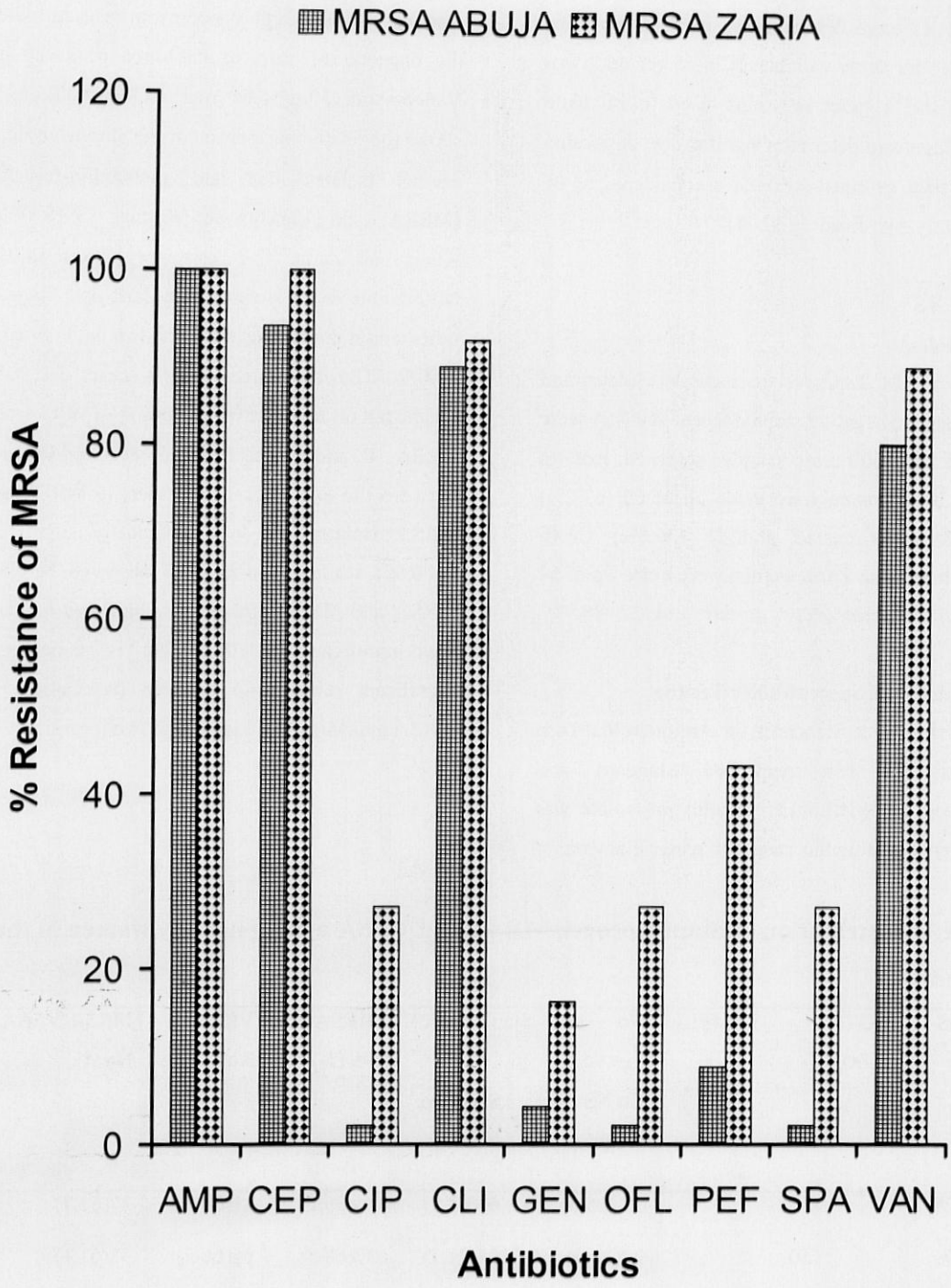

Figure 1: Resistance pattern of MRSA isolates from the two cities to other antibiotics.

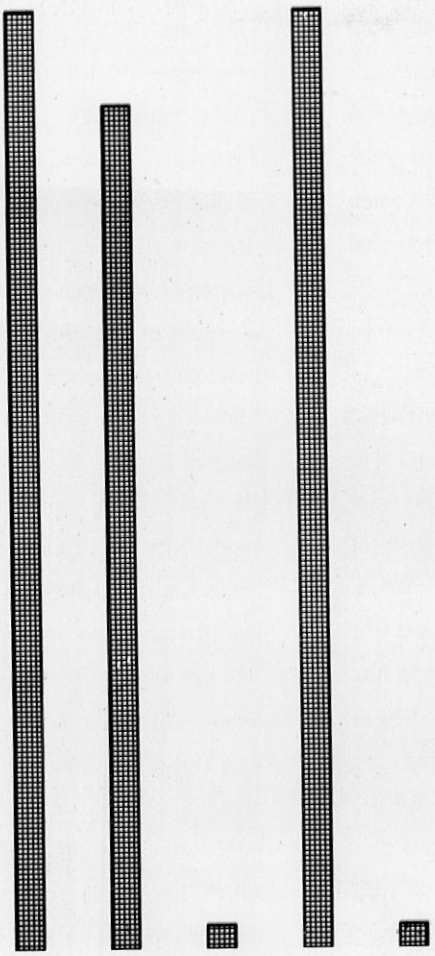

Figure 2: Resistance pattern of VRSA isolates from the two cities to other antibiotics

DISCUSSION

Infections caused by vancomycin-resistant *Staphylococcus aureus* (VRSA) are well documented and have been reported from Asia and the USA (2, 16-18). Our study reports the prevalence of MRSA isolates from healthy women that exhibited reduced susceptibility to vancomycin in Abuja and Zaria cities in Nigeria.

The result of our study showed a high prevalence rate of MRSA at 46 (76.7%) and 37 (68.5%) in Abuja and Zaria respectively, of which 37(80.4%) and 33 (89.2%) of the isolates from Abuja and Zaria respectively were resistant to vancomycin. Such a high prevalence of MRSA with high level of resistance in these environments where methicillin and vancomycin are rarely used for treatment of infections call for great concern as it is observed in the healthy (asymptomatic) women in these communities. This result is contrary to various reports of 100% susceptibility of MRSA infections to vancomycin (19,20). However our findings support the report by Olayinka *et al* (21) which had 57.7% VRSA and, the reports from Japan, France, United States, Korea and Germany of infections caused by MRSA strains with intermediate susceptibility to vancomycin (2,18,22,23).

All previous reports of patients who were infected or colonized with VRSA described a history of prolong vancomycin exposure (24). However the asymptomatic women volunteers in our investigation lived in communities where vancomycin is rarely used for treatment of infections or diseases and they were not on any antibiotics at the point of investigation. Therefore, concurrent or recent vancomycin exposure is not a prerequisite for the development of VRSA. Instead, it can be concluded that frequent use of other commonly available antibiotics provided sufficient selective pressure to promote colonization and/or infection with vancomycin resistant enterococci (25) and MRSA, eventually resulting in the emergence of VRSA (26).

These observed recent alarming increasing emergence of vancomycin resistance to MRSA strains might be due to acquisition of resistance determining genes like *van A, B, C* responsible for vancomycin resistance in enterococci (22,26,27) or a result of the thickening of the cell wall as reported by some authors (18,28).

The susceptibility pattern of the MRSA and VRSA isolates to other antibiotics revealed that they generally show high resistance to ampicillin, cephalexin and clindamycin but show low resistance to pefloxacin, ofloxacin, gentamicin, ciprofloxacin and sparfloxacin. This support the findings that MRSA isolates are generally resistant to other β-lactam antibiotics (29). The observed high resistance to clindamycin which is one of the antibiotics used in the treatment of MRSA infections (20) supports the report by Lu *et al* (30) which had 92.9% community-associated MRSA isolates showing resistance to clindamycin.

In this study, it was established that both the MRSA and VRSA isolates from both cities had over 70% susceptibility to ofloxacin, ciprofloxacin, sparfloxacin and gentamicin indicating that these antimicrobial agents could be used in the treatment of infections caused by MRSA and VRSA. This supports the previous reports where ciprofloxacin and gentamicin were used in the treatment of MRSA and VISA infections (10, 20).

The difference observed in the prevalence rates of MRSA and VRSA in two cites is highly significant ($p<0.05$) which suggest that there is an association between MRSA and VRSA strains. However, the difference observed in these strains between the two cities is not significant which suggests that geographical location is not a determining factor for the prevalence of these strains in this country.

The findings in this study suggest the need for establishing standard infection control precautions that will be effective in preventing the spread of this resistant pathogen. Thus, systematic surveillance of VRSA will enhance the ability of the public health and health care systems to rapidly recognise and aggressively contain infection and colonization due to this antibiotic resistant pathogen in the healthy population.

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