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Epidemiology of Vancomycin Resistant *Staphyloccus Aureus* among Clinical Isolates in a Tertiary Hospital in Abakaliki, Nigeria

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Abstract *Staphylococcus aureus* is a successful pathogen due to a combination of numerous bacteria immune-evasive strategies. The increased incidence of MRSA has led to more frequent use of vancomycin, the drug commonly relied upon as last resorts for treating MRSA infections. As a consequence, selective pressure was established that led to the emergence of strains of *S. aureus* and other species of *Staphylococci* with decreased susceptibility to vancomycin and other glycopeptides. The aim of our study was to determine the prevalence of vancomycin resistant *S. aureus* among clinical isolates in a tertiary health care facility in Abakaliki metropolis, the capital of Ebonyi State, South Eastern Nigeria. The susceptibility of S. aureus strains to vancomycin was determined by Kirby-Bauer disk diffusion technique as well as by tube dilution method. Among the 355 *S. aureus* isolates tested, 272 (76.6%) isolates were susceptible (zone diameter ≥ 15mm, MIC 0.5-2µg/ml) 64 (18.0%) isolates were intermediate (zone diameter ≤ 14mm, MIC 4-8µg/ml) while 19 (5.4%) isolates were resistant (zone diameter ≤ 14mm, MIC ≥ 16µg/ml). When subjected to statistical analysis, this prevalence rate was statistically non-significant ($p < 0.05$). But, nevertheless, clinically relevant considering the overall implication in the transfer of resistant gene.

Keywords: *vancomycin, resistance, Staphylococcus aureus, Abakaliki*

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1. Introduction

Staphylococcus aureus is a successful pathogen due to a combination of numerous bacteria immune-evasive strategies. It is a facultative anaerobic Gram-positive coccal bacterium which is frequently found as part of the normal flora of the skin and nasal passage. *S. aureus* has long been recognized as a major pathogen of hospital acquired infections. Over the last decade, methicillin resistant *S. aureus* (MRSA) strains have become endemic in hospitals worldwide. Also, it is now an endemic community pathogen in many geographical regions [1,2].

MRSA probably arose by a succession of mutations and the acquisition of the resistance plasmids. The resistance gene *mecA* and regulatory sequences that encode for production of a low- affinity penicillin- binding protein (PBP-2a) are not present in methicillin- sensitive strains. [3] the importance of MRSA strains is that in addition to being resistant to methicillin, most strains are also resistant to other β-lactam antibiotics, with the exception of glycopeptide antibiotics [4,5].

The increased incidence of MRSA has led to more frequent use of vancomycin, the drug commonly relied on for treating MRSA infections [6]. As a consequence, selective pressure was established that led to the emergence of strains of *S. aureus* and other species of *Staphylococci* with decreased susceptibility to vancomycin and other glycopeptides. [7] the first strain of *S. aureus* with reduced susceptibility to vancomycin and teicoplanin was reported in Japan in 1997 [8].

For many years after its initial use, vancomycin was traditionally reserved as a drug of last resort after other antibiotics has failed. This is largely as a result of its high toxicity. However, with the increase in the prevalence and endemicity of MRSA strains, its use became more frequent. The first clinical isolate of Vancomycin resistant *S. aureus* (VRSA) was reported in 2002 in The United States. [9] Presently VRSA has been isolated in different countries hence the burden has become a global phenomenon.

S. aureus strains are defined to be vancomycin resistant (VRSA) at minimum inhibitory concentration (MIC) ≥ 16µg/ml and vancomycin intermediate *S. aureus* (VISA) at MIC between 4-8µg/ml by the Clinical and Laboratory Standards Institute (CLSI). Also, the MIC for heterogenous VISA (hVISA) strains was defined by the presence of subpopulations of VISA at a rate of 1 organism per 10^5 to 10^6 organisms [10,11].

The aim of our study was to determine the prevalence of vancomycin resistant *S. aureus* among clinical isolates

in a tertiary health care facility in Abakaliki metropolis, the capital of Ebonyi State, South Eastern Nigeria.

2. Materials and Methods

2.1. Sample Collection

Bacterial isolates suspected to be *Staphylococcus aureus* obtained from various clinical specimens in Federal Teaching Hospital Abakaliki were collected between October 2012 and April 2013 and used for this study. A total of 355 *S. aureus* isolates were obtained.

2.2. Bacterial Characterization

The bacteria isolates were characterized with respect to their biochemical reactions and morphology on blood agar, MacConkey Agar and Cysteine Lactose Electrolyte Deficient (CLED) agar. Isolates suspected to be *S. aureaus* were further subjected to biochemical tests, after Gram-staining had confirmed them to be Gram-positive cocci arranged in clusters.

Biochemical tests that were performed on the isolates include; catalase test, coagulase test and growth on mannitol salt sugar.

2.3. Preparation of McFarlands Standard

0.5 McFarlands standard was prepared by adding 0.6ml of 1%(g/l) solution of Barium chloride to 99.4ml of Sulphuric acid.

2.4. Disk Diffusion

The susceptibility of strains to antibacterial agents was determined by the standard agar disk diffusion method. Strains were tested using the vancomycin antibiotic disc (30µg). Susceptibility of the isolates to others drugs were also performed. They include cefoxitin (30µg), penicillin (10µg), chloramphenicol (30µg), gentamicin (10µg), erythromycin (15µg), ciprofloxacin (10µg), nitrofurantoin (30µg), trimethoprim/ sulfamethaxole (30µg) and augmentin(30µg).

S.aureus isolates were suspended in normal saline to a turbidity equivalent of 0.5 MacFarland's standard, this was used as inoculum to inoculate Mueller-Hinton agar plates with the aid of sterile swabs. The antibiotic discs were placed on the surface of the media. Then the media plates were incubated at 35°C for 18hrs.

The diameters of the zone of the inhibition were measured and interpreted as either susceptible, intermediate or resistant using the National Committee On Clinical Laboratory Standards Methods (NCCLS) [11,12].

2.5. Determination of MIC

Minimum Inhibitory Concentrations of the *S. aureus* isolates to vancomycin were also determined. MIC of vancomycin was determined by agar dilution method. Gradient plates of Mueller- Hinton agar were prepared with vancomycin (0.5-30 µg/ml). Inoculation of the media plates were done by direct colony suspension method of 0.5McFarland equivalent inoculums prepared in sterile normal saline. Plates were incubated for 18hours at 35^0C and subsequently observed for any visible growth. Results were read and interpreted according to CLSI guidelines [11,12].

3. Result

The prevalence of VRSA among the population was performed by the Kirby-Bauer disk diffusion method and MIC. Results from both methods were correlated and reported. Among the 355 *S. aureus* isolates tested, 272 isolates were susceptible (zone diameter ≥ 15mm, MIC 0.5-2µg/ml) 64 isolate were intermediate(zone diameter ≤ 14mm,MIC 4-8µg/ml) while 19 isolates were resistant (zone diameter ≤ 14mm, MIC ≥ 16µg/ml).

Among the isolates that showed resistance to vancomycin, all were also resistant to cefoxitin (zone diameter ≤ 21mm). A total of 187 isolates were resistant to cefoxitin (MRSA), this gives a prevalence rate of 52.7% of MRSA among the *S. aureus* isolates studied.

The prevalence rate of vancomycin resistant *S. aureus* among the isolates was 5.3% (19 of 355). When subjected to statistical analysis, this prevalence rate was statistically non-significant ($p < 0.05$). It was observed that isolates that showed resistance to vancomycin and cefoxitin were also resistant to all other antibiotics tested (multidrug resistant strains).

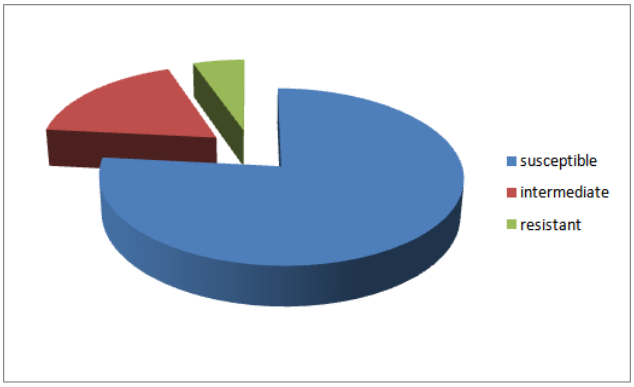

Figure 1. Pie chart showing the prevalence of strains that are susceptible, intermediate and resistant to vancomycin

4. Discussion

Two classes of vancomycin – resistant strains have been reported; vancomycin – resistant *S. aureus* that have a vancomycin MIC of 8mg/L and hetero-VRSA that spontaneously generates VRSA within the cell population. The nomenclature is based on the MIC breakpoints of the British Society for Antimicrobial Chemotherapy who defined the MIC of 8mg/L as resistant. The National Committee for Clinical Laboratory Standards (NCCLS) labels these strains vancomycin intermediate *S. aureus* or glycopeptide – intermediate *S. aureus* (GISA) in the USA [13,14].

In Nigeria, as in some other developing countries virtually all drugs are sold in local drug stores called 'chemists' in the local parlance. These stores make antibiotics readily available to the population without prescription and control. Also, the traders in these 'chemists' prescribe drugs to their customers with reckless abandon and worst still, there is high rate of empirical therapy prescription, whereby clinicians prescribe

antibiotics to patients without obtaining antibiotic sensitivity results from the medical laboratories. These factors increase the rate of drug abuse and consequently increase the rate of development of bacterial resistance to antibiotics in a geometric rate higher than that in developed countries.

Vancomycin resistant strains have been isolated in Japan, The USA, France, Korea, South Africa, Brazil and Scotland. Also, hetero – VRSA strains have been reported from many more countries, hence, the problem of glycopeptides resistance is global [14,15,16,17].

In our study, we observed a relatively high prevance rate of 5.3% of VRSA. While this high prevalence rate can be attributed to the high rate of indiscriminate abuse of antibiotics, significant rise of strains of *S. aureus* with reduced susceptibility to vancomycin, teicoplanin and oxacillin was found by Tiwari and Sen.[1] There is limited literature on the prevalence of VRSA in other places. It is our hope that more surveillance on the susceptibility pattern of *S. areus* to vancomycin will be carried out. The emergence of VRSA and VISA has been proposed to have been a consequence of building selective pressure of vancomycin.

One limitation of our study is the absence of isolation of the *Van A/Van B* genes by Polymerase Chain Reaction (PCR). However, in the study by Tiwari and Sen[1] these genes were absent in the VISA and VRSA strains isolated even in the presence of phenotypic resistance to vancomycin. Hence, the presence or absence of the resistant *van A/B* genes does not necessarily rule out that strains are not VRSA. Also, the phenotypic expression of VRSA and clinical failure of the drug *in vivo* lends credence to the weight of the burden of this resistance in *S. aureus.*

Vancomycin resistant *S. aureus* strains have increased the yearning of the world at large for the development of new drugs that will be effective in the treatment of multidrug resistant bacteria, *S. aureus* being one of them. Especially considering the widespread infection caused by *S. aureus* in developing countries.

The current study has exposed the presence of phenotypic vancomycin resistant *S. aureus* strains in the absence of PCR facility to perform genetic isolation of the *van A* and *van B* genes; it has therefore become necessary for further surveillance studies to be performed especially in developing countries where there is poor documentation of procedures and preservation of clinical data. The evidence of the phenotypic VRSA has pointed to its presence in Nigeria.

5. Conclusion

Vancomycin is a glycopeptide that is reserved for treatment when other antibiotics have failed especially as a result of MRSA. The mechanism of resistance of staphylococci to vancomycin is still unknown however the resistance gene is spreading as indicated by the presence of VRSA strains in many countries. While we hope that the prevalence does not increase, it is however necessary to impose restrictions on the availability of antibiotics and other prescription drugs to the public and develop new drugs to combat the menace of multidrug failure.

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