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Effect of Vancomycin on *Staphylococcus* species isolated from clinical specimens in South-Western Nigeria

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ABSTRACT: Bacteriological samples, wound swabs, ear swabs, pus, abscess, blood, urine, wound aspirate, high vaginal swabs, aural swab and conjuctival swabs, were obtained from seventy individuals (males and females), fifty coagulase positive *Staphylococcus* species and twenty coagulase negative species of *Staphykococcus*. The samples were drawn from patients attending University College Hospital (UCH), Ibadan; Obafemi Awolowo Teaching Hospitals, Ile-Ife, Ladoke Akintola University of Technology Teaching Hospital, osogbo and Ondo State Specialist Hospitals, Akure. Organisms isolated are: *Staphylococcus aureus* and *Staphylococcus epidermidis*. All isolates were characterized biochemically and their sensitivity against Vancomycin was tested using agar diffusion technique while minimum inhibitory concentration was also determined. All the isolates were susceptible to 30µg/disc of Vancomycin with diameter of zone of inhibition ranging from 15mm to 19mm. The minimum inhibitory concentration (MIC) ranged between 0.5 µg/ml and 4.0µg/ml for coagulase positive isolates and between 0.5µg/ml for coagulase negative isolates.

Key words: Staphylococcus, Vancomycin, Sensitivity, Resistance, Inhibition.

Introduction

Bacteria resistance to vancomycin has caused great concern among many health care professionals (Perl, 1999).

Characteristically, of 23 recognised *Staphylococcus* species, only three *S. aureus, S. epidermidis* and *S. saprophyticus* are recognised as being clinically important. *S. aureus* is a pathogen but the other two are regarded as opportunist or nosocomial pathogens. Several antibiotics have been found one time or the other to be effective against *Staphylococcus aureus* and *S. epidermidis* but many are now resistant against them. This resistance has been attributed to acquired plasmid for the antibiotic.

Vancomycin is a bactericidal antibiotic which is active against a variety of Gram-positive bacteria. Its primary aim is in the treatment of severe antibiotic resistant infection. Vancomycin hydrochloride is an ampholeric glycopeptide anti-microbial substance produced by the eneterococci, Vancomycin was first described in vitro by (Toala et al., 1969). Since then, there has been a remarkable increase in the incidence of vancomycin resistance among strains of eneterococci. Experimental evidence for the possible transfer of

vancomycin resistance from enterococci to *Staphylococcus aureus* has also been established according to Toala et al.

This study was therefore designed to evaluate the sensitivity patterns of some *Staphylococcus* species in clinical specimens to varying concentrations of vancomycin; to determine the minimum inhibitory concentration of vancomycin against *Staphylococcus* species; and to improve the standard of antibiotic treatment by updating clinicians knowledge on prospect of vancomycin.

The mode of action of vancomycin is by inhibition of bacterial cell wall synthesis by the glycopeptide. This is due to the formation of a complex between the antibiotic and the C-terminal D-alanine (D-ala) residues of peptidoglycan precursors (Barna et al., 1984). Formation of complexes at the outer surface of the cytoplasmic membrane prevents the transfer of precursor from the lipid carrier to the peptidoglycan by the transglycosidases. Vancomycin also inhibits the utilization of disaccharides and pentapeptide –P-phospholipid and all membrane function is also damaged. It exerts its bactericidal effect without a lag period. The minimum inhibitory concentration (MIC) of vancomycin has been reported to range from 8 to 16 µg/ml (Smith, 1999).

Materials and Methods

Staphylococcus aureus and *Staphylococcus epidermidids* were isolated from one hundred individuals. They were obtained from clinical specimens such as High Vaginal Swab (HVS), Wound swabs, Ear swabs, Conjuctival swabs, Abscess, Blood, Urine, Wound aspirates, Pus, Aural swabs. Samples were collected from patients attending University College Hospital (UCH), Ibadan, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo and Ondo State Specialist Hospital, Akure. The patients were males and females between ages of 14 and 60 years.

All the culture media and glasswares used were autoclaved at 121°C for 15 mins and various culture media prepared according to manufacturers specifications. The specimens were inoculated on Nutrient agar and Manitol salt agar and the plates were incubated at 37°C for 48 hours. The isolated *Staphylococcus aureus* and *Staphylococcus epidermidis* were identified biochemically using conventional techniques. All Gram-positive cocci and catalase positive isolates were tested for coagulase production.

A 30 µg portion of vancomycin disc was prepared using Whatman No. 1 filter paper perforated with 6mm sterile cork borer. Then 100 discs were placed in fifty's in 2 half once bottles and sterilized in a hot air oven at 1600°C for 1hour. Vancomycin powder (1.0g commercially prepared) was diluted in 10 and solution made to strength of 30 µg per disc that is each disc absorbing 0.02ml Vancomycin, and dried in a 35-37°C incubator for 1hour. The technique of Strokes et al., (1970) was used to determine antibiotic sensitivity. The susceptible and resistant inhibition zone diameter break points used in this study were greater than or equal to 15mm and less than or equal to 15mm respectively. Minimum inhibitory concentration (MIC) of vancomycin was determined by dissolving vancomycin powder to strength of 128µg/ml working solution aseptically. Ten tubes were arranged with tube 1 containing 0.125µg/ml vancomycin and 2 drops of overnight culture of test organism in peptone water was added to all tubes and incubated at 37°C overnight. The MIC was read as the least concentration of vancomycin to inhibit the visible growth of the organism (turbidity). Control test was also set up to confirm viability of the test organisms.

Results

Minimum Inhibitory Concentrations (MIC) and the zone of Inhibition (to 30µg of vancomycin) were determined for each isolate of *Staphylococcus* species. The results are presented in the Tables below. Of the seventy isolates obtained (fifty coagulase positive and twenty coagulase negative), there was no resistant strain; all the isolates were vancomycin-sensitive. Generally, the diameter oof the zone of inhibition ranges from 15mm-19mm (tables 1 and 2). The MIC ranges between 0.5µg/ml and 4.0 µg/ml for coagulase positive isolates and it ranges between 0.5 µg/ml and 2.0 µg/ml for coagulase negative isolates.

The MIC of vancomycin ranges from 0.5 to 4.0 µg/ml for *Staphyloccoccus aureus* with 24% isolates exhibiting 0.5 µg/ml while 76% shows 4.0 µg/ml. For *Staphylococcus epidermidis*, the MIC ranged from 0.5 to 2.0 µg/ml with 15% showing MIC of 0.5 µg/ml and 85% exhibiting MIC of 30 µg/ml.

Table 1: Distingushing features of some *Staphylococcus* species.

	S. aureus	***S. epidermis***	***S. saprophyticus***
Coagulase	+	-	-
Anaerobic growth and fermentation of glucose	+	+	-
Mannitol			
Acid aerobically	+	V	V
Acid anaerobically	+	-	-
Alpha-Toxin	+	-	-
Heat resistant endonucleases	+	-	-
Biotin required for growth	-	+	NT
Cell wall			
Ribitol	+	-	+
Glycerol	-	+	-
Protein A	+	-	-
Novobiocin Sensitivity (+)	**S**	**S**	**R**

(Joklik et al., 1984)

+	90% or more strain positive
-	90% or more strain negative
V	Some strains positive, some negative
NT	Not tested
S	Sensitive
R	Resistant
(+)	= R, MIC>2.0µg/ml

Discussion

In view of the incidence of vancomycin resistance among the enterococci and the possible transfer of vancomycin resistance from the affected group of organisms to various species of *Staphylococcus*, there has been concern that strains of *Staphylococcus* will emerge that are resistant to vancomycin. This study was however designed to investigate the effect of vancomycin on some *Staphylococcus* species from clinical specimens in South Western Nigeria. The result of this investigation shows that *Staphylococcus aureus* and *S. epidermidis* are susceptible to vancomycin and no resistant strain was observed. The present study conducted in South Western Nigeria conforms with that of (Ena, J., 1993) in which a seven-year audit of five glycopeptides and other drugs at the University Hospital in Iowa City, USA reveals that all isolates of *Staphylococcus* species were susceptible to vancomycin. From this study, all the seventy isolates of *Staphylococcus* species tested for sensitivity against 30 µg of vancomycin disc were susceptible. No resistant strain was identified.

The total sensitivity of *Staphylococcus* species to vancomycin may be due to the inhibition of cell wall synthesis by the glycopeptide, which is due to the formation of complex between the antibiotic and the C terminal D alanine (D-ala) residues of peptidoglycan precursor (Barna et al., 1984). Formation of complexes at the outer surface of the cytoplasmic membrane prevents the transfer of precursor from the lipid barrier to the peptidoglycan transglycosidases (Barna et al., 1984).

Table 2: Zones of inhibition to 30 µg of vancomycin with MIC of Coagulase positive isolates.

Isolate	Diameter of zone of inhibition (mm)	MIC/µg/ml	Isolate	Diameter of zone of inhibition (mm)	MIC µgml
1	17.0	1.0	26	17.0	0.5
2	18.0	1.0	27	18.0	0.5
3	15.0	1.0	28	15.0	1.0
4	17.0	1.0	29	16.0	1.0
5	17.0	1.0	30	18.0	2.0
6	17.0	2.0	31	18.0	0.5
7	17.0	1.0	32	18.0	0.5
8	18.0	2.0	33	16.0	1.0
9	18.0	0.5	34	19.0	1.0
10	16.0	0.5	35	18.0	0.5
11	17.0	2.0	36	16.0	1.0
12	15.0	2.0	37	16.0	1.0
13	15.0	2.0	38	17.0	1.0
14	16.0	1.0	39	16.0	0.5
15	17.0	2.0	40	18.0	1.0
16	19.0	4.0	41	15.0	0.5
17	19.0	1.0	42	17.0	2.0
118	19.0	1.0	43	16.0	2.0
19	19.0	2.0	44	17.0	1.0
20	17.0	2.0	45	15.0	2.0
21	17.0	4.0	46	17.0	0.5
22	16.0	0.5	47	19.0	1.0
23	17.0	1.0	48	19.0	1.0
24	17.0	2.0	49	18.0	1.0
25	16.0	2.0	50	16.0	2.0

Also, the findings of (Tonsberry, 1996) in which all *S. aureus* isolates were susceptible to vancomycin conform with results of this study. The effectiveness of this antibiotic can also be as a result of its ability to rapidly and tightly bound to organisms and the adequate penetration of the drug to its target receptor cell. Its contrast to this wor's result, a significant number of strains of *Staphylococcus* species heterogeneously resistant to vancomycin were isolated by (Keiichi et al., 1997) in Japan. They further stated that *Staphylococcus* is probably exchanging genetic material by various mechanisms, including transduction and cell-to-cell contact. Recent evidence is accumulating in favour of transfer of plasmid between *Staphylococcus aureus* and *Staphylococcus epidermidis*.

In conclusion, by virtue of this study, clinicians are better informed on handling of infections using vancomycin to discourage emergence of resistant strains. This further reiterates the usefulness of carrying on sensitivity tests prior to antibiotics prescription.

Table 3: Zones of inhibition to 30 µg of Vancomycin with MIC of Coagulase Negative isolates.

Isolate	Diameter of zones of inhibition (mm)	MIC/µg/ml
1	17.0	2.0
2	18.0	1,0
3	17.0	1.0
4	18.0	2.0
5	18.0	2.0
6	17.0	0.5
7	18.0	1.0
8	18.0	1.0
9	19.0	1.0
10	19.0	2.0
11	18.0	0.5
12	16.0	1.0
13	17.0	1.0
14	17.0	2.0
15	18.0	2.0
16	16.0	1.0
17	19.0	1.0
18	15.0	2.0
19	19.0	1.0
20	16.0	0.5

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