Original Article

An Evaluation of VRSA Isolates from Hospitalized Patient's in Karachi

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ABSTRACT

Objective: The aim of this study is to determine vancomycin resistance or susceptibility in patients visiting different hospitals in Karachi, Pakistan.

Place and Duration of study: Med Path Laboratories and Diagnostic Center, Gulshan-e-Iqbal Karachi & KESC Medical center, Karachi during Jan, 2013 to may, 2013.

Material and Methods: The present study deals with the distribution 100 isolates from various hospitals located in Karachi and studied by Kirby Bauer disk diffusion procedure at Med Path Laborites and Diagnostic Center KESC Medical Center.

Results: The study shows out of 100 isolates 20% were resistant, 2% were intermediate and 78% were sensitive.

Conclusion: Now there is an immediate need to slow down the spread of these strains (VRSA/VISA/VSSA) as much as possible with safety precautions and good infection control methods. The number of persons caring for the patient should be minimized and dedicated staff should be assigned to treat the VISA/VRSA patient. Implementation of the appropriate infection control precautions during patient care must be monitored.

Key Words: Vancomycin susceptible S. aureus. (VSSA), Vancomycin Intermediate S. aureus (VISA), Vancomycin resistant S. aureus (VRSA), Mean Inhibitory Concentration (MIC).

INTRODUCTION

A drastic situation has broken in by the emergence of resistant isolates of S.aureus to vancomycin in Karachi. Followed by the emergence of such strains in different parts of the world in recent past[1]. It is because of the reason that vancomycin was the final treatment of choice against MDRSA i.e. multidrug resistant S.aurueus or MRSA (methicillin resistant S.aureus).

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It was perceived that resistance to vancomycin would not be possible as the mechanism of resistance to this antibiotic was difficult to induce[2].

ANTIBIOTIC RESISTANCE:

Antibiotic resistance in bacteria has emerged as a medical catastrophe[3]. This results from the speed at which bacteria multiply and are spread, and the ease with which they can change their genetic material or acquire new genes[4]. They exert biochemical resistance by preventing entry of the drug, by rapidly extruding the drug, or by enzymatically inactivating the drug or altering its molecular aim[5].

EMERGENCE OF VANCOMYCIN RESISTANCE

Vancomycin has been the most reliable therapeutic agent against infections caused by methicillin-resistant Staphylococcus aureus (MRSA)[6].

However, in 1996 the first MRSA to acquire resistance to vancomycin was isolated from a Japanese patient[7]. Subsequent isolation of several vancomycin resistant S aureus (VRSA) strains from USA[8], France, Korea[9] South Africa, Hong Kong[10], Thailand[11], Michigan[12], Spain[13], Greece[14], Germany[15], Italy[16], United Kingdom[17] and Brazil has confirmed that the emergence of vancomycin resistance in S aureus is a global issue[18].

VANCOMYCIN

Vancomycin is a Glycopeptide antibiotic. Vancomycin first isolated in 1953 by Edmund Kornfeld[2,4] is a branched tricyclic glycosylated nonribosomal peptide produced by the fermentation of the Actinobacteria species Amycolatopsis orientalis (formerly designated Nocardia orientalis)[3]. Vancomycin exhibits atropisomerism — it has multiple chemically distinct rotamers owing to the rotational restriction of some of the bonds[19]. The form present in the drug is the thermodynamically more stable conformer and, therefore has more potent activity[5].

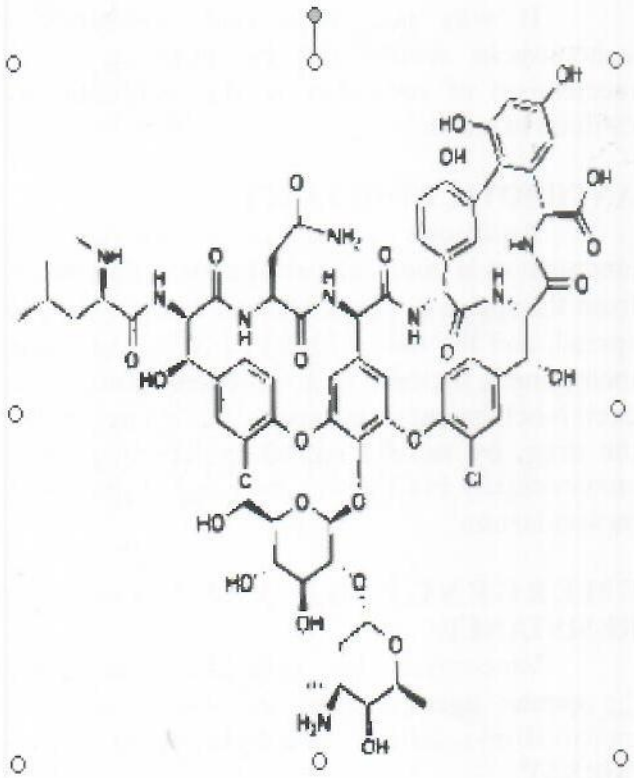


Fig.1 vancomycin structure

MECHANISM OF ACTION OF VANCOMYCIN

In order to exert an effect, vancomycin must reach the cytoplasmic membrane and bind with nascent cell wall precursors, thereby inhibiting their incorporation into the growing cell wall[20].

Vancomycin binds with the D-alanyl-D-alanine C terminus of the bacterial cell precursors, thereby preventing cross-linking by transpeptidation resulting in inhibition of cell wall production by attacking sites responsible for cell wall production[21].

MECHANISM OF RESISTANCE DEVELOPMENT AGAINST VANCOMYCIN

Cell wall synthesis and turnover are upregulated in VRSA isolates, leading to thicker and more-disorganized cell walls[7]. It has been proposed that the thicker, disorganized cell walls can actually trap vancomycin at the periphery of the cell, due to increased residues of the D-alanyl-D-alanine, thereby blocking its action[22]. It has been shown that vancomycin can be recovered intact from the cell walls of VISA and VRSA isolates, indicating that the antibiotic is not being inactivated but merely sequestered by the bacteria[23]. Further, it appears that resistant isolates have significantly less cross-linking in the peptidoglycan component of the cell wall[6].

It has been proposed in several researches that the transfer of genetic material among bacteria also contribute to the development of VRSA. In Patients co-infected or co-colonized with VRE (vancomycin resistant enterococci) and/or MRSA (Methicillin resistant S.aureus), the transfer of vanA gene from VRE to MRSA is more likely to occur leading to a VRSA strain[24].

The DNA sequence of the VRSA vanA gene was identical to that of a vancomycin-resistant strain of Enterococcus faecalis. The vanA gene is encoded within a transposon located on a plasmid carried by the VRSA isolate[25]. This transposon, Tn1546, confers vanA-type vancomycin resistance in enterococci[26,27].

CDC definitions of classifying isolates of S.aureus with reduced susceptibility to vancomycin are based on the laboratory breakpoints established by Clinical and laboratory standards institute (CLSI)

The CLSI breakpoints for S.aureus and vancomycin were modified in January 2010:

Vancomycin susceptible S.aureus (VSSA).
Vancomycin MIC = 4µg/ml.

Vancomycin Intermediate S.aureus (VISA)
Vancomycin MIC 8-16µg/ml.

Vancomycin resistant S.aureus (VRSA)
Vancomycin MIC =32 µg/ml[28].

MATERIAL AND METHODS:

No. of specimens = N = 100 (wound, pus, skin abscesses)

Test Culture = Staphylococcus aureus

Chemical/ Media/ Reagents

Gram staining kit, 0.5 Mc Farland's index, Catalase (hydrogen peroxide), Coagulase (human plasma), Nutrient agar, Blood agar, Mannitol salt agar, DNase agar, Agar dilution plates (Mueller Hinton broth), BHI broth, Micro broth dilution (micro titer plate), 500 mg vancomycin injection. For isolation and identification of S.aureus standard biochemical and cultural test including gram staining, catalase, coagulase, blood agar, Mannitol salt agar, DNase agar were performed. For separation of resistant and susceptible strains, Kirby Bauer disk diffusion procedure was adapted and performed i.e;

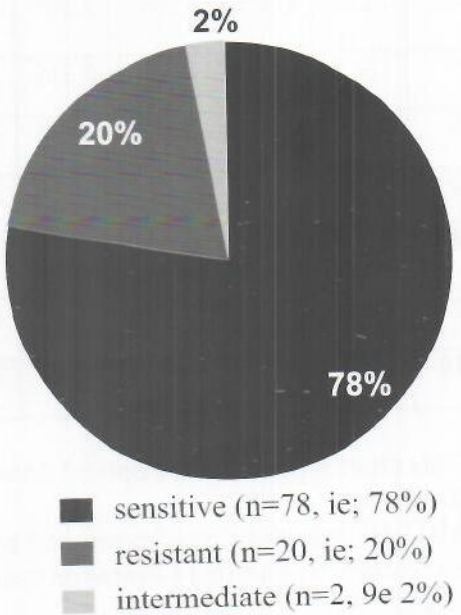
4-5 colonies of pure growth are transferred into a tube containing 4-5 ml of nutrient broth and incubated at 37°C until it achieves the turbidity of 0.5 Mc Farland's index (2-6 hours).

A lawn was prepared from inoculum using sterile cotton swabs on Mueller Hinton's agar plate. Antibiotic disks were placed on to the bacterial lawn with the help of forceps. The plates were inverted and incubated at 37°C for 24 hours. The zone of inhibition was observed around the disks and measured using verneir caliper. For evaluation of MIC's of isolated strains, microbroth dilution test was performed for which, Test bacteria were cultured in Brain heart infusion (BHI) broth at 37°C for 24 hours.

Antiobiotic stock solution -- 500 mg/liter,or 0.5 mg /ml was prepared. After that we added 100µl brain heart infusion (BHI) broth and 50 µl of antiobiotic solution to each respective well of the microtitre plate. Serial dilution of 50 µl from these wells was made to the wells of their respective rows. 50 µl from the last well of each row was discarded.

1 loop (10ul) amount of test organism (1 sample / a set of row) was inoculated in each well and incubated at 37°C for 18- 20 hours. Then observed for the growth by measuring turbidity. The lowest concentration of antibiotic sol, showing inhibition of growth is the MIC of the respective organism.

Analysis of resistant subpopulations of bacteria (population analysis) was done by spreading 50 µL of the starting cell suspension (prepared by diluting overnight cultures to an optical density [OD] of 0•3 at 490 nm) and its serial diluents over Brain heart infusion (BHI) agar plates containing various concentrations of vancomycin. The plates were incubated at 37°C for 48 h, and the number of mature colonies was counted. The number of resistant cells contained in 50 µL of the starting cell suspension was calculated and plotted on a semi-logarithmic graph. The maximum no. of isolates showed resistance from 9.7 µg/ml of vancomycin which was judged by the presence of turbidity in the microtitre plate well. And was also judged by observing the O.D of control and other antibiotic containing wells in the microtiter plate using ELISA at 470 nm. However these strains showed susceptibility to vancomycin at a concentration of 2500, 1250, and 625 µg/ml of antibiotic solution.



RESULTS

Total 100 clinical isolates of Staphylococcus aureus from hospitalized patients in Karachi were included in this study during January to May 2012. The biochemical and cultural tests performed confirmed the presence of Staphylococcus aureus in the samples.

The VRSA and VSSA were differentiated by the help of Kirby Bauer disk diffusion method using 30µg vancomycin disk. Result of this test confirmed 20 isolates as resistant by giving no zone around the colonies, 2 were intermediate and 78 were sensitive showing clear zone of inhibition around the colonies.

CALCULATION

(Vancomycin disk used = 30 µG)

MIC (i.e. Minimum inhibitory concentration) of the 10 resistant isolates was studied by the help of Micro broth dilution method accordiing to the NCCLS Standard 15 and the results were recorded using ELISA technique.

Since 1ml (or 1000 µl) of sol contain 50 mg of vancomycin

and 1 µl will have : 50/1000=0.05mg or 50 µg

thus, 50 µl will contain : 2500 µg of vancomycin.

means that the concentration of antibiotic in first wells is – 2500 µg and on serial dilution the pattern of concentration shall be as follows:-

DISCUSSION

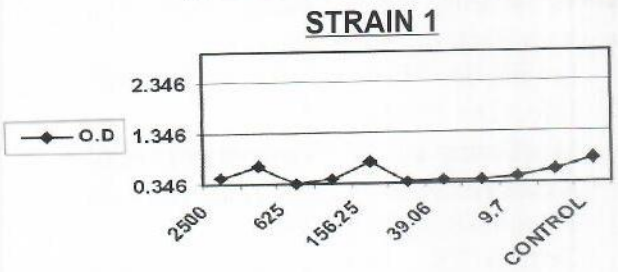
The isolated strains confirmed the presence of VRSA in Karachi, like U.S.A, Michigan, Penn Silvia, Saudi Arab, Japan, Kenya, and few other parts of the world in recent past[29]. The emergence of VISA/VRSA would signal the introduction of bacteria that are resistant to all currently available antibiotics. While the bacteria

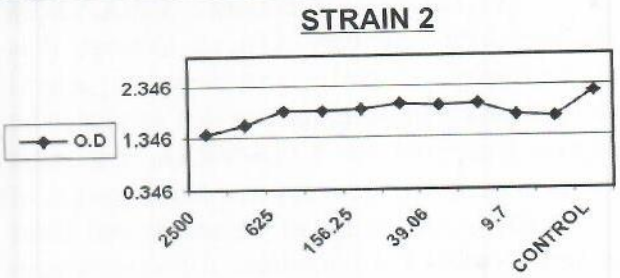
ANTIBIOTI CONC	100:50 2500µg	100:25 1250µg	100:12.5 625 µg	100:625 312.5µg	100:3.12 156.25µg	100:1.56 78.12 µg	100:0.78 39.06 µg	100:0.39 19.53 µg	100:0.19 9.7 µg	100:0.09 4.88 µg	CONTRO no antibiotic
Resistance											
Strain 1	0.490	0.724	0.346	0.430	0.774	0.381	0.422	0.428	0.470	0.608	0.8
Strain 2	1.397	1.579	1.854	1.855	1.894	1.971	1.931	1.992	1.760	1.719	2.2
Strain 3	1.772	2.246	2.382	2.261	2.395	2.324	2.277	2.192	2.306	2.361	2.5
Strain 4	1.221	1.993	2.154	2.235	2.240	2.320	1.986	2.204	2.204	2.137	2.53
Strain 5	1.955	2.080	1.906	2.398	2.347	2.224	2.518	2.196	2.944	2.190	2.51
Strain 6	2.026	2.261	2.305	2.456	2.321	2.347	2.358	2.139	2.331	2.288	2.91
Strain 7	1.939	2.214	2.222	2.384	2.394	2.381	2.380	2.244	2.275	2.241	2.65
Strain 8	1.787	1.984	1.355	1.573	2.083	2.076	2.077	1.874	2.422	1.647	2.78
Strain 9	1.517	2.020	2.352	2.285	1.968	2.310	2.287	1.958	1.694	2.128	1.95
Strain 10	2.277	2.265	2.039	2.365	2.366	2.000	1.517	2.020	2.252	2.211	2.97

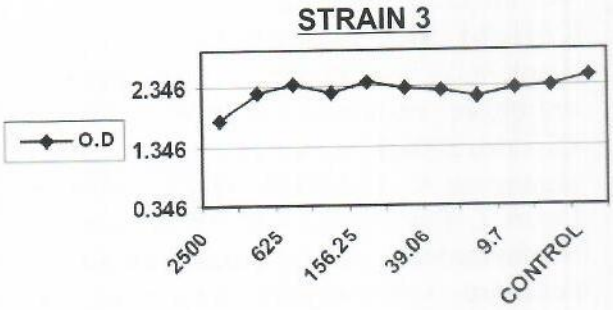
This chart represents the optical density of each strain of S.aureus after incubation of 24 hours by ELISA on 470 nm.

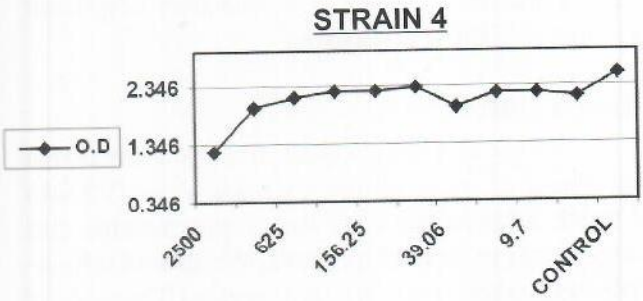
MIC of each strain was expressed by plotting the antibiotic concentration on x-axis and taking O.D of each respective strain (470nm) on y-axis

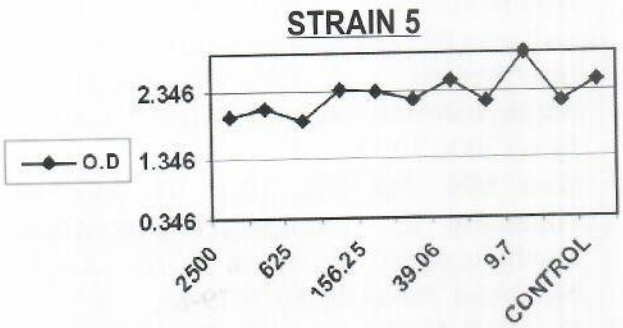
GRAPHICAL REPRESENTATION OF MIC OF EACH STRAIN

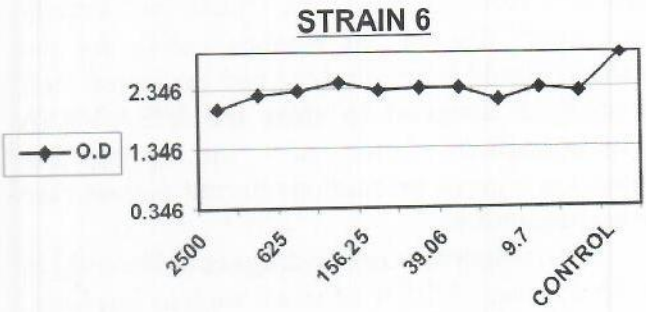


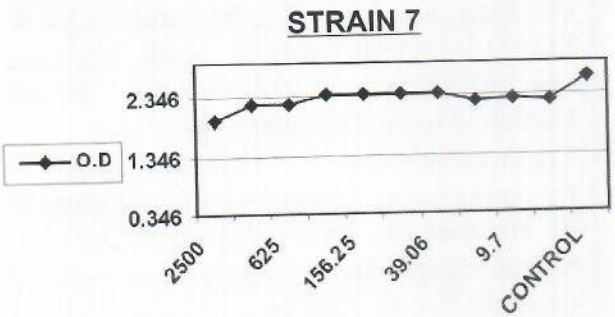


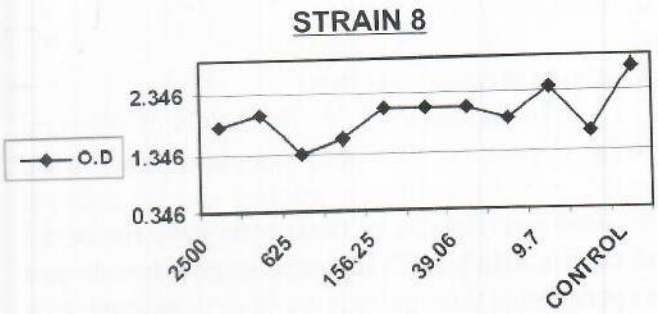


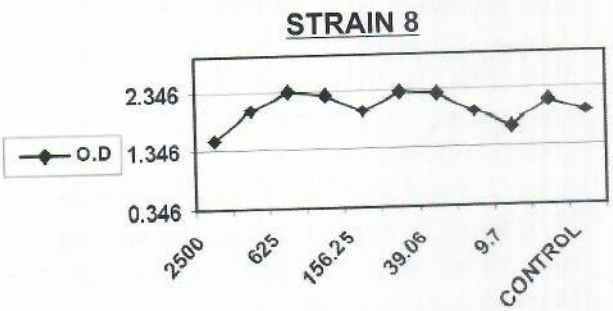


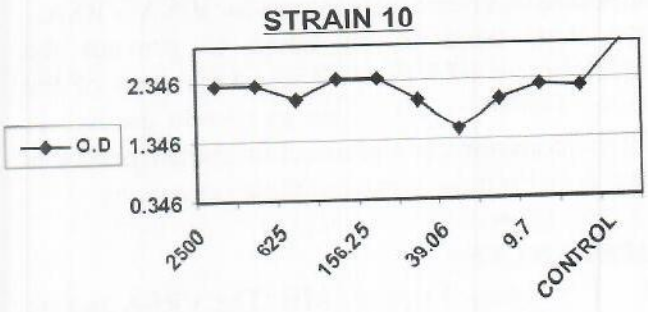












(X-axis represents antibiotic concentration in µg)

themselves may not be any more virulent than other staphylococcal infections, VISA/VRSA infections would be very difficult to treat. It is encouraging to note that experimental therapeutics are in development that appear to be effective treatment for VISA/VRSA, but steps need to be taken to prevent the development of VISA/VRSA. Awareness of the issues and strict adherence to current guidelines for vancomycin use and infection control practices may help limit the impact of these organisms[30].

CONCLUSION

There is an immediate need to slow down the spread of these strains (VRSA/VISA/VSSA) as much as possible with safety precautions and good infection control methods. We should not use antibiotic when they are not needed.The use of broad spectrum antibiotic must be strictly monitored. The no. of persons caring for the patient should be minimized and dedicated staff should be assigned to treat the VISA/VRSA patient.Implementation of the appropriate infection control precautions during patient care must be monitored.

Another alternative drugs could be used in order to treat a VRSA infection such as linezolid, trimethoprim sulfamethoxazole, quinupristin-dalfopristin , doxycycline.

RECOMMENDATIONS

To further evaluate the MICs of resistant isolates two of the gold standard tests were performed, Agar dilution method and Microbroth dilution test. Results of these tests gave the detail of each isolate's MIC. It is encouraging to note that experimental therapeutics are in development that appear to be effective treatment for VISA/VRSA, but steps need to be taken to prevent the development of VISA/VRSA. Awareness of the issues and strict adherence to current guidelines for vancomycin use and infection control practices may help limit the impact of these organisms

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