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Resistance Pattern of Clinical Isolates of Enterococcus SPP. Against Vancomycin and Various Selected Antimicrobials Over 4 Years in A Multicenter Tertiary Care Hospital in Karachi

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ABSTRACT

Background: Vancomycin is a glycopeptide antimicrobial usually reserved to serious and ampicillin resistant enterococcal infections. However, the emergence and propagation of vancomycin resistant enterococci (VRE) is becoming a serious threat to the lives since only limited choices are available to treat VRE infections. Previous reports on VRE in Pakistan have shown an increasing resistance of enterococci against many antimicrobial. In this study we have assessed the susceptibility pattern of enteroccoci against vancomycin and various other antimicrobials in the last 4 years. **Setting:** clinical laboratories of a multicenter tertiary care hospital in Karachi. **Period:** from the year 2012 to 2015 **Methods:** 3192 samples of *Enterococcus species* were reviewed for susceptibility against antimicrobials. **Results:** A total of 3192 samples were collected during this period, of this it was observed that vancomycin have antibiogram of 11 % with a sudden rise in 2014 to 15% and then a 13% resistant was observed in 2015. An alarming emerging resistance was seen against ampicillin and erythromycin and chloramphenicol as well whereas no resistance was observed against linezolid. **Conclusion:** The study showed high level of VRE along with high level of resistance against other antimicrobials. It is essential to determine and control the factors responsible for this augmentation of VRE.

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INTRODUCTION

Enterococci are part of the natural flora of the gut, oral cavity, and female genital tract.[1] However, over the last two decades it has emerged as a nosocomial pathogen, causing urinary tract infections, genital tract infections, and endocarditis which are mainly attributed to its colonizing capacity and its ability to exhibit multidrug resistance.[2,3] Studies worldwide have reported Enterococci species exhibiting high-level glycopeptides resistance, aminoglycoside resistance (HLAR) as well as β-lactamase production, among which the 'Vancomycin Resistant Enterococci' (VRE) are most prominent.[4,5,6] The two most commonly isolated enterococcal species from the clinical samples include *E. faecalis;* accounting up to 90 % whereas *E. faecium* accounts for 10 % of organisms.[7,8] The numbers of clinical infections caused by Enterococci species are consistently increasing and are considered a serious threat due to their intrinsic resistance against many commonly used antibiotics which may lead to therapeutic failures.[9] The rise in antibiotic resistance among Enterococci spp., specially against vancomycin, is presenting as a serious clinical and epidemiological threat.[10] Moreover, since 'Vancomycin-Resistant Enterococci' (VRE) from animal sources such as poultry and human foods of animal origin is affecting humans through colonization and infection, therefore a considerable level of VRE infections are found among people not associated with the health-care setting giving rise to the community-acquired infections as well.[11]

In the United States, the "Centers for Disease Control and Prevention" (CDC) approximates 30 % of hospital enterococcal infections as VRE.[12] According to the "European Antimicrobial Resistance Surveillance System", the prevalence ranges from 1 % to 30 % within the region and that in the United Kingdom (UK) it has been reported in the range of 20 % to 30 %.[13] Conversely a study

conducted in Northern India in 2016 reported the vancomycin resistance rate among the *Enterococcus* isolates to be 11.3 % but this was also comparatively higher when compared to other reports from same region.[14] VRE was reported in Pakistan for the first time in 2002.[15]

Since vancomycin is the preferred antimicrobial against infections caused by the *Enterococcus spp.* which are resistant to other antibiotics, the objective of this study was to estimate the current prevalence of vancomycin resistance in *Enterococcal spp.* in tertiary care hospital of Karachi and also to compare and establish the activity of other antibiotics against isolates from routine samples of blood, pus, sputum and urine etc.[16]

METHODOLOGY

Study Design: Descriptive study.

Period: 4 years, January 2012 to December 2015.

Setting: Microbiology Section, Clinical Laboratories, Ziauddin University Hospital, Karachi, Pakistan.

Sample collection: Three thousand one hundred and ninety-two (3192) consecutive isolates were selected for this study. All samples were collected with convenient sampling; duplicate sample were excluded from this study. 'Ethical Committee Approval' was obtained from the hospital. Information for this study was taken either from patients or from any other patient's relative.

Sample Processing and Identification

All the samples were collected in a sterile container either supplied from microbiological laboratory. Samples were inoculated on routine microbiological media including, 'Mac-Conkey's Agar', 'Chocolate Agar', 'Sheep Blood Agar', 'Colistin-Nalidaxic Acid Agar'. All inoculated plates were incubated in ambient air while 'Chocolate Agar' and 'Sheep Blood Agar' were incubated for 24-48 hours in a CO_2 incubator, by applying standard microbiological technique. Enterococcus species were identified by using conventional technique which include, 'Colony Morphology', 'Gram Staining', 'Catalase Test', 'Bile Esculin Fermentation Test' and 6.5 % NaCl.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out on 'Sheep Blood Agar' (Oxoid Ltd., England) with 'Modified Kirby Bauer's Disk Diffusion Assay' method according to the guidelines of "Clinical and Laboratory Standard Institution" (CLSI) guidelines.[16] 0.5% McFarland standard were prepared in Trypton broth (Oxoid Ltd., England) from organism colonies and inoculated on Sheep Blood agar. The antimicrobial disk was used, Vancomycin 30 µg, Teicoplanin 30 µg, Linezolid 30 µg, Erythromycin 15 µg, Ampicillin 10 µg, Levofloxacin 05 µg, Chloramphenicol 30 µg. All plates of Sheep Blood agar were than kept in 37^0C in CO_2 incubator for 24-48 hours. After 24-48 hours the zone of inhibition were recorded referring to the recommendations of CLSI guidelines.[18] "American type of Culture Collection" (ATCC) controls were used to check the quality of media and antibiotic disc before processing the patient samples. *Enterococcus faecalis* (ATCC 29212) strains were used as a control organism.

Statistical Analysis

"Statistical Package for Social Sciences" (SPSS) version-19.0, IBM Inc., USA was used to perform statistical analysis. The frequencies (N) of organisms, samples and antibiotic susceptibility pattern were calculated and expressed in percentages (%).

RESULTS

A total of 3192 samples were collected over a period of 4 years from year 2012 to year 2015 (see table 1).

Table 1: 4 years antibiogram of Enterococcus spp. against vancomycin and various other antimicrobials

Year Isolates	Enterococcus spp. (Enterococcus faecalis and *Enterococcus faecium*					
	Resistance (%) against selected antimicrobials					
	Ampicillin	Erythromycin	Chloramphenicol	Teicoplanin	Vancomycin	Linezolid
2012 (880)	36	19	35	11	11	0
2013 (779)	36	19	35	12	12	0
2014 (822	39	29	35	15	15	0
2015 (711)	46	36	36	13	13	0

The antibiogram resistance profile presents a grim situation of increasing antimicrobial resistance against all major antimicrobials used for gram positive infections except linezolid. There is a consistent increase in resistance seen against ampicillin and erythromycin in the last 4 years from 36 % to 46 % for ampicillin and from 19 % to whopping 36 % against erythromycin. On the other hand vancomycin and teicoplanin have similar antibiogram with 11 % resistance seen in 2012 to sudden jump to 15 % in year 2014. A 13 % resistance, however, is reported for the year 2015. During this course, fortunately, no isolate was found resistant to linezolid.

DISCUSSION

The increasing pattern of enterococcal resistance exhibited towards aminoglycosides along with the emergence of 'Vancomycin Resistance Enterococci' (VRE), has caused grave concerns among the physicians treating infections caused by these microorganisms.[19] It also poses the threat of increasing chances of MRSA due its ability to potentially transmit resistance within this organisms.[1]

This study has shown significant high level of VRE in the tertiary care hospital of Karachi, over a period of four years with a consistent increase per year from 11 % to 15 % with only the last year representing the resistance fell back to 13%. This is still alarmingly high since a study conducted in 2006 by Abdulla et. al. within Karachi reported a resistance of only 0.9 %.[20] A local study conducted in Rawalpindi in 2014 reported a very high frequency of 11.57 % of VRE in a tertiary care hospital and a recent report from Northern India published in 2016 also reported a frequency of 11.3 %.[14,21] The rate of VRE is significantly higher in our study and is indicative of the increasing trend of resistance shown by the Enteroccocus species against vancomycin, which is a cause of great concern.

Ampicillin is the drug of choice in enterococcal infections whereas chloramphenicol and teicoplanin are both considered a drug of choice in cases where Enterococcus had shown resistance against vancomycin.[22,23] However, in this study it has been observed that teicoplanin has shown similar resistance pattern as vancomycin throughout indicating the possible presence of Van A phenotype. Moreover, the high level of resistance shown specifically against chloramphenicol is a cause of concern as VRE has intrinsic resistance and the ability to develop cross resistance to most of the currently used antibiotics through mutation. This also leads to the threat of development of "Vancomycin Intermediate *Staphylococcus aureus"* (VISA) and "Vancomycin Resistant *Staphylococcus aureus"* (VRSA). The complete susceptibility of *Enterococcus spp*. towards linezolid is a positive finding indicating an alternative therapy for enterococcal infections but can only be reserved for last resort.

It is of utmost importance to control the spread of VRE by decreasing the imprudent use of vancomycin. The guidelines provided by 'Medical Microbiological and Infectious Disease Society of Pakistan' (MMIDSP) give clear indications where the use of vancomycin is justified. Also CDC has laid great emphasis on educating health care workers about the probable risk factors involved in the spread of VRE and the preventive measures that should be taken. It is the need of the hour that the fundamental factors involved in the augmentation of the VRE should be identified as a part of drug utilization review by the clinical pharmacists within our health care setup and steps should be taken in order to control these factors to reduce the rate of mortality and morbidity resulting directly from the spread of VRE.

CONCLUSION

In this study the occurrence of VRE has been found 11 % to 15 %, which is significantly high. It is essential to identify the factors responsible for this augmentation and to determine suitable interventions in order to control this situation.

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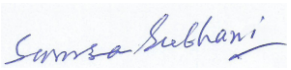
Samra Subhani Lecturer G.C University, Faisalabad	Proof Reading, Data Table	
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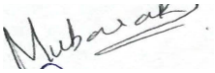
Dr. Mubarak Zeb Clinical Microbiologist, Ziauddin University Hospital, Karachi	Data Collection	
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