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Frequency and Resistance pattern of clinical isolates of Enterococcus Species in a Tertiary Care Hospital.

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Abstract:

Introduction: Hospital acquired infections are becoming a major concern for health care providers and especially nephrology patients where a lots of interventions and frequent admissions are observed. Objective of this study was to evaluate one of the emerging multidrug resistant enterococcus organisms with significant healthcare implications.

Methods: This was a retrospective study evaluating the isolates from different samples from May 2018 to Sep 2019. All isolates growing enterococci were identified and evaluated for sensitivity pattern.

Results: Out of 1500 samples 86 cases of Enterococci were isolated and 8 samples had resistance to Vancomycin. 2 isolates were also resistant to linezolid.

Conclusion: Vancomycin resistance among Enterococci isolates is a major concern for health care providers dealing with complex and prolonged hospital stay patients. Furthermore, the emergence of Linezolid resistance is a cause of concern highlighting the judicious use of antibiotics.

Key words: Enterococcus; cross infection; multidrug resistance; vancomycin; linezolid; Vancomycin-resistant Enterococci, urinary tract infection.

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Introduction

Enterococci are facultative anaerobic gram-positive cocci that previously belonged to Group D of Lancefield classification for Streptococci[1,] however currently these organisms are classified as separate genus with more than 35 identified species at present[2]. *Enterococcus faecalis* (90%) and *Enterococcus faecium* (10%) are the two most commonly isolated species of the genus Enterococcus[3,4]. Enterococci, normally present in the human intestine and the female genital tract have the ability to grow at a wide range of temperature, tolerate extreme conditions including extremes of pH[8], and have the ability to acquire resistance to multiple antimicrobial agents and transfer resistances to other pathogens e.g Methicillin resistant *Staphylococcus aureus* (MRSA)[9,11].

Infections caused by Enterococcus species are on the rise due to the development of resistance. [5]. Glycopeptide is the most commonly used class of antibiotics for the treatment against severe infections caused by Gram-positive pathogens, such as Enterococci. Acquired resistance against Glycopeptides

(vancomycin and teicoplanin) has limited their use for the treatment of enterococcus. Vancomycin introduced in 1958 is a cell wall inhibitor[12] works as a cell wall destabilizer by preventing the crosslinking of peptidoglycans leading to bacterial cell death by osmotic damage. The gram positive selectivity is due to the binding of vancomycin to the cell wall peptidoglycan precursor on the cell wall. [7]. Gram positive bacteria over the years due to frequent use of antibiotics acquire resistance by transposons and plasmids and are able to bypass the cell wall synthesis by incorporating the D-alanine-D-lactate pathway. [14-16].

Enterococci are leading cause of infections among inpatients. Such nosocomial infections are around 20-30% of all nosocomial infections in the USA[17]. Since 1986 with the appearance of the first VRE strains the first case of vancomycin-resistant *Enterococcus faecium* was reported in December 2002, from a patient admitted to the intensive care unit (ICU) of the Aga Khan University Hospital, Karachi, Pakistan[18]. The rationale of the study was to highlight the importance of multidrug resistant bacterial infections and especially enterococci. The objective of the current study was to identify the number of cases diagnosed and possible factors in our setting that can be improved. and also to determine the sensitivity pattern of VRE against commonly used antibiotics.

Methodology

This retrospective study was carried out at Fatima Memorial Hospital, Lahore, Pakistan (IRB # FMH-3-09-25-IRB1779) through medical records review from May 2018 to September 2019. The hospital being a tertiary care academic/teaching hospital is equipped with a fully functional microbiology lab with capacity to handle most of the clinical specimens.

Sampling was done from admitted clinically infected patients. Samples were collected according to the site of infection including blood, pus, and biological fluids and catheter tips. Standard culture techniques were used on Blood and MacConkey agar plates and for urine samples Cystine Lactose Electrolyte Deficient(CLED) agar was used. Later the isolates were identified by a positive Gram reaction, a negative catalase test and a positive bile aesculin hydrolysis test followed by their serological confirmation by agglutination with Group-D antiserum. Microbial Identification & Antibiotic Susceptibility Testing (AST) was done by use of the VITEK 2 compact system.

Data Analysis

All data was expressed in absolute numbers and percentage for microbial resistance and sensitivity. SPSS version 26 (New York, USA) was used for analysis.

Results

Out of 1500 different clinical samples, Enterococci were isolated in from 86 different specimens over a period of one year with 8 isolates being reported as VRE.

TABLE 1 Total number of VRE isolated from 1500 different cultured samples.

SPECIMEN	ISOLATED ENTEROCCI	ISOLATED VRE
Urine	55	4
CVP line tip	8	0
Blood	7	0
Pus	7	1
HVS	6	0
Ascitic fluid	1	1
Tracheal aspirate	1	2
TOTAL	**87**	**8**

The antibiogram resistance profile of isolated enterococci against major antibiotics is provided in Table 2.

TABLE 2: Resistance patterns of Vancomycin resistant enterococci.

ANTIBIOTIC	SENSITIVE	RESISTANT	% SENSITIVE	% RESISTANT
Vancomycin	0	8	**0**	100
Linezolid	6	2	**85**	15
Ciprofloxacin	1- I	7	**14**	86
Ampicillin	1	7	**14**	86
Augmentin	0	8	**0**	100
Tigecycline	3	5 - NT	**100**	0

I – intermediate resistance, NT - not tested

Discussion

Our study showed a prevalence of VRE as 9.19 % of all enterococci isolates over one year in a tertiary care hospital. The increasing pattern of enterococcal resistance against Vancomycin led to the emergence of Vancomycin Resistant Enterococci (VRE) as a serious concern in clinical routine[19]. It also poses threat of increasing vancomycin resistance in Methicillin Resistant Staph Aureus (MRSA) due its ability to potentially transmit resistance within this organisms[9-11]. A study conducted in the tertiary care hospital of Karachi, over a period of four years from 2012 to 2015 in which total of 3192 consecutive samples showed a prevalence of VRE from 11 % to 15 % with a consistent increase per year while only the last year representing the resistance falling back to 13% [20]. These results were replicated by Babar N. et al [22] who reported frequency of VRE to be 11.5 percent and a recent study from northern India published in 2016 also reported a frequency of 11.3 %.[21]

Ampicillin generally is considered the drug of choice for ampicillin-susceptible enterococci, including VRE. Nitrofurantoin, Fosfomycin, and doxycycline have intrinsic activity against enterococci, including VRE, and are possible oral options for VRE isolated from the urine[23]. In our study 85 % of the isolates were found to be sensitive to linezolid only. Tigecycline, the use of which should be evaluated on a case-by-case basis was tested on only 3 out of 8 cases. All 3 were found to be sensitive to Tigecycline.

Tigecycline does not have FDA approval for treatment of VRE[24] although it has been approved for treatment of complicated skin and skin structure infections and intra-abdominal infections, including infections in which vancomycin-susceptible *E. faecalis*were recovered. Thus, the use of tigecycline especially in bacteremia patients should be considered on case to case basis.

Emergence of Linezolid resistance is also of a major concern as highlighted recently by Nasir et.al. from Paksitan.[25] In our study we report 2 cases of linezolid resistance.

Conclusion

It is of utmost importance to control the spread of VRE by decreasing the imprudent use of vancomycin. The guidelines provided by national and international organizations 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, the utmost important being antimicrobial stewardship.

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