

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 were found to be resistant to Vancomycin. 2 of these isolates were resistant to linezolid as well.

Conclusion: Vancomycin resistance among Enterococci isolates is a major concern for health care providers dealing with complex and prolonged hospital stay patients. As an added note, emergence of Linezolid resistance shrinks the choice of antibiotics at the disposal of treating physicians.

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 Streptococci¹, however currently these organisms are classified as separate genus with more than 35 identified species at present². *Enterococcus faecalis* (90%) and *Enterococcus faecium* (10%) are the two most commonly isolated species of the genus Enterococcus^{3,4}. Enterococci, are residential organisms of intestines and the female genital tract. These are unique organisms which have the ability to tolerate a wide temperature and pH range⁸ and can acquire resistance to many antimicrobial agents. In addition, this organism have been shown to transfer resistance to other pathogens e.g Methicillin resistant *Staphylococcus aureus* (MRSA)^{9,11}.

Partly due to increasing healthcare burden and partly due to growing antimicrobial resistance enterococcal infections are on the rise⁵. Glycopeptide is the most commonly used class of antibiotics against severe infections caused by Gram-positive pathogens, such as Enterococci. There has been a

growing concern that acquired resistance against Glycopeptides (vancomycin and teicoplanin) will ultimately limit their use for the treatment of enterococcus. Vancomycin introduced in 1958 is a cell wall inhibitor¹² 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 USA¹⁷. Vancomycin-resistant enterococci (VRE) were first identified in 1986. In Pakistan, the first reported case of vancomycin-resistant *Enterococcus Faecium* was discovered in December 2002 from a patient admitted to the Aga Khan University Hospital, Karachi.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 elucidate the prevalence of VRE and possible factors in our setting that can be improved. Our effort is also aimed at determination of 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 patients who carried the diagnosis of sepsis. Culture samples were taken from clinically relevant sites and included: blood cultures, pus cultures, other biological fluids (e.g. urine) and vascular catheter tips. In our hospital we use, standard culture techniques were on Blood and MacConkey agar plates, however for urine samples Cystine Lactose Electrolyte Deficient (CLED) agar is routinely used. The usual algorithm followed is the identification by a positive Gram reaction, a negative catalase test, a positive bile aesculin hydrolysis test and lastly, 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 ENTEROCOCCI	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 collected data shows VRE prevalence at 9.19 % of all enterococci isolates over one year. Our findings highlights the increasing pattern of enterococcal resistance against Vancomycin as being reported elsewhere¹⁹. As noted above, it also poses risk of increasing vancomycin resistance in Methicillin Resistant Staph Aureus (MRSA) due to its unique ability to transmit resistance to this, already virulent, organism⁹⁻¹¹. A study conducted in the tertiary care hospital of Karachi from 2012 to 2015 showed a prevalence of VRE from 11 % to 15 % (out of a total samples of 3192) with a consistent increase per year²⁰. These results were replicated by Babar N. et al²² who reported frequency of VRE to be 11.5 percent. This seems to be a consistent trend in the locoregional microbiological profile since a recent study (2016) from northern India 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 urine²³. 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 VRE²⁴ 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. The injudicious use of vancomycin should be strongly discouraged.

It is imperative that all procedures carried out in hospitals be completely sterile. Healthcare workers should be educated about its appropriate use in accordance with both international recommendations and local guidelines issued by the Medical Microbiology and Infectious Diseases Society of Pakistan (MMIDSP), which clearly define the clinical situations in which vancomycin therapy is justified.

Emergence of Linezolid resistance is also of a major concern as highlighted recently by Nasir et.al. from Pakistan.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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