

Screening of Phenotypic Traits of Vancomycin-Resistant Enterococci (VRE) in a tertiary care centre

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Abstract: - **Background:** In recent years *Enterococcus* spp. have become second, commonest cause of nosocomial infections accounting for 34- 46%, due to multidrug-resistant and these pathogens usually affect patients who are debilitated by other, concurrent illnesses and undergoing prolonged hospitalization, causing significant mortality and morbidity. These organisms survive in hospital environment as they have intrinsic resistance to several commonly used antibiotics and can acquire resistance to other antibiotics, either by mutation or through the transfer of plasmids and transposons. The emergence of VRE is a cause of concern, as it is very difficult to control as VRE can spread from one person to another through contact with contaminated surfaces or equipment or through person to person spread, often via contaminated hands.

Methods: 191 Enterococcal isolates were identified processed according to standard protocols and speciation was based on facklam and collins conventional method, and was assessed by phenotypic tests, antibacterial susceptibility pattern by minimum inhibitory concentration (mic) for vancomycin was done by E- strip.

Results: Out of 639 stool samples ,191(30%) *Enterococcus* spp. were isolated, in which 12(6.3%) isolates were VRE, in them E. faecium 9(75%) E. faecalis 2(16.5%) E.gallinarum 1(8.5%) Maximum samples were from surgery wards 8(66.6%) followed by Medicine 4(33.4%) ward. Phenotypes by mic showed Van A 11(91.6%), Van C 1(8.4%).

Conclusions: This study indicates the 6.3% VRE colonization in stool samples are highlighted showing 1.8% glycopeptide resistance phenotypes were identified in stool samples in our region. And for us to support the low level of resistance is by educating on uncompromising “Antimicrobial stewardship policy” and “Hospital infection control practices” are crucial.

1. INTRODUCTION

Enterococcus spp. is opportunistic pathogens present in the oral cavity, gut and female genital tract in humans. *Enterococci* are classified as group D *Streptococci*. *Enterococci* are facultative anaerobes that are part of the normal intestinal flora in humans.^{1,2} *Enterococci*, recognized as opportunistic pathogens, natural inhabitants of the oral cavity, gut and the female genital tract in both humans and animals. *Enterococcus* spp. lives in vast quantities [10^5 - 10^8 cfu] per gram of feces in the human intestinal lumen and under most circumstances cause no harm to their hosts.³

Enterococcal infections may occur via the ability to persist in harsh environments due to their intrinsic properties, contribute largely to emergence of this organism as a Nosocomial pathogen.^{3,4} *Enterococcus* spp. are most commonly implicated in UTI, SSI, bacteremia, endocarditis and pelvic Infections.^{2,5} *Enterococci* are the third leading

cause of infections.⁷

The rapid emergence of antimicrobial resistance among *Enterococci* makes it difficult to treat the chronic infections.³ The process of invasion is usually facilitated by damage to host tissues, presence of bacterial virulence factors such as adhesins & antibiotic resistance that gradually assist in advancement and further survival in newly infected place. *Enterococci* utilize several virulence factors for adherence and colonization in the infection site by formation of cell aggregates such as biofilms, with interactions between host and *Enterococci* leads to the clinical manifestation of infection in target vital tissues.^{1,6}

VanA phenotype glycopeptides resistance, characterized by high level inducible resistance to both vancomycin and teicoplanin. Which is predominantly found in *E. faecium*, VanA-type resistance also occurs in *E. faecalis* and occasionally in other *Enterococcal* species.^{5,16}

VanB phenotype is characterized by low to moderate levels of vancomycin resistance but susceptibility to teicoplanin, and is found predominantly in *E. faecalis* and *E. faecium*.¹⁷ Several genes, including *vanA*, *vanB*, *vanC*, *vanD*, and *vanE*, contribute to resistance to vancomycin in *Enterococci*.⁸

Identification of VRE to species level aids in confirming whether an isolate has intrinsic (*vanC*) or acquired resistance (*vanA* or *vanB*). Knowledge of the type of resistance is critical for infection control purposes. *vanA* and *vanB* genes are transferable and can spread from organism to organism. In contrast, *vanC* genes are not transferable, have been associated less commonly with serious infections, and have not been associated with outbreaks.

For species differentiation, motility and pigment tests are easily performed and are important tests to distinguish among species phenotypically. *E. faecium* and *E. faecalis* are non-motile, whereas *E. gallinarum*, *E. casseliflavus* & *E. flavescens* are motile. *E. casseliflavus* & *E. flavescens* have a distinct yellow pigment. In addition to motility and pigment tests, an organism's susceptibility profile also helps differentiate *vanA* and *vanB* isolates from *vanC* isolates.⁸

Colonized patients carry VRE but do not have clinical signs or symptoms of infection. This distinction is important in VRE screening. Patients are usually colonized in the gastrointestinal tract and occasionally in the urinary tract. VRE colony counts are similar in the stool of colonized or infected patients. If a hospital VRE rate is based solely on VRE isolated from clinical cultures (infected patients), the facility may be adequately reporting its infection rate, but may be underestimating the true burden (and therefore potential transmissibility) of VRE in the facility. Screening for patients colonized by VRE provides information about potential sources of illness. The goal of screening is to identify as many colonized patients as possible so that infection control measures can be implemented to decrease transmission and reduce the number of patients infected with VRE.^{5,14}

MATERIALS AND METHODS

In present study all stool samples received by the Department of Microbiology in 7 years during from January 2013 to December 2020 in our tertiary care center were included in study.

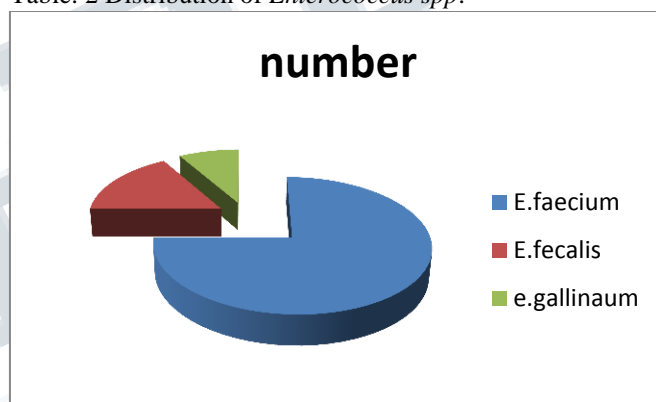
Total of 639 stool samples were inoculated on pure culture, out of which 191 *Enterococcus spp.* was isolated

and identified processed according to standard protocols and speciation was based on facklam and collins conventional method. Screening for VRE by inoculating stool specimens directly on bile esculin azide agar plates containing 6 µg/ml of vancomycin. Black colonies were identified as VRE and further confirmed as vancomycin resistant by an MIC E-strip method as per CLSI guidelines 2019.^{8,11}

RESULTS:

Out of 639 stool samples 191(30%) *Enterococcus spp.* were isolated, in which 12(6.3%) isolates were VRE and *E. faecium* 9(75%) *E. faecalis* 2(16.5%) *E.gallinarum* 1(8.5%).Maximum samples were from surgery wards 8[66.6%] *E. faecium* 6(75%), *E. faecalis* 1(12.5%)*E.gallinarum* 1(12.5%) followed by medicine 4(33.4%) ward *E. faecium* 3(75%), *E. faecalis* 1(25%).

Table: 2 Distribution of *Enterococcus spp.*



Out of 191 *Enterococcus spp.* isolates on pure culture had 12 VRE cases among which most common is *E. faecium* 9, *E. faecalis* 2 genotype VAN A which is acquired resistance and *E.gallinarum* 1, VAN C is intrinsic isolate, as we are already working on Hospital infection practices this data of VRE in our setting was a good eye opener for our clinicians and all other health care provider to know the significance of “Infection control practices” as simple as hand hygiene can prevent 90% of health care associated infections.

Infection control practices

- Keeping your hands clean to avoid getting sick and spreading germs that can cause infections
- Patients and their care givers should wash their hands with soap and water or use alcohol-based hand sanitizer, particularly:
 - after using the bathroom
 - before and after handling medical devices or caring for wounds before preparing food
- Frequently cleaning areas of common use such as bathrooms, doorknobs, switches, monitor screens...

etc that may become contaminated with VRE

- Wearing gloves if hands may come in contact with body fluids that may contain VRE, such as stool or bandages from infected wounds
- Always wash your hands after removing gloves.
- Informing healthcare providers if you or someone you care for, has VRE so that appropriate precautions can be taken to prevent spread.
- Hospital Infection Control Practices Advisory Committee (HICPAC) has made the following recommendations.
- Prudent use of vancomycin: Encouraging the appropriate use of oral and parenteral vancomycin is an important component of HICPAC recommendations. Other measures include formulary policies discouraging the use of third-generation cephalosporins and agents most likely to cause *C. difficile* colitis.
- Education of hospital staff: Continuous education programmes for health care workers should include information about the epidemiology of VRE and the potential impact of this pathogen on the cost and outcome of patient care.
- Effective use of the Microbiology laboratory: Early detection of patients colonized or infected with VRE is an essential component of any hospital programme designed to prevent nosocomial transmission of VRE.
- Implementation of infection control measures: Including the use of gloves and gowns and isolation of patients, as appropriate to specific conditions.
9,10,13,17,18

CONCLUSION:

Resistance to glycopeptides including vancomycin poses a therapeutic challenge to treating clinicians. Therefore, we undertook this study to “Screening of Phenotypic Traits of Vancomycin-Resistant *Enterococci* (VRE) in a tertiary care Centre” from all clinical stool samples to know its susceptibility pattern vancomycin which will help the clinician, to know the changing trend of *Enterococcus spp.*, to select appropriate therapeutic drugs, which aids in prevention of VRE and also provide appropriate control measures.

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