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COMPARITIVE EVALUATION OF PHENOTYPIC METHODS FOR VANCOMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF ENTEROCOCCI

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ABSTRACT:

Rate of Vancomycin resistance among Enterococci is increasing. This study was conducted to know the rate of vancomycin resistant enterococci and to compare the sensitivity and resistance of enterococci to vancomycin and to determine the minimum inhibitory concentration of both vancomycin sensitive and vancomycin resistance enteroccci.

Materials And Method:

In the current study, total 53 urinary isolates and 7 pus isolate of enterococccci strains were identified. The antibiotic susceptibility testing was determined for vancomycin using KirbyBauer disc diffusion and E-Strip test method. The interpretation was done according to CLSI guidelines and the results were compared with Vitek2.

RESULTS:

Out of 60 isolates, 58.3% of the isolates were identified as *E. faecalis* and 41.6% of the isolates were identified as *E. faecium*. 54 enterococcal isolates were sensitive to vancomycin and 6 isolates were vancomycin resistant. High level resistnce of enterococcal islotates towards tetracycin, ciprofloxacin and levofloxacin were observed. More numbers of enterococcal islotaes was observed in males than females. The enterococcal infections were more in in-patients, the infections caused by out-patients is very less.

CONCLUSIONS: Our study showed that the results obtained from disc diffusion and E-strip test were same. Discrepancy was observed when the results were compared to vitek results. By this we concluded that disc diffusion and E-Strip method can be widely used methods at peripheral laboratories where automated systems not available.

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INTRODUCTION:

Enterococci belong to the second biggest group reached in relation to microbial origin tracking. These are Gram-positive, Catalase-negative species and belongs to the Enterococcaceae family. It is a non–filamentous microorganism and is sometimes motile by scanty flagella. Enterococci species will have the ability to survive in the temperature ranging from 5-50 degree celcius. *E. faecium* and *E. faecalis* will have the capacity to grow in the broad range of pH with ideal being 7.5. Additionally, they can survive and even thrive in an environment containing 40% bile salts. *E. faecalis* can survive in the environment having 6.5% NaCl[1]. It is remarkable that the three Enterococcus species that were identified before 1950 are able to infect humans, given the widespread distribution of the Enterococcus genus in nature. *E. faecalis* and *E. faecium* are considered to be the most prevalent enterococci in human faeces and responsible for the all of enterococcal related illness. *E. faecium* infection was uncommon up to the mid nineteen nineties', when *E. faecalis* made up 90-95% of clinical specimens. Since then, more *E. faecium* isolates have been found, mostly as a result of antibiotic resistance, particularly to vancomycin and ampicillin[2]. Other species, including Enterococcus avium, gallinarum, casseliflavus, hirae, mundtii, and raffinosus, also have been isolated from human infection. Enterococcus gallinarum and Enterococcus casseliflavus infections are of special interest because of their intrinsic resistance to vancomycin, an antibiotic used to treat the aminoglycoside-resistant enterococcal infections that became problematic in the mid-1980s[3]. Most of the Enterococcus spp are commensal organisms, few are opportunistic human pathogen. *Enterococcus faecium* and *Enterococcus faecalis* are considered as the main important causative agents of nosocomial infections including, UTI, Endocarditis, Bacteremia, Neonatal Infection, Central Nervous System Infections and Abdominal and Pelvic Infections. Bloodstream infections caused by *Enterococcus faecalis* are connected with a significant rate of death. Recently, enterococci have drawn a lot of attention due to their astounding and rising antibiotic resistance, in addition to their growing importance in nosocomial infections. Because resistance enables enterococci to flourish in an environment with an abundance of antimicrobials, these two traits reinforce one another. The hospital setting provides medications that kill or suppress susceptible germs, giving resistant species a selection advantage, and the hospital also supplies the possibility for unsusceptibe enterococci to spread through the regular nosocomial routes[4]. A variety of cutting-edge methods for treating vancomycin-resistant enterococci [VRE] infections have been researched in experimental animal models, including β-lactam- β-lactam, β-lactam-glycopeptide, and β-lactam fluoroquinolone combinations. As there are chances of acquiring resistance to vancomycin through *VanA*, *VanB*, *VanC* genes [16]. Hence this study was undertaken to decide the rate of isolation of *Enterococcus spp* among pus and urine specimens received in the department of Microbiology for culture and sensitivity during the study period and also to compare vancomycin susceptibility profile of Enterococcus species using Vitek 2, disc diffusion ad E-strip.

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MATERIALS AND METHODS:

The current Laboratory based prospective study was held in the Department of Microbiology, JSS Hospital, Mysore for a duration of 3 months. All the urine and pus clinical samples received in the laboratory for culture and sensitivity and those yielding the growth of Enterococcus spp were taken in the study. Repeat specimens obtained from same patients or urine samples yielding mixed growth were excluded from the study. All the clinical samples were processed according to the Department Standard operating procedure and the isolates were subjected to phenotypic identification as follows:

Processing of Pus swabs:

Briefly, from two pus swabs that were received, one swab was subjected to direct microscopy, where grams and ZN staining was performed and the other swab was used for culture and inoculated on to Blood agar, MacConkey agar and CNA agar for isolation of pathogens. Depending on the growth, colony morphology, gram stain from the colony and biochemical reactions like catalase and Bile esculin agar test, Enterococcus spp was further included and processed.

Processing of urine samples:

A clean catch midstream urine sample was subjected to direct microscopy where wet mount examination was done for the presence of pus cells and organisms. Simultaneously, a drop of urine sample was transferred onto Urichrome agar by using a calibrated loop. Based on the colour of the colony and Vitek ID/ Manual ID, Enterococcus spp is further exposed to vancomycin susceptibility testing using Disc diffusion, E test and the vitek2 outcomes were compared.

Bile Esculin Test:

This is a selective as well as differential medium. It examines organisms' capacity to decompose esculin in the existence of bile. It's a term that's widely employed to describe representatives of the genus Enterococcus. The initial selective element in this agar is bile, which prevents Gram-positive bacteria save enterococci and several streptococci species from growing. Sodium azide is the second selective component. Esculin is the distinguishing component. The product esculetin is generated when an organism can dissolve esculin in the attendance of bile. Esculetin combines with ferric citrate to generate a phenolic iron complex that darkens to blackens the entire slant is considered as positive.

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Antibiotic Susceptibility Test:

The disc diffusion [DD] method was used to test susceptibility of antibiotic to vancomycin antibiotic, as mentioned by Clinical and Laboratory Standard Institute. The procedure entails applying antimicrobial vancomycin discs to the surface of agar that has already been sown with the bacteria under investigation. Then the plate containing organism and the disc is kept for incubation at 37-42℃ for 18-24 hours and observed for zone of inhibition. Those which showed a zone of ≥13 is considered as Sensitive and zone between 12-18 is considered as Intermediately sensitive and zone ≤12 is interpreted as Resistant to vancomycin.

E-Strip Test:

Ezy MIC was helpful for determining the quantitative antimicrobial susceptibility of bacteria. Following an overnight incubation, the system uses a predefined quantitative gradient to calculate the Minimum Inhibitory Concentration (MIC) in mcg/ml of various antimicrobial drugs against bacteria as tested on suitable agar media. Used only pure cultures that have passed a susceptibility test to Gram-staining. Transferred 4-5 comparable colonies using a wire, needle, or loop to a vitek2 tube that has been filled to the rim with peptone water, and then incubated for two hours at 35–37 °C until mild to moderate turbidity appears. Inoculum turbidity should be compared to the industry norm of 0.5 Mc Farland. Then, spin the plate at an angle of 600 degrees between each streaking after dipping a cotton swab into a sterilised inoculum and rotating the swab hard across the entire MHA agar surface three times. The plates should be incubated for 18–24 hours at 37–42°C. Read the MIC at the point on the strip where the ellipse crosses the MIC scale. Zone size interpretation for vancomycin on E-test is as follows:

Sensitive	**Intermediate**	**Resistance**
≤4	8-16	≥32

RESULTS:

The Rate Of Isolation Of Enterococci Among Urine Samples: Total 1488 urine samples were taken in the division of microbiology during the study period of March to May 2022, out of which 53 specimens have yielded the growth of enterococci which stands to be around 3.56% (53/1488). Out of which 56.6% (30/53) of the infections were found to be *Enterococcus faecalis* and 38.8% (23/53) of the infections were found to be *Enterococcus faecium.*

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Rate Of Isolation Of Enterococci Among Pus Samples: Total 295 pus samples were collected in the branch of microbiology during the study period of March to May 2022, out of which 7 specimens have yielded the growth of enterococci which stands to be around 2.37% (7/295). Out of which 71.42% (5/7) of the infections were found to be *Enterococcus faecalis* and 28.5% (2/7) of the infections were found to be *Enterococcus faecium*.

Of 60 samples included in the study, 56.6% (34/60) were collected from males and 43.3% (26/60) were from females, respectively. Majority 78.3% (46/60) of the participants were in-patients and 21.6% (13/60) were out-patients. Most of the Enterococcal infections were observed in the age group of >50.

Vitek2 results revealed that of 60 Enterococcal isolates, 8.3% (5/60) were resistant to vancomycin. 75% (45/60) samples were resistant to ciprofloxacin, 40% (24/60) samples were resistant to penicillin, 66% (40/60) samples were resistant to levofloxacin , 56.6% (34/60) samples were resistant to erythromycin, 5% (3/60) samples were resistant to linezolid, 10% (6/60) samples were resistant to teicoplanin, 86%(52/60) samples were resistant to tetracycline, 1.66%(1/60) samples were resistant to tigacyclin, 25%(15/60) samples were resistant to nitrofurantoin. No resistance to Daptomycin was noted in the current study.

Disc Diffusion Results: All the 60 isolates were subjected to disc diffusion and the interpretation was done accordance with guidelines of CLSI. Out of 60 isolates, 6 samples were resistant to vancomycin and 54 samples were sensitive to vancomycin

E-Strip Results: All the 60 isolates were subjected to E-Strip test and the interpretation was done according to CLSI guidelines. Out of 60 isolates 6 samples were resistant to vancomycin and 54 isolates were sensitive to vancomycin.

Discrepancy of vancomycin was noted in 1 isolate, where it was reported as intermediately sensitive and E-strip and disc diffusion showed resistant results.

DISCUSSION & CONCLUSION:

Enterococci Infections among healthcare have increasingly been linked to enterococci, which were once thought to be unharmful members of the gastrointestinal tract flora. They are now considered to be responsible for roughly 10% of all bacteremias worldwide, and in North America and Europe, they are the fourth and fifth most common causes of sepsis, respectively. Seema Sood et al (2008). In her studies mentioned that, Canada had the highest

enterococcal UTI detection rate (16.8%), followed by the US (12.5%) and Europe (11.7%)[5]. In our study the rate of infection of enterococci from urine specimen is 3.56% and from the pus samples is about 2.37%, out of which the rate of infection caused by *E. faecalis* is 58.6% and the rate of disease caused by *E. faecium* is 41.6%. Mounir M. et al (2018), in his study reported that 60-70% of the illnesses were caused by E. faecalis and 20-25% of the infections were caused by *E. faecium*. Jyoti Parameshwarappa et al (2013) in their study they have reported that 63.3% of the infections were caused by *E. faecalis* and 36.7% of the infections were caused by *E. faecium*[6]. Several studies have compared the performance of Vitek 2 system susceptibility testing along with other phenotypic tests for a single antimicrobial agent such as vancomycin, tetracycline, nitrofurantoin, or linezolid. In our study, Linzeolid was found to be the most outperformed drug than other agents such as vancomycin, penicillin, tigecycline, teicoplanin, and nitrofurantoin. Our study found that 90% of the isolates were vancomycin sensitive and 65% were sensitive to linezolid. These results were in consistent with an Egyptian investigation[7]. Out of 60 isolates collected in the study period 5 vancomycin resistant enterococci were found in vitek2, 6 isolates showed resistance to vancomycin on disc diffusion and E-Strip test, this discrepancy might be because of technical related issues where the inoculums did not match to 0.5Mcfarland turbidity either by Disc diffusion, E-strip or through Vitek2. Through this study, the authors would like to conclude that detection of MIC is mandatory for determining AST for any bacterial isolate. Disc diffusion test is not reliable for MIC. However, MIC detection can be done by various methods like Vitek2, E-Strip method and Broth dilution which have their own sensitivities. We found very few differences among the three compared methods. In the conclusion, Vitek2 method is always a feasible method because it not only gives antibiotic susceptibility pattern of minimum inhibitory concentration but also identifies the bacteria up to species level.

REFERENCES:

1. Bin-Asif H, Ali SA. The genus Enterococcus and its associated virulent factors. Microorganisms. 2019 Oct 29:109-30.

2. García-Solache M, Rice LB. The Enterococcus: a model of adaptability to its environment. Clinical microbiology reviews. 2019 Jan 30;32(2):e00058-18.

3. Riboulet E, Verneuil N, La Carbona S, Sauvageot N, Auffray Y, Hartke A, Giard JC. Relationships between oxidative stress response and virulence in Enterococcus faecalis. Microbial Physiology. 2007;13(1-3):140-6.

4. Hardie JM, Whiley RA. Classification and overview of the genera Streptococcus and Enterococcus. Journal of applied microbiology. 1997 Oct;83(S1):1S-1S.

5. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JD, Wengenack NL, Rosenblatt JE, Cockerill III FR, Smith T. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clinical microbiology reviews. 2006 Jan;19(1):165-256.

6. Parameswarappa J, Metri BV. Isolation, identification, and antibiogram of enterococci isolated from patients with urinary tract infection.

7.Rasha AA, Heba SE. Molecular characterization of enterococcus strains isolated from cases of neonatal sepsis in neonatal intensive care unit. African Journal of Microbiology Research. 2012 Nov 20;6(44):7206-11.