IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 1 Ver. 1 (January. 2019), PP 09-15
www.iosrjournals.org

Prevalence of Enterococcal Infection and the Antimicrobial Susceptibility Profile of the Organism with Special Reference to Vancomycin: A Study in a Rural Medical College Hospital in Eastern India.

DR. Hirak Jyoti Raj[1], DR. Munmun Das (Sarkar)[2*], Dr. Subrangshu Mondal[3].
[1]. *Assistant Professor, Dept. of Microbiology, Institute of Post-Graduate Medical Education and Research, Kolkata.*
[2]. *Associate Professor, Department of Microbiology, Calcutta National Medical College, Kolkata.*
[3]. *Consultant Microbiologist, CMRI, Kolkata.*
** Corresponding Author: Dr Munmun Das (Sarkar)*

Abstract: *Enterococci, once regarded as commensal flora are emerging as pathogen for multiple human infections. Their intrinsic resistance and properties of quick acquisition of resistance towards many in use and reserve antimicrobials draws the problem statement for these bacteria. The study eyes to determine the prevalence of Enterococccal infections and their antimicrobial susceptibility pattern to create a database and formulate guidelines for the empirical treatment. A prospective study was done over a period of 12 months in a rural tertiary care hospital enrolling patients having features of infections. Various samples were collected depending on clinical syndromes following criteria of inclusion and exclusion. Culture of samples was done followed by identification of resultant organisms including Enterococcus spp. as per standard guidelines. Antimicrobial susceptibility testing done for Enterococci isolates only by conventional disc diffusion method following CLSI guideline. Vancomycin resistance was tested by disc diffusion and Vancomycin screen agar method. In one year period a total 350 samples were cultured from different clinical cases. 173 of them were culture positive and 34 samples yielded Enterococus spp. Enterococus faecalis and Enterococus faecium isolates were 32 and two in number respectively. The overall prevalence of Enterococci was 9.71%. All Enterococci isolates were susceptible to Vancomycin, Linezolid, Teicoplanin. E. Faecalis was still susceptible towards Nitrofurantoin (86.2%), and HL Gentamicin (68.75%), HL Streptomycin (56%) but to a lesser extent to Fluoroquinolones, Chloramphenicol (21.86%) and Ampicillin (31.25%). E. faecium isolates were resistant to Fluoroquinolones , Chloramphenicol , HL Streptomycin (100%) and were susceptible to Nitrofurantoin, and HL Gentamicin, Ampicillin (50%). Vancomycin resistance among Enterococci was not an emerging problem in this geographic area. Prevalence of Enterococcus faecalis was higher than Enterococcus faecium. Enterococcus faecium was more drug resistant than Enterococcus faecalis.*

Key words: *Enterococci, Vancomycin, Prevalence, Resistance.*

Date of Submission: 18-12-2018 Date of acceptance: 03-01-2019

I. Introduction

The genus *Enterococcus* includes the Enterococcal members previously classified with the group D Streptococcus, and are Gram positive cocci that occur singly or arranged in pairs or as short chains.The so called 'Streptococcal group D antigen' is usually associated with the *Enterococcus spp*., but it isfound in the strains belonging to the *Streptococcus bovis* complex, as well as some *Leuconostoc, Pediococcus, Vagococcus* strains also. In human, enterococci are normal resident flora of the gastrointestinal and billiary tracts and, in lower numbers, of the vagina and male urethra[1]. Enterococcal population in the intestinal tract fluctuate according to the age and physiological condition of host.

25 distinct species are now included in the genus Enterococcus. Commonest being *E. Faecalis* followed by *E. faecium* to cause human infection. Though not considered to be highly virulent, their intrinsic resistance and ability to acquire resistance to several broad-spectrum antibiotics allow them to cause superinfections in patients already receiving antimicrobial therapy. *Enterococci* cause wide variety of infections namely Urinary tract infection (UTI), the commonest one, followed by bacteremia, endocarditis, intra-abdominal and pelvic infections, wound and soft tissue infections,neonatal sepsis, and very rarely meningitis[1].

Risk factors for development of Enterococcal bacteremia include structural abnormality or instrumentation of urinary tract, infection of other body sites like billiary tract, gastrointestinal tract or genitourinary tract infection, special conditions like advanced age, prematurity, immunosuppression, diabetes

mellitus, malignancy, renal insufficiency, congestive heart failure and deep seated infections[1]. These conditions are further synergised by long-term hospitalization and use of broad spectrum antibiotics having little or no anti-enterococcal activity (e.g., Cephalosporins).

Because of the diverse antimicrobial resistance mechanisms, both intrinsic and acquired as well, successful treatment and control of enterococcal infections are becoming increasingly difficult. Present options of antimicrobials for treating Vancomycin resistant *Enterococci* (VRE) include Linezolid, Quinupristine/Dalfopristin and Teicoplanin. Two newer antimicrobials came to act upon enterococcal strains including VREs namely Daptomycin, and Tigecycline[2]. Daptomycin is currently the Drug of Choice for VRE Strains.

The proposed study is an effort to find out to determine the prevalence of enterococccal infections and their antimicrobial susceptibility pattern in our hospital located in Burdwan so that guidelines for the empirical treatment of such infections can be formulated where facilities for culture do not exist.

II. Material And Methods

Within a span of one(01) year time, total 350 samples were collected comprising of 225 urine samples 45 blood samples (for culture) and 80 samples from suspected wound infections, diabetic foot ulcer, intra-abdominal abscess following strict inclusion and exclusion criteria and supplemented with a relevant questionnaire. Samples were collected following strict aseptic precaution to avoid any contamination following the guideline of standard text books[3,4,5]. Urine samples were cultured by semi-quantitative method in MacConkey's Agar and examined after overnight incubation to find out presence or absence of any growth of organism.Culture showing $\geq 10^5$ colony forming unit (cfu) of a single pathogen/ ml of properly collected mid stream clean-catch urine sample indicated infection. However, significant bacteruria is lacking in some cases of true UTI. Especially, in symptomatic patients or those on Antimicrobial therapy, a smaller number of bacteria (10^2 to 10^4 /ml) may signify infection. Urine specimens obtained by suprapubic aspiration or 'in- and-out' catheterization and in samples from a patient with an indwelling catheter, colony counts of $>10^2$ to 10^4/mL generally indicate infection[6].In case of two different types of colonies with no predominant one grown on a single plate,both the colonies were processed up till end.Samples were collected from ulcers, burn wounds, post-operative wounds, diabetic ulcer,trophic ulcer wounds through sterile cotton or dacron swabs from the patients admitted in the hospital and those who came to microbiology lab, after through explanation about the procedure and with proper consent from them. Before a representative sample was collected, any contaminating materials such as slough, necrotic tissue, dried exudates and dressing residue were removed by cleansing the wound with sterile normal saline.Two swabs were taken from the depth of wounds one for microscopy and the other for the seeding of culture plates. Following timely transport (As per guideline)swabs were processed within four hours. Each specimen was inoculated on to 5% Sheep Blood Agar, Nutrient Agar, and MacConkey Agar media and incubated aerobically at 37^o C for 18-24 hrs. and were examined for typical colonies.Suspected *Enterococcus* colonies were subcultured in MacConkey agar from mixed growth in order to obtain pure colony. Aspirate/ fluids from suspected intra-abdominal abscess sent to Dept. Of Microbiology with suggestive history to fit inclusion criteria were included in the present study and processed by both Gram's staining- microscopy and aerobic culture in MacConkey's agar and 5% Sheep blood Agar.Blood culture was indicated for suspected cases of *Enterococcal* bacteremia or endocarditis and the procedures were done following text book methodology[7].

2.1. Identification of Enterococci [1,3,8]:

Enterococcus looking colonies that showed Gram positivity, arranged in pair or tetrad and catalase negative were further confirmed by biochemical testing namely i) production of aesculin in 40% bile, ii) production of acetoin(VP +ve), iii) PYR and LAP positivity, iv) growth in pH 9.6, v) tolerance of 60^0C for 30 minutes vi) ability to grow in both 10^0 C and 45^0 C. Speciation of *Enterococci* isolates were done by tests for utilization and/or fermentation of mannitol, arabinose, pyruvate and raffinose. A typical strain of *Enterococcus faecalis* ferments mannitol, pyruvate and sorbitol but not arabinose and raffinose. On the contrary, *Enterococcus faecium* utilizes mannitol and arabinose but not pyruvate. Media and reagents for biochemical tests were procured from HI MEDIA, Mumbai. Tests were performed following standard text book procedures[1].

2.2. Antimicrobial susceptibility testing (AST):

AST of *Enterococci* isolates obtained was performed by Kirby Bauer Disc Diffusion Method in Muller-Hinton agar plates at 35^0 C as temperature for incubation. Incubation period was strictly 24 hours. Battery of antimicrobials chosen for AST, following CLSI guideline for AST comprised of High Level gentamicin (120µg), High Level streptomycin (300 µg), and ciprofloxacin (30 µg), levofloxacin (5 µg), ampicillin (30 µg), chloramphenicol(30 µg), vancomycin (30 µg), teicoplanin (30 µg), nitrofurantoin(300 µg) (for urinary isolates only), linezolid (30 µg), quinupristin/dalfopristine (20/10 µg). At least 24 mm gap was

maintained from centre to centre in between 2 discs. During performance of biochemical tests for identification, *Enterococcus faecalis* standard strain ATCC 29212 was used as control strain along with the isolates of *Enterococcus* obtained from the study samples. ATCC 29212 strain served as the control strain even at the time of AST.

2.3. Test for vancomycin resistance:

Vancomycin resistance in the present study was detected by:-

2.3.1) Conventional Disc Diffusion Method using Vancomycin (30 µg) disc. The disc is applied on a lawn of *Enterococcus spp*. A zone of inhibition (ZOI) ≥ 17mm was considered susceptible; whereas ZOI between14-17 mm and < 14mm was marked as intermediate sensitive & resistant respectively [9].

2.3.2)By using Vancomycin screen agar which is brain heart infusion agar supplemented with vancomycin (6 µg/ ml). Vancomycin screen agar is used for the screening of *Enterococci* resistant to vancomycin**.** In vancomycin screen agar plategrowth of *Enterococcus spp.* (≥ 1 colony) indicates the strain is resistant to vancomycin[10].

III. Result And Analysis

During the study period 350 different samples were collected from in outdoor and admitted in different wards of a rural tertiary care hospital of eastern India. Out of total clinical samples urine wasmaximum in number followed by different categories of wound swab/ pus/ body fluids.173 clinical samples were found to be culture positive. Among them 34 isolates were identified as of *Enterococci* comprising of 32 *E. faecalis* and two *E. Faceium* isolates. 31 isolates were obtained from urine samples (225 in number). Rest three isolates were recovered from wound infections including two isolated from diabetic foot wound swabs cultured.No *Enterococcus* was isolated from blood samples cultured in the study. So, the overall prevalence of *Enterococci* was being 9.71% among all samples. The same for urinary tract infection was being 13.77%. *Enterococcal* wound infection had the prevalence of 3.75% as calculated from the total number of relevant samples and yield of the organism under study from them. In AST, all *Enterococci* were susceptible to Vancomycin as tested by both vancomycin screen agar and Disc Diffusion method of Kirby-Bauer; Teicoplanin, another glycopeptides antibiotic and Linezolid. Both the *E. faecium* isolates were susceptible to Quinupristin/dalfopristin. Regarding other antimicrobials, susceptibility of *Enterococcus faecalis* isolates (n=32) showed following pattern (TABLE-1) – Ampicillin - 31.25%, gentamicin (high-level) - 68.75%, streptomycin (high-level) - 56.25%, chloramphenicol - 1.86%, ciprofloxacin-12.5%, levofloxacin-25%, nitrofurantoin-86.2%. All isolates are resistant to erythromycin. *E. faecium*(n=2) isolates are found to be more resistant than *E. faecalis* isolates (TABLE-1) as evident from following sensitivity profile:gentamicin (high level)-50%, nitrofurantoin-50%, ampicillin -50%. *E. faecium* isolates were resistant to all other antimicrobials used.

TABLE 1:- Antimicrobial susceptibility pattern of the isolates of *Enterococci*

	***E. faecalis*, n=32**		**E. faecium, n=02**	
Antimicrobials	Sensisitive	Resistant	Sensitive	Resistant
Ampicillin	10(31.25)	22(68.75)	01(50)	01(50)
Gentamicin	22(68.75)	10(31.25)	01(50)	01(50)
Streptomycin	18(56.25)	14(43.75)	00	02(100)
Chloramphenicol	07(21.86)	25(78.14)	00	02(100)
Erythromycin	00	32(100)	00	02(100)
Ciprofloxacin	04(12.5)	28(87.5)	00	02(100)
Levofloxacin	08(25)	24(75)	00	02(100)
Nitrofurantoin	25, out of 29(86.2)	04, out of 29 (13.8)	01(50)	01(50)
Vancomycin	32(100)	00	02(100)	00
Linezolid	32(100)	00	02(100)	
Teicoplanin	32(100)	00	02(100)	00
Quinupristin/ dalfopristine	-	-	02(100)	00

TABLE 2:-Patients' relevance between isolation of Enterococcus from their urine samples and history of catheterization and/ or instrumentation of their urinary tract.

Patients	Number of Enterococcus from patients with h/o urinary catheterisation/ instrumentation	Number of Enterococcus from patients with out h/o urinary catheterisation/ instrumentation	Total number of Enterococci isolates from urine samples
Male	05(41.66)	07(58.34)	12(100)
Female	05(26.32)	14(73.68)	19(100)
All patients	10(32.26)	21(67.74)	31(100)

*****Figures in the parenthesis indicate percentage.

Analysis:-It was not statistically significant (X^2= 0.799, p > 0.05; d.f=1).

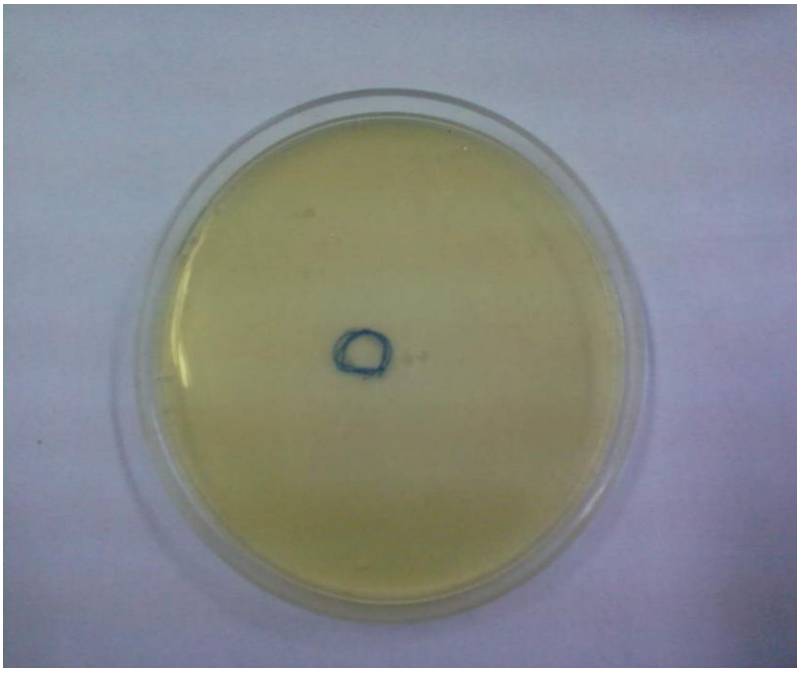

Fig 1: Vancomycin screen agar: Encircled area shows site of spot inoculation and No resultant growth after 24 hrs incubation (Susceptible)

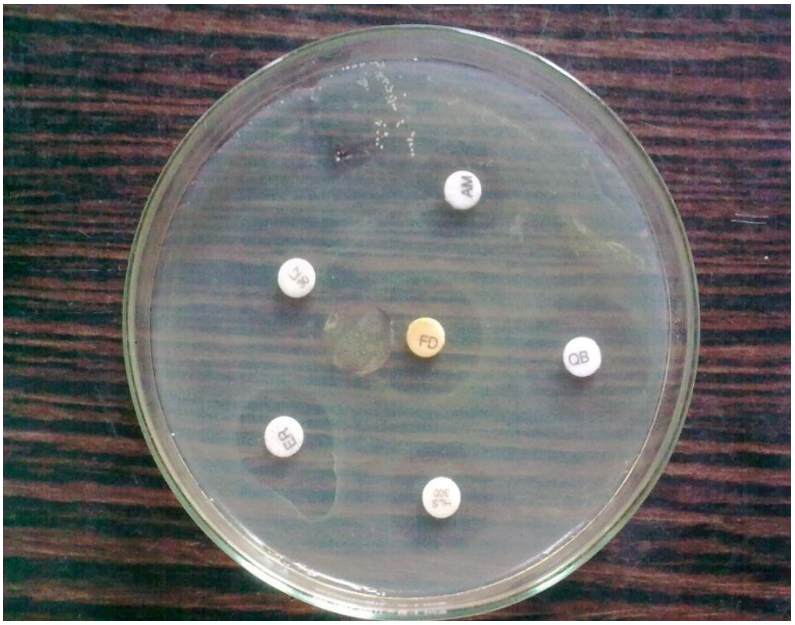

Fig 2: Susceptibility Profile of *Enterococcus faecalis (partially presented)*

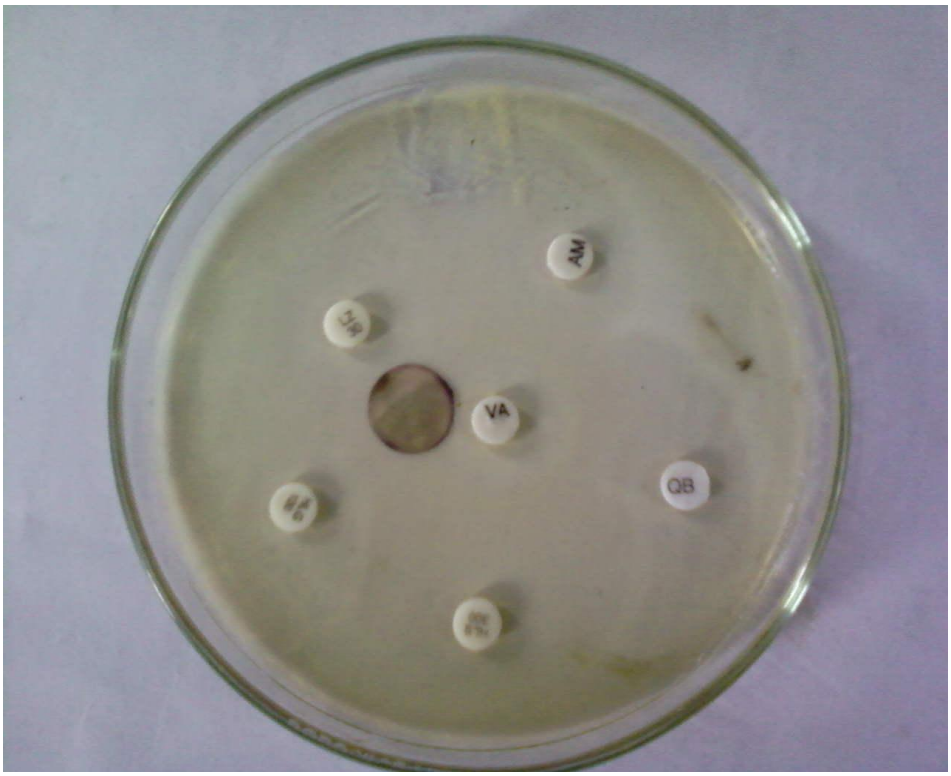

Fig 3: Susceptibility Profile of *Enterococcus faecalis (partially presented)*

IV. Discussion

Enterococci have not-clearly understood armamentarium consisting of Cytolysin/hemolysin, aggregation substance, extracellular surface protein (Esp) Sex pheromones, Gelatinase, AS-48 and extracellular superoxide thought to be participating as virulence factors[1,11]. Other factors include lipoteichoic acid that induces production of Tumour necrosis factor (TNF) & interferon, and coccolysin[1]. Resistance to several commonly used antimicrobials is a remarkable characteristic of most of the *Enterococcus* species. In addition to intrinsic resistance, *Enterococcus spp.* have acquired different genetic determinants that confer resistance to several other classes of antimicrobials including glycopeptides (Vancomycin). Acquired Vancomycin resistance in *Enterococcus* species corresponds to five different phenotypes, designated Van A, VanB, VanD, VanE, Van G [1].

Out of 350 clinical samples collected following strict inclusion and exclusion criteria, 225 consisted of urine, 80 pus/wound swab/body fluids from different body sites, 45 blood samples. Culture positivity was observed in 173 clinical samples (52.74%). Out of these 32 were those of *Enterococcus faecalis* and two *Enterococcus faecium* making a sum of 34 *Enterococci* isolates. So, the prevalence of *Enterococcal* isolates in this present study becomes 9.71%. This finding differs from that of K. O. Olawale of Osogbo, Nigeria, who found the prevalence to be 5.9% among different clinical samples in a similar study[12].Whereas, Desai et al had the finding of 22.19% out of total clinical samples in a similar study, which is higher than our present finding[13].The prevalence of *Enterococcus spp.* in urine samples is 13.77% which is nearly similar Karmarkar M.G. et al (10.27%)[14]. But it contradicts results of T. Butt et al of Pakistan (*Enterococci* prevalence in UTI ~4%)[15] and Desai P. et al (8.92%)[13]. Among the isolates obtained from wound infections/pus one was from wound infection and rest two were recovered from diabetic ulcers. The prevalence of *Enterococcal* wound infection in our study well correlates with study done by Dawood SE et al[16], but it does not corroborate with the study done by Cathey A. Petty et al in 2002 (isolation rate 12%)[17]. There is a unique finding that diabetic ulcers may show mixed growth on culture containing Gram – ve bacteria along with Gram + ve one like *Enterococci*. However, the role of Enterococci in such infection is still not clear. Blood samples were small in number due to strict exclusion criteria and no *Enterococcus spp* was isolated from blood. Most relevant species of *Enterococcus* which were isolated from the clinical samples in this study, were *E. faecalis* (94.28%) and *E. faecium* (5.72%). This finding well correlates with that of the study done by Gupta V. et al (*E. faecalis 96% and E. faecium 4%*)[18], T. Butt et al of Pakistan (*E. faecalis 90.28% and E. faecium 9.72%*) [15]. But the finding is dissimilar to that of Karmarkar et al[14]. No other species of *Enterococcus* was isolated in this study. Majority of isolation of Enterococci occurred from samples of hospitalized patients than those from outdoor patients. A major reason behind the survival of these organisms in hospital environment may be due to their virulence

factors and/ or being intrinsically resistant to commonly used antimicrobials and perhaps more importantly due to their ability to acquire resistance either by mutation or by receipt of foreign genetic material through plasmid and transposon[19].

In the present study 31.25% and 43.75% of *E. faecalis* isolates (n=32) were found to be resistant to gentamicin and streptomycin (both high level). Finding of the present study has been supported by the study of P. Mathur et al (26% isolates of *E. faecalis* having HLAR)[20] but not corroborating with that of Agarwal *et al*. showing a prevalence of HLGR in enterococci to be 7.8% whereas HLSR was reported to be 24.7%[21]; a study by Zervos et al showing a prevalence of 55% of HLGR in enterococci in an US centre[22], Karmarkar M.G et al (100% resistance to HLG among the isolates) [14], Gupta V et al (HLAR in 75% of the strains for gentamicin and in 69% strains for streptomycin)[18].Gentamicin -resistant enterococci (HLGRE) were frequently recovered in the AHEPA University Hospital, Thessaloniki, Greece, during a 15-month period (67/158, 42.4%)[23]. Because high level aminoglycoside resistant *Enterococci* often have plasmids which carry determinants encoding resistance to other antibiotics, these isolates often become multi-resistant. Regarding Erythromycin, P. Mathur et al. found 85% resistance[20], Gupta V et al. found 77.19% in their study[18]. In present study the resistance to erythromycin was 100%. So, erythromycin cannot be relied upon as an antimicrobial against *Enterococccci*. Almost 87.5% and 75% of *E. faecalis* isolates (n=32) in this study were resistant to Ciprofloxacin and Levofloxacin, respectively. In case of *E. faecium* strains (n=2), both are resistant to the drugs (Table 1). This finding was closely similar to that of P. Mathur et al.[20], Karmarkar et al.[14], but didn't match with the that of Makri A. et al[24]. Nitrofurantoin was used against the *Enterococci* isolates recovered from urine samples only. TABLE-1 showed sensitivity to Nitrofurantoin of *E .faecalis* (86.2%) and *E. Faecium* (50%) only; suggesting that it could be a drug of choice for treating enterococcal UTI, empirically. This finding was quite similar to that of T. Butt et al (88% of the *Enterococci* were sensitive to nitrofurantoin)[15] and Gupta V. et al (15.9% of the isolates are resistant)[18].In the present study all the isolates of *E. faecalis* (n=32) and *E. faecium* (n=02) were sensitive to Vancomycin. Antimicrobial susceptibility testing for Vancomycin was done by Kirby-Bauer disc diffusion method and vancomycin agar screen method as described under the section of Material and Methods. De A. et al followed the same methodology in their study and found 1.5% of the *Enterococci* isolates to be vancomycin resistant [25]. In a study, Taneja N. et al at PGI, Chandigarh, India, also adopted vancomycin agar screen method followed by MIC determination. They found 5.5% VRE strains among total *Enterococci* isolates[26]. Our finding corroborates with that of Makri et al. Out of their 116 strains, 88 were identified as *E. faecalis* and all were susceptible to vancomycin[24]. Study performed at an army hospital of Rawalpindi, Pakistan, reported 98.61% *Enterococci* isolates (n=144) to be susceptible to Vancomycin following the same methodology [15]. P. Mathur et al of AIIMS, New Delhi in a similar study with a total 444 isolates of *E. faecalis* found only 1% VRE strains[20]. Of late, an overall 1.82% vancomycin resistance in *Enterococci* has been reported by a group from Rajasthan, India[27]. But probably they did not adopt vancomycin agar screen methodology.

All the isolates of *E. faecalis* and *E. faecium* in the present study were sceptible to linezolid (disc diffusion method). This is similar to the finding of Makri A. et al and Gupta V. et al as referenced earlier. But *E. faecium* strains in the study of Makri A. et al were resistant to linezolid(11.7% isolates)[24].

All the isolates tested were susceptible to teicoplanin also. This was similar to Makri A. et al[24], Karmarkar et al[14], James W. Gray et al[28]and comparable to that of Gupta V. et al (2.1% isolates resistant)[18]. Both the *E. faecium* isolates were tested with quinupristin/ dalfopristin and found to be susceptible (100% susceptibility).

Summarily, the present study pointed out that *Enterococci* isolates from different clinical samples were quite low in comparison to other Gram positive bacteria and Gram negative intestinal pathogens. Fortunately, this study, done at the tertiary care hospital, did not show the *Enterococci* to be resistant to vancomycin. This might be due to precaution and measures taken to prevent emergence of this strain inthis hospital. So, it appeared from our study that Vancomycin Resistant *Enterococcus* (VRE) was not a significant problem in this geographical area. However, our study faced some limitations like short study period, small sample size for the study. As shown from different studies that VRE strains are mostly related to critical care unit, intensive care unit and ventilator associated infections. Most of the studies, which recovered VRE isolates, had either a larger sample size or a fairly big number of *Enterococci* isolates to begin with. A detailed project encompassing multicentre comprising of a large number of clinical samples spread over a longer period would clearly indicate the present status of Vancomycin resistance in *Enterococci*.

Acknowledgement

Faculty and staff of Dept of Microbiology, Burdwan Medical College, Burdwan.

References

[1]. Washington C. Winn Jr., Stephen D. Allen, William M. Janda, Elmer W. Koneman, GrayW.Procop, Paul C. Schreckenberger et al; Gram –Positive cocci Part-II: Streptococci, Enterococci, and the Streptococci like bacteria; Koneman'sColor Atlas and Textbook of Diagnostic Microbiology; 6th edition; USA; Lippincott Williams & Wilkins –A Wolters Kluwer Company; p700-4

[2]. Fauci,Braunwald,Kasper,Hauser,Longo,Jameson,Loscalzo; Sterptococcal and Enterococcal Infections; Harrison's principles of Internal Medicine ; vol-1 ,17th Edition, USA, McGraw Hill,2008;p 881-90

[3]. Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld; In: Infection of the urinary tract. Bailey & Scott's Diagnostic Microbiology. 12th edition: USA; Mosby Elsevier; 2007; p 842-55.

[4]. Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld; In: Bloodstream infection. Bailey & Scott's Diagnostic Microbiology. 12th edition: USA; Mosby Elsevier; 2007; p 778-97.

[5]. J.Colle/A.G. Fraser/B.P.Marmion /A.Simmons; In: Laboratory strategy in the diagnosis of Infective Syndrome, Lab control of antimicrobial therapy,eds. Mackie & McCartney, Practical Medical Microbiology, International Student Edition ; 14th edition USA; Churchill Livingstone,1996; p53-94, p151-78.

[6]. Fauci,Braunwald,Kasper,Hauser,Longo,Jameson; In: Urinary tract infection and Pyelonephritis; Harrison's principles of Internal Medicine; vol-II, 16th Edition, USA,McGraw Hill,2005;p 1715-21.

[7]. Betty A. Forbes, Daniel F. Sahm, AliceS.Weissfeld; In: Overview of bacterial identification Methods and Strategies. Bailey & Scott's Diagnostic Microbiology. 12th edition: USA; Mosby Elsevier; 2007; p 216-47

[8]. J.Colle/A.G. Fraser/B.P.Marmion /A.Simmons; Streptococcus and Enterococcus ; Mackie & McCartney, Practical Medical Microbiology, International Student Edition ; 14th edition USA; Churchill Livingstone,1996; 269-72

[9]. Clinical and laboratory standards institute. performance standards for antimicrobial susceptibility testing ; twenty first informational supplement. (m100-s21); Cls document. Pensylvania,Wayne ,pa,USA.2011.

[10]. Washington C. Winn Jr., Stephen D. Allen, William M. Janda, Elmer W. Koneman, Gray W. Procop, Paul C. Schreckenberger et al; Antimicrobial susceptibility testing; Koneman'sColor Atlas and Textbook of Diagnostic Microbiology; 6th edition; USA; Lippincott Williams & Wilkins –A Wolters Kluwer Company; p945-1021

[11]. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci.Clin Microbial Rev 1994;7;462-78(eta porer ref no. hobe)

[12]. KO Olawale, SO Fadiora, SS Taiwo; Prevalence of hospital acquired enterococci infections in two primary-care hospitals in Osogbo, Southwestern Nigeria; African Journal of Infectious Diseases>Vol 5, No 2 (2011)>Olawale

[13]. Desai P ; Pandit D ; Mathur M ; Gogate A; Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterized patients; Indian Journal of Medical Microbiology; 2001, Vol 19, issue 3, pp132-7.

[14]. Karmarkar M.G, Gershom Edwin S. & Mehta P. R. , Enterococcal infections with special reference to phenotypic characterization & drug resistance; Indian J Med Res 119 (Suppl) May 2004, pp 22-5.

[15]. T. Butt, M. J. Leghari, AMahmood; Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi. In-Vitro Activity of Nitrofurantoin in Enterococcus Urinary Tract Infection, JPMA 54: 466:2004.

[16]. Dowd SE, Wolcott RD, Sun Y, McGeehan T, Smith E, et al. (2008) Polymicrobial Nature of Chronic Diabetic Foot Ulcer Biofilm Infections Determined Using Bacterial Tag Encoded FLX AmpliconPyrosequencing (bTEFAP). PLoS ONE 3(10): e3326. Doi:10.1371/journal.pone.0003326; Editor: Christophe Egles, Tufts University, United States of America.

[17]. Cathy A. Petti, Linda L. Sanders, Sharon L. Trivette, Jane Briggs, and Daniel J. Sexton ;Postoperative Bacteremia Secondary to Surgical Site Infection; Clinical Infectious Diseases 2002; 34: p 305–8.

[18]. Gupta V, Singla N. Antibiotic susceptibility pattern of *Enterococci*. Journal of Clinical and Diagnostic Research [serial online] 2007 October [cited: 2007 Oct 1]; 5:385-9.

[19]. SeemaSood, MeenakshiMalhotra, B.K. Das &ArtiKapil; Enterococcal infections & antimicrobial resistance Revie article: Indian J Med Res 128, August 2008, pp 111-121.

[20]. Mathur P., ArtiKapil, Rachna Chandra, Pratibha Sharma &Bimal Das; Antimicrobial resistance in *Enterococcus faecalis*at a tertiary care centre of northern India ; Indian J Med Res 118, July 2003, pp 25-8.

[21]. Agarwal VA, Jain YI, Pathak A. Concomitant high level resistance to penicillin and aminoglycosides in enterococci at Nagpur, Central India. *Indian J Med Microbiol*1999; *17:* 85-7.

[22]. Zervos MJ, Kauffman CA, Therasse PM, Bergman AG, Mikesell TS, Schaberg DR. Nosocomial infection by gentamicin resistant *Streptococcus faecalis*. *Ann Intern Med* 1987; *106*: 687-91.

[23]. Tsakris A, Pournaras S, Maniatis AN, Douboyas J, Antoniadis A, Increasing prevalence of high-level gentamicin resistance among Enterococci isolated in Greece; Chemotherapy: 2001 Mar-Apr; 47(2): p86-9.

[24]. Makri A., Papavasileiou K., Iliadou H., Guahardo T., Gavalaki F., Oikonomopoulos G., Voyatzi A; Enterococcal urinary tract infections in children; Abstract number: P2223; 18 th European Congress of clinical microbiology and Infectious Disease; Barcelona,Spain,2008

[25]. De A, Bindlish A, Kumar S, Mathur M. Vancomycin resistant enterococci in a tertiary care hospital in Mumbai. Indian J Med Microbiol 2009; 27:375-6

[26]. NeelamTaneja, Phulan Rani, Rekha Emmanuel &Meera Sharma; Significance of vancomycin resistant enterococci from urinary specimens at a tertiary care centre in northern India, Indian J Med Res 119, February 2004, pp 72-4

[27]. Sood S, Gupta R. Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. Indian J Community Med 2012;37: 39-44

[28]. James W. Gray, David Stewart, Stephelen J. Pedler Species Identification and Antibiotic Susceptibility Testing of Enterococci Isolated from Hospitalized Patients; Antimicrobial Agents and Chemotherapy, Sept. 1991, p. 1943-5.

Dr Munmun Das (Sarkar). "Prevalence of Enterococcal Infection and the Antimicrobial Susceptibility Profile of the Organism with Special Reference to Vancomycin: A Study in a Rural Medical College Hospital in Eastern India."." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 1, 2019, pp 09-15.