Prevalence and Antibiotic Susceptibility Pattern of *Enterococcus* Species from Various Clinical Samples in a Tertiary Care Hospital in Kolkata

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ABSTRACT

Introduction: *Enterococcus* species has recently become the major nosocomial pathogen exhibiting resistance to many antimicrobials especially to vancomycin with increasing frequency. The aim of this study was determination of prevalence and susceptibility pattern of *Enterococci* in a tertiary care hospital.
Material and Methods: This study was done in the Department of Microbiology, Nilratan Sarkar Medical College, Kolkata, from January to December 2013.The samples included urine, blood, pus collected aseptically from patients suffering from urinary tract infection (UTI), septicaemia, pyogenic infections and their culture and antibiotic sensitivity were performed as per standard recommendations. Minimum inhibitory concentration (MIC) of vancomycin was determined by E test for all *Enterococci* isolates.
Results: The *Enterococci* isolated from 8153 clinical samples were 395, accounting for an infection rate of 4.8%. The maximum number of *Enterococcus* isolates obtained from urine 80% (318), followed by pus 16% (64), and blood 3.3% (13). The sensitivity pattern of the isolates showed an increased resistance to antibiotics like ampicillin, ciprofloxacin and gentamicin. Among the isolated *Enterococci* 3.8% (15) were vancomycin resistant. All the *Enterococci* were sensitive to linezolid
Conclusions: Various studies have shown an increase in the rate of infection and antibiotic resistance in *Enterococcus* species. There is also a change in pattern of antibiotic resistance in *Enterococcus* species with an increased isolation rate of VRE. The present method of VRE detection is not beyond doubt. It needs confirmation by MIC value.

Keywords: *Enterococci*, Antimicrobial susceptibility, Vancomycin resistant *Enterococci.*

INTRODUCTION

The genus *Enterococcus* are Gram-positive, ovoid shaped cocci, arranged in short chain or in pairs. Though they are normal flora of the intestinal tract, oral cavity and vagina, but have emerged as nosocomial pathogens.[1-3]

Enterococci have become increasingly important because of their ability to cause serious infections like endocarditis, bacteraemia, intraabdominal and urinary tract infection and due to their increasing resistance to different antimicrobials which include β lactam antibiotics, aminoglycosides and most importantly, glycopeptides like vancomycin. Serious *Enterococcal* infections are often refractory to treatment with a high mortality rate.[4-5]

Enterococcus infections have traditionally been treated with cell wall active agents (eg.penicillin or ampicillin) along with an aminoglycoside (streptomycin/gentamicin). Nowadays, increasing resistance to β lactam antibiotics and vancomycin and emergence of high level aminoglycoside resistance (HLAR) has led to failure of synergistic effect of combination therapy.

This emphasizes the need for their identification from various clinical specimens and to determine the accurate antimicrobial resistance patterns for *Enterococci* with special reference to vancomycin susceptibility, so that effective therapy and infection control measures can be initiated.[1,6]

The study was aimed to determination of prevalence and susceptibility pattern of *Enterococci* in a tertiary care hospital.

MATERIAL AND METHODS

The present study was conducted in the Department of Microbiology, Nilratan Sarkar Medical College, Kolkata, from January to December 2013.The sample specimens urine, blood, pus, were collected aseptically and their culture and antibiotic sensitivity were performed as per standard recommendations.

Clinically relevant samples were collected from patients admitted in the hospital due to various diseases including UTI, septicaemia, pyogenic infections. The total sample size was 8153, among which 5179 were urine, 1881 were pus, and 1093 were blood samples. For semi quantitative urine culture Cysteine Lactose Electrolyte Deficient Medium (CLED) was used. On MacConkey's agar and blood agar pus and blood samples were inoculated.

Enterococcus species were isolated from 395 samples. They were identified by using standard tests. Kirby Bauer disc diffusion method on 5% Mueller Hinton blood agar was used to determine the antimicrobial susceptibility along with a control strain of ATCC*E.faecalis*29212, as per CLSI guidelines.

MIC of vancomycin was determined by E test for all *Enterococci* isolates. A lawn culture of *Enterococci,* 0.5Macfarland's standard was made on 5% Mueller Hinton blood agar. The E strip obtained from Himedia was applied with an MIC scale. After an incubation period of 24 hours of at 37°C an elliptical zone of inhibition was observed. The antibiotic susceptibility pattern was interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines, 2007.[7-9] Ethical standards as per the Helsinki declaration of 1975 as revised in 2000 was followed while conducting the study.

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How to cite this article: Kheya Mukherjee, Debojyoti Bhattacharjee, Goutam Chakraborti, Shiv sekhar Chatterjee. Prevalence and antibiotic susceptibility pattern of *Enterococcus* species from various clinical samples in a tertiary care hospital in Kolkata. International Journal of Contemporary Medical Research 2016;3(6):1565-1567.

STATISTICS ANALYSIS

Microsoft Excel and Microsoft word (version 8.1) were use to generate the tables and figures. Results are based on descriptive statitics.

RESULTS

From various clinical samples, 395 *Enterococcus species* was isolated in a period of one year and the rate of infection was estimated to be 4.8% (table-1). Isolates were highest from urine 80% (318), followed by pus 16% (64) and blood 3.3% (13) (Figure-1,2). An increased resistance to ampicillin (70), ciprofloxacin (92), gentamicin (91.4) (Figure-3) was observed among the isolates. Among the *Enterococci*, 3.8% were Vancomycin Resistant (VRE). All the *Enterococci* were sensitive to linezolid (Figure-4).

DISCUSSION

The *Enterococcus*species recently isolated more frequently from clinical specimens as nosocomial pathogens. As per CDC (1993) data, the *Enterococci* are the second leading cause of nosocomial infection.[2] Their increasing resistance to many antimicrobial agents like β lactam antibiotics, aminoglycosides and most importantly glycopeptides like vancomycin have made it a important nosocomial pathogen.[6]

In our study, *Enterococci* were isolated from various clinical specimens with prevalence rate of 4.8%, which was lower than the study by Desai et al and higher than the study by S. Sreeja et al.[2,6]

Isolates were highest from urine (80%), followed by pus and blood. Most of the studies done on *Enterococci* support the same findings as *Enterococci* identified as the most frequent uropathogen.[1,10]

Penicillin along with aminoglycosides considered as treatment of choice, hence resistance of *Enterococci* against these antibiotics has important clinical implications. Our study showed that 70% of isolates were resistant to ampicillin, 92% to ciprofloxacin, 91.4% to gentamicin, which showed a drastic increase in resistance of the commonly used drugs, comparable to the study conducted by J. Parameswarappa et al, and Mendiratta et al.[1,11] This finding was also reported in some study.[12,13]

The most recent and important resistance in *Enterococci* is vancomycin resistance has been increasingly reported from all parts of the world. In our study 3.8% the isolates are VRE which showed significant similarity to results reported from other studies ranging between 1.7-20% in tertiary care hospitals in other parts of India.[1,5,6,14-16]

Study of Parameswarappa et al and Karmakar et al from India have reported higher percentage of VRE.[1,17]

The high prevalence of multidrug resistant *Enterococcal* infection in a tertiary care set up is due to excessive and indiscriminate use of broad spectrum antibiotics and high rate of patient transfer from peripheral centres.[1] The emergence ofVRE has been attributed to the inprudent use of vancomycin, the colonization pressure and noncompliance with the infection control measures.[6]

For long time, *Enterococci* were frequently considered as normal flora. Recently however due to its role in causing variety of infections in hospitalized patients and increasing resistance to different antibiotics has led to understanding the importance of *Enterococcus*.[2]

By increasing awareness regarding drug resistance and use of

Figure-1: E Test for VRE detection

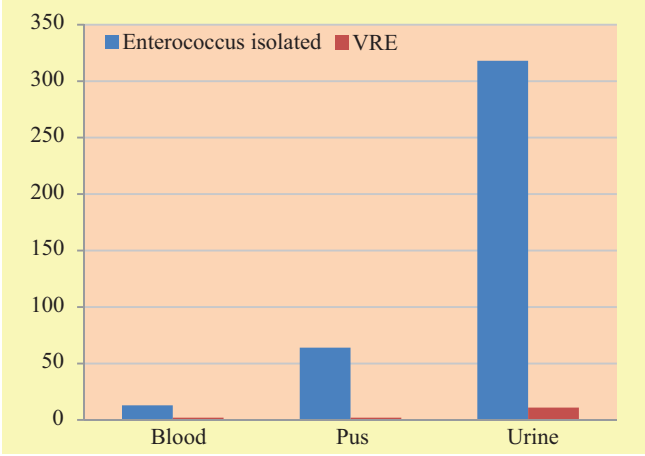

Figure-2: Enterococcus species isolated from different clinical samples

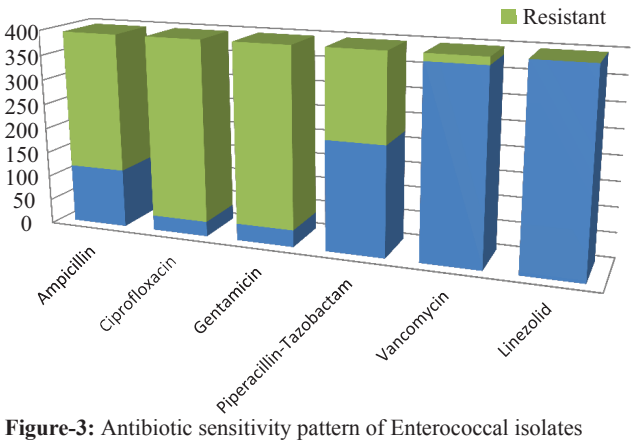

Figure-3: Antibiotic sensitivity pattern of Enterococcal isolates

Antibiotics	Ampicillin	Ciprofloxacin	Gentamicin	Piperacillin	Vancomycin	Linezolid
Sensitive (%)	30	8	8.6	56	96.2	100
Resistant (%)	70	92	91.4	44	3.8	0

Table-1: Antibiotic susceptibility pattern of *Enterococci*

proper antimicrobials, further emergence of VRE and multidrug resistant *Enterococci* can be reduced.[6]

CONCLUSIONS

Various studies have shown an increase in the rate of infection and antibiotic resistance in *Enterococcus* species. There is also a change in pattern of antibiotic resistance in *Enterococcus* species with an increased isolation rate of VRE. The present method of VRE detection is not beyond doubt. It needs confirmation by MIC value estimation and detection of resistance encoding gene by molecular techniques.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 11-04-2016; **Published online**: 09-05-2016