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Research Article

ANTIMICROBIAL RESISTANCE PATTERN OF ENTEROCOCCUS SPECIES WITH SPECIAL REFERENCE TO VANCOMYCIN RESISTANCE

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Abstract-Background: Enterococci are an important cause of hospital acquired infection and have become progressively more resistant to antibiotics. Vancomycin resistant enterococci are emerging as an important problem in hospitals worldwide leading to therapeutic failures. Therefore, this study was undertaken to estimate the prevalence of the enterococcus infection in our tertiary care hospital and to determine the antibiogram with special emphasis on vancomycin resistance.
Methods: From the period, January 2015-July 2015,225 isolate of enterococcus species were obtained from different clinical samples .The antibiotic susceptibility of these enterococcal isolates was performed by Kirby Bauer Disk Diffusion method and was further confirmed by Vitek 2C System.
Results: Out of these 225 enterococcal isolates, 168(74.7%) were identified *as Enterococcus fecalis* and 51 (22.7%) were *Enterococcal faecium*. The maximum no of isolates were from urine 122(54.2%) followed by the isolate from pus 41(18.2%). These isolates showed high level resistance to ampicillin, ciprofloxacin and high level gentamycin i.e. 58.2%, 47.6% and 43.6% respectively. The resistance to vancomycin in our study was found in only 10(4.4%) enterococcal isolates.
Conclusion: The study showed low level resistance to vancomycin amongst enterococcal isolates. Howeverthe presence of vancomycin resistant enterococci (VRE) along with increased rate of multidrug resistance amongst these isolates calls for regular surveillance of antimicrobial susceptibilities for the enterococcal isolates.

Keywords- Enterococci, Antibiotic Resistance, VRE

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Introduction

Enterococci form a part of the normal flora of intestinal tract, the oral cavity and vagina of humans. However in recent times it is emerging as an important nosocomial pathogen. The common infections associated with enterococci include urinary tract infections, endocarditis, bacteremia, catheter related infections, wound infection, intra-abdominal and pelvic infections [1]. The genus enterococcus include more than 13 species, although only few are implicated in human infections. *E. faecalis* and *E. faecium* are the most frequently isolated species amongst the clinical isolates, with both species responsible for about 95% of infections caused by enterococci [2,3]. The importance of Enterococcus has increased because of its resistance to many antimicrobials, including B-lactams, the aminoglycosides and most importantly, glycopeptides like vancomycin.[4] There has been a rapid increase in the incidence of infection and colonization of patients with vancomycin resistant enterococci (VRE). The widespread use of vancomycin and extended spectrum cephalosporins in the hospitals has resulted in this rapid increase in incidence of VRE over last two decades [5].

In health care settings, colonized patients are a potential source for the spread of organisms to the environment, health care workers and other patients. Enterococci have the tendency to transfer their antibiotic resistance genes to other bacteria, including methicillin resistant *Staphylococcus aureus* [6]. Also infections caused by VRE were found to be associated with adverse outcomes like extended length of hospital stay, increased hospitalization cost and increased mortality [3,7]. It is therefore, necessary to monitor the prevalent antibiogram pattern of enterococcal isolates including prevalence of VRE for proper treatment of patient as well as for controlling the bacterial resistance. Keeping this in mind the present study was undertaken to determine the prevalence of enterococcus from various clinical samples and to determine their anti-microbial resistance pattern with special reference to vancomycin susceptibility.

Materials and Methods

We studied 225 enterococcal strains isolated from various clinical samples of patients over a nine month period (January-July 2015) at Department of Microbiology, Hindu Rao Hospital & a large tertiary care hospital of North Delhi associated with NDMC Medical College. These isolates were obtained from various clinical samples including urine, pus, blood, catheter tips and other clinical materials. The enterococcal isolates were identified up to genus level by colony morphology, Gram stain, motility and biochemical tests using the standard microbiological techniques [2,8,9].

The susceptibilities of all isolates to different antimicrobial agents were tested by Kirby Bauer disc diffusion method on Muller Hinton agar (Hi-media, India) as per Clinical Laboratory Standard Institution guidelines.[10] The antibiotic disc tested were vancomycin (30ug), ampicillin (10ug), ciprofloxacin (5ug), high level gentamycin, linezolid (30ug). The results were recorded after 24 hours of incubation at 370C. Quality control strains of *E. fecalis* (ATCC 51299) was used to ensure the potency of each antimicrobial agent tested. Further identification of all the enterococcal isolates to their species level along with their antibiogram was confirmed by Vitek 2C (BioMe'rieux, France).

Results and Discussion:

Total of 225 enterococcal strains were isolated from different clinical samples as shown in [Table-1]. Of these 225 enterococcal isolates, 168(74.7%) were

identified as *E. faecalis*, 51(22.7%) as *E. faecium*, 2 (0.9%) as *E. avium*, 1(0.5%) as *E. gallinarium* and 3(1.3%) as *E. durans*. The maximum number of enterococcal isolates were obtained from urine (122,54.2 %) followed by that in pus (41,18.2%) and blood (27, 12%) as shown in [Table-1].

Table-1 *Distribution of Enterococcal Isolates in Various Clinical Samples*

Clinical Samples	No. of Enterococcal Species					Total(%)
	E. faecalis	*E.faecium*	*E.avium*	*E.gallinarium*	*E.durans*	
Urine	91	28	1	0	2	122(54.2)
Pus	32	8	1	0	0	41(18.2)
Blood	20	6	0	1	0	27(12)
Catheter Tip	6	5	0	0	0	11(4.9)
Others	19	4	0	0	1	24(10.7)
Total (%)	168(74.7)	51(22.7)	2(0.9)	1(0.5)	3(1.3)	225 (100)

These enterococcal isolates showed high resistance to two or more different classes of antimicrobial agents as depicted from [Table-2]. The *E. faecium* isolates showed comparatively higher resistance towards various antimicrobials tested. The vancomycin and high level gentamycin resistance was 7.8% and 51% respectively in *E. faecium* whereas it was 3.6% and 42.3 % respectively in *E. faecalis. E. gallinarium E. avium* and *E.durans* were fairly sensitive to most of the antibiotics tested. All the isolates are sensitive to linezolid.

Table- 2 *Resistance Pattern for Different Enterococcal Isolates*

Antimicrobial agents	*E. faecalis* (n=168)		*E. faecium* (n=51)		*Other species (n=6)*		Total (n=225)	
	S	R	S	R	S	R	S	R
Vancomycin	162(96.4)	6(3.6)	47(92.4)	4(7.8)	100	0	215(95.6)	10(4.4)
Linozolid	168(100)	0(0.0)	51(100)	0	6(100)	0	225(100)	0
Ampicillin	73(43.4)	95(56.5)	18(35.3)	33(64.7)	4(66.6)	2(33.4)	94(41.8)	131(58.2)
High Level Gentamicin	97(57.7)	71(42.3)	25(49)	26(51)	5(83.3)	1(17.7)	127(56.4)	98(43.6)
Ciprofloxacin	93(55.4)	75(44.6)	21(41.1)	30(58.9)	3(50)	3(50)	118(52.4)	107(47.6)

Discussion

As enterococcus species have now emerged as an important nosocomial pathogen, therefore it becomes important to know the changing patterns of the enterococcal infections and the antimicrobial susceptibility patterns of these isolates. In our study, maximum numbers of the enterococcus isolates were obtained from urine (54.2%), followed by pus (18.2%). This finding was in conformity with the other previous studies [1,11].

The majority of isolates in this study were *E. faecalis,* which caused about 74.7% infection followed by *E. faecium*, being responsible for about 22.7% infections. *E. avium*, *E. gallinarium* and *E. durans* accounted for only 2.7% of the isolates. This species distribution in our study is in accordance with the reports from different parts of the world, butin disagreement with reports from some other countries where *E. aecium* was predominant over *E.faecalis* [4,12,13,14]. An ICMR study by Jada *et al* reported 100% infections caused by *E. faecalis* [1].

In our study, out of the 225 enterococcal isolates, 58.2%, 47.6% and 43.6% were resistant to ampicillin, ciprofloxacin and high level gentamycin respectively which is comparative to the study by Biny Thapa *et al* [15]. However Jada *et al* reported78% isolates were resistant to penicillin, 82% to ciprofloxacin and 71% to high level gentamicin, which is higher according to our study [1].

The resistance rate to ampicillin was found as 58.2% in this study which is lower than that reported by Mathur *et al* (66%) and higher than that reported by Sreeja *et al* [4,16]. Since ampicillin is the drug of choice in the treatment of enterococcal infections, the relatively high resistance of isolates is of great concern. The detection of high level gentamycin resistance in 43.6% enterococcal isolates is a cause of concern, as it may signify the beginning of a major resistance problem by these organisms. Early detection of VRE is very crucial for timely management of at-risk patients apart from implementation of infection control programme to limit the spread of these nosocomial pathogens. Enterococci with vancomycin resistance are being reported from different parts of the world with increasing frequency, although the epidemiology of these microorganisms varies widely in different geographical areas [4,16,17]. The reports have shown increase in the vancomycin resistance from 0.3% in 1989 to 11% in 1996 among the hospital enterococcal infections [18]. In our study out of 225 isolates of enterococci, 10(4.4%) were found to be resistant to vancomycin. These results are slightly higher than those of Udo *et al* who detected VRE in 2.6% isolates at Kuwait [3]. However, our results are in disagreement with those of Khan *et al* and of Leven *et al* who reported the higher incidence i.e, 37% and 46.5% respectively of VRE In hospitalized patients [19,20]. Most of the isolates of *E. faecalis* in our study were susceptible to vancomycin (96.4%), which is in agreement with other Indian study [21]. This indicates that vancomycin still retains its therapeutic efficacy against majority of enterococcal isolates.

Conclusion:

Although in our study the prevalence of vancomycin resistance among enterococcal isolates was low, however their presence together with high level aminoglycosides resistance calls for regular surveillance studies for monitoring antibiotic sensitivity. Also there a need of coordinated efforts by various departments of the hospital to educate the hospital staff regarding the problem of drug resistance, vigilant use of antimicrobials by physicians and immediate implementation off appropriate hospital infection control measures. All these measures together can prevent the future emergence of VRE and can reduce the burden of multidrug resistant enterococci.

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