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Prevalence of Vancomycin Resistant Enterococci in Various Clinical Specimens in Tertiary Care Hospital in North Delhi

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ABSTRACT

Emergence of Vancomycin-resistant enterococci (VRE) is becoming a major problem in hospitals worldwide. This study was aimed to determine the occurrence, species prevalence and antibacterial resistance of Enterococci isolated in Jaipur Golden hospitals, Delhi. Sixty isolates of enterococcal species were recovered from various clinical samples and dentified to species level and were found to consist of E. faecalis (46.6%), E. faecium (35%), E. gallinarum (10%), E. avium (1.6%), E. hirae (1.6%), E. caecorum (1.6%), E. amnioenus (1.6%) and E. casseliflavus (1.6%) species. VRE accounted for 11.6% of the isolates and out of them 71.4% were E. faecium and 28.6% were E. gallinarum detected in urine, and blood specimens. seven (11.6%) out of 60 isolates were found to be VRE. This study illustrates the emergence of multidrug resistant enterococci along with increased rate of VRE. Thus there is a need for routine screening of bacterial isolates from clinical samples for VRE and regular surveillance should be conducted for the determination of risk factors.

KEYWORDS: VRE, Antibiotic resistance, Enterococci, enterococcus species, hospitalised patients

Introduction:

Over the past century enterococci were known as an intestinal commensal organism but now it has gained clinical significance and has become the second most common nosocomial pathogen worldwide associated with significant morbidity and mortality [1, 2]. They are capable to adapt to exposure to antibacterials maintaining low level resistance to aminoglycosides and intrinsic resistance to penicillins and a tremendous ability to acquire resistance to other antibacterials including high level resistance to aminoglycoside and glycopeptides [3,4,5]. In many resistant strains of gram positive bacterial infections, especially those caused by Enterococci vancomycin has been used as the drug of choice. In recent years, vancomycin-resistant enterococci (VRE) has been increasing rapidly in the incidence of infection and colonisation of patients due to widespread use of vancomycin in the hospitals. . This serious problem is emerging not only due to uncontrolled use of antibiotic but also because the resistance determinant can be transferred horizontally to other vancomycin-susceptible species. The resistance may be intrinsic or acquired via gene transfer [1,6,7]. The prevalence of VRE has dramatically increased worldwide [2]. A significant increase in the percentage of invasive nosocomial *Enterococcus* strains displaying high-level vancomycin resistance has been revealed by National Nosocomial Infection Surveillance (NNIS) system in the USA [8]. Previously the VRE strains were found to be sensitive to linezolid but it has been observed that enterococci are becoming resistant to linezolid also [9]. Various *enterococcus* species have been identified in which *Enterococcus faecalis* was the most common species associated with nosocomial infections, followed by *Enterococcus faecium*, and both species are responsible for about 95% of infections caused by enterococci [8]. VRE, especially *E. faecalis* and *E. faecium*, are prevalent in the hospitalised patients. Other *Enterococcus* species, *E. gallinarum, E. casseliflavus, E durans, E. avium,* and *E. hirae*, are isolated much less frequently and account for less than 5% of clinical isolates [1]. Infections caused by VRE were found to be associated with extended period of stay in hospital, increased cost of treatment and increased mortality [2,4]. Antibiotic resistance pattern of enterococci isolated from clinical specimens is very useful to get information about the prevalence of VRE and will be essential for preventing the spread of bacterial resistance. The aim of this study was to investigate the prevalence of antibacterial resistance in enterococci isolated from clinical samples in Jaipur Golden hospital, Delhi.

Material and method:

Enterococcus isolates

The study was conducted in the Microbiology laboratory, Jaipur Golden hospital, Delhi, India. Specimens were collected for this study over the period from September 2012 to April 2013 in which 60 strains of enterococci were isolated from various clinical specimens. They included 32 urine specimens, 16 blood samples, 7 pus swabs, 1 ascitic fluid, 1 drained pus, 1 plueral fluid, 1 semen, and 1 catheter tip, collected from in-patients and out-patients at hospital. The specimens were inoculated on blood and McConkey agar plates and incubated at 37˚C for 24-48 hours. Identification of enterococci was based on their growth characteristics on blood agar, Gram staining, the catalase reaction, ability to grow in 6.5% NaCl broth and bile esculin hydrolysis and biochemical tests using Rapid ID 32 Strep, mini-API (bioMerieux, Marcy Etoile, France). The study was approved by the Institutional ethical committee and the protocol was described to and every volunteer and a written consent was obtained from volunteers of this study and the data has been maintained properly in the hospital record.

Antimicrobial susceptibility testing

The susceptibilities of the isolates to penicillin, ampicillin, erythromycin, tetracycline, chloramphenicol, ciprofloxacin, levofloxacin, vancomycin, teicoplanin, nitrofurantoin, quinupristin- delfopristin, gentamicin, streptomycin antimicrobial agents were done by the clinical ATB ENTROC 5 strip, semi- automated system mini API. This strip was designed following NCCLS (CLSI) 2000 (1) committee recommendations while the susceptibility to norfloxacin, linezolid, amoxicillin- clauvalinic acid, piperacillin and sulbacin agents were done by Kirby-Bauer method using commercially prepared antibiotic discs of Hi-media laboratories (India) and was categorized as sensitive, intermediate and resistant as per NCCLS (2003) guidelines. The following concentrations were used: penicillin (8µg/ml), ampicillin (8 µg/ml), erythromycin (.5-4 µg/ml), tetracycline (4-8 µg/ml), chloramphenicol (8-16 µg/ml), ciprofloxacin (1-2

µg/ml), levofloxacin (2-4 µg/ml), vancomycin (4-16 µg/ml), teicoplanin (8-16 µg/ml), nitrofurantoin (32-64 µg/ml), quinupristin- delfopristin (1-2 µg/ml), gentamicin (500 µg/ml), streptomycin (1000 µg/ml), norfloxacin (10 µg), linezolid (30 µg), amoxicillin- clauvalinic acid (30 µg), piperacillin (100µg) and sulbacin (100µg).

Statistical analysis

Statistical analysis was carried out using the chi-squire test with a $p < 0.05$ set as a significance level.

Result:

Prevelence of enterococci in clinical specimens

A total of 60 enterococcal isolates were obtained from different clinical samples. Table 1 displays the sources and species identities of the 60 clinical enterococcal isolates. A total of 60 enterococcal isolates from various clinical samples were obtained from 32 urine cultures (53.3%), 16 blood cultures (26.6%), 7 pus (11.6), 1 ascitic fluid (1.6), 1 drained pus (1.6), 1 plueral fluid (1.6%), 1 semen (1.6%) and 1 cathater tip (1.6%). Eight different species were identified, of which *E. faecalis* was the most prevalent where as *E. faecium* being second. Out of 60 enterococcal isolates, 28 (46.6%) were identified as *E. faecalis*, 21 (35%) as *E. faecium*, 6 (10%) as *E. gallinarum,* 1 (1.6%) as *E. avium*, 1 (1.6%) as *E. hirae,* 1 (1.6%) as *E. caecorum,* 1 (1.6%) as *E. amnioenus* and 1 (1.6%) as *E. casseliflavus*. 31 out of them (51.7%) were females. *E. faecalis* was more common in males while *E. faecium* was more common in females. Out of 60 isolates 7 VRE (vancomycin resistant enterococci) were obtained in which 3 (42.9%) were obtained from urine and 4 (57.1%) were obtained from blood. A statistically significant difference was observed significant ($P<0.001$) among different species isolated from various clinical specimens by the application of Chi squire test.

Antibacterial resistance pattern

The distribution of antimicrobial susceptibility patterns of isolated enterococci is summarised in Table 2. The results show that resistance was most frequently observed with norfloxacin (78.3%), levofloxacin (78.3%), erythromycin (90%), ciprofloxacin (78.3%), penicillin (68.3%), quinupristin-delfopristin (86.7%), tetracycline (55%), ampicillin (53.3%), amoxicillin-clauvalinic acid (53.3%), piperacillin (53.3%), sulbacin (53.3%), nitrofurantoin (60%), chloramphenicol (56.7%), teicoplanin (16.7%). 11.6% of the isolates were resistant to vancomycin (MIC range: 4-16 µg/ml). However, none of them produced β-lactamase. The isolates were tested for their susceptibility to linezolid, a new oxazolidinone antibacterial that has been reported to have activity against Gram-positive cocci, including methicillin-resistant *S. aureus* and VRE. The only strain resistant to linezolid was *E. gallinarum* (1.7%). Out of 60 isolates 7 (11.6%) were VRE in which 5 (71.4%) were *E. faecium* and 2 (28.6%) were *E. gallinarum*. Among urinary isolates VRE accounted for 9.4 % of samples and among non- urinary isolates 14.3 % of the samples.

Discussion:

In the present study, we examined different clinical samples from 60 patients for the presence of VRE at Jaipur Golden hospital, delhi. The vast majority of the isolates in this study were *E. faecalis* which caused about 46.6% infection and *E. faecium* which was responsible for about 35% of infection, *E. gallinarium* was 10% while *E. avium*, *E. hirae, E. caecorum, E. amnioenus,* and *E. casseliflavus* were accounted for only 1.6% for each, which was comparable to the enterococcal species distribution in other studies [10]. Desai and colleagues isolated 202 enterococci from clinical specimens in which 49.50% were *E. feacalis*, 35.64% were *E. faecium*, 9.40%, *E. avium*, 2.47% were *E. hirae* and one isolate each of *E. gallinarum* and *E. casseliflavus* were the other number of Enterococcus species [11]. Udo and colleagues isolated 415 enterococci during their study. They found *E. faecalis* (85.3%), *E. faecium* (7.7%), *E. durans* (1.0%), *E. gallinarum* (0.5%), *E. avium* (1.2%), *E. casseliflavus* (4.0%) [8]. In our study, *E. faecalis* was the most common in urine sample while *E. faecium* was more common in blood specimen. In urine culture 59.3% were *E. faecalis* and 31.2 % were *E. faecium* while in the blood culture *E. faecalis* were 31.2% and *E faecium* were 50% which is in concordance to the study of Chaudhary and colleagues as well as Mohanty and colleagues [12, 13]. Recent studies showed an increasing incidence of enterococcal infections in tertiary care hospitals. Shinde and colleagues isolated 54 enterococci. Of them *E. faecalis* (87.03%) was the most commonly isolated species, followed by *E. faecium* (9.25%) and *E. durans* (3.7%) [14]. Salem and Colleagues found 69.2% *E. faecalis*, 11.3% *E. faecium*, 2.1% *E. avium*, 0.8% *E. hirae*, 1.3% *E. casseliflavus* and 1.3% *E. gallinarum* out of 206 enterococcal isolates in their study [15].

Of the 60 isolates, enterococcus species was found resistant to ampicillin (53.3%), erythromycin (90%), tetracycline (55%), chloramphenicol (56.7%), and ciprofloxacin (78.3%), levofloxacin (78.3%) and vancomycin (11.6%). Salem-Bekhit and colleagues found the resistant isolates to erythromycin (64.1%), tetracycline (66.5%), chloramphenicol (34.5%) and ciprofloxacin (49.9%)[15]. Udo and colleagues resulted the isolates resistant to erythromycin (63%), tetracycline (60%) and chloramphenicol (40%) out of 415 isolates in their study [8]. In these both studies, frequency of resistance to tetracycline was slightly higher while resistance to erythromycin and chloramphenicol was lower than that reported in our study. These both studies were performed in Riyadh and Kuwait respectively. In our study, 90% of isolates were resistant to erythromycin, which is higher than what was reported from other countries such as Riydh, kuwait and Lebanon(59%) [10]. In India, the prevalence of VRE has been reported to be between 0- 30 percent [16-21]. In this study, VRE were found in 7 (11.6%) patients in which 5 isolates were identified as E. faecium (71.4%) and 2 isolates were identified as E. gallinarum (28.6%). E. faecalis were 100% susceptible. All the patients of VRE were admitted in ICU except the 2 isolates E. gallinarum in which one was found in urine sample of a female patient and the other one was found in the blood of male patient. 57.1% VRE were reported in blood and 42.9% VRE were reported in urine specimens. 57.1% VRE were isolated from female patients while 42.9% VRE isolated from male patients. Medical records of these seven patients were reviewed and their clinical features were determined. The patients were from 26 to 87 years old except two which were 1 to 2 years old. However, our results are in the agreement to reports stating higher percentage of E. faecium (2.6%) among VRE isolates. Out of 415 isolates at Kuwait hospitals, in which 1.9% were E. faecalis and 12.5% were E. faecium [8] while in the other study, 8 (3.9%) VRE out of 206 isolates were found, in which 1.8% were E. faecalis and 18.5% were E. faecium [15].

In this study, all of the isolates were susceptible to linezolid except one, which was *E. gallinarum*. 1.7% isolates were resistant to linezolid. This isolate was also resistant to high level gentamicin. It was reported in the urine sample of a female patient who was admitted in ICU. In one study, 2% of isolates were resistant to linezolid in which 1% were E. faecalis, 0.5% were E. gallinarum and 0.5 % were E. casseliflavus [22].

E. gallinarum and *E. casseliflavus* are motile enterococci and primarily they were found in the gastrointestinal tract of poultry, in foods, and in domestic fowls. human clinical specimens and rarely are in human clinical specimens. Now they are playing their role in causing invasive infections in humans, especially immunocompromised or chronically ill patients, and sometimes are nosocomially acquired [8]. In our study, *E. gallinarum*, a rare enterococcal species in human infections, was encountered 10% thereby emphasising the need to speciate the genus *Enterococcus* encountered in human infections. VRE infections are on rise and their percentage will increase in future if appropriate steps are not taken in time. So, more rational and restricted use of antimicrobial agents is needed in order to minimize such infection.

Table 1: ENTEROCOCCUS SPECIES ISOLATES FROM VARIOUS CLINICAL SAMPLES (n =60) (%)

Samples	E. faecalis	E. faecium	E. gallinarum	E. avium	E. hirae	E. caecorum	E. amnioenus	E. casseliflavus	Total (%)	VRE
Urine	19 (59.3)	10 (31.2)	3 (9.3)	-	-	-	-	-	32 (53.3)	3(9.3)
Blood	5 (31.2)	8 (50)	2 (12.5)	-	-	1(6.2)	-	-	16 (26.6)	3 (18.7)
PUS	2 (28.5)	2 (28.5)	-	1 (14.2)	1 (14.2)	-	-	1 (14.2)	7 (11.6)	-
Ascitic fluid	-	1 (100)		-	-	-	-		1 (1.6)	
Fluid (drained pus)	-	-	1 (100)	-	-	-		-	1 (1.6)	-
Plueral fluid	-		-	-	-	-	1 (100)		1 (1.6)	
Semen	1 (100)	-	-	-	-	-	-		1 (1.6)	
Cathater tip	1 (100)	-	-	-	-	-	-		1 (1.6)	
Total	28 (46.6)	21 (35)	6 (10)	1 (1.6)	1 (1.6)	1 (1.6)	1 (1.6)	1 (1.6)	60 (100)	6 (10)

P<0.001, VRE: vancomycin resistant enterococci

Table 2: ANTIMICROBIAL RESISTANCE DISTRIBUTION IN VARIOUS ENTEROCOCCUS SPECIES

Antibiotics (No. of strains Resistant for)	E. faecalis	E. faecium	E. gallinarum	E. avium	E. hirae	E. caecorum	E. amnioenus	E. casseliflavus
Penicillin (41)	10(24.4)	21 (51.2)	6 (14.6)	1 (2.4)	1(2.4)	1 (2.4)	-	1 (2.4)
Ampicillin (32)	4 (12.5)	20 (62.5)	5 (15.6)	1(3.1)	-	1 (3.1)	-	1 (3.1)
Erythromycin(54)	26 (48.1)	19 (35.1)	6 (11.1)	1(1.8)	-	1 (1.8)	-	1 (1.8)
Tetracyclin (33)	19 (57.6)	8 (24.2)	4 (12.1)	-	-	1 (3.0)	-	1 (3.0)
Chloramphenicol (34)	17(50)	13 (38.2)	3 (8.8)	-	1 (2.9)	-	-	-
Ciprofloxacin (47)	20 (42.5)	20 (42.5)	6 (12.8)	-	-	1 (2.1)	-	-
Levofloxacin (47)	20 (42.5)	20 (42.5)	6 (12.8)	-	-	1 (2.1)	-	-
Vancomycin (7)	-	5 (71.4)	2 (28.6)	-	-	-	-	-
Teicoplanin (10)	2 (20)	6 (60)	2 (20)	-	-	-	-	-
Nitrofurantoin (36)	11 (30.5)	16 (44.4)	5 (13.9)	1 (2.8)	1 (2.8)	1 (2.8)	-	1 (2.8)
Quinupristin	-							
Delfopristin (52)	24 (46.1)	19 (36.5)	5 (9.6)	1 (1.9)	1 (1.9)	1 (1.9)	-	1 (1.9)
Norfloxacin (47)	20 (42.5)	20 (42.5)	6 (12.8)	-	-	1 (2.1)	-	-
Linezolid (1)	-	-	1 (100)	-	-	-	-	-
Amoxicillin	-							
Clauvalinic acid (32)	4 (12.5)	20 (62.5)	5 (15.6)	1 (3.1)	-	1 (3.1)	-	1 (3.1)
Piperacillin (32)	4 (12.5)	20 (62.5)	5 (15.6)	1 (3.1)	-	1 (3.1)	-	1 (3.1)
Gentamicin (H) (45)	18 (40)	20 (44.4)	5 (11.1)	1 (2.2)	-	1 (2.2)	-	-
Sulbacin (32)	4 (5)	15 (75)	2 (10)	1 (5)	-	1 (5)	-	-
Streptomycin (57)	26 (45.6)	21 (36.8)	6 (10.5)	1 (1.7)	1 (1.7)	1 (1.7)	-	1 (1.7)

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