ORIGINAL RESEARCH PAPER

Medical Microbiology

PHENOTYPIC CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF ENTEROCOCCUS AT A TERTIARY CARE HOSPITAL IN WESTERN UTTAR PRADESH

KEY WORDS: Enterococcus, E. faecalis, E. faecium, Vancomycin-resistant Enterococci (VRE), Antimicrobial resistance

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ABSTRACT

Background: Enterococcus has emerged as a significant opportunistic pathogen, particularly in hospitalized and immunocompromised individuals. E. faecalis and E. faecium are commonly found to be associated with nosocomial infections. The special concern is their striking feature of intrinsic and acquired resistance to multiple antibiotics. Aim and Objectives: To determine the frequency, species distribution, and antimicrobial resistance patterns of Enterococcus in a tertiary care hospital in Western Uttar Pradesh. Methods: This retrospective study was conducted over one year (January 2023-December 2023) including the clinical samples submitted for bacterial culture and antibiotic sensitivity testing. Isolates suspected to be Enterococcus were identified using standard microbiological techniques and confirmed using the Vitek 2 compact system. Antibiotic susceptibility testing was performed as per CLSI 2023 guidelines using the Kirby-Bauer disc diffusion method and minimum inhibitory concentration (MIC) of certain antibiotics was determined by Vitek 2 AST- P628 card Results: Out of 7012 culture-positive samples, 690 (9.8%) yielded Enterococcus species, with the majority isolated from urine (73.5%), followed by pus (12.5%) and blood (10.4%). E. faecalis was the predominant species (66.23%), followed by E. faecium (32.89%). High resistance rates were observed against ciprofloxacin (71.6%), gentamicin (74.8%), and penicillin (61%). Vancomycin resistance was noted in 10% of isolates, predominantly from indoor patients. Among VRE isolates, high-level gentamicin resistance was seen in 93%, while linezolid showed the highest sensitivity (90%). Conclusion:The study highlights a concerning emergence of multidrug-resistant Enterococcus, including vancomycin-resistant strains. Regular surveillance and strict antibiotic stewardship are crucial for effective infection control and prevention of further resistance development.

INTRODUCTION

Enterococci are Gram positive, non-spore forming bacteria belonging to the family Enterococcaceae. They reside as commensals in the gut of a variety of hosts including humans (1). These are facultative anaerobes and do not produce catalase. They can tolerate wide range of temperature from 10 C to 45 C. The genus comprises about 54 species, namely Enterococcus faecalis, E. faecium, E. casseliflavus, E. gallinarum, E. durans, E.avium, etc. (2). Moreover, they can grow in the presence of 6.5% NaCl and hydrolyze esculin in the presence of 40% bile. These characteristics differentiate them from streptococci (3). These bacteria are known to be relatively non-pathogenic in healthy individuals, but they may behave as pathogens in immunocompromised patients and bears a potential to cause nosocomial infections in hospitalized patients (4). The common species which cause human infections are E. faecalis (80-90 %) and E. faecium (5-10%)(4), both can survive in hospital environments and colonize patients, causing infections such as bacteremia, urinary tract infections, , endocarditis, surgical wound infections, neonatal sepsis etc (5). Risk factors associated with enterococcal infections includes prior administration of antibiotics, prolonged hospital stay, operative procedures, and underlying diseases like malignancy, HIV and diabetics. Enterococci exhibit resistance to a variety of antimicrobial agents through intrinsic and acquired mechanisms. They may acquire resistance through van associated genes (vanA, vanB, vanD, vanE, vanG, vanL); of which vanA and vanB are the most common genotypes in clinical isolates (6,7). Vancomycin was once considered as a drug of choice for multidrug resistant Gram-positive bacteria, however in the recent years, there has been development of resistance to this supposed to be the last resort antibiotic. Studies have reported an escalation in the incidence of vancomycin resistant enterococcal infections among hospitalized patients (6,8). To the best of our knowledge, limited data is available from north India, regarding the prevalence of enterococcal infections and their antibiotic susceptibility patterns. Hence, the present study had been undertaken to identify and speciate the clinical isolates of Enterococcus and to determine their antibiotic resistance profile at our healthcare centre in western part of Uttar Pradesh.

MATERIALS AND METHODS

The retrospective study was carried out in the department of Microbiology of a tertiary care centre in Uttar Pradesh, over a time of one year, from January 2023 to December 2023. Our laboratory records were analyzed for data collection. All the clinical samples received for culture and sensitivity, over the study duration were included. All the samples were processed for aerobic bacterial culture as per standard techniques by conventional methods (9) or by automated BACTEC System (BD) as per the requisition. Appearance of non-hemolytic translucent colonies on blood agar and tiny pink colonies on MacConkey agar colonies after 24 hours of aerobic incubation at 37oC, raised the suspicion of Enterococcus. The isolate was placed in the genus conventionally by culture smear and biochemical tests, that included negative catalase test, fermentation of aesculin on nutrient agar with 4% bile, ability to grow in broth containing 6.5% NaCl and positive PYRase test. Speciation was done by fermentation test of mannitol and arabinose (10). The isolates were subjected to confirmation by automated Vitek 2 compact system (BD). Antimicrobial sensitivity testing was done as per CLSI guidelines 2023, by Kirby Bauer's disc diffusion method on Muller Hinton agar supplemented with 5% sheep blood. The minimum inhibitory concentration (MIC) of certain antibiotics was determined by Vitek 2 AST-P628 card (11).

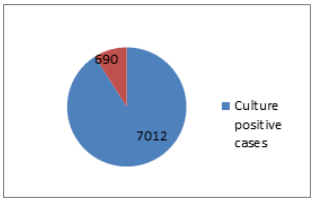

Figure 1- Frequency of isolation of Enterococcus species in culture positive cases (n=7012)

Table 1- Age and Gender wise distribution of Enterococcus culture positive cases (n=690)

Age (years)	Total(690)	Male (275)	Female (415)
0-10	22	9	13
11-20	44	18	26
21-30	165	68	97
31-40	82	34	48
41-50	101	35	66
51-60	120	47	73
61-70	93	39	54
>70	63	25	38

Table 2- Sample-wise isolation of Enterococcus species

Clinical samples	Number of enterococci recovered (n=690)	% of enterococci recovered
Urine	507	73.5
Pus	86	12.5
Blood	72	10.4
Peritoneal fluid	11	1.6
Bile	8	1.2
Synovial fluid	6	1.0

Table 3- Distribution of Enterococcus species (n=690)

Isolate	Number	%
E. faecalis	457	66.23
E. faecium	227	32.89
E. gallinarum	5	0.72
E. casseliflavus	1	0.14%

Table 4- Antimicrobial susceptibility pattern among Enterococcus isolates (n=690)

Antibiotic disc (µg)	Resistant		Sensitive	
Antibiotic disc (µg)	number	%	number	%
Penicillin G (10 units)	421	61.0	269	39.0
Ampicillin (10µg)	414	60.0	276	40.0
Amoxicillin-clavulanate(20/10 µg)	384	55.6	306	44.4
Ciprofloxacin (5 µg)	494	71.6	196	28.4
Levofloxacin (5 µg)	433	62.7	257	37.3
Gentamicin (120 µg)	516	74.8	174	25.2
Chloramphenicol (30µg)	227	33.0	463	67.0
Vancomycin (30 µg)	70	10.0	620	90.0
Teicoplanin (30 µg)	59	8.6	631	91.4
Linezolid (30 µg)	7	1.0	683	99.0

Figure 2- Vancomycin Resistant Enterococci (VRE): Indoor versus outdoor cases

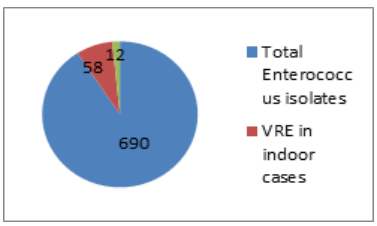

Table 5- Sample-wise isolation of VRE species

Clinical samples	Number of VRE screened (n=70)	% of VRE screened
Urine	48	69
Pus	2	3.0
Blood	20	29

Table 6- Antimicrobial susceptibility profile among VRE isolates (n=70)

Antibiotic disc	Resistant		Sensitive	
Antibiotic disc	number	%	number	%
Teicoplanin (30µg)	54	77	16	23
Linezolid (30µg)	7	10	63	90
HLG (120µg)	65	93	5	07
Chloramphenicol (30µg)	14	20	56	80

RESULTS

We present the retrospective data analysis over a period of year. As per our laboratory records, out of total 7012 culture positive cases, Enterococcus spp. were recovered from 690 cases (9.84%) (Figure-1). The isolation of Enterococcus spp. was higher in females (415, 60%) as compared to males (275, 40%), and most commonly affecting the younger to middle aged group (21-30 years and 51-60 years) (Table-1). The isolates were recovered predominantly from urine (73.5%), followed by pus (12.5%), blood (10.4%), peritoneal fluid (1.6%), bile (1.2%), and synovial fluid (1.0%) (Table-2). Looking at species level isolation, 457 (66.23%) isolates were of E. faecalis, 227 (32.89%) were of E. faecium, 5 (0.72%) were of E. gallinarum and 1 (0.14%) was of E. casseliflavus (Table-3). Majority of these isolates recovered from indoor cases (519, 75%) as compared to outdoor cases (171, 25%).Antimicrobial susceptibility testing showed maximum sensitivity to linezolid (99%), followed by teicoplanin (91%) and vancomycin (90%), chloramphenicol (67%), amoxicillin-clavulanate (44%), ampicillin (40%), penicillin (39%), levofloxacin (37%), ciprofloxacin (28%), high level gentamicin (25%)(Table-4). Tigecycline was found to be sensitive in 98% of non urinary cases (n=183). Sensitivity to nitrofurantoin and fosfomycin was detected in 83% and 85% of urinary cases, respectively (n=507).

Vancomycin resistant enterococci (VRE) were isolated from 70 (10%) cases, among them 40 (57.2%) were E. faecium, 24 (34.3%) were E. faecalis, 5 (7.1%) were E. gallinarum and 1 (1.4%) was E. casseliflavus. Out of 70 cases of VRE, 58 (82.9%) from in-patient departments (Figure-2). VRE was isolated mostly from urine samples, accounting to 69% cases of UTI, which was followed by isolation from blood (29%) and pus (3%)(Table-5). Antibiogram of VRE isolates showed that 54 isolates (77%) were also resistant to teicoplanin, thus reflecting their reduced susceptibility to other glycopeptide. Resistance to HLG was detected in 65 (93%) isolates of VRE. However, VRE isolates showed high sensitivity against linezolid (90%) and chloramphenicol (80%) (Table-6). Tigecycline was found to be sensitive in 97% of non urinary cases (n=22). Nitrofurantoin and fosfomycin were sensitive in 96% and 94% urinary cases (n=48) respectively.

DISCUSSION

Enterococcus species has procured attention as an emerging nosocomial pathogen of concern in recent years due to their increasing resistance to antimicrobials(12). In current study, the frequency of isolation of enterococci from various clinical samples was found to be 9.84%, which was comparable with a study done by Phukan et al., where isolation rate was reported to be 7.47% (13). On the other hand, our isolation was much higher than studies done in south west India (2.3%) (14), Bangladesh (3.2%), Egypt (3.3%), Ethiopia (3.5%), Asian pacific (3.6%) and Kenya (0.22%) (15). Conversely, our isolation rate was quite lower than reported from Iran 28.5% (5) and Saudi Arabia 31.71% (3). This variation in the results could be due distinctiveness in the geographical areas and target populations. In present study, E. faecalis was the commonest amongst the enterococci isolated from clinical samples. Our observation was consistent with the data reported by various authors in from different regions (5,13,14,16, 17,18). However, in a study done in South India in 2021, E. faecium has been reported as the most commonly isolated species from blood culture (19). In our study, majority of enterococci (75%) were recovered from

inpatients cases, thus pinpointing their potential as nosocomial pathogens. Our data collaborates with various other studies that has also reporting maximum isolation of enterococci from hospitalized patients (3,13,15,17). These findings emphasize the need of effective infection control measures. Majority of our isolates were obtained from urine (73.5%), followed by pus (12.5%) and blood (10.4%). These findings were in consistent with the findings of other studies, which showed maximum isolation of enterococci from urine and pus (3,13, 17,18). However, in a study done by Sreeja S et al., majority of isolates (43%) were recovered from pus (14), while Ferede et al., reported highest isolation from urine and blood (15). These observations depicts that enterococci are one of the common causes of urinary tract infections, wound infections and blood stream infections. In our study, we found female predominance in acquiring enterococcal infections, which was in concordance with other studies (15,16,18). This could be justified due to the fact that females are more prone to get urinary tract infections as compared to males. In contrast, slightly higher rate of enterococcal infections in males has been reported by other authors (3). Antimicrobial sensitivity pattern of enterococci showed multidrug resistance. Isolates resistant to penicillin and ampicillin accounted to be 61% and 60%, respectively. Resistance to ciprofloxacin was seen in 71.6% of isolates This data was in consistent to the reported resistance rate against these said antimicrobials in previous studies (3,20). These findings were higher than that reported by Sreeja et al., Phukan et al. and Adhikari et al. in their studies (13,14,16). Studies across have also documented 100% resistance against ampicillin (21). Our data reflected 74.8% resistance to high level gentamicin (HLG), which discourages the synergistic bactericidal effect of a cell wall active drug and aminoglycoside. Moreover, varying percentage resistance to HLG ranging from 44.08% to 82.7% have been observed in previous studies (5,14,15,17,18, 22). Fortunately, most of the enterococcal isolates in current study were sensitive to linezolid (99%), followed by teicoplanin (91%) and vancomycin (90%). These findings were in agreement with the observations made by Gupta et al. (23). Moreover, high sensitivity to linezolid has also been addressed in other studies conducted in India and Bagladesh (24, 25). 83% of our urinary isolates were found to be sensitive to nitrofurantoin (16, 17). Tigecycline was sensitive in 98% of non urinary isolates, as also favoured by previous studies (26, 27, 28). In this present study, the isolation rate of Vancomycin resistant enterococci (VRE) was found to be 10%, that was in consistent with results of other studies (29, 30, 31). In contrast, high rates of VRE ranging from 18.75% to 75% have been reported in different studies (3,13,18,20). VRE infection has been attributed to different risk factors including inappropriate use of antibiotics, previous hospitalization, critical illness, patient transfer, underlying diseases, urinary catheter, contact with VRE patients (20). In our set-up, underlying chronic illnesses and impaired immune status were the common factors predisposing to acquisition to infection with VRE. As per our data, all the VRE isolates were of E. faecalis . This correlates with the other studies where VRE was found to be more often associated with E. faecalis (5,13). In contrast , E. faecium (57.2%) was the commonest species among VRE isolates, as reported by other authors. (5). In our healthcare setting, majority of VRE isolates were found to be associated with urinary tract infections and blood stream infections, which collaborates with other workers (3, 13). An upsurge of VRE associated urinary tract infections was observed in our settings. Nitrofurantoin (96%) appeared to be a good option for treating urinary infection associated with VRE as per our data. In the present study, 10% of our VRE isolates were found to be linezolid resistant. This is in agreement with findings of another study where the antibiogram had shown maximum sensitivity of VRE isolates to linezolid (90%) (19), but reduced sensitivity to HLG (7%) and teicoplanin (23%). VRE and linezolid resistant enterococci are also an emerging global concern as there is limited option for treatment available (19). At our setup, glycopeptides and linezolid were found to be effective for treating enterococcal infections. Tigecycline was found to be an alternative choice for treating multidrug resistant enterococci and VRE in non urinary cases.

CONCLUSION

Our study highlights the emergence of healthcare associated multidrug resistant enterococcal infections, thus limiting the therapeutic options. Enterococcus species, particularly E. faecalis and E. faecium, were frequently isolated from urinary and blood samples with a substantial proportion showing multidrug resistance. The spread of VRE poses a serious issue, as vancomycin has been regarded as the last-resort antibiotic for multidrug-resistant Gram-positive infections. The escalating resistance to commonly used antibiotics underscore the urgent need for regular monitoring of antimicrobial resistance trends and rational usage of antibiotics. Emphasis should be laid on adoption of infection control measures and effective antibiotic stewardship programs to allay the threat of this drug resistant bug.

LIMITATIONS

The data would have been more assertive if the study had been carried out for a prolonged time span. Due to limited resources, molecular detection of resistance genes, especially van A and van B, could not be done.

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