ACTA MICROBIOLOGICA BULGARICA Volume 37 / 3 (2021)

Epidemiological Profile, Speciation and Antibiogram of Enterococcus Species in the Era of Resistance

Dhanashree Padmanabh Inamdar[1*], Anuradha Basavaraju[1], Malatesh Undi[2]

[1]*Department of Microbiology, Mamata Medical College and Hospital, Khammam, Telangana, India*
[2]*Department of Community Medicine, Karwar Institute of Medical Sciences, Uttara Kannada, Karnataka, India*

Abstract

Enterococcus species have now emerged as leading causes of nosocomial infections. Speciation and antibiotic susceptibility testing (AST) are needed for these organisms due to their increasing resistance to various antibiotics. As geographic diversity contributes to varied resistant patterns in these organisms, the present study was conducted with the objective to analyse the prevalence, clinicodemographic profile, and speciation of enterococci from various clinical samples, and to assess their antimicrobial resistance (AMR) pattern at our setting. A prospective observational study was carried out for a period of six months at a tertiary care hospital in Telangana, India. Clinical samples which showed growth of pathogens during the study period were included. *Enterococcus spp.* were identified by appropriate biochemical tests followed by AST. 106 *Enterococcus spp.* were isolated among 1864 samples with growth during the study period. Prevalence of enterococcal infections was 5.6%. Male population, age distribution (31-40 yrs), gynaecology ward inpatients, and urine samples had significant (p value <0.05) enterococcal isolation. *Enterococcus faecalis* (61.3%) was the commonest isolate. High-level streptomycin (HLS) resistance was 33.01% and high-level gentamicin (HLG) resistance was 37.7%. Although resistance to commonly used antibiotics was high, vancomycin resistance was only 6.6%, and linezolid resistance was 0% at our setting. The clinicodemographic profile of patients has to be scrutinized when dealing with enterococcal infections. There is geographic variation in the resistance patterns of enterococci, which needs to be addressed before institution of definitive therapy. Speciation not only helps epidemiology, but also guides in tracking resistance patterns emerging among them.

Keywords: *Enterococcus spp*., antibiotic susceptibility testing (AST), antimicrobial resistance (AMR), vancomycin, vancomycin resistant Enterococcus (VRE).

Резюме

Ентерококите са водещи причинители при вътреболничните инфекции. Поради тяхната нарастваща резистентност към различни антибиотици е необходимо тестиране на специфичността и чувствителността им към антибиотици (AST). Тъй като географското разнообразие допринася за различни модели на резистентност при тези микроорганизми, настоящото проучване е проведено с цел да се анализира разпространението, клинично-демографския профил и видовата характеристика на ентерококи от различни клинични проби и да се оцени моделът на тяхната антимикробна резистентност (AMR). За период от шест месеца е проведено проспективно обсервационно проучване в болница за третични грижи в Телангана, Индия. Включени са клинични проби, които показват растеж на патогени през изследвания период. Видовете ентерококи са идентифицирани чрез подходящи биохимични тестове, последвани от AST. Изолирани са 106 вида ентерококи от общо 1864 проби. Разпространението на ентерококовите инфекции е 5.6%. Половото и възрастовото разпределение (31-40 години), пациентите в гинекологичното отделение и пробите от урина показват значително достоверно (p <0.05) изолиране на ентерококи. Най -често срещаният изолат е *Enterococcus faecalis* (61.3%). Резистентността към HLS (високо ниво на стрептомицин) е 33.01%, а резистентност към HLG (високо ниво на гентамицин) е 37.7%. Въпреки че резистентността към често използваните антибиотици е висока, резистентността към ванкомицин е само 6.6%, а резистентността към линезолид е 0%. Клинично-демографският профил на пациентите трябва да се изследва

* Corresponding author: id–dhanashreeltmmc@gmail.com

внимателно при наличие на ентерококови инфекции. Съществуват географски различия в моделите на резистентност на ентерококите, които трябва да бъдат разгледани преди въвеждане на окончателна терапия. Видовата характеристика не само помага на епидемиологията, но е и ръководеща при проследяването на възникващите модели на резистентност.

Introduction

Group D *Streptococci* includes Gram-positive cocci, of which the genus *Enterococcus* is clinically significant. These organisms are normal residents of the gastrointestinal, biliary and urogenital tracts in humans (Ross, 2006; Rasovic, 2018). With 19 species within this genus, *Enterococcus faecalis* contributes to the majority of infections - up to 80-90%, followed by *Enterococcus faecium* 10-15%, *Enterococcus avium*, *Enterococcus casseliflavus, Enterococcus durans,* and *Enterococcus gallinarum* (Udo *et al*., 2003; Upadhyaya *et al*., 2009). This speciation helps not only in evidence-based diagnosis, but is particularly useful with regard to treatment, as beta lactamases are now widespread in this genus and glycopeptide resistance to vancomycin can result in therapeutic failure in treating urinary tract infections, hospital acquired bacteraemia and endocarditis caused by this organism (Gordon *et al*.,1992; Low *et al*., 2001). Although *Escherichia coli* is the most common pathogen causing community acquired or nosocomial urinary tract infections (UTI), the Centers for Disease Control and Prevention (CDC) mention *Enterococcus spp.* as the second most common causative agent of nosocomial UTI next to it in a survey (Oberoi and Aggarwal, 2010; Aljicevic *et al*., 2019). Treating enterococcal infections is one of the greatest challenges faced by clinicians of 21st century. This is due to the increasing resistance in this organism, which can be intrinsic or acquired. The drug used for empirical treatment of enterococcal infections is usually beta-lactam antibiotic. Vancomycin is clinically indicated in serious infections when the patient does not respond to preliminary antibiotics like beta-lactams or has beta-lactam allergy (Kristich *et al*., 2014). Thus, antibiotic susceptibility testing (AST) is obligatory, as it guides in initiating antibiotics and aids in evidence-based treatment. *VanA* gene cluster, which is carried on transposons or via conjugative plasmids, is responsible for vancomycin resistance, which can otherwise be inducible also (Salem-Bekhit *et al*., 2012).

Many literature sources make reference to the emergence of glycopeptide resistance like vancomycin-resistant enterococci (VRE) and high-level aminoglycoside resistance (HLAR) along with methods to detect the resistance genes involved. Studies of antimicrobial susceptibility patterns are now referred to as antimicrobial resistance (AMR) studies due to the huge shift from susceptible to resistant bacterial populations (Jaiswal *et al*., 2017; Naruka *et al*., 2019). The World Health Organisation (WHO) has declared antimicrobial resistance (AMR) as one of the top ten global threats facing humanity, which requires urgent collaboration at multisectoral level. This issue has its grave consequences on patients, as it increases the cost, length of hospital stays, and causes adverse outcome of patient recovery (Schouten *et al*., 2000). Not only the presence of beta-lactamase genes, van genes circulating in *Enterococcus* contribute to resistance, but also different geographic locations have varied resistance patterns due to diverse antibiotic prescribing policy by general practitioners and disparity in empirical treatment being initiated for infections caused by this microbe (Gangurde *et al*., 2014). Thus, there is a need to know their resistance pattern so that early treatment can be initiated, as VRE poses a great challenge in clinical settings. Hence, the present study was undertaken with the aim to check the antimicrobial resistance (AMR) pattern in enterococci at our setup along with clinicodemographic profile, prevalence, and its speciation from various clinical samples. The aim was to study the prevalence, clinicodemographic profile, speciation of Enterococci from various clinical samples and to assess the antibiogram in those isolates.

Materials and Methods

Institutional ethical committee clearance was obtained before the start of the study. A prospective observational study was carried out in the Department of Microbiology, Mamata Medical College and Hospital, Khammam, Telangana, from June 2019 to January 2020, which caters patients mainly belonging to rural background.

Sample processing

Clinical samples collected in this period were subjected to Gram stain, ZN stain and special stains wherever required. All samples were inoculated on routine media like blood agar and MacConkey agar for bacterial culture and incubated at 37°C for 18-24hrs. Chocolate agar was incubated for 48-72 hrs in a candle jar. Genus's identification along with speciation was done by routine and special biochemical tests for all isolates. *Enterococcus spp*.

was identified by colony morphology, catalase test, growth in 6.5% NaCl broth, PYR test and bile esculin test. Further speciation was performed by motility, sugar fermentation test with mannitol, arabinose, raffinose, lactose, sucrose, deamination of arginine on Moellers decarboxylase broth and pigment production on white Dacron swab (Ross, 2006).

Antibiotic susceptibility testing

This was carried out by Kirby Bauer disc diffusion method on Muller Hinton agar (MHA) plates with broth turbidity matching 0.5 McFarland standard as per CLSI 2019-2020 guidelines. Antibiotic discs required for testing AMR testing were procured from Himedia (Mumbai, India). ATCC *E. faecalis* 25912 was used for quality control (QC) check on MHA plates. Plates were inoculated and incubated at 37°C for 16-18 hrs for all antibiotics except for vancomycin, where incubation was extended to 24 hrs. The plates were read with reflected light a few inches above black background except for vancomycin, which was read under transmitted light. The area showing no obvious, visible growth to unaided eye was considered as zone diameter. Those isolates which showed intermediate and resistant zones with vancomycin were further subjected to Epsilometer (E MIC strip) test procured from Himedia (Mumbai, India) on Muller Hinton agar plates for 24 hrs and results were reported as per MIC values

Reporting based on CLSI guidelines:

The following antibiotics were interpreted as per the Clinical and Laboratory Standards Institute (CLSI, 2019).

Group A = penicillin and ampicillin (for all clinical isolates)

Group B = vancomycin and linezolid (for all clinical isolates)

Group C = high-level gentamicin resistance (HLG), high-level streptomycin resistance (HLS), doxycycline (for all clinical isolates).

Group U = ciprofloxacin, levofloxacin, nitrofurantoin (Primarily/used only for urinary isolates) and fosfomycin (for urinary isolates of *E. faecalis* only).

HLG and HLS resistance was detected when the organism showed no zone, was inconclusive for 7-9 mm, and susceptible if >10 mm diameter was measured on AST (mentioned separately as Table 3J in CLSI).

Statistical analysis

Data were coded and entered into Microsoft Excel 2019 (v16.0) (Microsoft, 2019). The data were analysed using SPSS Statistics for Windows, version 16.0 (SPSS Inc., Chicago, Ill., USA) (SPSS Inc, 2007). The results were described using mean standard deviation and percentages. A chi-square test of independence was performed to examine the relation between categorical variables. The relation between these variables was considered statistically significant if $p<0.05$.

Results

Epidemiological profile

Among the 1864 culture positive samples during the study period, 106 *Enterococcus spp*. were isolated. Prevalence of the *Enterococcus spp*. in our institute was 5.6%. A chi-square test of independence showed that there was a significant association between sex and enterococcal isolates, χ^2 (1, N=1864)=10.631, p=0.0011, with male population showing increased isolation. There was also a significant association between age and enterococcal isolates, χ^2 (5, N=1864)=12.01, p=0.03467, with the 31-40 yrs age group showing more isolation. Though the enterococcal isolates did not differ significantly by overall clinical settings (outpatient department (OPD)/inpatient department (IPD)), χ^2 (1, N=1864)=2.21, p=0.1370, there was a significant association between different inpatient wards and enterococcal isolates, χ^2 (4, N=1864)=92.36, p <.0001, showing gynaecology patients harbouring more infection. There was a significant association between the type of sample and enterococcal isolates, χ^2 (3, N=1864)=26.710, p <.0001. Among the 106 enterococcal isolates obtained, urine samples (86, 81.13%) had the highest enterococcal isolates followed by pus (17, 16.04%), blood (2, 1.89%), and ET aspirate (1, 0.94%) (Table 1).

Speciation

Overall *E. faecalis* (61.3%) was the predominant species isolated in our setting followed by *E. faecium* (25.4%), *E. gallinarum* (3.7%), *E. raffinosus* (2.8%), *E. durans* (2.8%), *E. casseliflavus* (2.8%), and *E. avium* (0.9%) (Table 2).

Figure 1 depicts the distribution of various enterococcal isolates in clinical samples. *E. faecalis* was the most predominant isolate from urine, pus, blood, and ET aspirate followed *E. faecium*.

Antibiogram

Figure 2 depicts the antimicrobial resistance (AMR) pattern of enterococcal isolates to various antimicrobial agents. Resistance to penicillin was 86.7% (92/106) and to ampicillin was 83.9% (89/106). None of the isolates was resistant to linezolid (0/106). Nitrofurantoin was tested for urinary

Table 1. Clinicodemographic profile of patients (n=1864)

Characteristics		Frequency	Negative† (%)	Positive† (%)	χ2* (df)	p-value*
Sex						
Male		1102	1017 (92.29)	65 (5.9)	10.631 (1)	0.0011**
Female		762	741 (97.24)	41 (2.76)		
Age (in completed years)						
<18		43	38 (88.37)	05 (11.63)	12.01 (5)	0.03467**
18-30		93	85 (91.40)	08 (8.60)		
31-40		822	781 (95.01)	41 (4.99)		
41-50		618	591 (95.63)	27 (4.37)		
51-60		176	160 (90.91)	16 (9.09)		
>60		112	103 (91.96)	09 (8.04)		
Department						
OPD		995	931 (93.57)	64 (6.43)	2.211 (1)	0.1370
IPD		869	827 (95.17)	42 (4.83)		
IPD 1	Paediatric ward	11	09 (81.82)	02 (18.18)	92.36 (4)	< 0.00001**
IPD 2	Gynaecology ward	478	462 (96.65)	16 (3.35)		
IPD 3	Medicine ward	351	339 (96.58)	12 (3.42)		
IPD 4	Surgery ward	19	11 (57.89)	08 (42.11)		
IPD 5	Orthopaedics ward	10	06 (60.00)	04 (40.00)		
Samples						
Urine		1098	1012 (92.17)	86 (7.83)	26.710 (3)	< 0.00001**
Pus		691	674 (97.54)	17 (2.46)		
Blood		71	69 (97.18)	02 (2.82)		
ET aspirate		04	03 (75.00)	01 (25.00)		

Note: † for *Enterococcus* isolation, *Chi-square test for independence, **statistically significant ($p<0.05$). Total samples with growth - 1864, *Enterococcus spp*. Isolated - 106

Table 2: Distribution of *Enterococcus spp.* in various samples

	E. faecalis	*E. faecium*	*E. gallinarum*	*E. raffinosus*	*E. durans*	*E. casseliflavus*	*E. avium*
Urine (n=86)	54	21	2	3	3	2	1
Pus (n=17)	9	5	2	-	--	1	
Blood (n=2)	1	1	-	-	-	-	
ET†aspirate (n=1)	1	-	-	-	-	-	
Total=106	65 (61.3)	27 (25.4)	4 (3.7)	3 (2.8)	3 (2.8)	3 (2.8)	1 (0.9)

†ET= Endotracheal

isolates only, with 30.23% (26/86) of the isolates showing resistance. Fosfomycin was tested for urinary isolates of *E. faecalis* only, which showed 29.62% (16/54) resistance. Fluoroquinolones and tetracycline resistance was high, with ciprofloxacin 71.6% (76/106), levofloxacin 67.9% (72/106), and doxycycline 69.8% (74/106) at our setting. High-level streptomycin (HLS) resistance was 33.01% (35/106) and high-level gentamicin (HLG) resistance was 37.7% (40/106) at our setting.

Image 1 shows antibiotic susceptibility testing done as per CLSI guidelines for *E. faecalis* on Muller Hinton agar plate (100 mm) by Kirby Bauer disc diffusion method with six antibiotic discs.

Although resistance to commonly used antibiotics was high in our setting, one interesting ob-

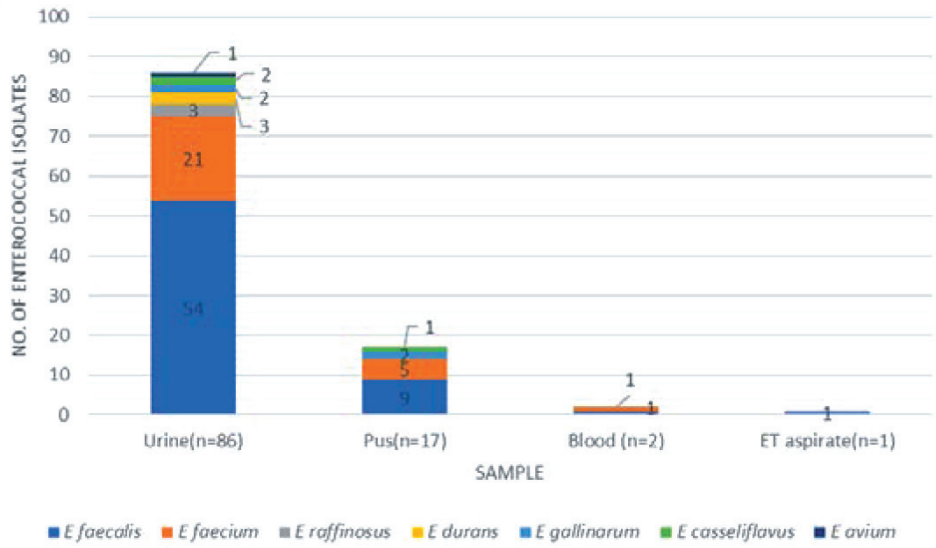

Fig. 1. Distribution of *Enterococcus spp.* isolated from various samples

	P	AMP	VA	LZ	CIP	LE	HLG	HLS	DO	FO	NIT
RESISTANT	92	89	3	0	76	72	40	35	74	16	26
INTERMEDIATE	0	0	4	0	6	7	0	0	5	0	10
SENSITIVE	14	17	99	106	24	27	66	71	27	38	50

Fig. 2. Antibiogram of *Enterococcus spp*., P=Penicillin, AMP=Ampicillin, VA=Vancomycin, LZ=Linezolid, CIP=Ciprofloxacin, LE=Levofloxacin, HLG=High level gentamicin, HLS=High level streptomycin, DO=Doxycycline, FO=Fosfomycin, NIT=Nitrofurantoin.

servation was the resistance to vancomycin, which was unexceptionally low. On a total of seven (7) isolates, of which three isolates showed complete resistance on disc diffusion method and four isolates showed intermediate resistance pattern, vancomycin E MIC strip test was performed. All isolates had Minimum Inhibitory Concentration (MIC) breakpoint of ≥ 32µg/ml, indicating vancomycin resistance. Vancomycin resistant Enterococcus (VRE) isolation at our setting was 6.6% (7/106).

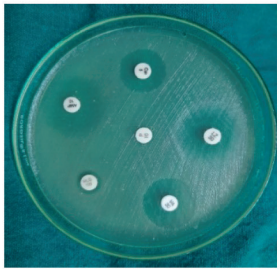

Image 1: Antibiotic susceptibility testing (AST) of *E. faecalis* on Muller Hinton agar plate by Kirby Bauer disc diffusion method.

Thus, VRE was much less (6.6%) compared to HLG (37.7%) and HLS (33.01%) resistance at our setting. *E. faecium* showed the highest vancomycin resistance followed by *E. faecalis.*

Discussion

The epidemiological pattern of enterococci varies with different geographic locations (Gangurde *et al*., 2014). Once susceptible, this genus has now acquired widespread resistance with many literature sources mentioning antimicrobial resistance patterns, characterization and phenotyping methods as a title (Jaiswal *et al*., 2017; Naruka *et al*., 2019). The prevalence of enterococci in our study was 5.6%, which was high compared to Mukherjee *et al*. (2016), which was 4.8% and Sreeja *et al*. (2012), which was 2.3%, and less compared to Desai *et al*. (2001), which was 22.19 % and Jada and Jayakumar (2012), which was 15.46%.

The present study showed significant distribution of enterococcal isolation with sex (p val-

ue<0.05), with male patients showing higher isolation. Studies done by Ferede *et al*. (2018) and by Salem Bekhit *et al*. (2012) showed no significant association of sex with enterococcal isolation, although male population was predominant in their study. The 31 to 40 years age group was associated with increased enterococcal isolation, showing a significant p value (<0.05) in the present study in contrast to studies done by Balan *et al*. (2016), which had 21-40 years age group affected, and Ferede *et al*. (2018), where >59 years age group was more commonly affected. IPD and OPD patient distribution was insignificant although gynaecology ward patients in the present study had higher isolation of enterococcal isolates followed by medicine ward and surgery ward with a statistically significant p value (<0.05). Although the distribution among IPD and OPD was insignificant, nosocomial infection by enterococcus was observed in the present study as among IPD patient distribution was significant. Medical ward patients had higher enterococcal isolation in the study done by Ferede *et al*. (2018), which contrasts with the present study. Isolation of enterococci was the highest in urine samples followed by pus, blood, and endotracheal (ET) aspirates. This study is comparable to studies done by Karmarkar *et al*. (2004), Anbumani *et al*. (2005) and Mukherjee *et al*. (2016) where Enterococci were isolated predominantly from urine samples. Thus, the clinicodemographic profile contributes significantly when dealing with *Enterococcus spp*. at any clinical setup, which is routinely ignored.

E. faecalis was the predominant isolate in the present study followed by *E. faecium, E. gallinarum, E. raffinosus, E. durans, E. casseliflavus, and E. avium*, which is similar to the study done by Salem-Bekhit *et al*. (2012), and Mukherjee *et al*. (2016). *E. faecium* was reported to be predominant species in a study done by Aberna in 2018, which contrasts to the present study.

The *Enterococcus spp*. are intrinsically resistant to many antibiotics. They also develop resistance very quickly compared to other organisms, resulting in increased nosocomial infections (Morris *et al*., 1995). Among 106 isolates tested in the present study, resistance to beta lactams, fluoroquinolones, tetracyclines(doxycycline) was 86.7%, 71.6%, and 69.8%, which was high compared to other studies (Mendiratta *et al*., 2008; Parameswarappa *et al*., 2013). High-level resistance to streptomycin and gentamicin was 33.01% and 37.7%, respectively, which was also considerably higher compared to other studies (Mittal *et al*., 2016; Maradia *et al*., 2017). The incidence of nitrofurantoin (30.23%) and fosfomycin (29.62%) resistance was comparatively less in urinary isolates, which is comparable to the study done by Butch *et al*. in 2011 but contrasts the study by Balan et al. in 2016. None of the strains was resistant to linezolid, which is comparable to most of the studies conducted in India (Srivastav *et al*., 2013; Lavanya *et al*., 2016). In 1986, the first case of VRE was observed and reported from Europe (Leclercq *et al*., 1988). Since then, many cases of VRE are being increasingly reported from many parts of the world. India also started reporting VRE from the early 20th century, when few cases were observed in the beginning and later on their number increased (Vidyalaxmi *et al*., 2012). In the present study, the most notable finding was VRE, which was 6.6%, which contrasts with most of the recent studies published on enterococci but similar to the study done by Balan *et al.* (2016), Ferede *et al*. (2018), Asgin *et al*. (2020), and Gupta *et al*. (2020). One of the reasons in the present setting which may contribute to this antimicrobial pattern may be due to hospital antibiogram policies prescribing lesser use of vancomycin, which could be due to its non-affordability for the patients. Thus, geographic location matters for AMR in enterococci due to the local prescribing policy based on affordability for patients. If followed properly, mandatory AST, speciation, antimicrobial stewardship along with infection control practices play a significant role for reducing resistance in Enterococcus – a process which has a long way to go.

Conclusion

There was 5.6% prevalence of enterococcal isolation in the present study, significant association of clinicodemographic profile of patients with enterococcal infections and high level of resistance to routinely used antibiotics with the exception of vancomycin and linezolid. Routine screening of all enterococcal isolates as per CLSI is recommended due to evolving resistance in this organism by multiple mechanisms and due to widespread geographical variation.

References

Aberna, R. A., K. Prabhakar (2018). Antimicrobial susceptibility pattern for *Enterococcus* species colonizing the GIT of hospitalized and community patients. *Indian J. Microbiol. Res*. **5**: 290-294.

Aljicevic, M., O. Amina, R. Velma, M. Sabina, A. Amila (2019). Antimicrobial susceptibility/resistance of *Escherichia coli* among the outpatients with urinary tract infections in Mostar. *Acta Microbiol. Bulg*. **35**: 141-146.

Anbumani, N., M. Thangam, J. Kalyani, M. Mallika (2005). Isolation, distribution, and prevalence of various species of enterococcal isolated from clinical specimens in a tertiary care hospital. *Indian J. Pathol. Microbiol*. **48**: 534-537.

Asgin, N., O. Baris (2020). Antibiotic resistance and molecular epidemiology of vancomycin-resistant Enterococci in a tertiary care hospital in Turkey. *Infect. Drug Resist.* **13**: 191-198.

Balan, K., A. V. Sangeetha, L. Abirami, T. S. Vijayalakshmi, D. D. Sheila (2016). Characterization and *in vitro* susceptibility pattern of Enterococci. *Clin. Biomed. Sci*. **6**: 24-27.

Butch, M., S. S. Akcay, A. S. Inan, S. Aksaray, S. Engin, G. Calisici (2011). *In vitro* susceptibility of Enterococci strains isolated from urine samples. *J. Infect. Chemother*. **17**: 575-578.

Clinical and Laboratory Standards Institute (2019). Performance standards for antimicrobial susceptibility testing. Twenty Nineth informational supplement ed. M**100**-S29.

Desai, P. J., D. Pandit, M. Mathur, A. Gogate (2001). The prevalence, identification, and the distribution of various species of enterococci which were isolated from clinical samples, with special reference to the urinary tract infections in catheterized patients. *India J. Med. Microbiol*. **19**: 132-137.

Ferede, Z. T., D. T. Kassu, G. D. Solomon, G. Y. Addisu (2018). Prevalence and antimicrobial susceptibility pattern of *Enterococcus* species isolated from different clinical samples at Black Lion specialized teaching hospital, Addis Ababa, Ethiopia. *BMC. Res. Notes* **11**: 793-796.

Gangurde, N., M. Mane, S. Phatale (2014). Prevalence of multidrug resistant *Enterococci* in a tertiary care hospital in India: a growing threat. *Open J. Med. Microbiol*. **4**: 11-13.

Gordon, S., J. M. Swenson, B. C. Hill, N. E. Pigott, R. R. Facklam, R. C. Cooksey, C. Thornsberry, W. R. Jarvis, F. C. Tenover (1992). Antimicrobial susceptibility pattern of common and unusual species of Enterococci causing infection in the United States. Enterococcal Study Group. *J. Clin. Microbiol*. **30**: 2373-2378.

Gupta, S., P. Srivastava, S. Yadav, S. N. Tayade (2020). Vancomycin-resistant enterococci causing bacteriuria in hospitalized patients from Northwest India. *J. Datta Meghe. Inst. Med. Sci. Univ*. **15**: 421-425.

Jada, S., J. Karthika (2012). Prevalence of *Enterococcus* species from various clinical specimens in Shri Sathya Sai Medical College & Research Institute with special reference to speciation and their resistance to vancomycin. *Int. J. Med. Clin. Res.* **3**: 154-160.

Jaiswal, S., A. Singh, R. K. Verma, D. P. Singh, S. Kumari (2017). Characterization, speciation and antimicrobial resistance pattern of *Enterococcus* species isolated from clinical specimens at a rural tertiary care hospital. *Int. J. Res. Med. Sci*. **5**: 3484-3487.

Karmarkar, M. G., E. S. Gershan, P. R. Mehta (2004). Enterococcal with special reference to phenotypic characterization and drug resistance. *Indian J. Med. Res*. **119**: 22-25.

Kristich, C. J., L. B. Rice, C. A. Arias (2014). Enterococcal Infection -Treatment and Antibiotic Resistance. In: Gilmore, M. S., D. B. Clewell, Y. Ike, N. Shankar (Eds.). Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014.

Lavanya, T., Kamalasekaran, C. Jhansi (2016). Antibiotic susceptibility pattern of enterococcal isolates from a tertiary care hospital. *Innov. Pharm. Pharmacother.* **3**: 730-732.

Leclercq, R., E. Derlot, J. Duval, P. Courvalin (1988). Plasmid-mediated resistance to vancomycin and teicoplanin resistance in *Enterococcus faecium. N. Engl. J. Med*. **319**: 157-161.

Low, D., N. Keller, A. Barth, R. Jones (2001). Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of Enterococci: results from the Sentry antimicrobial surveillance program, 1997–1999. *Clin. Infect. Dis*. **32**: 133–145.

Maradia, M. R., K. Mehta, K. Prajapati, V. Minesh, S. Pranay, V. Mahendra (2017). Prevalence of multidrug-resistant *Enterococcus* species isolated from urine samples in a tertiary care hospital, Western India. *Int. J. Med. Sci. Public. Health* **6**: 715-719.

Mendiratta, D. K., H. Kaur, V. Deotale, D. C. Thamke, R. Narang, P. Narang (2008). Status of high-level aminoglycoside resistant *Enterococcus faecium* and *Enterococcus faecalis* in a rural hospital of central India. *Indian J. Med. Microbiol*. **26**: 369-371.

Mittal, S., P. Singla, A. Deep, B. Kiran, S. Rama, G. Meenu, C. Uma (2016). Vancomycin and high-level aminoglycoside resistance in *Enterococcus* spp. in a tertiary health care centre: a therapeutic concern. *J. Pathog*. **2016**: 8262561.

Morris, J. G., D. K. Shay, J. N. Hebden, R. J. McCarter, B. E. Perdue, W. Jarvis, J. A. Johnson, T. C. Dowling, L. B. Polish, R. S. Schwalbe (1995). Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establishment of endemicity in a university medical center. *Ann. Intern. Med.* **123**: 250-259.

Mukherjee, K., B. Debojyoti, C. Goutam, C. Shivsekhar (2016). Prevalence and antibiotic susceptibility pattern of *Enterococcus* species from various clinical samples in a tertiary care hospital in Kolkata. *Int. J. Contemp. Med*. Res. **3**: 1565-1567.

Naruka, H. S., A. E. Chand, H. Meena (2019). Prevalence of various *Enterococcus* species and their antibiotic resistance pattern among urinary isolates in tertiary care center in South-Eastern Rajasthan. *Int. J. Med. Microbiol. Trop. Dis*. **5**: 18-22.

Oberoi, L., A. Aggarwal (2010). Multidrug resistant enterococci in a rural tertiary care hospital – a cause of concern. *J. K. Sci*. **12**: 157-158.

Parameswarappa, J., V. P. Basavaraj, C. M. Basavaraj (2013). Isolation, identification and antibiogram of *Enterococci* isolated from patients with urinary tract infection. *Ann. Afr. Med*. **12**: 176-181.

Rasovic, M. B. (2018). Potential and constraints of use of indigenous *Enterococci* in Dairy Industry. *Acta Microbiol. Bulg*. **34**: 18-24.

Ross, P. W. (2006). *Streptococci* and *Enterococci.* In: Colle J. G., B. P. Marmion, A. G. Fraaser, A. Simmons (Eds). Mackie and McCartney Practical Medical Microbiology. Ed. 14, pp. 268-272.

Salem-Bekhit, M. M., I. Moussa, M. M. Muharram, F. K. Alanazy, H. M. Hefni (2012). Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. *Indian J. Med. Microbiol*. **30**: 44-51.

Schouten, M. A., J. A. Hoogkamp-Korstanje, J. F. Meis, A. Voss (2000). Prevalence of vancomycin resistant enterococci in Europe. *Eur J. Clin. Microbiol. Infect. Dis*. **19**: 816-822.

Sreeja, S., B. P. R. Sreenivasa, A. G. Prathab (2012). The prevalence and the characterization of the Enterococcus species from various clinical samples in a tertiary care hospital. *J. Clin. Diagn. Res*. **6**: 1486-1488.

Srivastava, P., M. Raman, P. S. Nirwan, S. Meeta, S. S. Dahiya (2013). Prevalence and antimicrobial susceptibility of *Enterococcus* species isolated from different clinical samples in a Tertiary Care Hospital of North India. *Natl. J. Med. Res*. **3**: 389-391.

Udo, E. E., N. Al-Sweih, O. A. Phillips, T. D. Chugh (2003). Species prevalence and antibacterial resistance of *Enterococci* isolated in Kuwait hospitals. *J. Med. Microbiol*. **52**: 163-168.

Upadhyaya, G. P. M., K. L. Ravikumar, B. Umapathy (2009). Review of virulence factor of *Enterococcus*: an emerging nosocomial pathogen. *Indian J. Med. Microbiol*. **27**: 301-305.

Vidyalakshmi, P. R., R. Gopalakrishnan, V. Ramasubramanian, K. A. Ghafur, P. S. Nambi, M. A. Thirunarayana (2012). Clinical, epidemiological, and microbiological profile of patients with vancomycin-resistant *Enterococci* from a Tertiary Care Hospital. *J. Glob. Infect. Dis*. **4**: 137-138.