International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Prevalence, Virulence Factors and Antibiotic Susceptibilities of Enterococcal Isolates from Various Clinical Specimens in Coimbatore, Tamil Nadu

Palanisamy Manikandan[1, 2, 3]

[1]Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Majmaah University, Kingdom of Saudi Arabia
[2]Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, Tamilnadu, India
[3]Greenlink Analytical and Research Laboratory India Private Ltd., Coimbatore, Tamilnadu, India

Running title: Isolation of enterococci from various clinical specimens

Abstract: ***Aim: To determine and analyze the prevalence, antibiotic susceptibilities and virulence factors of enterococci isolated from various clinical specimens. Methods: The microbiological records of all patients infected with enterococcal species were reviewed. Information on specimen type, age, sex, seasonal variation, coexisting infections, results of each diagnostic techniques and in vitro antibacterial susceptibility patterns were recorded and analyzed. Culture characteristics and virulence factors of the isolates such as hemolytic pattern and gelatinase production were also studied. Results: Of3486 total cases reported during the study period, 1848 (53%) and 1638 (47%) were found to be male and female respectively. Urine specimen was the predominant clinical specimen received.A total of 827 (23.7%) bacterial isolates were identified and 35.6% (295) and 64.3% (532) were notedto be gram-positive and gram-negative pathogens respectively.S. aureuswas the most prevalent gram positive isolate in this region. More number of Enterococcus spp (30; 48.3%) was isolated from pus specimen. 61 (98.4%)enterococcal isolates were determinedto be susceptibleto linezolid, teicoplanin, vancomycin and amoxicillin. Conclusion: Prospectiveregion basedlarge-scale studies would certainly bring out a supportive database for an appropriate treatment of enterococcal infections.***

Keywords: enterococci, susceptibility, infections, antibiotic, vancomycin

1. Introduction

Enterococci are normal gastrointestinal flora of mammals and other warm blooded animals and can also be found in soil, on plants, in water and in several food products. They are also commensals of the upper respiratory tract, biliary tract and vagina of humans that survive well outside the host.[1]Along with pathogenic enterococci, these commensals can also cause opportunistic infections in immunocompromised patients.[1-3]There are various virulence factors associated with enterococcal infections like cytolytic toxin, aggregation substance, gelatinase, extracellular surface protein and extracellular superoxide production.[2]

Enterococci are one of the major causes of urinary tract infections (UTI's)[4], nosocomial infections and endocarditis.[5] Treatment of enterococcal infections in humans is becoming difficult as they acquire rapid resistance to a wide range of antibiotics and become multi drug resistant against antibiotics such as beta-lactams, glycopeptides and aminoglycosides like Gentamycin and Vancomycin.[6,7]Enterococci acquire resistance very often through the transfer of plasmids and transposons, chromosomal exchanges and mutations. Glycopeptide resistant enterococci were first isolated in Europe in 1986 and shortly thereafter in USA, and over the past decade they have become a world wide problem. For example, Vancomycin (a glycopeptide antibiotic), is a cell wall antibiotic that represents the last line of defense against many multiple drug resistant gram- positive pathogens.[3] Therefore, the epidemiological knowledge of different enterococcal infections caused by pathogenic as well as multidrug resistant strains will help clinicians for first line treatment and effective control of transmission of this pathogen.

Against this background, an experimental study was conducted in Coimbatore to determine and analyze the current prevalence, antibiotype and virulence factors of enterococci isolated from various clinical specimens.

2. Materials and methods

Specimens

This retrospective study was conducted in Coimbatore for a period of five months to determine the incidence, demography (Sex and age of infected patents), seasonal variation, and predisposing factors of enterococcal infections. Culture proven microbiology records of patients with enterococcal infections reported at the Microbiological Laboratories (Micro Labs, Coimbatore, India), were scrutinized and reviewed further for the study.

Microbiological methods

The microbiological records of all patients infected with enterococcal species were reviewed. Information on specimen type, age, sex, seasonal variation, coexisting infections, results of each diagnostic techniques and *in vitro* antibacterial susceptibility patterns were recorded and analyzed. During the five-month study period 3486 various clinically suspected various specimens were received from patients and processed for microbiological investigations.

Culture methods involved direct inoculation of specimens on 5% sheep blood agar, chocolate agar, MacConkey Agar, potato dextrose agar, thioglycollate and brain-heart infusion broths. The inoculated potato dextrose agar was incubated at 25°C to isolate fungi and examined daily up to 3 weeks. To isolate bacteria, inoculated sheep blood agar plates were incubated under aerobic and anaerobic conditions; chocolate agar was incubated with 5% carbon dioxide, and thioglycollate and brain-heart infusion broths aerobically at 37°C. The isolated enterococcal species were further confirmed and all laboratory methods were followed by standard microbiological procedures.[8,9]

Antibiotic susceptibility patterns were studied for the confirmed isolates of enterococci by agar disc diffusion method[10]following guidelines by Clinical &Laboratory Standards Institute (CLSI 2012).[11] The panel of antibiotics included were Ceftazadine (30 µg), co-trimoxazole (25 µg), Gentamycin (10 µg), Cephalexin (30 µg), Amikacin (30 µg), norfloxacin (10 µg), ceftazidine (30 µg), ciprofloxacin (5 µg), netilin (30 µg), ofloxacin (10 µg), imipenem (10 µg), nalidixic acid (30 µg), linezolid (30 µg), teicoplanin (30 µg), vancomycin (30 µg),chloramphenicol (30 µg), amoxicillin (10 µg), erythromycin (15 µg), cloxacillin (1 µg).

Evaluation of virulence factors

Hemolysin

The pus isolates were assayed for the production of cytolysin toxin. Different blood agar plates (of sheep and human bloods)wereused to detect cytolysin production by the test isolates. All the test isolates were inoculated, the plates were incubated at 37°C and examined for β-hemolysis (clear zone of hydrolysis)after 24 hours and 48 hours.[12]

Gelatinase production

Production of the virulence factor gelatinase enzyme was assayed among the test enterococcal isolates by conducting the gelatinase liquefaction test as described previously.[13] A positive culture (*Bacillus* sp.), negative culture (*E.coli*) and an uninoculated tube were kept as control along side. Following 24 hours incubation at 37°C, the results were examined.

Estimation of extracellular proteins

For the estimation of both whole cell and extra cellular (with out concentration) proteins, Lowry *et al.*, 1951 methodology was employed.[14]

Separation of whole cell protein

The whole cell and extracellular proteins were separated using SDS-PAGE[15] and the protein profiles were compared.

3. Results

The total number of cases reported during the study period was 3486, of which 1848 (53%) and 1638 (47%) were found to be male and female respectively. The specimens received during the study period included blood, urine, pus, semen, swab, sputum and fluid. While urine specimenwas found to be the predominant specimen and yielded more culture positivity (51.1%),fluid category was received at low number andwith a culture positivity of 1.2%.Upon microbiological analyses, a total number of 827 (23.7%) bacterial isolates was identified. Of which,35.6% (295) and 64.3% (532) were found to be gram-positive and gram-negative bacteria respectively(Table 1). More number of pathogenic isolates was reported from male and its preponderance was obvious.Amongthe gram-positive and gram-negative organisms, *S. aureus*and*E. coli* were determinedto be more prevalent in this region respectively. Of 827 bacterial pathogens isolated, as much as 281 (62.8%)*E. coli*were from urine specimen alone. Male patients were often infected with *S. aureus*and *E. coli* bygram positive and gram-negative organisms respectively. Similarly, among female patients *Streptococcus* sp. and *E. coli* were reported as common causative agents.

Of62*Enterococcus* spp. isolated from 3468 various clinical specimens, 40 (64.5%) and 22 (35.4%) were determined to be from male and female patients respectively. Number of persons between the age group of 60 and above was more (Table 2). More number of *Enterococcus* spp (30; 48.3%) was isolated from pus specimen.

Of the 18 test antibiotics representing different groups tested, 61 (98.4%)enterococcal isolates were susceptible to linezolid, teicoplanin, vancomycin, amoxicillin and 61.3%, 64.5%%, 66.1%, and 80.6% of strains were identifiedto be resistant to gentamycin, co-trimoxazole,nalidixic acid and amikacin respectively (Figure 1).

Almost all species of enterococci noted to produce yellow colored colonies on BHIA and theirgrowth was observed in 6.5% NaCl. Though 4 isolates of the study, did not show hemolysis on sheep blood agar, 58 (93.5%)strains confirmed β-hemolytic pattern. Similarly, majority of the enterococci of this study proved marked gelatinase activity. Protein profiles of extracellular and whole cell proteins from selected isolates were not highly resolved.

4. Discussion

Enterococci are Gram-positive facultative anaerobic cocci, which usually present as a normal flora in the alimentary tract of humans. They are capable of living in a variety of stress environments, including those of high temperature conditions.[2] Multidrug resistant isolates of enterococci are the leading cause of human infections worldwide.[16]Also, high-level gentamycin and vancomycin resistant enterococcal (VRE) isolates are serious problems in clinical settings, which limit the treatment options against the infections.[17]

In this present study, a total of 62 enterococcal isolates identified fromdifferent specimens excluding fecal samples. In contrast to our findings, in a study conducted by Silverman *et al*[18]from 250 fecal samples alone, 107 enterococcalisolates were determined. Therefore, the incidence of enterococci in the fecal samples was uncertain**.**The antibiogramagainst the test isolates revealed linezolid, teicoplanin and vancomycin as the most promising drugs. Gentamycin and nalidixic acid were less successful and many isolates exhibited multiple drug resistance particularly to Ceftriaxone, Cotrimoxazole, Gentamycin, Amikacin, Norfloxacin, Netilin, Amoxycillin, Erythromycin and Cloxacil.Leven*et al*[19]have reported an increased

prevalence of glycopeptide resistant enterococcal (GRE) isolates of intestinal origin. In a similar study, the *in-vitro* susceptibilities of 4208 enterococcifrom patients in 27 European countries towards 16 antibiotics were determined and that high level of resistance to gentamycin reported to be varied by country and species.[20] In ananother study conducted in North Rhine-Westphalia, Germany as much as 730 enterococcalisolates were tested for their antibiotic resistance against various antibiotics. It was reported with resistance rates to ampicillin (7.4%), high-level gentamycin (15.0%) & streptomycin (27.9%), ciprofloxacin (37.9%) vancomycin (1.5%) and teicoplanin (1.5%).[21]

Remarkably, the study confirmed, one enterococcal isolate resistant to vancomycin as well as to 3 more antibiotics such as amikacin, erythromycin and Cloxacil. The prevalence of vancomycin resistant enterococci (VRE) was noted to be less among pus derived enterococcal isolates and not significant when compared with other findings. Karmarkar*et al*., (2004) have reported as much as 12 VRE isolates from 52 enterococci. In a multicenter study, 778 VRE had been isolated from 28 USA medical centers and this accounted for 75% of the total enterococcal isolates collected during the study period.[22]A survey of environmental surfaces in the clinical microbiology laboratory to determine the prevalence of vancomycin resistant enterococci and multi drug resistant enterobacteriaceae during a routine working day revealed a total of 20(10%) VRE and 4(2%) MDRE for 193 surfaces tested.[23]Similarly, the investigation of VRE species particularly *E. faecium* from 728 bed tertiary care hospital revealed a prevalence of a total of 413 VREF isolates from urine (52%) wounds (16%) blood (11%) catheter tips (6%) and other sites (15%).[24]

Cytolysin produced by enterococci is capable of lysing different cell types particularly the erythrocytes of human, horse, sheep, rabbit etc. They also show bactericidal activity against a wide range of gram-positive and gram-negative bacteria. These two phenomena play a major role in the progression of enterococcal infections.[25]On blood agar medium, the pattern of hemolysis formed by enterococci varies at strain or species or general level. Therefore, it becomes imperative that the analysis of RBC lysis by enterococci is much more important in the characterization of clinical enterococcal isolates. In this study, 4 (6.4%) cultures did not show hemolysis on sheep blood agar. The remaining 58 (93.5%) cultures showed β-hemolytic pattern.

Cytolysin consists of two components; lysine L and activator A and the cytolysin genes are carried on a plasmid or are integrated into the bacterial chromosome. The possibilities of such a plasmid transfer from one organism to other are inevitable. The production of cytolysin by enterococci has been shown to significantly worsen the severity of endocarditis and endophthalmitis in animal models as well as contribute to the severity of enterococcal disease in humans.[26]

All the enterococcal isolates of this study showed marked gelatinase activity. Gelatinase is an important virulence factor in the determination of pathogenicity and also plays a notable role in inflammatory process during infection by enterococci. It also aggravates the condition ofinfection like endocarditis as the rate of gelatinase production among enterococcal isolates does vary and hence, the assay for the level of excretion of gelatinase is significant.[25]

The extracellular and supernatant protein contents of pus enterococcal specimens were extracted and estimated. As the protein profiles of the extracellular and whole cell proteins were clearly resolved, no conclusion could be derived. However, the protein profiles of extracellular proteins from the 14 pus enterococcal isolates did show relatively a prominent band present uniformly in all lanes upon sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This may be suspected that excretion of this particular type of protein could be a strong phenotypic feature common for all the enterococcal isolates and could even be a toxic protein determining the virulence factor among the enterococci. The analyses of whole cell proteins using SDS-PAGE helps in molecular typing of pathogenic isolates.[27] Hence, based on the protein profiles, the enterococcal isolates could be grouped to a good extent so as to identify the most common clonal types that are spreading and causing diseases frequently among communities or in a hospital environment.[28]

While growth was observed with all pus isolates of enterococci on BHIA, only one isolate showed yellow pigment production. Yellow pigment production is one of the key tests done to identify *Enterococcus* spp. Almost all species of enterococci are known to produce yellow colored colonies on BHIA. However, few species do not produce this pigment and form white coloured colonies on BHIA and hence it is an important test in speciation of enterococci.[29]Also, the growth of test enterococci was observed in 6.5% NaCl for all cultures. As most of the common bacterial genera do not grow in extreme concentrations of sugar and salt but enterococci, this test is inevitable in the confirmation of this genus.[30]

From this study, it was imperative that the enterococcalisolates exhibited resistance to the most commonly used antibiotics such as gentamycin, co-trimoxazole,nalidixic acid and amikacin. Studying and exploring region based data on the prevalence, virulence factors and antibiotic susceptibility of enterococcal infections would certainly enable the clinicians to understand real magnitude of the problem.

5. Acknowledgement

Author thanks Dr. KanesanPanneerSelvam and Ms. Anjali, Department of Microbiology, M. R. Government Arts College, Mannargudi, Tamil Nadu for data collection and technical support.

References

[1] Saffari F, Dalfardi MS, Mansouri S, Ahmadrajabi R. Survey for Correlation between Biofilm Formation and Virulence Determinants in a Collection of Pathogenic and Fecal Enterococcus faecalis Isolates. Infection & Chemotherapy. 2017;49:176-183.

[2] Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology. 2009;155:1749-57.
[3] O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015;8:217-30.
[4] Poh CH, Oh HM, Tan AL. Epidemiology and clinical outcome of enterococcalbacteraemia in an acute care hospital. J Infect. 2006;52:383-6.
[5] Malani, Preeti N. and Kauffman, Carol A. and Zervos, Marcus J. 2002. EnterococcalDisease, Epidemiology, and Treatment, p 385-408. In Gilmore, Michael S. and Clewell, Don B. and Courvalin, Patrice and Dunny, Gary M. and Murray, Barbara E. and Rice, Louis B. (ed), The Enterococci. doi:10.1128/9781555817923.ch10
[6] Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014. Available from http://www.ncbi.nlm.nih.gov/books/NBK190424/ PubMed PMID: 24649510.
[7] Kristich CJ, Rice LB, Arias CA. Enterococcal Infection—Treatment and Antibiotic Resistance. 2014 Feb 6. In: Gilmore MS,Clewell DB, Ike Y, Shankar N, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014-. Available from http://www.ncbi.nlm.nih.gov/books/NBK190420/ PubMed PMID: 24649502.
[8] Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott's Diagnostic Microbiology. 11th ed. Mosby, St. Louis 2002.
[9] Collee JG, Fraser AG, Marmion BP, Simmons A, Eds. Mackie & McCartney Medical Microbiology. 14th ed. Churchill Livingstone, New york 1996.
[10] Bauer AW, Kirby WWM, Sherris TC, Truck M. Antibiotic susceptibility tests by standard single disc method. Am J Clin Path 1966;45:493-6.
[11] Performance standards for antimicrobial disk susceptibility tests. In: National Committee for Clinical Laboratory Standards. Approved standard. 11th edition. Clinical and Laboratory Standards Institute; 2012. Vol 32, No 1. M02-A11.
[12] Franz CM, Muscholl-Silberhorn AB, Yousif NM, Vancan- neyt M, Swings J, Holzapfel WH. Incidence of virulence factors and antibiotic resistance among Enterococci isolated from food. Appl Environ Microbiol 2001;67:4385-9.
[13] Marra A, Dib-Hajj F, Lamb L, Kaczmarek F, Shang W, Beck- ius G, Milici AJ, Medina I, Gootz TD. Enterococcalviru- lence determinants may be involved in resistance to clinical therapy. DiagnMicrobiol Infect Dis 2007;58:59-65.
[14] Lowry OH, rosebrough NJ, farr AL, randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.
[15] Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.
[16] Prabaker K, Weinstein RA. Trends in antimicrobial resistance in intensive care units in the United States. CurrOpinCrit Care 2011;17:472-9.
[17] Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. ClinMicrobiol Rev 2000;13:686-707.
[18] Silverman J, Thal LA, Perri MB, Bostic G, Zervos MJ. Epidemiologic evaluation of antimicrobial resistance in community-acquired enterococci. JClinMicrobiol. 1998;36:830-2.
[19] Ieven M, Vercauteren E, Descheemaeker P, van Laer F, Goossens H. Comparison of direct plating and broth enrichment culture for the detection of intestinal colonization by glycopeptide-resistant enterococci among hospitalized patients. J ClinMicrobiol. 1999;37:1436-40.
[20] Schouten MA, Willems RJ, Kraak WA, Top J, Hoogkamp-Korstanje JA, Voss A. Molecular analysis of Tn1546-like elements in vancomycin-resistant enterococci isolated from patients in Europe shows geographic transposon type clustering. AntimicrobAgentsChemother. 2001;45:986-9.
[21] Mascini EM, Jalink KP, Kamp-Hopmans TE, Blok HE, Verhoef J, Bonten MJ, Troelstra A. Acquisition and duration of vancomycin-resistant enterococcal carriage in relation to strain type. JClinMicrobiol. 2003;41:5377-83.
[22] Zhanel GG, Hoban DJ, Karlowsky JA. Nitrofurantoin is active against vancomycin-resistant enterococci. AntimicrobAgentsChemother. 2001;45:324-6.
[23] Paule SM, Trick WE, Tenover FC, Lankford M, Cunningham S, Stosor V, Cordell RL, Peterson LR. Comparison of PCR assay to culture for surveillance detection of vancomycin-resistant enterococci. JClinMicrobiol. 2003;41:4805-7.
[24] Bischoff WE, Reynolds TM, Hall GO, Wenzel RP, Edmond MB. Molecular epidemiology of vancomycin-resistant Enterococcus faecium in a large urban hospital over a 5-year period. J ClinMicrobiol. 1999;37:3912-6.
[25] Hickey RM, Twomey DP, Ross RP, Hill C. Production of enterolysin A by a raw milk enterococcal isolate exhibiting multiple virulence factors. Microbiology. 2003;149:655-64.
[26] Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D, Goossens H. Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of Enterococcus faecium. J ClinMicrobiol. 2004;42:4473-9.
[27] Huys G, D'Haene K, Collard JM, Swings J. Prevalence and molecular characterization of tetracycline resistance in Enterococcus isolates from food. Appl Environ Microbiol. 2004;70:1555-62.
[28] Semedo T, Almeida Santos M, Martins P, Silva Lopes MF, Figueiredo Marques JJ, Tenreiro R, BarretoCrespo MT. Comparative study using type strains and clinical and food isolates to examine hemolytic activity and occurrence of the cyl operon in enterococci. J ClinMicrobiol. 2003;41:2569-76.
[29] Nallapareddy SR, Duh RW, Singh KV, Murray BE. Molecular typing of selected Enterococcus faecalis isolates: pilot study using multilocus sequence typing

and pulsed-field gel electrophoresis. J ClinMicrobiol. 2002;40:868-76.

[30] Kapoor L, Randhawa VS, Deb M. Antimicrobial resistance of enterococcal blood isolates at a pediatric care hospital in India. Jpn J Infect Dis. 2005;58:101-3.

Table 1: Total number of bacterial/ yeast pathogens confirmed from various clinical specimens during the study period

Type of Clinical Specimen	Organisms Isolated	Total Number	Percentage
Urine (51.1%)	*Staphylococcus* sp.	14	3.5%
	Streptococcus sp.	9	2.2%
	*Enterococcus*spp	20	5.0%
	E. coli	281	69.7%
	Klebsiella sp.	33	8.2%
	Pseudomonas sp.	20	5.0%
	Enterobacter sp.	3	0.7%
	Proteus sp.	11	2.7%
	Haemophilus sp.	1	0.2%
	Candida sp.	7	1.7%
	Non-fermenting gram negative bacilli	4	1.0%
	Total	**403**	**100.0%**
Blood (7.6%)	*Staphylococcus* sp.	18	28.6%
	E. coli	3	4.8%
	Klebsiella sp.	4	6.3%
	Salmonella sp.	30	47.6%
	Non-fermenting gram negative bacilli	8	12.7%
	Total	**63**	**100.0%**
Pus (31.4%)	*Staphylococcus* sp.	110	42.3%
	Streptococcus sp.	24	9.2%
	*Enterococcus*spp	30	11.5%
	E. coli	36	13.8%
	Klebsiella sp.	13	5.0%
	Pseudomonas sp.	20	7.7%
	Enterobacter sp.	7	2.7%
	Proteus sp.	5	1.9%
	Candida sp.	4	1.5%
	Non-fermenting gram negative bacilli	11	4.2%
	Total	**260**	**100.0%**
Swab (9.3%)	*Staphylococcus* sp.	14	18.2%
	Streptococcus sp.	23	29.9%
	*Enterococcus*spp	5	6.5%
	E. coli	4	5.2%
	Klebsiella sp.	11	14.3%
	Pseudomonas sp.	2	2.6%
	Enterobacter sp.	2	2.6%
	Proteus sp.	8	10.4%
	Haemophilus sp.	1	1.3%
	Candida sp.	1	1.3%
	Non-fermenting gram negative bacilli	6	7.8%
	Total	**77**	**100.0%**
Semen (1.7%)	*Staphylococcus* sp.	1	7.1%
	Streptococcus sp.	2	14.3%
	*Enterococci*spp	7	50.0%
	E. coli	3	21.4%
	Proteus sp.	1	7.1%
	Total	**14**	**100.0%**
Sputum (3.2%)	*Staphylococcus* sp.	5	18.5%
	Streptococcus sp.	8	29.6%
	E. coli	1	3.7%
	Klebsiella sp.	5	18.5%
	Pseudomonas sp.	5	18.5%
	Proteus sp.	1	3.7%
	Haemophilus sp.	1	3.7%
	Non-fermenting gram negative bacilli	1	3.7%
	Total	**27**	**100.0%**
Fluids (1.2%)	*Staphylococcus* sp.	4	40.0%
	Streptococcus sp.	2	20.0%
	Klebsiella sp.	1	10.0%
	Enterobacter sp.	1	10.0%
	Proteus sp.	1	10.0%

	Non-fermenting gram negative bacilli	1	10.0%
	Total	**10**	**100.0%**

Table 2: Various age group of patients- received during the study period

Age group in years - all patients								
< 1	**1-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**> 60**	**Total**
412 (11.8%)	200(5.7%)	126 (3.6%)	900 (25.8%)	534 (15.3%)	804 (23.1%)	170 (4.9%)	340 (9.8%)	**3486**
Age group in years - Patients with enterococcal infections								
< 1	**1-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**> 60**	**Total**
5 (8%)	5(8%)	8(13%)	10 (16%)	11 (18%)	11 (18%)	-	12 (19%)	**62 (2%)**

Table 3: Virulence factors of enterococcal isolates from different clinical specimens

Hemolysis pattern in 5% sheep blood agar		**Number of isolates**	**Percentage**
	Complete Hemolysis (β)	58	93.5
	NoHemolysis (γ)	4	6.4
Gelatinase production			
	Positive	62	100
	Negative	--	--
Protein concentration			
	0.0 - 0.1	1	
	0.1 - 0.2	2	
	0.2 - 0.3	17	

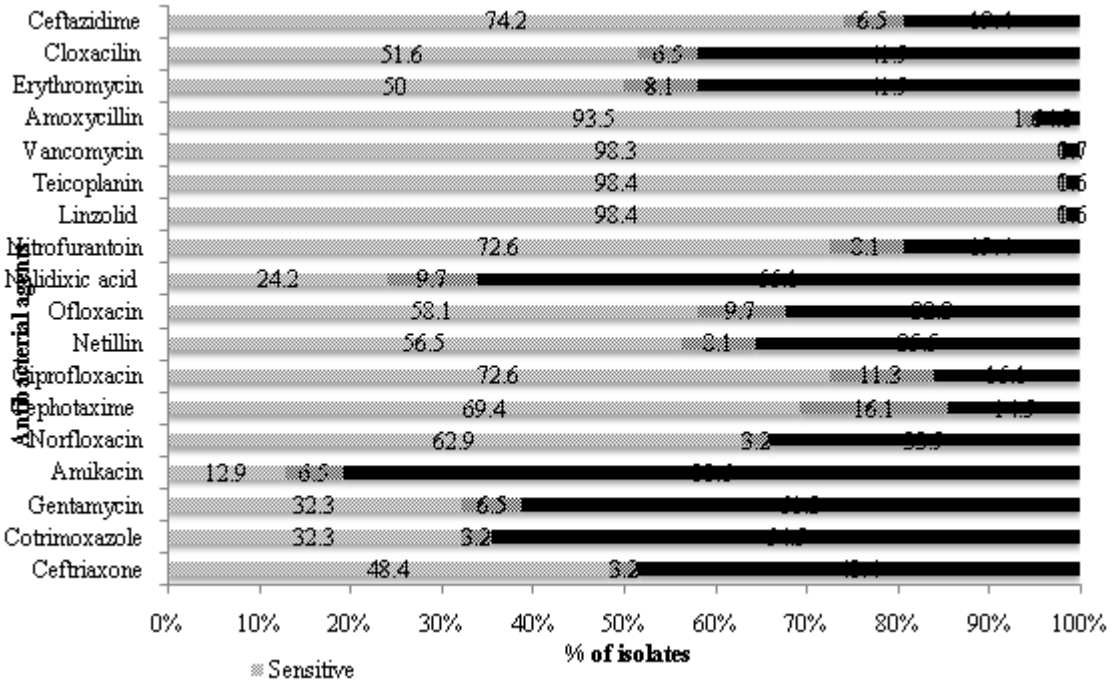

Figure 1: Antibiotic susceptibility patterns of enterococcal isolates