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Antimicrobial susceptibility pattern of enterococcus species isolated from patients at holy family hospital, Rawalpindi

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Abstract
The present study was conducted to evaluate the antimicrobial susceptibility pattern of enterococcus species in urine, pus, Catheters, HVS and fluids isolated from patients at Holy Family Hospital Rawalpindi**.** All sample received in laboratory were processed. All urine samples were inoculated on CLED agar and the remaining (pus, Catheters, HVS and fluids) were inoculated on Blood agar and MacConkey agar. All isolates were then identified by conventional method by Gram stain, Catalase and Cephradinedisk. During 6 months study periods from march to august 2016, 100 Enterococcus positive cultures and sensitivity were analysed. 70% isolates were from females and 30% from males. The highest sensitivity, 100% for (Vancomycin, Teicoplanin) and 92% for Nitrofurantion was observed in the present study and the resistance pattern of isolates showed highest resistance 100% to Cephradine.

Keywords: enterococcus species, susceptibility pattern, holy family hospital

1. Introduction
Enterococci have emerged over the last decade as one of the most important nosocomial pathogen worldwide, responsible for increasing number of episodes of bacteremia, endocarditis, meningitis, urinary tract infection and soft tissue infection. 19 species within genus are recognized [1] The *Enterococcus* isolated from human infection may include *E. faecalis, E. faecium, E. casseliflavus, E. durans, E. gallinarum and E. hirae*. Among the *Enterococcus i*solated, *E. faecalis* account for 90% and *E. faecium* account for 10% [2].
Enterococci were originally classified as enteric gram-positive cocci and later included in the genus *Streptococcus*. In the 1930s, with the establishment of the Lancefield serological typing system, *Enterococci* were classified as group D streptococci and were differentiated from the *non-Enterococci group D streptococci* such as *Streptococcus bovis* by distinctive biochemical characteristics [3].
Enterococci have been documented to be the third most prevalent pathogens in nosocomial bloodstream infections (BSIs) in the United States and are associated with 5%–15% of cases of bacterial endocarditis [4].
Enterococci have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. The emergence of high level resistance to aminoglycosides (HLAR), β lactam antibiotics and to vancomycin by some strains, together with multi drug resistance led to failure of synergistic effects of combination therapy [5].
Enterococci tolerate a wide variety of growth conditions, including temperatures of 10 °C to 45 °C, hypotonic, hypertonic, acidic, or alkaline environments Sodium azide and concentrated bile salts which inhibit or kill most microorganisms, are tolerated by enterococci and used selective agent in agar-based media. As facultative organisms, enterococci grow under reduced or oxygenated conditions. Enterococci are usually considered as strict fermenters because they lack Kreb's cycle and respiratory chain [6].
Although enterococci have been considered of relatively low virulence, these organisms can cause serious infections, including endocarditis. In addition, urinary tract infections are commonly caused by enterococci, particularly among hospitalized patients.

Although enterococci have generally been regarded as having limited virulence, their intrinsic resistance to antibiotics and the ease with which they adapt to their environment and acquire resistance to antibiotics provide them with distinct survival advantages over other more susceptible bacterial pathogens [7]. The increasing importance of these bacteria is largely due to their resistance to many antimicrobial agents, including Beta-lactam antibiotics, glycopeptides, and amino glycosides [8]. Enterococci have been implicated in approximately 10% of all UTIs and in up to approximately 16% of nosocomial UTIs. Enterococcus is a common constituent of gastrointestinal tract which can colonize other areas [9].

The present study was conducted to evaluate the antimicrobial susceptibility pattern of enterococcus species in urine, pus, Catheters, HVS and fluids isolated from patients at Holy Family Hospital Rawalpindi

2. Materials and Methods

The present study was carried out over a period of 6 months from March to august 2016, in the microbiology section of the Holy Family Hospital Rawalpindi Pakistan. The aim of the study was to determine the current resistance and sensitivity of enterococcus species in outdoor patients (OPD) as well as indoor patients from different wards of the hospital. Clinical samples *viz*. urine, pus, Catheters, HVS and fluids were collected aseptically, from patients of Holy Family Hospital.

One hundred Enterococcus positive cultures were identified. Out of these urine sample were (n=59) Catheters (n=17) Pus (n=13) HVS (n=08) Drain Fluids (n=03). All samples were streaked on pre-incubated MacConkey' sagar (CM7-OXOID) and blood agar(CM55 and SR50- OXOID) plates and urine on CLED agar(CM301-OXOID) plates within 5 hours of sample collection and were kept under incubation at 37 °C for 48 hrs. Colonies appeared were further confirmed by colony morphology on MacConkey's agar, Blood agar, CLED, Gram staining, Catalase test and Cephradine for differentiation from Streptococcus species. Each enterococcal isolates were tested by using Muller Hinton agar (MHA) by Kirby-Bauer disc diffusion method as per CLSI guidelines. The following antibiotics were tested.

Vancomycin (30µg), Ciprofloxacin (30µg), Gentamicin (10µg), Tazocin (40µg), Teicoplanin (30µg), Cephradine (30µg), Imipenem (10µg) Nitrofurantoin (30µ) Augmentin (30µg). Further incubated at 37 °C for 24 hours and examined for zone of inhibition. Zone was measured and results were interpreted as sensitive, intermediate and resistant according to the recommendations of CLSI zone sizes.

2.1 Procedure of gram staining

The principal stain used for microscopic examination of bacteria.

2.2 Reagents used

Crystal violet, Lugolsiodine, Acetone (decolorizer), Safranin or methylene blue (counter stain)

2.3 Procedure

1. The smear was fixed to the slide to preserve & kill the bacteria and to avoid washing away during staining steps.
2. The slide passed 3 times through the flame of Bunsen burner and the slide was allowed to cool.
3. The smear was flooded with crystal violet for 1 min.
4. The smear was flooded with Gram's iodine for 30-60 sec. Iodine acts as a mordant; chemically bound the crystal violet to the bacterial cell wall.
5. Smear was rinsed with water to remove excess stain.
6. The smear was dripped with decolorizer (acetone) across the slide for few sec (thicker smears required more prolonged decolorization)
7. The smear was rinsed with water to remove excess decolorizer.
8. The slide was flooded with Safranin solution.
9. The slide was allowed to stand for 20-30 sec.
10. The slide was rinsed with water to remove excess stain.
11. Slide was Blot dried.
12. Slide was examined microscopically at 100X using oil immersion [10].

2.4 Interpretation

Gram-positive bacteria	-	violet
Yeast cell	-	violet
Gram-negative bacteria	-	pink or red
Neutrophils	-	red
Epithelial cells	-	pale red (Cheesbrough, 2005).

2.5 Catalase Test

Catalase test is commonly used to differentiate Streptococci (catalase negative) for Staphylococci (catalase positive)"

The Catalase enzyme breaks down the Hydrogen peroxide into H_2O and O_2 most aerobic and facultative bacteria are catalase-positive, with the exception of Streptococcus**.**

2.6 Procedure

- 1 to 2 drops of hydrogen peroxide solution were poured onto a glass slide.
- Using a wire loop colonies of the test organism were removed and immersed in hydrogen peroxide solution.
- Immediate bubbling was checked.

2.7 Interpretation

- Positive: Rapid appearance of gas bubbles
- Negative: No bubbles

2.8 Controls

- **Positive Control:** Staphylococcus species
- **Negative Control:** Streptococcus species (Cheesbrough, 2005).

2.9 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed by Kirby-Bauer modified disc diffusion method.

The bacterial colonies were streaked onto Muller-Hinton agar (Oxiod) plates with the help of sterilized wire loop. The antibiotic discs were dispensed on the surface of the media at different positions and the plates were incubated aerobically at 37 C for 24 hours. Grades of sensitivity recognised are sensitive, intermediate and resistant by comparison of zone of inhibition as indicated as defined by CLSI.

3. Results

The present study was conducted to find out the current resistance pattern of Enterococci in admitted as well as outdoor patients. A total of 100 Enterococcus positive samples were isolated from various clinical samples during the six months study period.

Among these 100 patients 30% were male and the remaining 70% were female. (Table 1, Fig 1)

Among these 37% isolates were from OPD and 63% were

from different Wards of hospital. (Table 2, Fig 2)

The major source of Enterococcus strains isolated was urine (59%), followed by Catheter (17%), pus (13%), HVS (8%) and Drain Fluid (3%). Table 3, Fig 3)

The patients were divided into different age groups which includes the patient ages from 10- 20 years (8%), from 21-30 years (31%), from 31-40 years (22%). from 41- 50 years (11%), from 51-60years (18%) and more than 60 years (10%). (Table 4, Fig 4)

Of these isolates 37% were from OPD, 16% were from Obstetrics, 24% were from Medicine, 11% were from ICU and 12% from Surgery. (Table 5, Fig 5)

The antibiotic sensitivity pattern shows that 0%, 20.8%, 20.9%, 24.3%, 46.3%, 46. 4%, 47. 9%, and 49% sensitivity pattern was shown by. Cephradine, Ciprofloxacin, Tetracyclin, Gentamicin, Augmentin, Ampicillin, Imipenem and Tazocin respectively. While the highest sensitivity, 100% for (Vancomycin, Teicoplanin) and 92% for Nitrofurantion was observed in ours work. (Table 6, Fig 6).

The resistance pattern of isolates showed highest resistance 100%, 79.2%, 79.1%, 75.7%, 53.7%, 53.6%, 52.1% and51% resistance to Cephradine, Ciprofloxacin, Tetracyclin, Gentamicin, Augmentin, Ampicillin, Imipenem and Tazocin respectively while contrary to these the minimum resistance was 0% (Vancomycine, Teicoplanin) and 08% (Nitrofurantion). (Table 6, Fig6).

Table 1: Distribution of patients according to t gender.

Gender	Number	Percentage
Male	30	30%
Female	70	70%
Total	100	100%

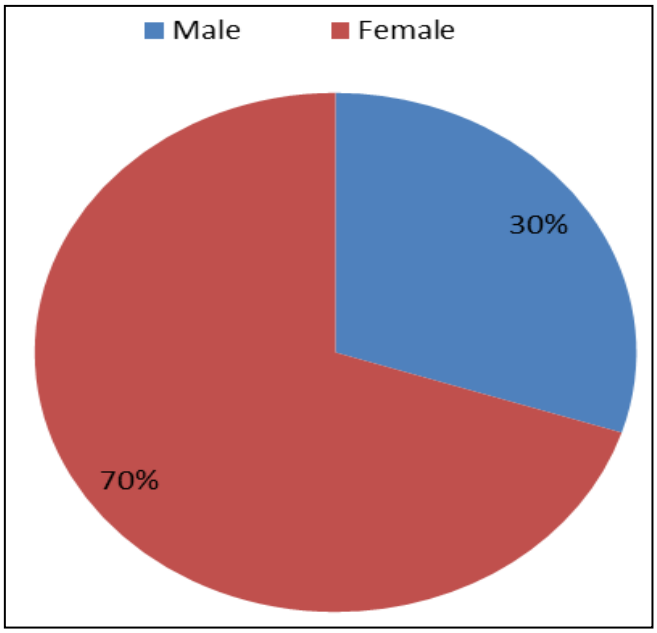


Fig 1: male and female wise distribution

Table 2: Location–wise distribution

Location	Total	Percentage
OPD	37	37%
Wards	63	63%

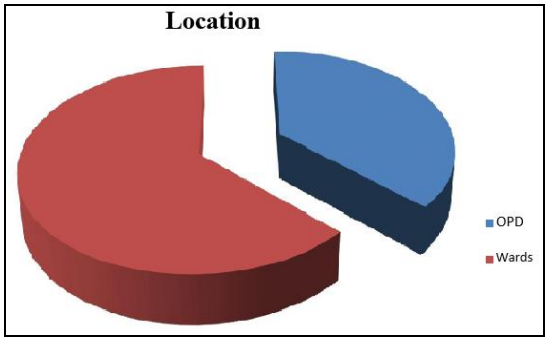


Fig 2: location wise distribution

Table 3: Showing sample distribution

Sample	Male/%	Female /%	Total /%
Urine	15/25.4	44/74.5	59/59%
Catheter tip	7/41.1	10/58.8	17/17%
Pus	6/46.1	53.8	13/13%
HVS	0/0	13/100	8/8%
Fluid	2/66.6	1/33.3	3/3%

Table 4: Age- wise distribution of Enterococcus isolates

Age group(years)	No. of isolates	Percentage
10-20	8	8%
21-30	31	31%
31-40	22	22%
41-50	11	11%
51-60	18	18%
More than 60	10	10%

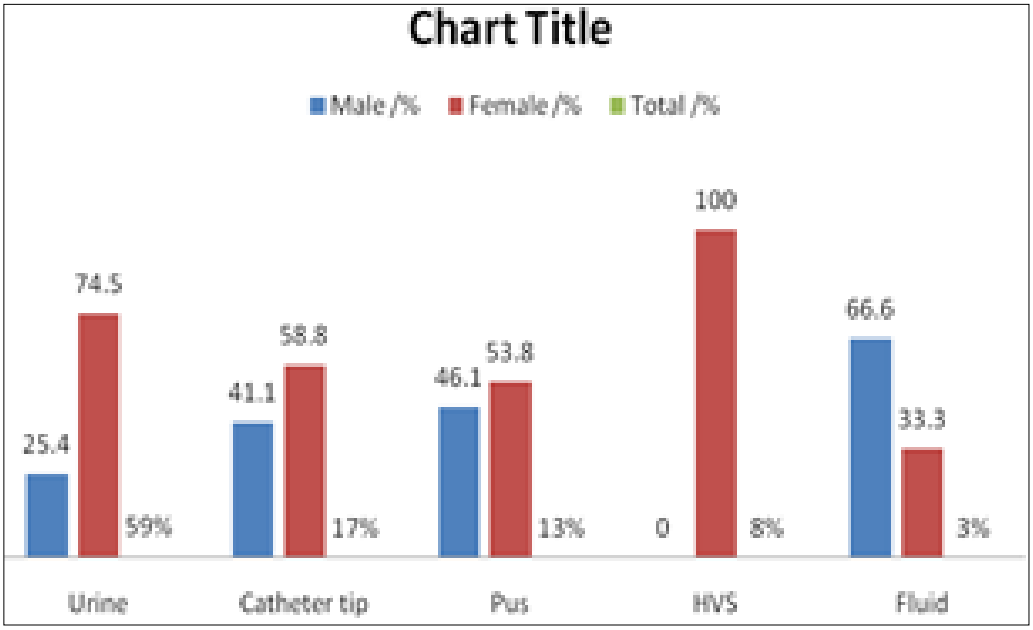


Fig 3

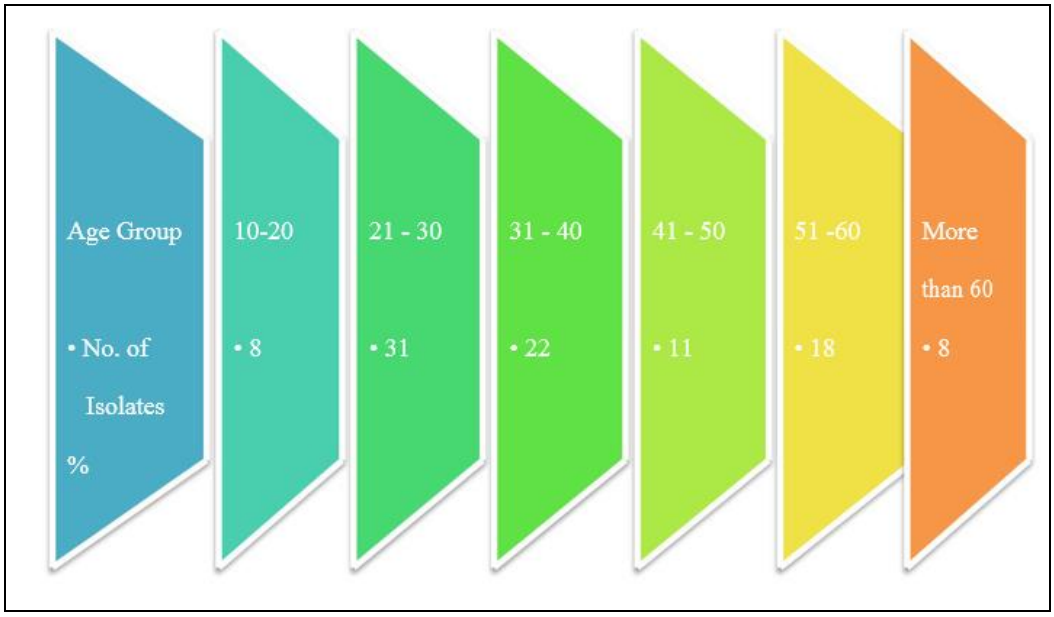


Fig 4

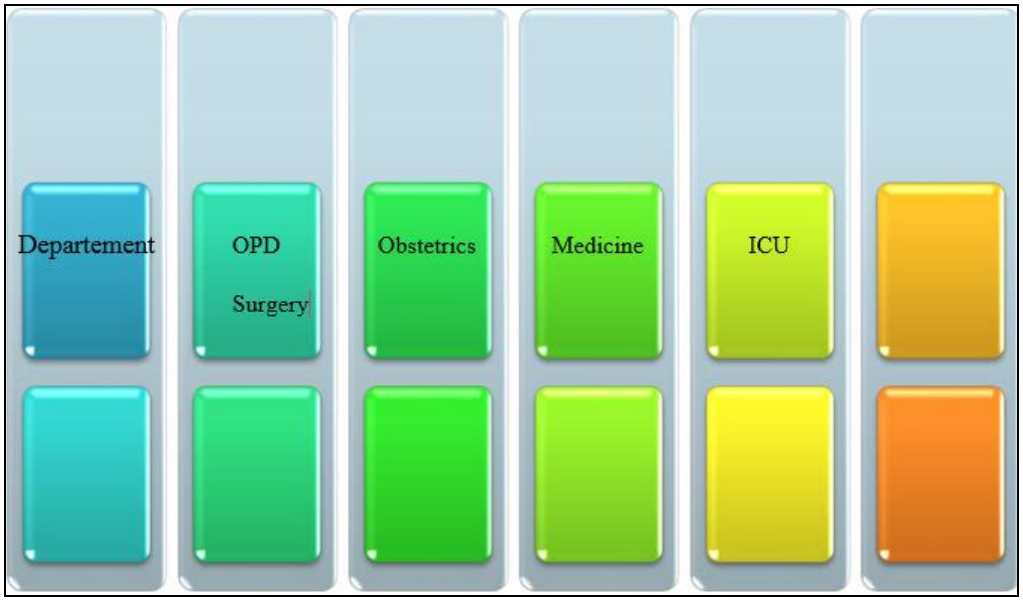


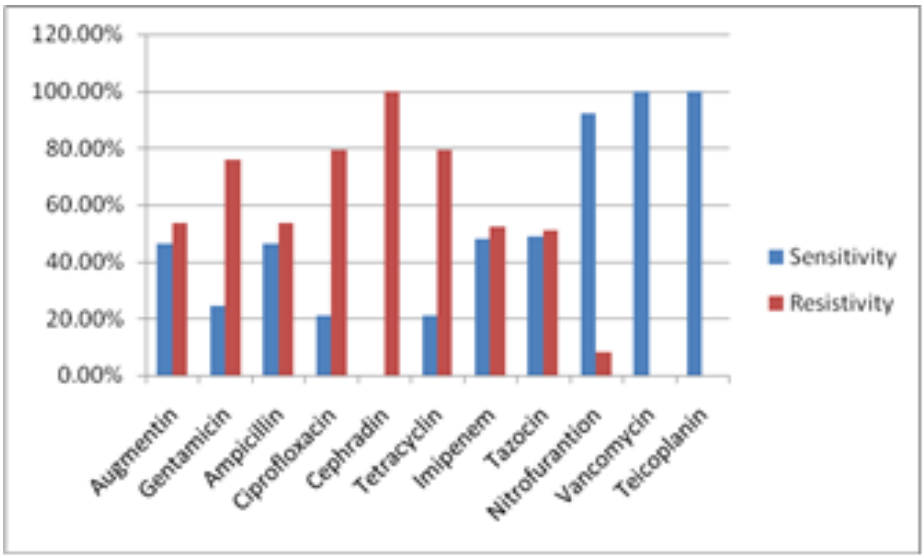
Fig 5

Table 5: Distribution of Enterococcus isolates in various departments

Department	No. of isolates	Percentage
OPD	37	37%
Obstetrics	16	16%
Medicine	24	24%
ICU	11	11%
Surgery	12	12%

Table 6: shows the sensitivity pattern of Enterococcus against different antibiotics

S/no	Antibiotic type	% sensitivity	% resistivity
1	Augmentin	46.3%	53.7%
2	Gentamicin	24.3%	75.7%
3	Ampicillin	46.4%	53.6%
4	Ciprofloxacin	20.8%	79.2%
5	Cephradin	00%	100%
6	Tetracyclin	20.9%	79.1%
7	Imipenem	47.9%	52.1%
8	Tazocin	49%	51.0%
9	Nitrofurantion	92%	8.0%
10	Vancomycin	100%	0%
11	Teicoplanin	100%	0%



4. Discussion

Despite the fact that enterococci have been considered to be relatively low virulent, in the past few years these organisms, among all nosocomial pathogens, have emerged as a significant concern. Data indicates that incidence of nosocomial enterococcal infections have been increasing. According to recent surveys, *Enterococci* remain in the top 3 most common pathogens that cause nosocomial infections [11].

In this study we observed a high percentage growth (59%) in urine samples. Among these high percentage (70%) were female patients similar to (Oluremi BB *et al* 2011) [12].

The urinary tract was the most common site of Infections, which often occurred after instrumentation of the patient's urinary tract.

Enterococcus with high level Gentamcin resistance were common (24.3%) similar to (Gordon S *et al* 1992) [13].

The problem of treatment and control of enterococcal infection is underscored by the high prevalence of nosocomial isolates and their ability to acquire resistance to the limited number of useful antimicrobial agents available in the treatment of enterococcal infections, [13].

In our study high numbers of Enterococci were isolated from urine sample followed by pus which is similar to our studies. [14-15].

The three types of resistance of most significance in the enterococci are high-level resistance to the aminoglycosides, ampicillin resistance caused by beta lactamase production, and glycopeptides resistance including vancomycin resistance. Conjugal transfer of VanA-type vancomycin resistance genes from enterococci to other Gram-positive bacteria has been accomplished *in vitro.*

The appearance of plasmid-mediated transferable resistance to major antibiotic classes emphasizes, once more, not only on the necessity for more discriminate use of new drugs but also for continuous efforts to find or design antimicrobial agents. Thus, we suggest intensified actions to promote more the rational use of antibiotics in health care settings, more surveillance studies in order to monitor changes in enterococcal resistance patterns and the adoption of measures to prevent the spreading of genetically related resistance isolates.

Identification of enterococcal isolates to the species level in the clinical microbiology laboratory is useful because it can help predict patterns of antimicrobial susceptibility, particularly to penicillins. In serious clinical diseases (e.g., bloodstream infections or meningitis), idetification to the species level and determination of high-level aminoglycoside resistance to gentamicin and streptomycin should be strongly encouraged because of the differences in antimicrobial susceptibilities between E. faecium and *E. faecalis* [12].

5. Conclusion

Present study concluded that mostly female patients were infected as compared to male i.e. 70% and mostly of these patients were from wards. Due to emergence of antibiotic resistance strain =s of bacteria, present study was also designed for antibiotic susceptibility of enterococcus species. Present study revealed that tazocin showed the highest sensitivity pattern to enterococcus species i.e. 49% as compared to other antibiotics. While 100% of resistivity was shown to cephradine by enteroccouus species. Present study concluded that antibiotic showing sensitive patterns such as Augmentin, Ampicillin, Imipenem and Tazocin shiuld be prescribed by physicians rather than other resistive antibiotics. For controlling of such increase in resistive strains of enterococcus awareness should be brought in people.

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