Original Research Article

Prevalence and Antibiotic Susceptibility Pattern of Enterococcus Spp. Isolated from Urine Samples

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Abstract: Enterococci are the most common cause of healthcare-associated urinary tract infections. Enterococcus spp. were isolated from each of Urine and differences for Enterococcus contamination in urine were determined. Because of its unimportant commensals, Enterococci have developed into diverse, deadly diseases due to the routinely prescribed antibiotics for the treatment of nosocomial UTIs are less effective, the developing antibiotic resistance is concerning. This study aimed to determine the prevalence and antibiotic susceptibility patterns of different enterococcal species isolated from urine samples and to observe the prevalence of microbes in urine samples among the different types of patients. The frequency of enterococci isolation from hospitalized patients' urinary tract has risen. This cross-sectional study was conducted from March 2021 to December 2021 at the Department of Microbiology, AMZ Hospital Dhaka, Bangladesh. A total of 439 urine samples were collected and 96 (21.87%) samples showed growth of different isolates, in growth isolates 29 (30.20%) samples were Enterococcus spp. In this study, the sample was sensitive against Linezolid (95.55%), Vancomycin (89.67%), Penicillin (68.98%), Gentamycin (68.96%), Imepenum (55.17%). From our study revealed that different strains of Enterococcus spp. are resistant to drugs at different rates.

Keywords: Enterococcus spp, E.Coli, Linezolid, Penicillin, UTI.

1. Introduction

Enterococci are part of the normal intestinal flora of humans and animals. They have been long recognized as important human pathogens and are becoming increasingly so. The genus Enterococcus includes more than 17 species, although only a few cause clinical infections in humans. Since the beginning of the antibiotic era, they have posed major therapeutic challenges, including the need for synergistic combinations of antibiotics to successfully treat enterococcal infective endocarditis (IE)[1]. Enterococcus spp. are facultative anaerobic organisms that can survive temperatures of 60℃ for short periods and grow in high salt concentrations. In the laboratory, enterococci are distinguished by their morphologic appearance on Gram stain and culture (gram-positive cocci that grow in chains) and their ability to hydrolyze esculin in the presence of bile, their growth in

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6.5% sodium chloride, their hydrolysis of pyrrolidonyl arylamidase and leucine amino peptidase, and their reaction with group D antiserum[2]. Before they were assigned their genus, they were classified as group D streptococci. Enterococcus faecalis and Enterococcus faecium are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. Other enterococcal species known to cause human infection include Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus, and Enterococcus mundtii. E.faecium is responsible for most vancomycin-resistant enterococci (VRE) infections[3].

The majority of enterococci infections are UTIs, which account for 10% of all UTIs and up to 16% of nosocomial UTIs. Enterococcus species have been linked to these diseases. High mortality rates and metastatic abscesses in numerous organs are usually linked to enterococci bacteremia. A significant contributor to endocarditis, enterococcus is thought to be responsible for about 20% of instances of native valve bacterial endocarditis and for about 6-7% of cases of prosthetic valve endocarditis. Due to restrictions on bactericidal antibiotic therapy for Enterococci infections, especially when brought on by vancomycin-resistant Enterococcus (VRE), endocarditis continues to be among the most challenging to treat Enterococcal infections. Concern over the role of the Enterococcus species in endodontic, implant, and medical device-associated infections is also on the rise[4,5].

Urine is a liquid by-product of metabolism in humans and many other animals. Urine flows from the kidneys through the ureters to the urinary bladder. Urination results in urine being excreted from the body through the urethra. The cellular metabolism generates many by-products that are rich in nitrogen and must be cleared from the bloodstream, such as urea, uric acid, and creatinine. These by-products are expelled from the body during urination, which is the primary method for excreting water-soluble chemicals from the body. A urinalysis can detect nitrogenous wastes in the mammalian body[6,7]. Urinary tract infections (UTIs) are frequently caused by enterococci, especially among hospital inpatients. Although enterococci isolation is uncommon in simple illnesses, it can happen more commonly in elderly men after urinary catheterization (21%)[8]. Due in large part to antibiotic resistance, the bacteria traditionally linked to urinary tract infections (UTI) are changing many of their characteristics. To reduce the occurrence of resistance and stop its spread, empiric treatment will evolve throughout the following few years. The underlying host variables that worsen UTI, such as age, diabetes, spinal cord damage, or catheterization, have an impact on the genesis of UTI as well. As a result, complicated UTIs have a more varied etiology, and hosts with underlying anatomical, metabolic, or immunologic diseases are susceptible to substantial disease from organisms that seldom cause illness in healthy patients[9].

2. Materials and Methods

Total of 439 (287 males and 152 female) study participants were included in this study. It was a cross-sectional study conducted from March 2021 to December 2021 at the Department of Microbiology, AMZ Hospital Dhaka, Bangladesh. All consecutive Enterococci infection suspected patients were included in the study**.** Samples were collected aseptically & carried them in a sterile container.

2.1 Isolation, Identification, Antibiotic Sensitivity and Biochemical Test

The collected samples were incubated overnight at 37℃ on Cystin Lactose Electrolytes Deficient agar (CLED, Oxide, Basingstoke, UK) using a calibrated wire loop (0.001 mL). Significant colony counts were defined as >105 CFU/mL. The culture plates that showed no development underwent a second 48-hour incubation. Utilizing common microbiological methods such as Gram staining, colony morphology, and biochemical testing (Oxoid), the pure isolated bacterial colonies were identified. Species identification was carried out using classical biochemical methods and Analytical Profile Index (API) identification strips, where required, using recommended guidelines[10]. Using the Kirby Bauer disc diffusion method and Müller-Hinton agar and blood agar (Oxoid), the CLSI 2018 recommendations for assessing antibiotic susceptibility were followed. Reference strains of S. aureus (ATCC 25923), E. coli (ATCC 25922), and P. aeruginosa (ATCC 27853) were employed for the quality control. The discs containing antibiotics were purchased from Oxoid. The following antibiotic discs and dosages (g) were used for both Gram-positive and Gram-negative bacteria[11]. Amoxicillin (30), azithromycin (15), ceftriaxone (30), ciprofloxacin (05), doxycycline (30), linezolid (30), vancomycin (30), ceftrazidime (30), contrimoxazole (25), impenum (10), levofloxacin (05), penicillin (10). The zone of inhibition of antibiotics was measured and interpreted according to the CLSI 2018 guidelines[11].

2.2 Data Analysis

Data were analyzed with the help of the software SPSS (Statistical Package for Social Sciences) version 23 and Microsoft Excel 2016. The results were expressed as mean ±SD (standard deviation), percentage.

3. Results

A total of 439 urine samples were collected from different sex and age groups among them 287 males and 152 females. Among the male patient's maximum case were from age group 30-45 (90), followed by age group 18-29 (83) and age group >45 (69). For female patient's maximum cases were form age group 30-45 (49) and followed by age group >45 (43).

Table 1 Age group distribution with gender

Age group	Male (n)	Female (n)
<18	45	23
18-29	83	37
30-45	90	49
>45	69	43
Total	**287**	**152**

From the tested samples, 343 (78.13%) samples showed no growth, and 96 (21.87%) samples showed growth of different isolates. Enterococcus spp was 29 (30.20%) from a total of 96 growth samples. The results of the gram stain show gram-positive cocci arranged in pairs or short chains (Figure 1).

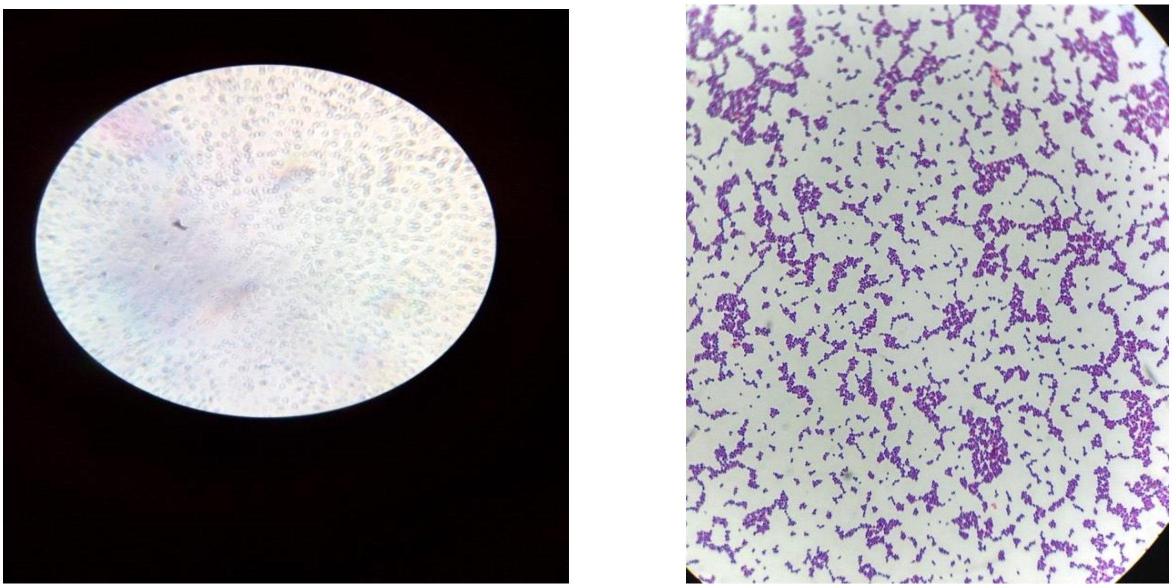

Figure 1 Gram Stain Results status

Table 2 results of biochemical test

SL NO.	Test Name	Result
1.	Catalase	Negative (-)
2.	Coagulase	Negative (-)
3.	Oxidase	Negative (-)
4.	Citrate	Negative (-)
5.	Urease	Negative (-)
6.	Indole	Negative (-)
7.	MR	Negative (-)
8.	VP	Positive (+)
9.	Gas	Negative (-)
10.	TSI	Negative (-)

Table 2 shows positive result only for the VP test but negative for other biochemical tests performed on the samples.

Table 3 Result of antibiotic sensitivity

SL NO.	Name of antibiotics	Concentration	Percentage of Sensitive Strains	Percentage of Resistant Strains
1.	Amoxicillin	30 μg	20.68	62.10
2.	Azithromycin	15 μg	34.48	65.52
3.	Ceftriaxone	30 μg	17.26	62.06
4.	Gentamycin	10 μg	68.96	13.80
5.	Ciprofloxacin	05 μg	48.27	41.39
6.	Doxycycline	30 μg	31.04	55.17
7.	Linezolid	30 μg	95.55	4.45
8.	Vancomycin	30 μg	89.67	6.89
9.	Ceftrazidime	30 μg	20.68	79.32
10	Cotrimoxazole	25 μg	44.84	34.48
11.	Imepenum	10 μg	55.17	31.03
12.	Levofloxacin	05 μg	62.06	31.05
13.	Penicillin	10 μg	68.98	20.68

R=Resistance *S=Sensitive* *I=Intermediate*

The high rate of resistance to ceftrazidime (79.32%), azithromycin (65.52%), and ceftriaxone (62.06%). Other antibiotics resistance is low (Table 3). The prevalence of Enterococcus spp in different clinical samples was calculated by using a single population proportion formula, a 50% prevalence.

4. Discussion

Urinary tract infection (UTI) is described as the health condition that has association between clinical signs and symptoms and detection of pathogenic microorganisms in the urine, bladder, urethra, kidney and prostate[12]. UTIs are the second most common bacterial infection affecting individuals of different ages worldwide[13]. Globally, an estimated 50% of women have UTIs at least once in their lifetime and UTIs are particularly more common in those aged 16–64 years[13,14]. Prevalence of UTIs is very low among boys but can be observed in the first year of life particularly in those with anatomical or functional abnormalities[15]. Antimicrobial resistance represents one of the main threats to healthy ecosystems. In recent years, among the multidrug-resistant microorganisms responsible for nosocomial infections, the Enterococcus species have received much attention.

Indeed, Enterococcus have peculiar skills in their ability to acquire resistance genes and to cause severe diseases, such as endocarditis[16]. Enterococci are members of the normal intestinal microflora in humans and animals, and they are common in environments affected by animal and human fecal material. These organisms are not considered primary pathogens, but due to their ability to acquire high-level resistance to antimicrobial agents, enterococci have emerged as nosocomial pathogens worldwide[17]. Some strains are resistant to many antibiotics, but antibiotic resistance alone cannot explain the virulence of enterococci[18]. The differentiation of apparently safe and non-safe enterococci strains is not simple, especially due to effective horizontal gene transfer mechanisms[19].

A total of 439 urine samples were collected from different sex and age groups. From the tested samples, 343 (78.13%) samples showed no growth, and 96 (21.87%) samples showed growth of different isolates. Enterococcus spp was 29 (30.20%) from a total of 96 samples. These 96 samples were used for this project. 96 pure colonies were isolated and these pure colonies were used for this study analysis. When Biochemical tests were performed for identify the characteristics of these 96 isolates, the isolates were observed and most of them were given similar results to Enterococcus spp. Some isolates were given different results. When Gram stains were performed the gram-positive cocci were isolated under the microscope. Some were given-gram negative results because some species of Enterococcus were gram negative. The species didn't confirm that this study was identified. Because PCR and other confirmation tests were not performed. The antibiotic sensitivity test results were given sensitive and resistant results. In this study, the sample was sensitive against Linezolid 95.55%, Vancomycin 89.67%, Penicillin 68.98%, Gentamycin 68.96%, and Imepenum 55.17%. Others were given mixed results like Ciprofloxacin 48.27%, Cotrimoxazole 44.84%, and Azithromycin 34.48% sensitive result. The zones of inhibition were measured by millimeter-scale horizontally and vertically.

In a Brazilian study carried out in 2009, only 38% were susceptible to ciprofloxacin[20]. Our study also revealed results with quinolones as only 42% of total enterococcal isolates were susceptible to ciprofloxacin. One study in Pakistan they found the tested specimens, 290 (36.1%) had significant bacterial growth and 147 (50.7%) of the strains were isolated from female patients. The frequently identified isolates were Escherichia coli (68.9%), followed by Klebsiella pneumoniae (8.9%) and Staphylococcus aureus (6.7%). The highest percentages of resistance have been observed against tested antibiotics. The majority of the isolates were extended spectrum β-lactamase producers (85.2%) and multidrug-resistant (98.3%). They observed that Gram-negative bacteria were the main cause of UTIs where the predominant microorganism was E. coli[21]. One study in Italy, a total of 3236 isolates of Enterococcus faecalis (82.2%) and Enterococcus faecium (17.8%) were collected from urine cultures, blood cultures, catheters, respiratory tract, and other samples. E. faecium showed a high resistance rate against ampicillin (84.5%), ampicillin/sulbactam (82.7%), and imipenem (86.7%), while E. faecalis showed the highest resistance rate against gentamicin and streptomycin high level, but both were highly sensitive to such antibiotics as tigecycline and vancomycin[22]. Infections commonly caused by Enterococci Include Urinary tract infection, Bacteremia, Intra-abdominal and pelvic infection, Skin, soft tissue and wound infection, and Occasionally endocarditis or meningitis[23].

A similar rising trend of enterococcal resistance to various antibiotics was noted in another Indian study by Shinde in a tertiary care hospital in Mumbai[24]. One study concluded that Enterococci have emerged from being harmless commensals to versatile lethal pathogens. The rising antibiotic resistance is worrisome as the commonly used antibiotics for the treatment of nosocomial UTIs are less effective. Injudicious usage of antibiotics must be curtailed and local antibiotic policies must be formulated.

5. Conclusion

In this study, the Enterococcus spp were isolated in urine samples. This organism was observed to be available in the human urinary tract. Enterococcus spp has become a problem of the world as an emerging nosocomial infection and multi-drug resistance bacteria. Among several species which belong to the genus

Enterococcus, E. faecalis is the most common isolate of human Enterococcal infection. The study showed that the rate of isolated Enterococcus spp has variable degrees of resistance to the antibiotics. The presence of a high percentage multidrug-resistant Enterococcus spp in our study should be considered as an alarm and further study on a large scale is needed.

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Conflicts of Interest

The author hereby declares that there are no conflicts of interest concerning this paper.

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