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# THE PREVALENCE, ISOLATION, AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ENTEROCOCCUS SPECIES FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

## *PREVALENSI, ISOLASI, DAN UJI KEPEKAAN ANTIMIKROBA PADA SPESIES ENTEROCOCCUS DARI BERBAGAI SAMPEL KLINIS DI RUMAH SAKIT PERAWATAN TERSIER*

**Original Research Article**
*Penelitian*

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**A B S T R A C T**

***Background:*** *Enterococcus is routinely considered a low-grade pathogen. The synergistic action of Enterococci with other bacteria increases the risk of infection. Enterococci are currently the next most common cause of healthcare-associated infections after E. coli. Greater understanding is needed regarding Enterococcus stress survival, virulence, and resistance patterns to assess the complexity of disease-causing Enterococcus.* ***Purpose:*** *Analyze the prevalence of Enterococcus and assess the antibiotic sensitivity pattern of Enterococcus.* ***Method:*** *A descriptive cross-sectional study was designed and carried out in the Department of Microbiology at Bangladesh University of Health Sciences, Dhaka, over a period of 3 months. Bacterial culture and sensitivity were the methods employed for microbiological examination.* ***Result:*** *A total of 558 bacterial strains were isolated, among which the growth of Enterococcus spp. was 27 (4.83%). The prevalence of Enterococcus spp. among different samples was 4.83%. The number of highly sensitive strains ranged from 66.66% to 77.77% for antibiotics, namely gentamycin, ampicillin, amoxicillin, and meropenem. Moderately high sensitivity to levofloxacin (29.62%) and low sensitivity to doxycycline (14.81%) were also analyzed.* ***Conclusion:*** *The study recommends that antibiotics should be used after proper laboratory procedures are undertake and it should be selected based on antimicrobial susceptibility tests.*

**A R T I C L E  I N F O**

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**A B S T R A K**

**Latar belakang:** *Enterococcus* merupakan patogen yang dianggap tingkat rendah. Tindakan sinergis *Enterococci* dengan bakteri lain dapat meningkatkan risiko infeksi. Saat ini, *Enterococci* merupakan penyebab paling umum dari infeksi di layanan kesehatan setelah *E. coli*. Pemahaman yang lebih luas mengenai kelangsungan hidup, virulensi, dan pola resistensi *Enterococcus* diperlukan untuk menilai kompleksitas penyakit yang disebabkan oleh *Enterococcus*. **Tujuan:** Menganalisis prevalensi dan menilai uji kepekaan antibiotik pada *Enterococcus*. Metode: Penelitian ini menggunakan metode deskriptif *cross-sectional* yang dilakukan di Departemen Mikrobiologi, Ilmu Kesehatan Universitas Bangladesh, Dhaka, selama jangka waktu 3 bulan. Pemeriksaan mikrobiologi dilakukan dengan metode kultur dan uji kepekaan bakteri. **Hasil:** Sebanyak 558 strain bakteri berhasil diisolasi dan menghasilkan pertumbuhan *Enterococcus* spp. sebesar 27 (4,83%). Prevalensi *Enterococcus* spp. pada sampel yang berbeda sebesar 4,83%. Jumlah strain yang sangat sensitif pada antibiotik gentamisin, ampisilin, amoksisilin, dan meropenem berkisar antara 66,66% sampai 77,77%. Selain itu, juga dianalisis sensitivitas pada levofloxacin (29,62%) yang memiliki sensitivitas cukup tinggi dan doksisiklin (14,81%) dengan sensitivitas rendah. **Kesimpulan:** Penelitian ini merekomendasikan penggunaan antibiotik dapat dilakukan setelah melakukan prosedur laboratorium yang tepat dan harus dipilih berdasarkan uji kepekaan antimikroba.

**Kata kunci:**
Spesies *Enterococcus* , Prevalensi, Uji kepekaan

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## INTRODUCTION

*Enterococci* are normal inhabitants of the gastrointestinal tract and biliary tracts of humans and animals (Huycke *et al*., 1998; Kwit *et al*., 2023). Sometimes, they are present in small numbers in the vagina and male urethra. The synergistic action of *Enterococci* with other bacteria increases infection (Afonina *et al*., 2018; Seputiene *et al*., 2012; Zaheer *et al*., 2020). Urinary tract infections are the most common periodic infections resulting from *Enterococcus* (Marino *et al*., 2021; Mishra *et al*., 2022). Post-surgery wound infections rank second. *Enterococcus* is routinely considered a low-grade pathogen. The capability of *Enterococcus* species to survive in a range of unfavorable environments permits numerous paths of cross-contamination of *Enterococci* in human disease (Zaheer *et al*., 2020), combining those from food, environmental (Ferguson *et al*., 2013; Kim *et al*., 2022; Monteiro *et al*. 2023), and hospital sources (Kwit *et al*., 2023; Ramos *et al*., 2020). The bacteria have come into view as an expanding and significant cause of nosocomial infection in recent decades (Hufnagel *et al*., 2004; Spengler *et al*., 2009.; Yadav *et al*., 2017). *Enterococci* are currently the next most familiar source for healthcare-associated infections after *E.coli* (Govindarajan *et al*., 2022; Billington *et al*. 2014; Marino *et al*., 2021; Esmail *et al*., 2019). They exhibit both intrinsic and acquired resistance to antibiotics (Hollenbeck and Rice, 2012; Yoshino, 2023). Acquired resistance in *Enterococci* occurs either through mutations in DNA or through the acquisition of new DNA (Coombs *et al*., 2014; Yadav *et al*., 2017). Resistance to a broad range of antibiotics enhances the emergence of *Enterococci* as a cause of nosocomial infection (Tuncay and Sancak, 2022). Greater understanding is needed regarding *Enterococcus* stress survival, virulence, and resistance patterns to assess the complexity of disease-causing *Enterococcus* (Kim *et al*., 2022; Miller *et al*., 2014). The virulence factors that increase pathogenicity not only appear in increasing numbers among various clinical isolates but are also associated with more severe clinical presentations (Seputiene *et al*., 2012).

Therefore, the involvement of certain *Enterococcus* traits in virulence is proven by a greater incidence in nosocomial isolates. Significant control of multiple drug-resistant *Enterococcus* requires better contact isolation in hospitals along with the patient care environment, sensible use of antibiotics, and continuous surveillance. Overall, this study aims to analyze the prevalence of *Enterococcus* and assess the antibiotic sensitivity pattern of *Enterococcus*. The expected impact of the study results from various factors influencing the prevalence of *Enterococcus* with corresponding recommendations.

## MATERIAL AND METHOD

A descriptive cross-sectional study was designed and carried out in the Department of Microbiology at Bangladesh University of Health Sciences, Dhaka, Bangladesh, for three months. The study population included patients attending both the *Outpatient Department* (OPD) and *Inpatient Department* (IPD) of a general hospital who consented to participate in the study.

This research was conducted on pus and urine samples. Pathogens in pus specimens are the causative agents of infectious diseases affecting the skin, liver, lungs, brain, eyes, and joint cavities. Urine samples can also help in the early detection of serious diseases such as kidney disease, diabetes, liver disease, and urinary tract infections. Bacterial culture and sensitivity was the microbiological examination method. The research instruments included analytical balance, autoclave, hot air oven, incubator, laminar airflow, wire loop etc. Data regarding age and gender were collected, and categorical and numerical data were summarized using numbers, frequencies, and percentages.

### Examination of specimen

Collection and transport of pus and urine: Special care was taken to avoid contaminating the specimen with commensal organisms from the skin before an antiseptic dressing was applied by using a sterile technique, up to 5 mL of pus from a drainage tube was collected and transferred to a leak-proof container.

The first midstream urine passed by the patient at the beginning of the day was collected for examination. The specimen was the most concentrated and therefore the most suitable for culture, microscopy, and biochemical analysis. A sterile, dry, wide-necked, leak-proof container was given to the patient to collect a 10 - 20 mL specimen. The container was labeled with the date, the patient's name and number, and the time of collection.

### Culture

On the first day, specimens were cultured on Blood and MacConkey agar immediately after collecting the sample and incubated for 18-24 hours at 37$^{o}$C. Gram staining was performed, and a routine examination of urine revealed a probable number of pus cells. On the second day, the media were examined for colony morphology. Catalase and coagulase tests were performed for growth on blood agar, with *Staphylococcus aureus* testing positive for both catalase and coagulase tested positive. Motility indole urease, triple sugar iron, Cimon citrate, and oxidase test were performed for growth on MacConkey. *E.coli* was identified as citrate-negative and motile. *Klebsiella* spp. was identified as citrate-positive and non-motile. *Pseudomonas* spp. was identified as oxidase test positive. Proteus was urease

and indole positive. *Acinetobacter* spp. was lactose-fermenting, catalase-positive, non-motile, oxidase-negative, and aerobic gram-negative coccobacilli characteristics. *Citrobacter* spp. was identified as catalase, citrate, H2S, motility positive, and indole-negative. *Enterococci*, growth on blood agar was found with circular colonies of 1-2 mm in diameter, and there was no growth on MacConkey. *Enterococci* are gram-positive bacteria stirring as pairs or short chains (Namikawa *et al*., 2017). A catalase test was performed. In the case of a catalase negative result, the bile esculin test, growth in 6.5% NaCl with trypticase soy broth and bacitracin sensitivity biochemical tests were performed. On the third day, the results of the biochemical tests were analyzed. *Enterococcus* was identified as gram-positive cocci in chains, catalase-negative, bile esculin-positive, and growth in trypticase soy broth with 6.5% NaCl (Mishra *et al*., 2022).

Antimicrobial susceptibility testing was carried out using modified *Kirby-Bauer* disc diffusion techniques, as recommended by the Clinical and Laboratory Standard Institute. The turbidity of the suspension was assessed in comparison with 0.5 McFarland standards. *Mueller-Hinton* agar plates were used for the antimicrobial susceptibility test. The antimicrobial-impregnated disks of ampicillin, amoxicillin, cefixime, ceftriaxone, ceftazidime, cefuroxime, gentamicin, doxycycline, levofloxacin, and meropenem were placed using sterile forceps, positioned away from each other to avoid overlapping zones of inhibition. Interpretation was performed according to the manufacturer's guidelines. The categorization of sensitivity or resistance to antibiotics was done based on a range of zones of inhibition. The specific range for each antibiotic was as follows: ampicillin (disk potency -10 microgram, susceptibility ≥17, resistant ≤13), amoxicillin (disk potency -20 microgram, susceptibility ≥18, resistant ≤13), cefixime (disk potency -5 microgram, susceptibility ≥19, resistant ≤15), ceftriaxone (disk potency -30 microgram, susceptibility ≥27, resistant ≤24), ceftazidime (disk potency -30 microgram, susceptibility ≥18, Resistant ≤14), cefuroxime (disk potency -30 microgram, susceptibility ≥18, resistant ≤14), gentamicin (disk potency -120 microgram, susceptibility ≥15, resistant ≤12), doxycycline (disk potency -30 microgram, susceptibility ≥16, resistant ≤12), levofloxacin (disk potency -5 microgram, susceptibility ≥19, resistant ≤15), and meropenem (disk potency -10 microgram, susceptibility ≥16, resistant ≤13).

## RESULT

Demographic data collected based on gender and age are shown in Tables 1 and 2. Among the participants, 10 (37%) were male, and 17 (63%) were female. The ages of the participants were categorized into three groups (0 - 20, 20 - 40, >40). The highest number of participants, both male and female, belonged to the >40 years group.

**Table 1**. Gender distribution of the participants

| Gender | Frequency | Percentage (%) |
|---|---|---|
| Female | 17 | 63 |
| Male | 10 | 37 |
| **Total** | **27** | **100** |

**Table 2**. Age groups of the participants

| Age | Female | Percentage (%) | Male | Percentage (%) |
|---|---|---|---|---|
| 0 - 20 | 1 | 5.9 | 1 | 10 |
| 20 - 40 | 2 | 11.76 | 0 | 0 |
| >40 | 14 | 82.35 | 9 | 90 |
| **Total** | **17** | **100** | **10** | **100** |

Table 3 demonstrates that a total of 558 bacterial strains were isolated, with the predominant isolate being *E. coli,* accounting for 196 (35.12%) of the total. This was followed by *Klebsiella* spp. at 132 (23.65%), *Staphylococcus aureus* at 70 (12.54%), *Pseudomonas* spp. at 62 (11.11%), *Proteus* spp. at 35 (6.27%), *Enterococcus* spp. at 27 (4.83%), *Citrobacter* at 25 (4.48%), and Acinetobacter spp. at 11 (1.97%).

**Table 3**. Pattern of bacteria isolated (n = 558)

| Bacteria | Number | Percentage (%) |
|---|---|---|
| *E. coli* | 196 | 35.12 |
| *Klebsiella* spp. | 132 | 23.65 |
| *Pseudomonas* spp. | 62 | 11.11 |
| *Enterococcus* spp. | 27 | 4.83 |
| *Staphylococcus aureus* | 70 | 12.54 |
| *Proteus* | 35 | 6.27 |
| *Acinetobacter* spp. | 11 | 1.97 |
| *Citrobacter* spp. | 25 | 4.48 |
| **Total** | **558** | **100** |

Table 4 demonstrates that out of 558 organisms, *Enterococcus* spp. was 27 (4.83%). Sixty percent of *Enterococcus* was isolated from the pus sample. The highest number of isolates was from pus with 16 (60%) similar to what Sreeja *et al.* (2012) reported.

**Table 4**. Number and percentage of *Enterococcus* spp. identified from different samples

| Sample | Number | Percentage (%) |
|---|---|---|
| Urine | 11 | 40 |
| Pus | 16 | 60 |
| **Total** | **27** | **100** |

The prevalence of *Enterococcus* spp. was calculated as (27/558)*100 = 4.83%. The antimicrobial sensitivity patterns of the isolates are shown in Table 5. The number of highly sensitive strains, ranging from 66.66% to 77.77% – namely gentamycin, ampicillin, amoxicillin, meropenem – was analyzed. Additionally, moderately high sensitivity to levofloxacin (29.62%) and low sensitivity to doxycycline (14.81%) were examined.

**Table 5**. Antimicrobial susceptibility pattern of different drugs (n =27)

| Antibiotic | Sensitive (number) | Percentage (%) |
|---|---|---|
| Ampicillin | 20 | 74.07 |
| Amoxicillin | 21 | 77.77 |
| Ceftriaxone | 0 | 0 |
| Cefixime | 0 | 0 |
| Cefuroxime | 0 | 0 |
| Ceftazidime | 0 | 0 |
| Doxycycline | 4 | 14.81 |
| Gentamycin | 18 | 66.66 |
| Levofloxacin | 8 | 29.62 |
| Meropenem | 21 | 77.77 |

## DISSCUSSION

A total of 558 bacterial strains were isolated, with *Enterococcus* spp. accounting for 27 (4.83%) of them. Most of the participants were in the >40 age group, with the predominant age range being 40 - 60 (Billington *et al*., 2014). The higher infection rate among the elderly (37.03% for males and 62.96% for females) was attributed to older individuals having increased exposure to the external environment, coupled with a history of receiving treatment from various healthcare facilities (Moghimbeigi *et al*., 2018). This history served as a source for transmitting the infection. Shifts in the frequency of *Enterococcus* populations can arise from mutable changes in environmental conditions over time as a result of antibiotic treatment that delineates individual selective settings in hospitals (Tedim *et al*., 2015; Horner *et al*., 2021). Moreover, immunity tends to decrease with age, facilitating the colonization of these bacteria (Hufnagel *et al*., 2004). In developing countries like Bangladesh, females usually receive treatment from government healthcare settings due to lower cost. This was the main reason for achieving the higher Enterococcal infection rate among females. However, anatomically, females are more prone to development.

The epidemiology of Enterococci is not implicit as prominent differences exist among species of resistant isolates originating from numerous geographic locations. The present study demonstrates that the prevalence of *Enterococcus* spp. among different samples was 4.83%, which surpasses the rates reported in India (2.3%), Ethiopia (3.5%), and the Asia-Pacific region (3.6%) (Ferede *et al*., 2018; Low *et al*., 2001; Paul et al., 2017). The percentages of isolates showing extreme sensitivity ranged from 66.66% to 77.77%, specifically to gentamycin, ampicillin, amoxicillin, and meropenem. Moderate high sensitivity to levofloxacin (29.62%) and low sensitivity to doxycycline (14.81%) were also investigated can be seen in Table 6.

**Table 6**. Percentage of antimicrobial agent sensitivity in different studies

| Antimicrobial Agent | Present study | Ferede *et al.* (2018) | Paul *et al.* (2017) | Low *et al.* (2001) |
|---|---|---|---|---|
| Ampicillin | 74.07% | 20% | 21.5% | 60% |
| Gentamycin | 66.66% | 40% | 55.8% | 60% |
| Doxycycline | 14.81% | 26.7% | - | 40% |

The comparison of antimicrobial susceptibility testing in this study with other previous studies is presented in Table 6. High-sensitivity ampicillin showed 74.07% susceptibility, similar to the research of Low *et al.* (2001), who reported 60% susceptibility in the Asia-Pacific region. On the other hand, the studies by Ferede *et al*. (2018) and Paul *et al*. (2017) analyzed 20% and 21.5% susceptibility, respectively. The susceptibility of gentamycin was 66.66%, showing similarity to the studies by Paul *et al*. (2017) and Low *et al*. (2001), where their susceptibility rates were 55.8% and 60%, respectively. Once again, the susceptibility of doxycycline was 14.81%, indicating low sensitivity. This did not align with the findings of Low *et al*. (2001) at 40% for the Asia-Pacific region but was similar to the research by Ferede *et al*. (2018) at 26.7%.

The findings of the present study recommend that antibiotics should be used after undertaking proper laboratory procedures, and should be selected based on antimicrobial susceptibility tests. Antibiotics should be prescribed depending on experience and adjusted or changed according to the susceptibility report. It should also be recalled that certain drugs should be reserved and used only in cases of treatment failure. Continuous surveillance of all other drugs should be performed on drug-resistant proportions (Sabouni *et al*., 2016, Siddig *et al*., 2022). Resistance against cefixime, ceftazidime, and ceftriaxone has increased (Ahmed et al., 2020 Siddig *et al*., 2022). Currently, the first-line drugs ampicillin, amoxicillin, and meropenem are convenient but should be given after a proper culture sensitivity test (Yoshino, 2023).

## CONCLUSION

The observations of the present study are very concerning for developing countries such as Bangladesh. The first limitation of this study is the small sample size. Second, the findings are not generalizable at all times due to the short research period. These limitations present a new platform for further research. In the future, research will try to determine *Enterococcus*'s stress survival pattern to assess the complexity of disease-causing *Enterococcus*. It was challenging to collect data from participants.

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