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Original Article

Antibiotic Resistance in *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* Causing Urinary Infections in Central Vietnam

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Abstract

Background: Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and are becoming increasingly complicated by the emergence of multidrug-resistant pathogens. *Enterococcus faecalis* and *Pseudomonas aeruginosa* are important pathogens of UTIs, particularly in hospitalized patients. Their rising resistance to multiple antibiotic classes poses a serious challenge to treatment and infection control, especially in low-income and middle-income countries, including Vietnam. Accordingly, this study aimed to determine the frequency of *E. faecalis* and *P. aeruginosa* in UTIs and assess their antibiotic-resistance status at Da Nang C Hospital, Central Vietnam, in 2022.

Methods: This retrospective cross-sectional study was conducted on 299 bacterial strains isolated from the urine specimens of patients diagnosed with UTIs at Da Nang C Hospital in 2022. These strains were identified through culture analysis, and their antibiotic susceptibility was assessed using the disk-diffusion method (Kirby-Bauer test).

Results: *E. faecalis* and *P. aeruginosa* were found in 10.03% and 8.7% of cultures, respectively. All isolates of *E. faecalis* were completely susceptible to linezolid, and most of them were susceptible to rifampin (93.8%) and vancomycin (86.2%). In addition, many of these isolates were susceptible to penicillin G, ampicillin, doxycycline, and chloramphenicol (67.9–77.8%). Resistance to ciprofloxacin, tetracycline, levofloxacin, and erythromycin was detected in 68–88.9% of isolates. Regarding *P. aeruginosa*, 53.8% to 66.7% of cultures were resistant to levofloxacin, gentamicin, ciprofloxacin, tobramycin, ofloxacin, and nitrofurantoin. However, *P. aeruginosa* was highly susceptible to piperacillin/tazobactam (87.5% of isolates), imipenem (75%), cefoperazone/sulbactam (72.7%), and cefepime and meropenem (64%). Eventually, multidrug resistance was observed in 56.7% and 34.6% of *E. faecalis* and *P. aeruginosa* isolates, respectively.

Conclusion: The bacteria isolated in this study exhibited resistance to many commonly used antibiotics at various levels. Our findings demonstrated the critical need for more stringent antibiotic stewardship guidelines and routine surveillance in order to enhance patient outcomes and prevent the spread of resistant bacteria.

Keywords: *Enterococcus faecalis*, *Pseudomonas aeruginosa*, Urinary tract infection, Antibiotic resistance

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Introduction

Urinary tract infections (UTIs) are prevalent among individuals of all ages worldwide. *Enterococcus faecalis* and *Pseudomonas aeruginosa* bacteria are common causes of these infections in Vietnam and other countries (1-3). Although UTIs have a lower mortality rate than other infections, they can lead to sepsis, thereby prolonging the duration of hospitalization and increasing treatment costs (4). Numerous studies have reported the severe consequences of infections caused by *E. faecalis* and *P. aeruginosa*, owing to their complex pathogenesis and the difficulty of selecting appropriate antibiotics for treatment (5,6).

Gram-positive *E. faecalis* is increasingly recognized as a major cause of hospital-acquired infections due to its intrinsic and acquired resistance mechanisms. According to evidence, its prevalence in UTIs ranges from 4.3% to 33.3% and from 2.7% to 6% in the north and south of Vietnam, respectively (7-12). High resistance rates have been reported, especially to tetracycline (81%–91.4%)

and quinolones, such as levofloxacin (50–71.4%) and ciprofloxacin (35.7%). In contrast, resistance to linezolid remains low (3.6%), and no resistance has been detected to vancomycin, nitrofurantoin, fosfomycin, or daptomycin (7,8,10,11). Similar concerns have been raised by statistics from other countries; in Iran, the highest rate of resistance is to cotrimoxazole, vancomycin, and amikacin, while the lowest rate has been reported against nitrofurantoin (8.3%). Contrarily, in Germany, *E. faecalis* has led to 16.1% of UTI cases in male outpatients, with rising resistance to ciprofloxacin (13,14). These results highlight the necessity of continuous monitoring to direct the proper use of antibiotics and prevent the emergence of multidrug-resistant (MDR) strains.

Pseudomonas aeruginosa, a ubiquitous gram-negative organism, is associated with severe UTIs and exhibits high-level resistance to multiple antibiotic classes. Reports from both high-income and low-to-middle-income countries have documented increasing prevalence and resistance of these organisms in UTIs. In Vietnam, the prevalence of *P. aeruginosa* ranges from 7.9% to 15%, with resistance exceeding 50–70% for most antibiotics, including complete resistance to ciprofloxacin, norfloxacin, and ticarcillin/clavulanic acid. Moreover, resistance to cefepime and amikacin has been 36.7% and 22.7%, respectively (12,15-18). Studies from India and Iraq have reported similar findings (prevalence rates of 6.3% and 14%), with *P. aeruginosa* identified as one of the most drug-resistant pathogens. Although there was high susceptibility to meropenem, imipenem, amikacin, and tobramycin, and resistance to cefixime, cefotaxime, and ceftriaxone persisted, the majority of isolates were MDR (19,20). Recent isolates of *P. aeruginosa* in UTIs in Korea have demonstrated a high carbapenem resistance rate of 46.7% (21).

These bacterial species are often resistant to most antibiotics, including newly developed broad-spectrum agents (22,23). The emergence of MDR bacterial infection has become a global concern, imposing a significant burden on clinical practice (24). Accordingly, accurate and rapid identification of the etiology of UTIs and their antibiotic-resistance patterns can facilitate more targeted antibiotic selection, enhance treatment efficiency, reduce economic burden, and help limit the spread of antibiotic resistance (25). However, a limited number of studies have focused on evaluating *E. faecalis* and *P. aeruginosa* in central Vietnam. Therefore, this study aims to determine the prevalence of these bacteria in UTIs at Da Nang C Hospital, Vietnam, and to investigate the extent of their antibiotic resistance. Understanding the patterns of antibiotic resistance in *P. aeruginosa* and *E. faecalis* may help clinicians more effectively treat individuals who require antibiotics.

Materials and Methods

Sample Collection

Overall, 299 cultures of bacteria isolated from the urine samples of patients diagnosed with UTIs at Da Nang C Hospital were collected from January 2022 to December 2022. Midstream urine samples were collected for culture within 2 hours.

Quantitative Culture of Bacteria in Urine

According to the Health Ministry of Vietnam (26), there are several diagnostic criteria for UTIs. (*a*) If the bacterial count is lower than 10 colonies per plate ($< 10^4$ CFU/mL), the result is reported as "no significant bacterial growth detected." (*b*) If the culture yields 10–100 colonies per plate (10^4–10^5 CFU/mL), further actions depend on clinical presentation. A repeat urine sample and quantitative culture are recommended for asymptomatic patients. On the other hand, identification and susceptibility testing should be performed in symptomatic patients with 1–2 bacterial species. In addition, the CFU count is reported with a recommendation for repeat urine collection and culture if symptoms are ambiguous and gram staining reveals low leukocyte and bacterial counts (*c*). Further, pathogen identification and antimicrobial susceptibility testing are required if bacterial growth exceeds 10^5 CFU/mL with 1–2 dominant species (*d*). Finally, the sample is considered contaminated if cultures show polymicrobial growth (>2 species) in cases 2 and 3, and a properly collected urine specimen is required accordingly.

Bacterial Identification in Infected Urine Samples

Bacterial identification was performed using samples that reached clinically significant levels, as detailed in the previous part. Colony morphology was examined following cultivation on blood agar and MacConkey agar (selective for gram-negative bacteria; Merck, Germany) at 37 ˚C after 24 hours. Gram-positive bacteria collected in blood agar and gram-negative bacteria collected in MacConkey agar were identified using the Vitek 2 GP ID card and Vitek 2 GN ID card, respectively.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the disk-diffusion method (Kirby-Bauer test). Antibiotic discs were placed on Mueller-Hinton agar, and the inhibition zone diameter was measured according to the Clinical Laboratory Standards Institute protocol (2018). Penicillin G, ampicillin, rifampin, ciprofloxacin, levofloxacin, erythromycin, nitrofurantoin, linezolid, vancomycin, chloramphenicol, doxycycline, and tetracycline were antibiotics tested for *E. faecalis*. Ticarcillin/clavulanic acid, piperacillin/tazobactam, cefoperazone/sulbactam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, netilmicin, tobramycin, ciprofloxacin, levofloxacin, ofloxacin, clindamycin, and nitrofurantoin were tested for *P. aeruginosa*. The evaluation of MDR bacteria followed the criteria established by Magiorakos et al (22), defining MDR as resistance to at least one antibiotic in each of at least three different antimicrobial classes.

Statistical Analyses

The obtained data were transferred and managed using WHONET microbiology laboratory database software, while data analysis and data processing were performed using Excel.

Results

Distribution of Bacteria Causing Urinary Tract Infections in Hospital Departments

Bacteria that cause UTIs were detected in all departments of Da Nang C Hospital. These bacteria were most frequently isolated from the General Internal Medicine Department (27.1% of isolates), followed by surgery (23.7%), nephrology and urology (23.1%), intensive care unit (ICU) and stroke center (17.4%), and other departments (8.7%).

Prevalence of Enterococcus faecalis and Pseudomonas aeruginosa Causing Urinary Infections

Among the 299 bacterial strains causing UTIs in our study, *Escherichia coli* was the most prevalent (57.2%), followed by *Klebsiella pneumoniae* (17.7%), *E. faecalis* (10.0%), *P. aeruginosa* (8.7%), *Proteus mirabilis* (3.3%), and *Acinetobacter baumannii* (2.3%), while *Staphylococcus aureus* (0.7%) was the least common bacterium (27). Although *E. coli* and *K. pneumoniae* are the most common pathogens isolated in UTIs, *E. faecalis* and *P. aeruginosa* present more clinical challenges due to their complex resistance mechanisms and association with nosocomial infections. These characteristics make them important targets for detailed investigation in antimicrobial resistance surveillance and infection control strategies.

Table 1 presents the distribution of bacteria isolated from the departments. *E. faecalis* was more frequently isolated from the Department of Nephrology and Urology, as well as the ICU and the Stroke Center. *P. aeruginosa* was most commonly obtained from the Department of Surgery, followed by the Department of General Internal Medicine and Urology.

Antibiotic Resistance of Enterococcus faecalis

Enterococcus faecalis exhibited high resistance rates (68%–88.9%) to ciprofloxacin, tetracycline, levofloxacin, and erythromycin (Figure 1). However, *E. faecalis* remained fully sensitive (100%) to linezolid. Importantly, 17 out of 30 *E. faecalis* isolates (56.7%) were classified as MDR.

Antibiotic Resistance of Pseudomonas aeruginosa

For *Pseudomonas aeruginosa*, resistance to tetracycline, levofloxacin, gentamicin, ciprofloxacin, tobramycin, ofloxacin, and nitrofurantoin was observed in 53.8–66.7% of the isolates (Figure 2). Notably, 9 out of 26 *P. aeruginosa* isolates (34.6%) were categorized as MDR.

Discussion

Prevalence of Enterococcus faecalis and Pseudomonas aeruginosa Causing Urinary Tract Infections at Da Nang C Hospital

Among 299 bacterial strains isolated from patients with UTIs across various departments, *E. faecalis* and *P. aeruginosa* were identified in 30 (10.03%) and 26 (8.69%) cases, respectively, which aligns with the results of a study performed in northern Vietnam (17), reporting an *Enterococcus* isolation rate of 10.57% but a higher *P. aeruginosa* rate (14.15%). In contrast, another study in Vietnam recorded lower isolation rates of *E. faecalis* (5%) and *P. aeruginosa* (8%) (28). In other countries, while *P. aeruginosa* may account for around 10–14% of UTIs in certain groups, *E. faecalis* prevalence appears to be more variable, occasionally lower than 10% in uncomplicated UTIs but higher in specific subgroups (29-33). While the reported prevalence varies, multiple studies confirm that *E. faecalis* and *P. aeruginosa* are significant causative agents of UTIs in hospitalized patients. The observed differences could be attributed to variations in study design, patient populations, clinical settings (community vs. hospital-acquired infections), and regional infection control practices.

In this study, *E. faecalis* and *P. aeruginosa* were isolated from patients in all departments. The rate of both bacterial species isolated from patients with UTIs at our ICU was higher than reported at hospitals in northern Vietnam (34). Higher detection rates for ICU patients likely reflect their severe clinical conditions and usually suppressed immune responses, thereby increasing susceptibility to illness (35-37). Urinary catheter placement in the surgery department is also a risk for hospital-acquired UTIs (38).

Antibiotic Resistance Among Enterococcus faecalis Isolates

Enterococcus faecalis is a gram-positive coccus, naturally resistant to several antibiotic classes, including cephalosporins and low-level aminoglycosides. The resistance of this bacterium to antibiotics significantly limits therapeutic options in clinical practice (39). In our study, *E. faecalis* isolates exhibited a high resistance rate (68%–88.9%) to ciprofloxacin, tetracycline, levofloxacin, and erythromycin. In contrast, the results revealed high susceptibility to linezolid, rifampin, and vancomycin,

Table 1. Distribution of *E. faecalis* and *P. aeruginosa* in Urine Samples Across Hospital Departments

Bacterium	General internal medicine	Endocrine urology	Surgery	Intensive care unit and stroke center	Other departments	Total
Enterococcus faecalis	5 (16.7)	8 (26.7)	6 (20)	8 (26.7)	3 (10)	30 (100)
Pseudomonas aeruginosa	5 (19.2)	5 (19.2)	12 (46.2)	2 (7.7)	2 (7.7)	26 (100)

Data are expressed as Number (%).

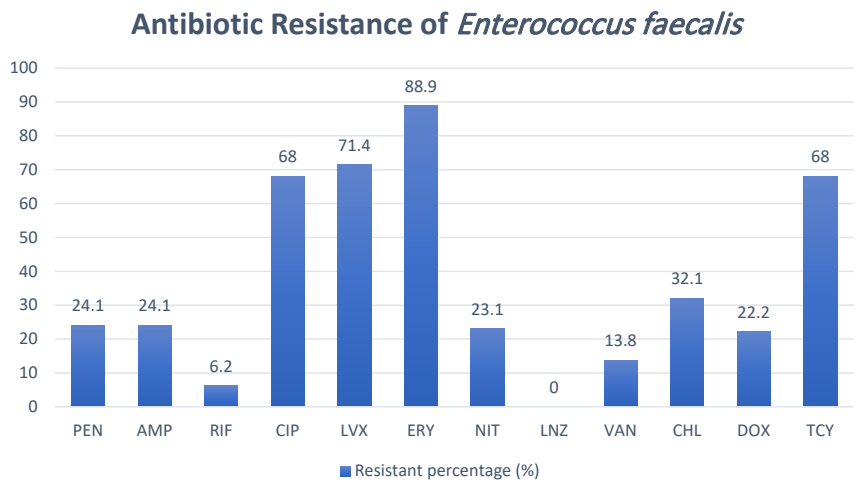

Figure 1. Antibiotic Resistance of *Enterococcus faecalis* Isolates. *Note*. The number in each column indicates the percentage of isolates resistant to each antibiotic. PEN: Penicillin G; AMP: Ampicillin; RIF: Rifampin; CIP: Ciprofloxacin; LVX: Levofloxacin; ERY: Erythromycin; NIT: Nitrofurantoin; LNZ: Linezolid; VAN: Vancomycin; CHL: Chloramphenicol; DOX: Doxycycline; TCY: Tetracycline

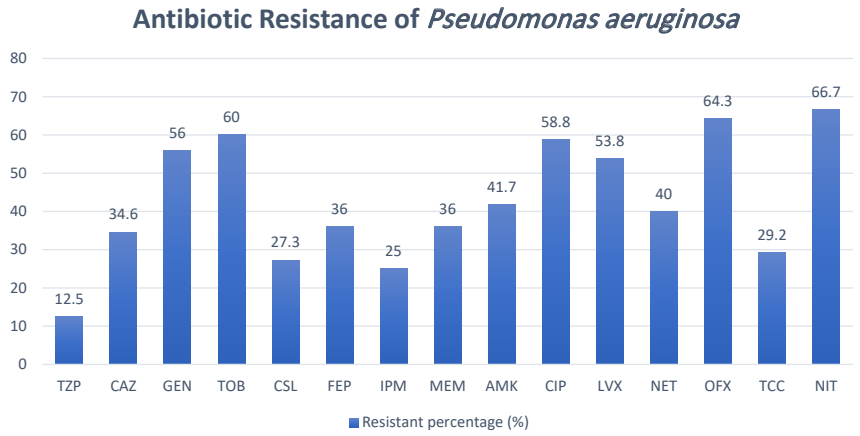

Figure 2. Antibiotic Resistance of *Pseudomonas aeruginosa* Isolates. *Note*. The number in each column represents the percentage of isolates resistant to each antibiotic. TCC: Ticarcillin/clavulanic acid; TZP: Piperacillin/tazobactam; CSL: Cefoperazone/sulbactam; CAZ: Ceftazidime; FEP: Cefepime; IPM: Imipenem; MEM: Meropenem; AMK: Amikacin; GEN: Gentamicin; NET: Netilmicin; TOB: Tobramycin; CIP: Ciprofloxacin; LVX: Levofloxacin; OFX: Ofloxacin; CLI: Clindamycin; NIT: Nitrofurantoin

but moderate susceptibility to penicillin G, ampicillin, doxycycline, and chloramphenicol. Our results conform to those of the study performed by Nguyen and Binh at a hospital in northern Vietnam, where *Enterococcus* spp. demonstrated > 50% resistance to macrolides, tetracyclines, and fluoroquinolones (28). Similarly, a study in the north-central region of Vietnam reported resistance rates of 73.5% to ciprofloxacin and levofloxacin (17). Compared to a study conducted in northern Vietnam, our study showed higher resistance to fluoroquinolones (ciprofloxacin: 68%, levofloxacin: 71.4%), potentially due to differing local prescription practices for outpatients, including many elderly patients who often visit C Hospital for medical examinations. Internationally, a higher rate of resistance to vancomycin was reported in Iran (14). A recent systematic review has revealed a gradual increase in antibiotic resistance by *E. faecalis* worldwide, including resistance to linezolid (40). However, both vancomycin and linezolid remained efficacious in our study and in Vietnam as a whole (7,8,10,11), suggesting that differences in clinical antibiotic-prescribing practices between countries influence selective pressure and patterns of antibiotic resistance.

Antibiotic Resistance Among Pseudomonas aeruginosa Isolates

Pseudomonas aeruginosa displayed varying levels of resistance to all antibiotics tested in our study. High resistance rates (> 50% of isolates) were observed against levofloxacin, gentamicin, ciprofloxacin, tobramycin, ofloxacin, and nitrofurantoin. These results are consistent with our previous findings (41). The resistance rates of *P. aeruginosa* to ceftazidime, cefepime, and piperacillin/tazobactam were higher than those reported in northern Vietnam (28). Contrarily, our study confirmed a lower frequency of resistance compared to a study in southern Vietnam (10), where resistance rates to ceftazidime, ciprofloxacin, cefepime, gentamicin, imipenem, meropenem, piperacillin/tazobactam, and nitrofurantoin exceeded 77%. While high susceptibility to meropenem,

imipenem, amikacin, and tobramycin was observed in India and Iraq (19, 20), our findings demonstrated relatively high resistance rates to these antibiotics (25%–60%), which is in line with the pattern reported in Korea (21). This discrepancy may be attributed to selective antibiotic pressure influenced by clinical prescribing practices, infection control, and microbial stewardship in individual countries. The aggressive use of empirical antibiotics in hospitals may drive the selection of more resistant P. aeruginosa strain.

Multidrug Resistance of Enterococcus faecalis and Pseudomonas aeruginosa

Out of 30 *E. faecalis* isolates, 17 (56.7%) were MDR (resistant to three or more antibiotic classes). The rate of MDR of *E. faecalis* in our study was higher than that in Romania (47.61%) (36) and lower than that in Egypt (74.6%) (42). Additionally, 34.6% of our *P. aeruginosa* isolates were identified as MDR. This MDR rate is lower than the frequencies of around 50% reported at a hospital in Pakistan in 2016 and 2019 (22,30), and much lower than the 77.2% reported in northern Vietnam from 2014 to 2021 (43). Overall, the frequency of MDR *P. aeruginosa* bacteria was lower compared to that reported in some national and international studies, implying that many isolates exhibited resistance to one or two drug classes, but were not concurrently resistant to ≥ 3 antibiotic classes as defined for MDR. The lower reported MDR rate may potentially be attributed to the preponderance of samples from non-critical patients in the surgery department rather than the ICU. Therefore, thorough profiling is necessary to precisely assess the clinical impact of resistance. In general, disparities in hospital-level infection control procedures, access to broad-spectrum antibiotics, study population, and antibiotic stewardship may all possibly contribute to the different MDR prevalence in the individual regions of both bacteria.

This study has provided updated data on the prevalence and antibiotic resistance of *E. faecalis* and *P. aeruginosa* in UTIs in Vietnam. Our results highlight multidrug resistance in *E. faecalis*, for which there is limited information in Vietnam. Our results further support the importance of regular surveillance and targeted antimicrobial stewardship to effectively manage infections caused by *E. faecalis* and *P. aeruginosa*. Nevertheless, this study was limited to a single hospital, which may not fully represent the prevalence and antibiotic resistance patterns of *E. faecalis* and *P. aeruginosa* in the region. Accordingly, it is recommended that further researchers perform multicenter studies with larger sample sizes to better represent regional trends. These should incorporate molecular techniques to identify resistance genes.

In this study, demographic information (including age and gender) was not collected, thereby limiting our ability to perform subgroup analyses. Nevertheless, our main goal was achieved, which was to concentrate on the microbiological traits and patterns of antibiotic resistance.

Conclusion

Our study highlights *E. faecalis* and *P. aeruginosa* as the significant causative agents of UTIs at Da Nang C Hospital, Central Vietnam. Both pathogens displayed high levels of antibiotic resistance, particularly to fluoroquinolones and β-lactam antibiotics. The detection of many MDR strains underscores the urgent need for continuous surveillance and effective antibiotic stewardship programs.

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Authors' Contribution

Conceptualization: Nguyen Thi Doan Trinh, Truong Thi Thu Suong, Na T. D. Tran.
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Visualization: Nguyen Thi Doan Trinh, Nguyen Huy Hoang, Hoang Thi Minh Hoa, and Na T. D. Tran.
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Writing–review & editing: Truong Thi Thu Suong, Nguyen Thi Doan Trinh, Nguyen Huy Hoang, Hoang Thi Minh Hoa, Na T. D. Tran.

Competing Interests

The authors declare that they have no conflict of interests.

Data Availability Statement

The data used in this study were obtained from the WHONET surveillance system at Da Nang C Hospital, Da Nang, Vietnam. Due to patient confidentiality, ethical restrictions, and institutional regulations, the dataset cannot be shared publicly. Thus, access to the data requires permission from Da Nang C Hospital and the Ministry of Health of Vietnam.

Ethical Approval

The main ideas and framework of this study were authorized by the Ethics Committees of the Da Nang C Hospital and Da Nang University of Medical Technology and Pharmacy, Vietnam (54/CT-HĐĐĐ). All patients or their parents provided written informed consent before starting the study. The personal information of all patients was kept confidential.

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