Antimicrobial resistance and virulence-associated genes of *Enterococcus faecalis* and *Enterococcus faecium* clinical isolates in Central Vietnam

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Abstract

Introduction: *Enterococcus faecalis* and *E. faecium* are prevalent pathogens in community and healthcare settings, often resistant to multiple antibiotics. This study aimed to assess the prevalence of virulence factors, drug resistance, and genetic determinants in clinical isolates in central Vietnam. **Materials & Methods:** 72 *Enterococcus* spp. isolates from patients at Hue Central Hospital and Hue University of Medicine and Pharmacy Hospital were analyzed. Bacteria identification was implemented by biochemical tests and PCR technique, and the antibiotic susceptibility testing was determined by using disk diffusion method. **Results:** Antibiotic resistance rates were as follows: erythromycin (50.8%), ciprofloxacin (50%), penicillin (42%), high-level gentamicin (34.7%), ampicillin (30.6%), tetracycline (28.5%), vancomycin (11.1%), and nitrofurantoin (7.1%). Fosfomycin showed 100% sensitivity. Multi-drug resistance was observed in 27.8% of *Enterococcus faecalis*/*E. faecium* isolates, with asa1 gene prevalence at 80.6% in *E. faecalis* and gelE at 74.2%, with hyl gene at 6.4%. 64.3% of *E. faecalis* strains carried both asa1 and gelE genes, primarily in pus and urine samples, notably high in MDR *E. faecalis* strains. **Conclusion:** This study highlights the prevalence of antibiotic resistance and virulence genes in clinical *Enterococcus* spp. strains, emphasizing the need for infection control and treatment strategies.

Keywords: *Enterococcus faecalis, Enterococcus faecium*, virulence genes, antibiotics resistance.

1. INTRODUCTION

Enterococcus spp. are Gram-positive cocci naturally occurring in the human and animal gastrointestinal tract, as well as in feces, food, soil, and wastewater [1], [2]. Previous studies suggested that enterococci played a minor role in disease causation. However, in recent years, *Enterococcus spp*. has garnered significant attention as a notable hospital-acquired pathogen. They have become one of the leading causes of healthcare-associated infections, with mortality rates in bloodstream infections reaching up to 50%. Infections primarily occur in hospitalized patients undergoing treatments such as pelvic and abdominal infections, urinary tract infections, wound infections, bloodstream infections, endocarditis, and meningitis [1]. Among these, *Enterococcus faecalis* and *Enterococcus faecium* are the main pathogens, contributing to a wide range of clinical [2]. Besides hospital-acquired infections, *Enterococcus spp*. is also responsible for 5-20% of cases of community-acquired endocarditis [1].

The incidence of infections caused by *Enterococcus spp.* is rapidly increasing due to their antibiotic resistance and virulence traits [3], [4]. Natural and acquired resistance characteristics associated with this bacterial genus allow enterococci to resist several antibiotic classes, including β-lactams, aminoglycosides, and glycopeptides, making the treatment of these infections challenging [1,2]. *E. faecium* exhibiting vancomycin resistance is classified by the World Health Organization (WHO) as a high-priority pathogen, necessitating the development of new antibacterial therapies. In Europe, the rate of antibiotic resistance among *Enterococcus spp.* ranks third after *Escherichia coli* and *Staphylococcus aureus* [1]. The mortality and economic burden posed by vancomycin-resistant enterococci (VRE) are significant, with over 54,500 hospitalizations, 5,400 deaths, and $539 million in healthcare costs annually in the United States alone [5]. In Vietnam, a study on antibiotic resistance among gram-positive bacterial pathogens causing urinary tract infections at the Huu nghi General Hospital in Nghe An by Que Tram Anh et al. 2022 found that *E. faecium* had the highest resistance rate (40.7%), exhibiting 100% resistance to several antibiotics including ampicillin, penicillin, ciprofloxacin, and levofloxacin. *E. faecalis* ranked second (33.0%), with a resistance rate of 63.3% to quinolones [6]. Due to this resistance, clinicians face

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challenges in selecting effective antibiotic regimens for hospitalized patients or those with healthcare-associated infections. To assist clinicians in selecting effective first-line antibiotics, it is essential to accurately assess the drug-resistance capabilities of bacterial pathogens isolated from patients.

Enterococcus spp. cause human infections through various virulence factors, including secreted and surface-expressed toxins. *E. faecalis* and *E. faecium* possess diverse virulence factors such as adhesive proteins like *asa1*, *cylA* (cytolysin), *gelE* (gelatinases), and *hyl* (hyaluronidase), which have been identified using molecular biology techniques in recent years. These virulence factors contribute to bacterial invasion, colonization, and infection in the host body [7]. However, in Vietnam, there are currently limited studies utilizing molecular techniques to identify and detect the virulence factors of these bacteria.

2. MATERIAL AND METHODS

2.1. Study design

Cross-sectional descriptive study and laboratory experimental study

2.2. Study location and period

Department of Microbiology, Hue University of Medicine and Pharmacy Hospital from August 2022 to August 2023.

2.3. Study subjects

72 *Enterococcus spp*. strains were isolated from clinical samples of patients treated at Hue Central Hospital and Hue University Medicine and Pharmacy Hospital.

2.4. Research methods

Isolation and identification of Enterococcus spp.

Clinical samples such as pus, urine, blood, and other body fluids will be cultured on suitable media according to the standard procedures of the laboratory. Identification belonging to *Enterococcus spp.* will be based on characteristics such as Gram staining and biochemical properties such as negative catalase test, positive Bile-Esculin test, and positive PYR test. *Enterococcus spp.* bacterial strains will be stored in BHI medium supplemented with 15% Glycerol at -80°C until further identification by PCR and other tests.

Enterococcus faecalis and Enterococcus faecium identification

Total DNA of *Enterococcus spp.* strains are extracted using the boiling method from the biomass of the culture in a nutrient agar medium [8]. *Enterococcus faecalis* and *Enterococcus faecium* are identified using multiplex PCR technique with specific primer pairs for *ddl* gene. The PCR reaction mixture includes 0.2µM of each primer, 12.5µl of 2× Master Mix-Tracking Dye, 1µL of total DNA, and nuclease-free water to make a total volume of 25µL. The thermal cycling conditions consist of an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 54°C for 1 minute, extension at 72°C for 1 minute, and a final extension step at 72°C for 10 minutes using Veriti PCR System (Applied Biosystems, USA). The PCR products are analyzed by electrophoresis on 1% agarose gel in 1×TAE buffer stained with GelRed™ and visualized using a 100bp DNA ladder [9].

Table 1. Primers for *E. faecalis and E. faecalis* identification [9]

Primer	Sequences (5'-3')	Gene	Product size (bp)
$ddl_{E.\ faecalis}$ *E1* - F	5' ATCAAGTACAGTTAGTCTT 3'	*ddl*	941 bp
$ddl_{E.\ faecalis}$ *E1* - R	5' ACGATTCAAAGCTAACTG 3'		
$ddl_{E.\ faecium}$ *E1* - F	5'-TTGAGGCAGACCAGATTGACG-3	*ddl*	658 bp
$ddl_{E.\ faecium}$ *E1* - R	5'-TATGACAGCGACTCCGATTCC-3'		

Antibiotic susceptibilities testing by the disk diffusion method:

Enterococcus spp. strains isolated were tested for sensitivity to nine antibiotics using the Kirby-Bauer disk diffusion method according to the laboratory's SOP, and the guidelines of the Vietnamese Ministry of Health [10,11]. The antibiotics used in the study included ampicillin (10µg), penicillin (10 units), high-level gentamicin (120µg), vancomycin (30µg), erythromycin (15µg), tetracycline (30µg), nitrofurantoin (300µg), ciprofloxacin (5µg), and fosfomycin (200µg). Fosfomycin was only tested with *E. faecalis* isolated from urine specimens. Erythromycin was not tested against strains isolated from urine specimens. Tetracycline, nitrofurantoin, ciprofloxacin, and fosfomycin were only tested

against uropathogenic strains. The results were interpreted for sensitivity and resistance according to the Clinical and Laboratory Standards Institute (CLSI) - M100 2020 edition [12].

Amplification of virulence genes of Enterococcus spp.

The presence of virulence genes such as *asa1, gelE,* and *hyl* in *E. faecalis* and *E. faecium* strains was determined using multiplex PCR with specific primers as listed in Table 2 . The PCR reaction mixture included 0.2µM of each primer, 12.5µl of 2× Master Mix-Tracking Dye, 2µL of total DNA, and nuclease-free water to make a total volume of 25µL. The thermal cycling conditions consisted of an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 1 minute, and a final extension step at 72°C for 10 minutes using Veriti PCR System (Applied Biosystems, USA) [13]. The PCR products were analyzed by electrophoresis on 1% agarose gel in 1×TAE buffer stained with GelRed™ and visualized using a 100bp DNA ladder.

Table 2. Primer sequences for amplifying the virulence genes *asa1, gelE,* and *hyl* of *E. faecalis* and *E. faecalis* [13]

Primer	Sequences (5'-3')	Gene	Product size (bp)
asa1-F	5' CACGCTATTACGAACTATGA 3'	*asa1*	375 bp
asa1-R	5' TAAGAAAGAACATCACCACGA 3'		
gelE-R	5' TATGACAATGCTTTTTGGGAT 3'	*gelE*	213 bp
gelE-R	5' AGATGCACCCGAAATAATATA 3'		
hyl-F	5' ACAGAAGAGCTGCAGGAAATG 3'	*hyl*	276 bp
hyl-R	5' GACTGACGTCCAAGTTTCCAA 3'		

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 17.0). Probability values (p) of < 0.05 were considered statistically significant.

3. RESULTS

3.1. Isolation and identification of *Enterococcus spp.*

From pus, urine, blood, and other body fluids, 72 strains of *Enterococcus spp.* were isolated and preliminarily identified based on their biochemical characteristics. *Enterococcus* isolates were sourced from a variety of samples, with pus samples comprising the majority at 56.9% of the total. Urine samples followed, contributing 19.4%, while blood samples constituted 5.6%. Other fluid samples made up 18.1% of the isolates. All strains were species-identified using a multiplex PCR technique with specific primer pairs for the *ddl* gene. The results showed that out of 72 strains, 31 strains had PCR products approximately 941bp in size, identified as *E. faecalis* (43.1%), and 41 strains had PCR products approximately 658bp in size, identified as *E. faecium* (56.9%) (Figure 1).

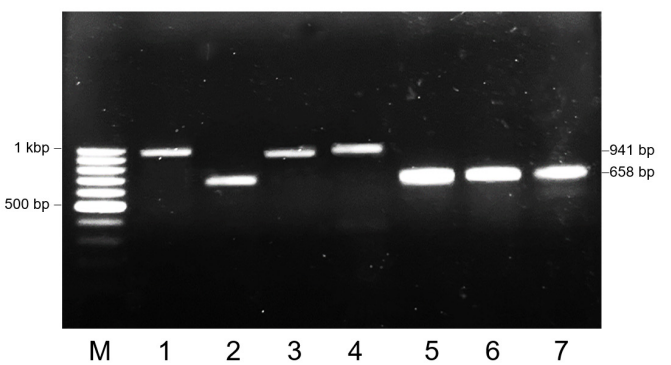

Figure 1. The results of agarose gel electrophoresis for species identification using *ddl* gene of *E. faecalis* and *ddl* gene of *E. faecium*.M: 100bp DNA ladder; 1: *E. faecalis* ATCC 29212; 2: *E. faecium* NEQAS; 3-7: Clinical strains isolated and identified based on biochemical characteristics as *Enterococcus spp*.

3.2. Antibiotic resistance rate of isolated *E. faecalis* and *E. faecium*

The prevalence of *Enterococcus* isolates resistant to erythromycin was 50.8%, to ciprofloxacin 50%, to penicillin 42.0%, to high-level gentamicin 34.7%, to ampicillin 30.6%, to tetracycline 28.5%, to vancomycin 11.1%, and nitrofurantoin 7.1%, while none were resistant to fosfomycin (Figure 2). Among these, *E. faecalis* showed resistance to ciprofloxacin at 50%, to tetracycline at 40%, to high-level gentamicin at 35.5%, to erythromycin at 28.5%, to penicillin at 12.9%, to vancomycin at 9.7%, to ampicillin at 6.5%, and none to fosfomycin and nitrofurantoin. For *E. faecium* strains, resistance rates were 62.1% to erythromycin, 53.7% to penicillin, 50% to ciprofloxacin, 50% to tetracycline, 48.8% to ampicillin, 34.1% to high-level gentamicin, 25.0% to nitrofurantoin, and 12.2% to vancomycin (Table 3).

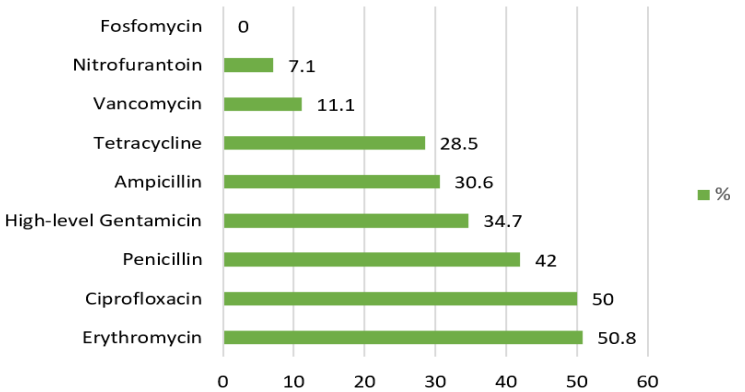

Figure 2. Distribution of antibiotic resistance among *Enterococcus spp.* strains in the study

Table 3. The resistance rate of *E. faecalis* and *E. faecium* to antibiotics

Antibiotic group	**Antibiotics**	**Resistance**			
		E. faecalis		***E. faecium***	
		n = 31	%	n = 41	%
β-Lactam	Ampicillin	2	6.5	20	48.8
	Penicillin	4	12.9	22	53.7
Aminoglycoside	High-level Gentamycin	11	35.5	14	34.1
Glycopeptid	Vancomycin	3	9.7	5	12.2
Macrolit	Erythromycin	6	28.5	23	62.1
Tetracycline	Tetracycline	4	40.0	2	50.0
Nitrofurantoin	Nitrofurantoin	0	0	1	25.0
Quinolon	Ciprofloxacin	5	50.0	2	50.0
	Fosfomycin	0	0	-	-

The antibiotic susceptibility results also revealed that out of 72 *Enterococcus* spp. strains isolated in the study, 20 strains (27.8%) showed multidrug resistance. Among them, 4 strains of *E. faecalis* and *E. faecium* each exhibited multidrug resistance.

3.3. Distribution of the virulence genes

All *Enterococcus spp.* strains were subjected to multiplex PCR to confirm the absence or presence of at least one of three virulence genes, with respective PCR product sizes of 375bp for *asl1*, 213bp for *gelE*, and 276bp for *hyl (Figure 3).* In our study, the highest proportion *of E. faecalis* carried the *asa1* gene (80.6%), followed by the *gelE* gene (70.1%), and the *hyl* gene had the lowest proportion (6.4%). Additionally, the proportion of *E. faecalis* carrying both *asa1* and *gelE* genes was high (64.3%), while those not carrying any gene accounted for 3.6%. For *E. faecium*, the highest proportion carried the *gelE* gene (53.7%), followed by the *asa1* gene (39.0%), and the *hyl* gene was found in 7.3% of strains. Moreover, the proportion of *E. faecium* carrying both *asa1* and *gelE* genes was 29.3%, while those not carrying any gene accounted for 31.7% (Table 3).

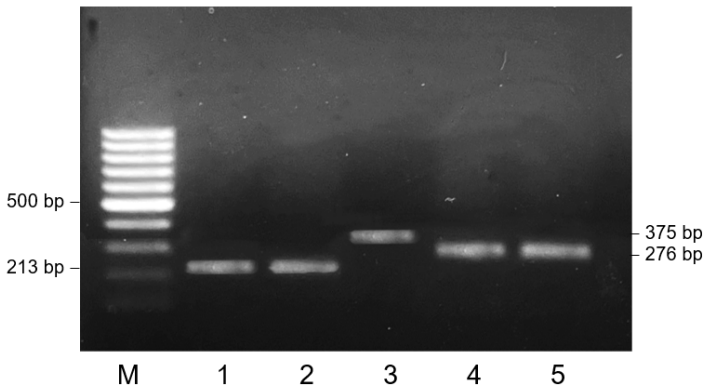

Figure 3. PCR product electrophoresis results for identifying the 3 genes *asa1 (375bp)*, *gelE (213bp)*, and *hyl (276bp)*. M: 100bp DNA ladder; 1, 3*: E. faecalis* ATCC 29212; 4: *E. faecium* NEQAS QC strain; 2, 5: isolated clinical strains.

Table 4. Distribution of *Enterococcus spp.* harboring virulence genes

Carrying virulence genes	*E. faecalis*		*E. faecium*	
	n = 31	%	n = 41	%
asa1	25	80.6	16	39.0
gelE	22	70.1	22	53.7
hyl	2	6.4	3	7.3
asa1, gelE	18	64.3	12	29.3
asa1, hyl	1	3.6	2	4.9
No carrying	1	3.6	13	31.7

There is a statistically significant correlation between ampicillin sensitive and the presence of the *asa1* and *gelE* genes, indicating that *Enterococcus* strains carrying both genes tend to be more susceptible to ampicillin compared to those lacking these genes (*p<0.05*). Similarly, there is a statistically significant correlation between penicillin sensitive and the presence of the *gelE* gene, suggesting that *Enterococcus* strains isolated carrying the *gelE* gene tend to be more susceptible to penicillin compared to those without this gene (p<0.05). Furthermore, there is a statistically significant correlation between gentamicin resistance and the presence of the *asa1* gene, indicating that Enterococcus strains isolated without the *asa1* gene tend to be more susceptible to gentamicin compared to those carrying the *asa1* gene (p<0.05) (Table 4).

Table 5. The correlation between virulence genes and antibiotic resistance rates of isolated *Enterococcus spp.* strains.

Genes \ AB		Ampicillin			Penicillin			Gentamicin			Vancomycin			Erythromycin		
		I/R	S	*p*	I/R	S	*p*	I/R	S	*p*	I/R	S	*p*	I/R	S	*p*
asa1	(+)	8	33	0.009	12	29	0.097	19	22	**0.038**	13	28	0.239	30	1	0.227
	(-)	15	16		15	16		7	24		6	25		26	1	
gelE	(+)	8	36	0.001	10	34	**0.001**	12	32	0.254	12	32	0.232	34	2	0.335
	(-)	15	13		17	11		13	15		6	22		22	0	
hyl	(+)	1	4	0.553	2	3	0.905	2	3	0.851	2	3	0.474	5	0	0.683
	(-)	22	45		25	42		24	43		17	50		52	1	

(AB: antibiotics, I: intermediate, R: resistant, S: sensitive)

4. DISCUSSIONS

This study illustrates the prevalence of *Enterococcus* isolates collected from various clinical specimens, with pus samples dominating at over 50% (56.9%), followed by urine samples (19.4%), blood samples (5.6%), and other fluid specimens (18.1%). Among these, *E. faecalis* and *E. faecium* were predominantly isolated from pus samples (20.8% and 36.1%, respectively), followed by urine samples (13.9% and 5.6%, respectively). Our findings align closely with those of Ayan Aden Moussa et al. (2019), who reported similar proportions of *Enterococcus* isolates from pus, blood, urine, and other specimens [14]. However, there are disparities compared to the study by Meiji Soe Aung et al. (2023), where urine samples constituted the majority (78.5%), followed by vaginal secretions (13.8%) and other specimen types (7.7%) [15]. These differences may stem from variations in sample collection times, different disease models across countries, and the patient population, with our study focusing mainly on patients treated in the Department of Surgery, hence the prevalence of pus samples. Additionally, urine cultures yielding *Enterococcus* demonstrate a relatively high proportion, ranking second after pus samples in our study, underscoring the bacteria's role in urinary tract infections. *Enterococcus* predominantly colonizes the gastrointestinal tract, with approximately 10^8 bacteria per gram of stool. These strains can adhere to urothelial tissue through surface proteins, leading to recurrent urinary tract infections. Moreover, urinary tract infections can recur through the perineal urethral route, particularly in immunocompromised patients [16].

According to our research, *E. faecium* was predominant in the study population, accounting for 56.9% of isolates, consistent with Thean Yen Tan et al. (2017), who found 3.7 times more *E. faecium* (n = 141) than *E. faecalis* (n = 38) [17]. Sara Ping et al. (2021) in Texas detected 55 *E. faecium* strains (63.2%) and 32 *E. faecalis* strains (36.8%) using PCR [18]. Similarly, Quế Anh Trâm (2022) in Nghệ An Province identified *E. faecium* as the most prevalent (40.7%), followed by *E. faecalis* at 33.0% [6]. Our findings align with global studies, including Wink Phyo Thu et al. (2019) in Thailand and Laos, with *Enterococcus* spp. prevalence at 53%, of which 66% were *E. faecium* and 34% *E. faecalis* in Thailand and 84.0% *E. faecium* and 16.3% *E. faecalis* in Laos. Félix Carrasco Calzada et al. (2023) in Uganda noted a higher prevalence of *E. faecium* infections (65.3%, n = 32) compared to *E. faecalis*, while most infections in a Spanish grade II hospital were *E. faecalis* (92.7%, n=51) [19].

The resistance rates of *E. faecalis* and *E. faecium* to erythromycin were 50.8%, ciprofloxacin 50%, penicillin 42.0%, and high-level gentamicin resistance 34.7%. Resistance rates to ampicillin were 30.6%, tetracycline 28.5%, vancomycin 11.1%, nitrofurantoin 7.1%, and fosfomycin 0%. Our results are consistent with Martin Georges et al. (2022), with Enterococcus sensitivity to nitrofurantoin, ampicillin, and gentamicin at 90%, 84.1%, and 63.6%, respectively, but lower sensitivity to tetracycline and erythromycin [13]. Grace Mwikuma et al. (2023) reported higher resistance rates to erythromycin, tetracycline, ciprofloxacin, ampicillin, nitrofurantoin, penicillin, and vancomycin, compared to our study. Nguyen Thi Nhung et al. (2021) found similar resistance rates of Enterococcus spp. to ciprofloxacin and fosfomycin, but higher resistance to erythromycin and tetracycline [4]. Our data showed that among the strains studied, *E. faecalis* exhibited resistance rates to ciprofloxacin (50%), tetracycline (40%), gentamicin (35.5%), erythromycin (28.5%), penicillin (12.9%), vancomycin (9.7%), ampicillin (6.5%), fosfomycin (0%), and nitrofurantoin (0%) [20].

The prevalence of multidrug-resistant (MDR) *E. faecalis*/*E. faecium* in our study population was 27.8%. Specifically, the MDR rate for *E. faecalis* was 5.6%, while for *E. faecium*, it was 22.2%. Majda Golob et al. (2019) reported a similar rate of 30.5%, but noted that 29.6% of *E. faecalis* strains and 73.3% of *E. faecium* strains isolated clinically were MDR [21].

The prevalence of the *asa1* gene among *E. faecalis* was highest (80.6%), followed by the *gelE* gene (70.1%), and the *hyl* gene had the lowest prevalence (6.4%). Additionally, 64.3% of *E. faecalis* carried both *asa1* and *gelE* genes, while 3.6% did not carry any gene. For *E. faecium*, the prevalence of the *gelE* gene was highest (53.7%), followed by the *asa1* gene (39.0%), and the *hyl* gene had a prevalence of 7.3%. Furthermore, 29.3% of *E. faecium* carried both *asa1* and *gelE* genes, while 31.7% did not carry any gene.

Our analysis showed that the virulence genes *asa1* and *gelE* are commonly found in *E. faecalis*, as demonstrated by various studies. Specifically, Jing-xian Yang et al. (2015) reported prevalence rates of *asa1* at 100%, *gelE* at 71.4%, and *hyl* at 0% in *E. faecalis* [[22]. Similarly, Marlos Barbosa-Ribeiro et al. (2016) found prevalence rates of *asa1* and *gelE*

genes at 60% and 75%, respectively. Mohammad Reza Arabestani et al. (2016) found *asa1* to be the most common factor among *E. faecalis* strains (97%), although the *hyl* gene appeared relatively high at 56.6%. Additionally, in *E. faecium* strains, the *asa1* gene had the highest prevalence rate (100%), while the *hyl* gene had a prevalence rate of 71.6%. The occurrence of both virulence genes in these two bacteria in our study is higher than in previous studies.

Related studies, such as that by Ali Jahansepas et al. (2017), reported prevalence rates of virulence genes *asa1*, *gelE*, and *hyl* in *E. faecalis* at 74.4%, 88.0%, and 1.6%, respectively. However, the prevalence rates of these genes in *E. faecium* were higher than in our study, at 71.4%, 86.7%, and 77.1%, respectively, with up to 80% not carrying any gene. Similarly, Meiji Soe Aung et al. (2023) reported prevalence rates of virulence genes *asa1*, *gelE*, and *hyl* in *E. faecalis* at 59.2%, 58.2%, and 0%, respectively, and in *E. faecium* at 0%, 0%, and 11.1%, respectively [23].

Limited studies are addressing the correlation between antibiotic resistance of *E. faecalis* and *E. faecium* carrying virulence genes. Recent research has shown that in *E. faecalis*, the presence of the *asa1*, *esp*, and *cylA* genes is significantly associated with resistance to erythromycin, gentamicin, and tetracycline. Conversely, in *E. faecium*, the presence of the esp and *hyl* genes is significantly associated with resistance to ampicillin, ciprofloxacin, erythromycin, and gentamicin. Another study by Ali Jahansepas et al. (2022) found that the proportions of E. faecalis carrying virulence genes asa1 and gelE resistant to the gentamicin resistance gene were 75% and 87.5%, respectively [24]. This indicates a complex relationship between the resistance of *E. faecalis* and *E. faecium* strains carrying virulence genes.

5. CONCLUSION

The resistance rates of both *E. faecalis* and *E. faecium* to various antibiotics were as follows: erythromycin (50.8%), ciprofloxacin (50%), penicillin (42%), high-level gentamicin (34.7%), ampicillin (30.6%), tetracycline (28.5%), with comparatively lower resistance observed for vancomycin (11.1%) and nitrofurantoin (7.1%), while fosfomycin showed 100% sensitivity. In the study population, the proportion of MDR (multi-drug resistant) *Enterococcus faecalis/E. faecium* was 27.8%, with *E. faecalis* exhibiting an MDR rate of 5.6% and *E. faecium* at 22.2%.

The prevalence of the *asa1* gene in *E. faecalis* was the highest at 80.6%, followed by *gelE* at 74.2%, with the lowest rate observed for the *hyl* gene at 6.4%. Moreover, a significant proportion of *E. faecalis* strains, totaling 64.3%, carried both the *asa1* and *gelE* genes. Virulence gene distribution according to specimen type was primarily concentrated in pus and urine samples. Additionally, among MDR *E. faecalis* strains, those harboring the virulence genes *asa1* (75%) and *gelE* (100%) exhibited notably high proportions.

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