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Research Article

Prevalence and antibiotic resistance of Enterococci in pork from supermarkets, retail markets, and slaughterhouses in Hanoi

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Abstract

The use of antibiotics in livestock, combined with unsanitary slaughter conditions, not only increases the risk of food poisoning but also facilitates the transmission of antibiotic-resistant bacteria from food to humans. This study aimed to assess the contamination status and antibiotic resistance rates of Enterococci bacteria in pork samples collected from supermarkets, retail markets, and slaughterhouses in Hanoi between August 2019 and June 2020. Enterococci strains were isolated from pork samples, identified, and the antibiotic resistance genes were determined using PCR, while antibiotic resistance was assessed by the Kirby-Bauer method. Results showed that all 96 pork samples (100%) purchased from retail markets, supermarkets, and slaughterhouses were contaminated with Enterococci. Of the 588 strains isolated from the 96 pork samples, 259 strains (44%) were identified as *E. faecalis* and 97 strains (16.5%) as *E. faecium*. *E. faecalis* exhibited a higher rate of multidrug resistance compared to *E. faecium* ($p<0.05$). The proportion of *E. faecalis* resistant to five or more antibiotics was 42.9%, while for *E. faecium*, it was 26.8%. Additionally, 23.6% of *E. faecalis* were resistant to six antibiotics, and 2.7% were resistant to seven antibiotics tested in this study. These results indicate a potential risk of multidrug-resistant Enterococci being transmitted from contaminated food to humans during food consumption.

Keywords: *antibiotics, antibiotic resistance, Enterococci.*

1. INTRODUCTION

Vietnam's rapidly growing population and economy have driven a surge in demand for animal-derived foods, with pork leading as the most produced and consumed meat at 32 kg per person annually, far outpacing beef, chicken, and duck. However, much of the pork supply comes from small-scale farms and informal slaughterhouses, where inadequate infrastructure, equipment, and water hygiene increase the risk of contamination by pathogenic bacteria. Compounding this issue, the widespread use of antibiotics in Vietnamese livestock farming through unregulated sales or as feed additives has fueled the rise of antibiotic-resistant bacteria and residues in food, posing serious threats to public health [1, 2].

While acute food poisoning from bacteria like *Escherichia coli*, *Listeria*, *Campylobacter*, and *Salmonella* remains a concern, Enterococci are increasingly recognized for their broader risks beyond foodborne illness. These resilient bacteria thrive in harsh conditions and are commonly found in the gastrointestinal tracts of humans and animals [3], making their presence in food a key indicator of fecal contamination [4]. Recently,

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their robust growth has positioned Enterococci as a major cause of multidrug-resistant infections [5, 6]. They exhibit natural resistance to certain antibiotics, including β-lactams, some aminoglycosides, and cephalosporins, and can transfer resistance and virulence genes to other gut microbes or pathogens, amplifying their threat [7]. For instance, aminoglycoside resistance genes like *aac(6')-Ie-aph(2'')-Ia* produce enzymes that confer high-level resistance to gentamicin, while *ant(6)-Ia* and *aph(3')-IIIa* drive resistance to streptomycin and kanamycin. Genes such as *aac(6')-Ii* and *ant(4')-Ia*, often found in *E. faecium*, further contribute to multidrug resistance [15].

Strains like *Enterococcus faecalis* and *Enterococcus faecium* are major contributors to hospital-acquired infections, including endocarditis, bacteremia, urinary tract infections, and meningitis, and rank as the second-leading cause of hospital-associated bacteremia in the United States [8]. Research has shown that antibiotic-resistant Enterococci in animal-derived foods can be transmitted to humans [9-11]. Although global studies have explored Enterococci prevalence and resistance in food, comprehensive data on multidrug-resistant Enterococci in Vietnam's food supply are scarce. To address this gap, we conducted a study titled "Prevalence and Antibiotic Resistance of Enterococci in Pork from Supermarkets, Retail Markets, and Slaughterhouses in Hanoi, 2019–2020," with two primary objectives: (1) To assess the extent of Enterococci contamination in pork from selected supermarkets, retail markets, and slaughterhouses in Hanoi; and (2) To evaluate the antibiotic resistance profiles of Enterococci strains isolated from these pork samples.

2. MATERIALS AND METHODS

2.1. Study subjects

This study focuses on fresh pork samples collected from retail markets, supermarkets, and slaughterhouses in Hanoi. Inclusion criteria required samples to be from pork slaughtered on the same day, containing skin, lean meat, and fat. Specialty pork (e.g., free-range or wild boar), imported pork, or frozen pork were excluded.

2.2. Research methods: Cross-Sectional Descriptive Study

2.2.1. Sample size

The sample size was calculated using the formula:

$$N = \boldsymbol{Z^2_{(1-\alpha/2)}} \frac{\boldsymbol{p(1-p)}}{\boldsymbol{(d)^2}}$$

Where:

α: Statistical significance level, set at 0.05 (95% confidence level), corresponding to

$Z_{(1-\alpha/2)} = 1{,}96$.

d: Margin of error, set at 0.1.

p: Estimated proportion of pork samples contaminated with Enterococci, set at 0.5 due to the lack of published data on Enterococci prevalence in Vietnam. This yielded a sample size of N = 96 samples.

2.2.2. Sampling locations, timeframe, and procedure

Samples were purposively collected from supermarkets, retail markets, and slaughterhouses across eight inner-city districts of Hanoi: Ha Dong, Thanh Xuan, Hoang Mai, Dong Da, Hai Ba Trung, Ba Dinh, Cau Giay, and Hoan Kiem. Sampling occurred from August 2019 to June 2020, following a standardized procedure to ensure representativeness and prevent cross-contamination:

Supermarkets: Two supermarkets were randomly selected per district, yielding 32 samples (2 supermarkets × 8 districts × 2 samples). Each sample (200 – 250 g) was taken from fresh pork trays in the raw meat section, packaged on the day of collection.

Retail markets: Two markets were randomly selected per district, yielding 32 samples (2 markets × 8 districts × 2 samples). Each sample (200 – 250 g) was collected from fresh pork stalls in the morning, immediately after the meat was displayed for sale. Vendors cut the samples using their knives, and research staff transferred them into sterile polyethylene bags using sterile tongs.

Slaughterhouses: Eight slaughterhouses in Hanoi were sampled, yielding 32 samples (8 slaughterhouses × 4 samples). Each sample (200 – 250 g), consisting of skin, lean meat, and fat, was cut directly from freshly slaughtered pork within 2 hours of slaughter using sterile knives on the slaughter line.

All samples were placed in sterile polyethylene bags, labeled, stored in coolers at 4°C, and transported to the laboratory within 2–3 hours of collection.

2.2.3. Analytical Methods

Isolation of Enterococci: Enterococci were isolated following the method of Mayer, Domig, and Kneifel (2003) [12]. Colonies grown on Slanetz-Bartley agar (Merck, Germany) at 44°C for 48 hours, appearing pink, maroon, or dark red with a narrow white halo, were identified as typical *Enterococcus* colonies. From each Slanetz-Bartley agar plate, 3–6 typical colonies were tested for resistance to gentamicin and vancomycin on Mueller-Hinton agar (Oxoid, UK) supplemented with 500 mg/L gentamicin (Sigma-Aldrich, USA) or 16 mg/L vancomycin (Sigma-Aldrich, USA).

Species identification: *Enterococcus* colonies resistant to gentamicin and/or vancomycin were identified to species level using multiplex PCR. *E. faecalis* and *E. faecium* were identified using a multiplex PCR kit (Qiagen, Germany) as described by Charlene R. Jackson et al. (2003) [13]. Primers were sourced from IDT, USA, with sequences listed in **Table 1**.

Antibiotic resistance testing: The antibiotic resistance profiles of isolated *E. faecalis* and *E. faecium* strains were determined using the Kirby-Bauer disk diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (2013) [14].

Detection of aminoglycoside resistance genes: High-level gentamicin-resistant *E. faecalis* and *E. faecium* strains were analyzed for aminoglycoside resistance genes using the method of S. B. Vakulenko et al. (2003) [15].

Controls: Positive controls (*E. faecalis* ATCC 29212 and *E. faecium* ATCC 35667) and a negative control (sterile distilled water) were used during isolation, PCR, and antibiotic susceptibility testing to ensure result reliability.

Table 1. *Primer sequences for PCR reactions*

Species/genes	Sequences (5' - 3')	Size (bp)
E. faecalis	ACTTATGTGACTAACTTAACC TAATGGTGAATCTTGGTTTGG	360
E. faecium	GAAAAAACAATAGAAGAATTAT TGCTTTTTTGAATTCTTCTTTA	215
aac(6')-Ie-aph(2")-Ia	CAGGAATTTATCGAAAATGGTAGAAAAG CACAATCGACTAAAGAGTACCAATC	369
aac(6')-Ie-aph(2")-Ia	CAGAGCCTTGGGAAGATGAAG CCTCGTGTAATTCATGTTCTGGC	348
aph(2")-Ib	CTTGGACGCTGAGATATATGAGCAC GTTTGTAGCAATTCAGAAACACCCTT	867
aph(2")-Ic	CCACAATGATAATGACTCAGTTCCC CCACAGCTTCCGATAGCAAGAG	444
aph(2")-Id	GTGGTTTTTACAGGAATGCCATC CCCTCTTCATACCAATCCATATAACC	641
aph(3')-IIIa	GGCTAAAATGAGAATATCACCGG CTTTAAAAAATCATACAGCTCGCG	523
ant(4')-Ia	CAAACTGCTAAATCGGTAGAAGCC GGAAAGTTGACCAGACATTACGAACT	294

2.2.4. Data processing and analysis

Data were compiled and organized using Microsoft Excel. Statistical analyses were conducted with SPSS version 22. The Chi-square test (χ^2) and Fisher's Exact Test were applied to compare the rates of multidrug resistance and the presence of resistance genes across groups, with statistical significance set at $p < 0.05$. Findings were presented through tables and graphs for clarity.

3. RESULTS AND DISCUSSION

3.1. Prevalence of Enterococci contamination in pork from supermarkets, retail markets, and slaughterhouses in Hanoi, 2019

The average Enterococci contamination level across 96 pork samples was 4.65 log CFU/g. Samples from retail markets showed the highest contamination, averaging 4.89 log CFU/g, followed by slaughterhouse samples at 4.61 log CFU/g, and supermarket samples with the lowest at 4.44 log CFU/g (**Figure 1**).

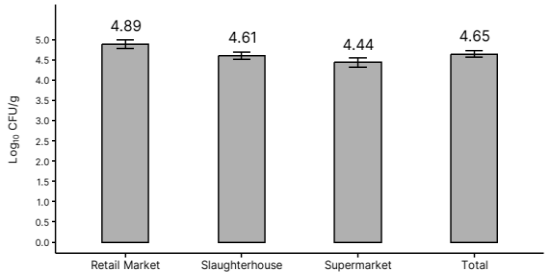


Figure 1. *Levels of Enterococci Contamination in Pork (log CFU/g)*

Of the 96 samples, 89 (92.7%) were contaminated with gentamicin-resistant Enterococci. Retail markets and slaughterhouses had a 100% prevalence of gentamicin-resistant Enterococci, while supermarkets showed a lower rate of 78%. For vancomycin resistance, 11.5% of samples were contaminated with vancomycin-resistant Enterococci, with retail markets exhibiting the highest prevalence (15.6%), followed by both slaughterhouses and supermarkets at 9.4%. Detailed results are presented in **Table 2**.

Table 2. *Prevalence of Enterococci resistant to gentamicin (500 mg/L) and vancomycin (16 mg/L)*

Resistant to Antibiotics	Retail Markets n (%)	Slaughterhouses n (%)	Supermarkets n (%)	Total n (%)
Gentamicin	32 (100)	32 (100)	25 (78.0)	89 (92.7)
Vancomycin	5 (15.6)	3 (9.4)	3 (9.4)	11 (11.5)
Both	5 (15.6)	3 (9.4)	3 (9.4)	11 (11.5)

Using multiplex PCR, 588 Enterococci strains isolated from 96 pork samples were identified: 259 strains (44.0%) were *E. faecalis*, 97 strains (16.5%) were *E. faecium*, and 232 strains (39.5%) belonged to other *Enterococcus* species. The isolation rate of *E. faecalis* was 44.4% in both retail markets and supermarkets, and 43.3% in slaughterhouses. For *E. faecium*, the rates were 16.9% (retail markets), 20.2% (slaughterhouses), and 11.8% (supermarkets). These results, shown in **Table 3**, revealed no statistically significant differences across sampling locations ($p > 0.05$, Chi-square test).

Table 3. *Multiplex PCR results for species identification*

Species		Retail Markets (n=207)	Slaughterhouses (n=203)	Supermarkets (n=178)	Total (n=588)	*p* (χ^2-test)
E. faecalis	n	92	88	79	259	> 0.05
	%	44.4	43.3	44.4	44.0	
E. faecium	n	35	41	21	97	
	%	16.9	20.2	11.8	13.4	
Other Enterococci	n	80	74	78	232	
	%	38.7	36.5	43.8	39.5	
Total	n	207	203	178	588	
	%	100.0	100.0	100.0	100.0	

The average Enterococci contamination level across the 96 pork samples was 4.65 log CFU/g, higher than the 3.8 log CFU/g reported by Koluman et al. (2009) in retail food in Turkey [16]. In Vietnam, a study by Ngo Hoang Hai Tuan et al. (2021) on 671 pork samples from retail channels in northern Vietnam reported an average total bacterial count (TBC) of 6.51 log CFU/g in traditional markets, significantly higher than the 4.65 log CFU/g for Enterococci in this study, indicating severe overall microbial contamination at retail points [17]. Additionally, Hoang Minh Duc et al. (2024) reported a 41.67% prevalence of *Enterococcus* spp. in meat samples, with pork at 46.67% (14/30) and chicken at 36.67% (11/30), notably lower than the rates in this study [18]. Our study, conducted from August 2019 to June 2020, differs in timing from Hoang Minh Duc's study (February 2022 to June 2022). Variations in sampling seasons and years may have influenced Enterococci contamination levels.

The E. faecalis prevalence (44.0%) aligns closely with Aslam et al. (2012) in Canada (50%), while the E. faecium rate (16.5%) is lower than their reported 20%, reflecting regional and temporal differences in species distribution [19].

3.2. Antibiotic resistance profiles of *E. faecalis* and *E. faecium*

E. faecalis strains exhibited high resistance to tetracycline (TET, 84.2%) and quinopristin-dalfopristin (QD, 81.8%). Resistance rates for erythromycin (ERY), chloramphenicol (CHL), high-level gentamicin (GEN), linezolid (LZD), and ciprofloxacin (CIP) were 59.5%, 45.2%, 35.5%, 32.4%, and 21.6%, respectively. Most *E. faecalis* strains remained susceptible to tigecycline (TGC), ampicillin (AMP), and nitrofurantoin (NIT), with resistance rates of 4.6%, 2.7%, and 2.3%, respectively. Resistance to teicoplanin (TEC) and vancomycin (VA) was minimal, both at 0.8% (see **Table 4**). No statistically significant differences in *E. faecalis* resistance rates were observed across retail markets, supermarkets, and slaughterhouses ($p > 0.05$, Chi-square test and Fisher's Exact Test).

Table 4. *Antibiotic resistance rates of E. faecalis to 12 antibiotics*

Antibiotics	% resistant *E. faecalis* (%)								p (χ^2-test, Fisher's Exact Test)
	Retail Markets (n=92)		Slaughterhouses (n=88)		Supermarkets (n=79)		Total (n=259)		
	n	%	n	%	n	%	n	%	
Erythromycin	61	66.3	50	56.8	43	54.4	154	59.5	> 0.05
High level gentamicin	34	37.0	31	35.2	27	34.2	92	35.5	> 0.05
Ciprofloxacin	18	19.6	23	26.1	15	19.0	56	21.6	> 0.05
Tetracycline	78	84.8	68	77.3	72	91.9	218	84.2	> 0.05
Quinopristin - dalfopristin	77	83.7	69	78.4	64	81.0	210	81.8	> 0.05
Vancomycin	2	2.2	0	0.0	0	0.0	2	0.8	> 0.05
Nitrofurantoin	4	4.3	1	1.1	1	1.3	6	2.3	> 0.05
Ampicillin	3	3.3	0	0.0	4	5.1	7	2.7	> 0.05
Linezolid	25	27.2	27	30.7	32	40.5	84	32.4	> 0.05
Tigecycline	8	8.7	3	3.4	1	1.3	12	4.6	> 0.05
Chloramphenicol	41	44.6	42	47.7	34	43.0	117	45.2	> 0.05
Teicoplanin	0	0.0	1	1.1	1	1.3	2	0.8	> 0.05

The tetracycline resistance rate (84.2%) for *E. faecalis* in this study exceeds the 68.42% reported by Hoang Minh Duc et al. (2024) for *Enterococcus* spp. in pork and poultry from Gia Lam, Hanoi [18]. The low vancomycin resistance rate (0.8%) aligns with findings from Hoang Minh Duc et al. (2024). Compared to international data, the tetracycline resistance rate is similar to the 85% reported by Aslam et al. (2012) in retail meat in Canada, reflecting the global prevalence of tetracycline resistance [19]. In contrast to clinical samples, the high-level gentamicin resistance rate (35.5%) is notably higher than the 20% observed by Que Anh Tram (2022) in Nghe An, Viet Nam, highlighting differences between food and clinical environments [21].

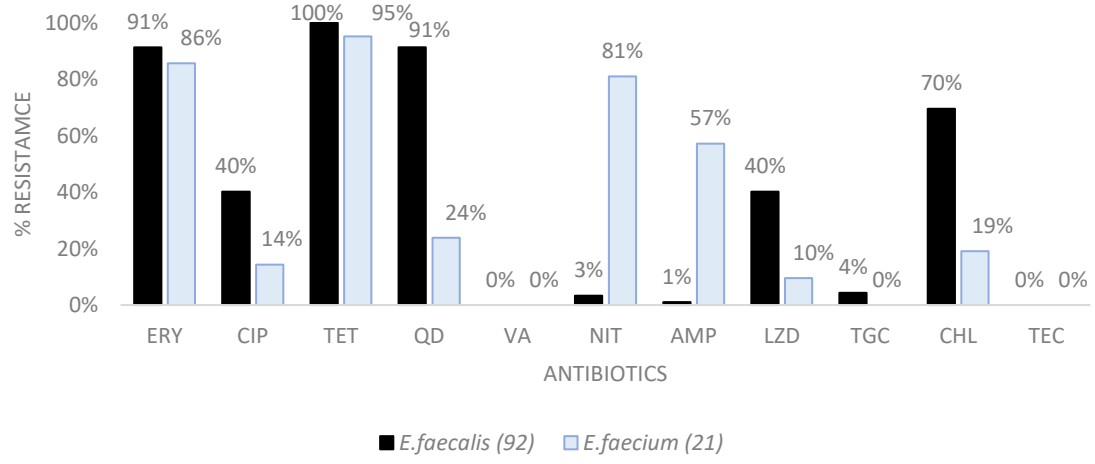
Data from **Table 4** indicate that *E. faecium* exhibited high resistance to tetracycline (TET, 83.5%) and erythromycin (ERY, 68.0%). Resistance to ampicillin (AMP) and nitrofurantoin (NIT) was also substantial, at 58.8% and 47.4%, respectively. Resistance rates for high-level gentamicin (GEN) and quinupristin-dalfopristin

(QD) were 21.6% and 18.6%, while resistance to linezolid (LZD), ciprofloxacin (CIP), and chloramphenicol (CHL) each stood at 10.3%. Most *E. faecium* strains remained susceptible to vancomycin (VA), with a resistance rate of 2.1%, and no resistance was detected for tigecycline (TGC) or teicoplanin (TEC). Statistically significant differences in resistance rates for GEN and QD were observed across sample sources (p = 0.013 and p = 0.005, Chi-square and Fisher's Exact Tests), with supermarkets showing the lowest GEN resistance rate (0.0%) (**Table 5**).

Table 5. *Antibiotic resistance rates of E. faecium to 12 antibiotics*

Antibiotic	% Resistant *E. faecium* kháng (%)								P (χ^2-test, Fisher's Exact Test)
	Retail Markets (n=92)		Slaughterhouses (n=88)		Supermarkets (n=79)		Total (n=259)		
	n	%	n	%	n	%	n	%	
Erythromycin	27	77.1	26	63.4	13	61.9	66	68.0	> 0.05
High level gentamicin	10	28.6	11	26.8	0	0.0	21	21.6	0.013
Ciprofloxacin	4	11.4	4	9.8	2	9.5	10	10.3	> 0.05
Tetracycline	28	80.0	37	90.2	16	76.2	81	83.5	> 0.05
Quinopristin-dalfopristin	1	2.9	12	29.3	5	23.8	18	18.6	0.005
Vancomycin	0	0.0	2	4.9	0	0.0	2	2.1	> 0.05
Nitrofurantoin	22	62.9	25	61.0	10	47.6	57	58.8	> 0.05
Ampicillin	15	42.9	24	58.5	7	33.3	46	47.4	> 0.05
Linezolid	5	14.3	3	7.3	2	9.5	10	10.3	> 0.05
Tigecycline	0	0.0	0	0.0	0	0.0	0	0.0	
Chloramphenicol	4	11.4	5	12.2	1	4.8	10	10.3	> 0.05
Teicoplanin	0	0.0	0	0.0	0	0.0	0	0.0	

Most high-level gentamicin-resistant E. faecalis and E. faecium strains also showed resistance to TET (100% and 95%) and ERY (91% and 86%) (see **Figure 2**). E. faecalis exhibited significantly higher resistance to QD and CHL compared to E. faecium, with rates of 91% versus 24% for QD and 70% versus 19% for CHL. Resistance to CIP and LZD was also higher in E. faecalis (both 40%) than in E. faecium (14% for CIP, 10% for LZD). Conversely, E. faecium showed greater resistance to NIT (81%) and AMP (57%), while E. faecalis was nearly fully susceptible to these antibiotics. Only 4% of E. faecalis strains were resistant to TGC, and no E. faecium strains showed TGC resistance. All high-level gentamicin-resistant E. faecalis and E. faecium strains were susceptible to VA and TEC.



Note: Erythromycin (ERY); High-level of gentamicin (GEN); Ciprofloxacin (CIP); Tetracycline (TET); Quinopristin-dalfopristin (QD); Vancomycin (VA); Nitrofurantoin (NIT); Ampicillin (AMP); Linezolid (LZD); Tigecycline (TGC); Chloramphenicol (CHL); Teicoplanin (TEC)

Figure 2. *Comparison of high-level gentamicin resistance in Enterococcus faecalis and Enterococcus faecium*

3.3. Multidrug resistance rates of *E. faecalis* and *E. faecium*

Both *E. faecalis* and *E. faecium* exhibited high rates of multidrug resistance (MDR), defined as resistance to three or more antibiotics. *E. faecalis* showed a significantly higher MDR rate compared to *E. faecium* ($p < 0.05$). Specifically, 42.9% of *E. faecalis* strains were resistant to five or more antibiotics, compared to 26.8% of *E. faecium* strains. Notably, 23.6% of *E. faecalis* strains were resistant to six antibiotics and 2.7% to seven, while for *E. faecium*, 7.2% were resistant to six antibiotics, 2.1% to seven, and 3 out of 97 strains (3.1%) were resistant to 8 of the 12 antibiotics tested (see **Table 6**).

Table 6. *Multidrug resistance rates of Enterococci*

Number of Antibiotics		0	1	2	3	4	5	6	7	8
E. faecalis (n=259)	n	10	41	36	24	37	43	61	7	0
	%	3.9	15.8	13.9	9.3	14.3	16.6	23.6	2.7	0.0
E. faecium (n=97)	n	6	14	12	20	19	14	7	2	3
	%	6.2	14.4	12.4	20.6	19.6	14.4	7.2	2.1	3.1
Total (n=356)	n	16	55	48	44	56	57	68	9	3
	%	4.5	15.4	13.5	12.4	15.7	16.0	19.1	2.5	0.8

Compared to global studies, the MDR rate for *E. faecalis* (42.9% resistant to ≥5 antibiotics) is lower than the 58% reported by Aslam et al. (2012) but significantly higher than the 35% observed by Daniel et al. (2015) in Southeast Asia, positioning this study's findings at an intermediate level within the region [19, 20]. A study by Usui et al. (2014) examined antibiotic susceptibility of *E. faecalis* (n=117) and *E. faecium* (n=180) isolated from poultry in three Southeast Asian countries (Vietnam, Indonesia, and Thailand) in 2014. Compared to our findings, their MDR rate (≥3 antibiotics) for *E. faecalis* was lower (81.5% vs. 100% in Vietnam), and for *E. faecium*, the MDR rate (79.4%) was notably lower than the 95.5% reported by Usui et al. (2014) in Vietnam [22].

3.4. Prevalence of aminoglycoside resistance genes in *E. faecalis* and *E. faecium*

The PCR results highlight the significant presence of aminoglycoside resistance genes among gentamicin-resistant *Enterococcus* strains isolated from pork samples, with distinct patterns observed between *E. faecalis* and *E. faecium*. One *E. faecium* strain was found to carry five aminoglycoside resistance genes. Among the 113 gentamicin-resistant strains, 111 (98.2%) harbored the *aac(6')-Ie-aph(2'')-Ia* gene. Notably, 62% of *E. faecalis* strains carried a combination of three resistance genes: *aac(6')-Ie-aph(2'')-Ia*, *ant(6)-Ia*, and *aph(3')-IIIa*. In contrast, nearly half (47.6%) of gentamicin-resistant *E. faecium* strains carried four resistance genes: *aac(6')-Ie-aph(2'')-Ia*, *ant(6)-Ia*, *aph(3')-IIIa*, and *aac(6')-Ii* (see **Table 7**).

Table 7. *Combinations of aminoglycoside resistance genes detected*

Number of resistant genes	*aac(6')-Ie-aph(2'')-Ia*	*aac(6')-Ii*	*ant(6)-Ia*	*aph(3')-IIIa*	*ant(4')-Ia*	*E. faecalis* (N=92)		*E. faecium* (N=21)	
						n	%	n	%
5	+	+	+	+	+	0		1	1.8
4	+	+	+	+	-	0		**10**	**47.6**
	+	+	+	-	-	0		4	19.0
3	+	+	-	+	-	0		1	1.8
	+	-	+	+	-	**57**	**62**	1	1.8
	-	+	+	+	-	0		1	1.8
2	+	+	-	-	-	0		1	1.8
	+	-	+	-	-	8	8.7	2	9.5
	+	-	-	+	-	22	23.9	0	
1	+	-	-	-	-	4	4.3	0	
0	-	-	-	-	-	1	1.1	0	

Note: "+" indicates presence of the gene; "-" indicates absence.

The results demonstrate a high burden of aminoglycoside resistance genes in gentamicin-resistant from pork, with *E. faecium* exhibiting greater genetic diversity and complexity in resistance profiles compared to *E. faecalis*. These findings emphasize the need for targeted interventions to curb antibiotic resistance in food production and prevent its spread to human populations.

4. CONCLUSIONS

This study revealed that 100% of pork samples collected from slaughterhouses, retail markets, and supermarkets in Hanoi were contaminated with Enterococci, predominantly *E. faecalis* (44.0%) and *E. faecium* (16.5%). This universal contamination points to systemic hygiene failures across the pork supply chain, likely due to poor sanitation during slaughter and handling. As opportunistic pathogens and indicators of fecal contamination, Enterococci in food pose risks of foodborne illness and resistance gene transfer.

Both *E. faecalis* and *E. faecium* exhibited high resistance to tetracycline (84.2% and 83.5%), erythromycin (59.5% and 68.0%), high-level gentamicin (35.5% and 21.6%), and ciprofloxacin (21.6% and 10.3%), driven by widespread antibiotic use in Vietnamese pig farming (**Tables 4 and 5**). However, susceptibility to vancomycin (0.8% and 2.1% resistance), tigecycline (4.6% and 0%), and teicoplanin (0.8% and 0%) remains high, preserving these as critical treatment options. Two-thirds of strains showed multidrug resistance (MDR, ≥3 antibiotics). These findings underscore the urgent need for stricter antibiotic regulations and enhanced surveillance to curb resistance spread from pork to humans.

Five of seven aminoglycoside resistance genes were detected using PCR in gentamicin-resistant strains, with *aac(6')-Ie-aph(2")-Ia* present in 98.2% of 124 resistant isolates. Notably, 62% of *E. faecalis* carried a three-gene combination (*aac(6')-Ie-aph(2")-Ia*, *ant(6)-Ia*, *aph(3')-IIIa*), while 47.6% of *E. faecium* had four genes, including *aac(6')-Ii*. These profiles suggest mobile genetic elements facilitate resistance spread.

These findings indicate a significant risk of antibiotic-resistant Enterococci transmission from pork to humans, potentially complicating clinical treatments. The high MDR rates and resistance gene prevalence underscores the need for stricter antibiotic regulations in livestock and enhanced food safety surveillance to curb the spread of resistant strains. Further research is critical to monitor resistance trends and inform public health interventions.

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