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Original article

Antimicrobial susceptibility profiles of *Escherichia coli* and *Enterococcus* spp. isolated from companion animals in Indonesia

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Abstract

This study aimed to investigate the antimicrobial susceptibility profile in *Escherichia coli* and *Enterococcus* spp. isolated from healthy cats and dogs in Indonesia. A total of 186 rectal swab samples were individually collected from June to October 2025 at 13 animal clinics from three regions. Bacterial isolation was performed using selective agar media, and the species was confirmed by polymerase chain reaction. In this study, we obtained 71 of 164 (43.3%) antimicrobial-resistant (AMR) *E. coli* and 53 of 94 (56.4%) AMR enterococci by disc diffusion test. The highest resistance of *E. coli* was observed for ampicillin (36.6%), followed by streptomycin (21.3%), tetracycline (15.9%), cefazolin (13.4%), nalidixic acid (12.8%), cefotaxime (11.0%), gentamicin (11.0%), aztreonam (8.5%), doxycycline (8.5%), chloramphenicol (7.9%), sulfamethoxazole-trimethoprim (6.7%), amoxicillin-clavulanic acid (3.7%), ciprofloxacin (3.0%) and enrofloxacin (2.4%). On the other hand, the highest resistance of enterococci was identified for tetracycline (24.5%), followed by high-level streptomycin (21.3%), erythromycin (21.3%), ciprofloxacin (10.6%), enrofloxacin (9.6%), penicillin (7.4%), linezolid (6.4%), ampicillin (5.3%), fosfomycin (4.3%) and chloramphenicol (3.2%). Multidrug-resistant (MDR) *E. coli* was detected in 44 of 164 (26.8%) isolates, which were dominated by extraintestinal pathogenic strains (phylogroup B2 and D), while MDR enterococci were detected from 13 of 94 (13.8%) isolates. Our study offers valuable insights into the resistance profiles of Indonesian companion animals. A continuous monitoring strategy for AMR is needed to control the development of AMR bacteria in companion animals.

Keywords: antimicrobial resistance, companion animals, *Escherichia coli*, enterococci, Indonesia

Introduction

In recent years, the bond between humans and their pets has strengthened, as evidenced by the rapid growth of pet-related industries and veterinary services, even in rural and peri-urban regions of Indonesia (https://ditjenpkh.pertanian.go.id). These trends indicate a growing level of public concern for animal health and welfare. Nevertheless, the misuse of antimicrobials remains a significant challenge. As the number of companion animal owners continues to rise globally, many pet owners self-medicate their pets, which is facilitated by the widespread availability of drugs through pharmacies and online platforms. Using antimicrobials without professional supervision increases the risk of incorrect dosing, adverse effects, and antimicrobial resistance resulting from improper antibiotic use (Konno et al. 2025). The presence of antimicrobial-resistant (AMR) bacteria in companion animals is a significant public health concern because these organisms can be transmitted between companion animals and humans through close physical contact, shared living spaces, and environmental contamination (Sukmawinata et al. 2020).

Escherichia coli and *Enterococcus* spp., which represent Gram-negative and Gram-positive bacterial groups, respectively, are widely used as indicator organisms in AMR surveillance. This is due to their ubiquity, capacity to acquire and transfer resistance determinants, and their relevance in both clinical and community settings (Sato et al. 2020, Sukmawinata et al. 2020). Therefore, understanding the resistance profiles of these two key indicator bacteria is essential for mapping resistance dynamics in companion animals. Indonesia is underrepresented in AMR companion animal data. This study aims to characterize the AMR patterns of *E. coli* and *Enterococcus* spp. isolates from cats and dogs, and also to assess the correlation structure of resistance phenotypes to identify potential co-resistance patterns. These patterns could inform AMR risk prediction and guide antimicrobial stewardship strategies.

Materials and Methods

Sampling

A total of 186 rectal swab samples were obtained from 144 healthy cats and 42 healthy dogs at 13 animal clinics across three regions in Indonesia (Fig. 1) from June to October 2025. Specifically, 103 samples (92 from cats and 11 from dogs) were collected from six clinics in the first region (Jakarta, Bogor and Depok City), 47 samples (46 from cats and one from a dog) were collected from two clinics in the second region (Bukittinggi and Solok City), and 36 samples (7 from cats and 29 from dogs) were collected from five clinics in the third region (Kupang City). We enrolled pet animals that visited the clinics during the study period provided they were clinically healthy, as assessed by a veterinarian, and met the following criteria: no fever, cough or runny nose; free from mites and fungus; not pregnant or breastfeeding; and given worming medication prior to the visit. Additionally, owners had to provide informed consent, regardless of the purpose of the visit, and the animals had to have received no antibiotic treatment for at least one month. Basic background information, including age, sex, species and breed, was recorded.

Isolation and identification

Rectal swab samples were collected in 0.1% buffered peptone water (Oxoid CM0509B, Basingstoke, Hampshire, UK) and stored in a cooler box during transport to the Laboratory of Veterinary Public Health, School of Veterinary Medicine and Biomedical Science, IPB University for analysis. Isolation and identification of *E. coli* and enterococci were conducted based on Sato et al. (2020) and Sukmawinata et al. (2020), respectively, with minor modifications. A loop of the swab sample was streaked onto a Tryptone Bile Glucuronic (TBX) agar (Himedia M1591, Dindori, Nashik, India) and incubated at 37ºC for 24 hours. Presumptive *E. coli* was indicated by bluish colonies on the TBX agar. If no presumptive *E. coli* colonies appeared, the swab samples were enriched using Tryptone Soya Broth (TSB; Himedia M1876, Dindori, Nashik, India) and incubated at 37ºC overnight, and were further cultured onto TBX agar for *E. coli* isolation. To isolate *Enterococcus* spp., the samples were subcultured into Brain Heart Infusion (BHI; Oxoid BO1230B, Basingstoke, Hampshire, UK) broth containing 6.5% NaCl and incubated at 42ºC for 24 hours. The samples were then streaked onto Slanetz and Bartley Medium (SBA; Himedia M612, Dindori, Nashik, India) agar and incubated at 42ºC for 24-48 hours. The colonies that appeared as brown colonies on SBA agar were considered presumptive *Enterococcus* spp. At least two single colonies of both *E. coli* and *Enterococcus* spp. from each sample were purified using Tryptic Soya Agar (TSA; Himedia M1968, Dindori, Nashik, India). The colonies were then kept frozen in TSB containing 20% glycerol at -20ºC until further analysis. Genomic DNA was extracted with the boiling method by heating bacterial suspensions in nuclease-free water at 95ºC for 10 minutes. Presumptive *E. coli* isolates were confirmed to the species and

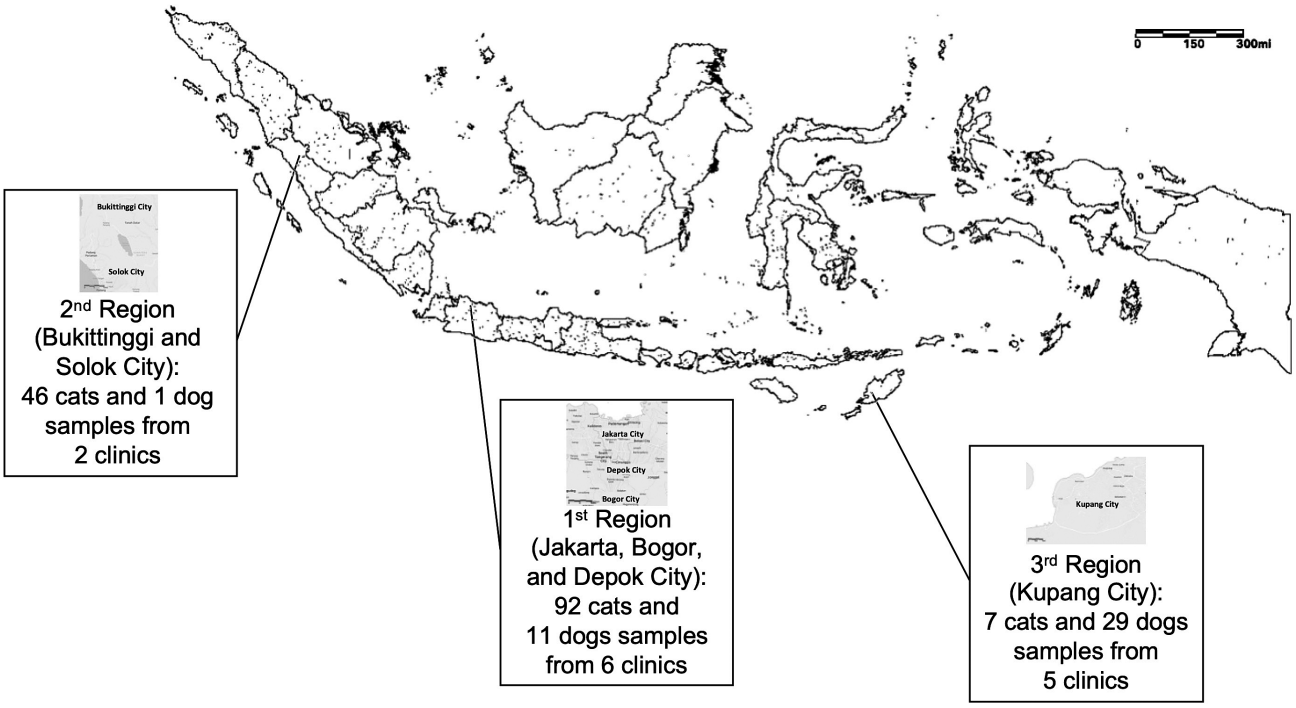

Fig. 1. Sampling location in this study.

phylogroup levels using polymerase chain reaction (PCR) (Doumith et al. 2012), while *E. faecalis* and *E. faecium* isolates were confirmed by targeting the *ddl* gene (Kariyama et al. 2000).

Antimicrobial susceptibility testing

The Kirby-Bauer method was used to performed for antimicrobial susceptibility testing (AST) on *E. coli* and also on *E. faecalis* and *E. faecium*. *E. coli* isolates were tested against sixteen antibiotics including ampicillin 10µg (AMP), amoxicillin-clavulanic acid 30 µg (AMC), cefotaxime 30 µg (CTX), aztreonam 30 µg (ATM), meropenem 10 µg (MEM), streptomycin 10 µg (S), gentamicin 10 µg (CN), nalidixic acid 30 µg (NAL), ciprofloxacin 30 µg (CIP), chlorampenicol 30 µg (C), tetracycline (TE), doxycicline (DO), sulfamethoxazole-trimethoprim 1.25 µg and 23.75 µg, respectively (SXT), and colistin 10 µg (CT) which were provided by the Oxoid company; cefazolin 30 µg (CZ) and enrofloxacin 10 µg (EX) were provided by the Himedia company. *E. faecalis* and *E. faecium* were tested against twelve antibiotics, which were consisted of penicillin 10 µg (P), ampicillin 10 µg (AMP), erythromycin 15 (E), ciprofloxacin (CIP), chloramphenicol 30 µg (C), tetracycline 30 µg (TE), doxycycline 30 µg (DO), fosfomycin 50 µg (FOS), linezolid 30 µg (LZD) provided from the Oxoid company, and high-level streptomycin 300 µg (HLS), enrofloxacin 10 µg (EX), and vancomycin 30 µg (VA) from the Himedia company. *E. coli* ATCC 25922 and *E. faecalis* ATCC 51299 were used for quality control purposes. Antimicrobial susceptibility results were primarily interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023). When CLSI breakpoints were unavailable, HLS results for *Enterococcus* spp. were interpreted using the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2023). EX results were evaluated based on CLSI VET01 (CLSI, 2015). CT results for *E. coli* were interpreted using the criteria proposed by Galani et al. (2008): resistant ≤11 mm and susceptible ≥14 mm. *E. coli* isolates showing resistance to CTX were investigated for the presence of the bla_{CTX-M} gene using PCR (Sukmawinata et al. 2019). Multidrug-resistant (MDR) bacteria were defined as an isolate that showed resistance to at least three classes of antibiotics (Magiorakos et al. 2012).

Statistical analysis

The proportion of susceptibility profiles was recorded and visualized using Microsoft Excel (version 16.70). Logistic regression analysis was conducted using RStudio (version 2022.12.0+353) to assess associations between host factors (age, sex and species) and AMR. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by exponentiating regression coefficients. Statistical significance was determined at $p<0.05$. The presence of significant differences in the percentages of sensitivity levels (Susceptible, Intermediate and Resistant) for each antibiotic, the proportions of resistance among pets and regions, the phylogroup of *E. coli*, and the enterococci species was analysed using Chi-Square or Fisher's Exact Test in

Table 1. Variable associated with the occurrence of antimicrobial-resistant bacteria isolated from Indonesian companion animals.

Variable[a]	Category	AMR isolation (%)	OR (95% CI)	p-value
E. coli (n=164)				
Age group	Young (≤ 2 years)	32 (19.5)		Ref.
	Adult (2-6 years)	36 (22.0)	2.72 (1.44–5.20)	0.002*
	Senior (>6 years)	3 (1.8)	1.38 (0.27–5.74)	0.666
Sex	Male	42 (25.6)	1.03 (Not estimable)[#]	0.987
	Female	29 (17.7)		Ref.
Species	Cat	57 (34.8)	1.06 (0.50–2.23)	0.886
	Dog	14 (8.5)		Ref.
Enterococci (n=92)				
Age group	Young (≤ 2 years)	31 (33.7)		Ref.
	Adult (2-6 years)	20 (21.7)	0.95 (0.39–2.31)	0.91
	Senior (>6 years)	2 (2.2)	0.33 (0.04–2.33)	0.27
Sex	Male	29 (31.5)	0.65 (0.26-1.57)	0.34
	Female	24 (26.1)		Ref.
Species	Cat	39 (42.4)	0.68 (0.24-1.82)	0.45
	Dog	14 (15.2)		Ref.

OR – Odds Ratio
CI – Confidence Interval 95%
[a] – *E. coli* and enterococci were obtained from 164 and 92 companion animal's rectal swab samples, respectively.
* significant ($p<0.05$)
[#] Not estimable due to instability in the model
Ref. = Reference category

RStudio. The strength of the correlation among antimicrobial profiles was visualized using a Phi correlation coefficient heatmap in RStudio. To increase analytical stability and capture broader shifts in susceptibility patterns, isolates categorized as resistant or intermediate were grouped as non-susceptible. Positive correlations are defined as r-values close to 1, and negative correlations as r-values close to -1.

Results

The isolation rates of *E. coli* and enterococci were 88.2% (164/186) and 49.5% (92/186), respectively. One representative isolate per bacterial species was then selected from each sample for antimicrobial susceptibility test (AST), resulting in a total of 164 *E. coli* isolates and 94 enterococci isolates (a total of *E. faecalis* and *E. faecium* isolates) being analyzed in this study. In particular, *E. coli* was isolated from 87.5% (126/144) of cats and 90.5% (38/42) of dogs, while enterococci were isolated from 50.0% (72/144) of cats and 52.4% (22/42) of dogs. Species identification revealed that the tested enterococci isolates consisted of 75 (79.8%) *E. faecalis* and 19 (20.2%) *E. faecium*. Logistic regression analysis showed that adult animals had significantly higher odds of carrying resistant *E. coli* isolates compared to young animals (OR 2.72, 95% CI 1.44-5.20, p=0.002); however no significant association was observed between age group and resistant enterococci (Table 1). No significant associations were observed for sex and species.

Antimicrobial resistance in *E. coli*

We obtained 71 of 164 (43.3%) AMR *E. coli* isolates using the disc diffusion test. The highest resistance in *E. coli* was then identified to AMP (36.6%), followed by S (21.3%), TE (15.9%), CZ (13.4%), NAL (12.8%), CTX (11.0%), CN (11.0%), ATM (8.5%), DO (8.5%), C (7.9%), SXT (6.7%), AMC (3.7%), CIP (3.0%) and EX (2.4%). No *E. coli* isolates showed resistance to MEM or CT in this study. The analysis also revealed that phylogroup B2 (44.5%; 73/164) had the highest proportion among the *E. coli* isolates, followed by B1 (31.7%; 52/164), A (14.0%; 23/164), and D (9.8%; 16/164). Furthermore, the combination of phylogroups B2 and D exhibited a higher resistance rate to all antibiotics than the combination of phylogroups A and B1 strains. Specifically, *E. coli* in phylogroup B2 were significantly more resistant to DO than B1 (p=0.047), and almost significantly more resistant to AMP (p=0.084) and TE (p=0.101). To our knowledge, the resistance rate to TE was significantly higher in the

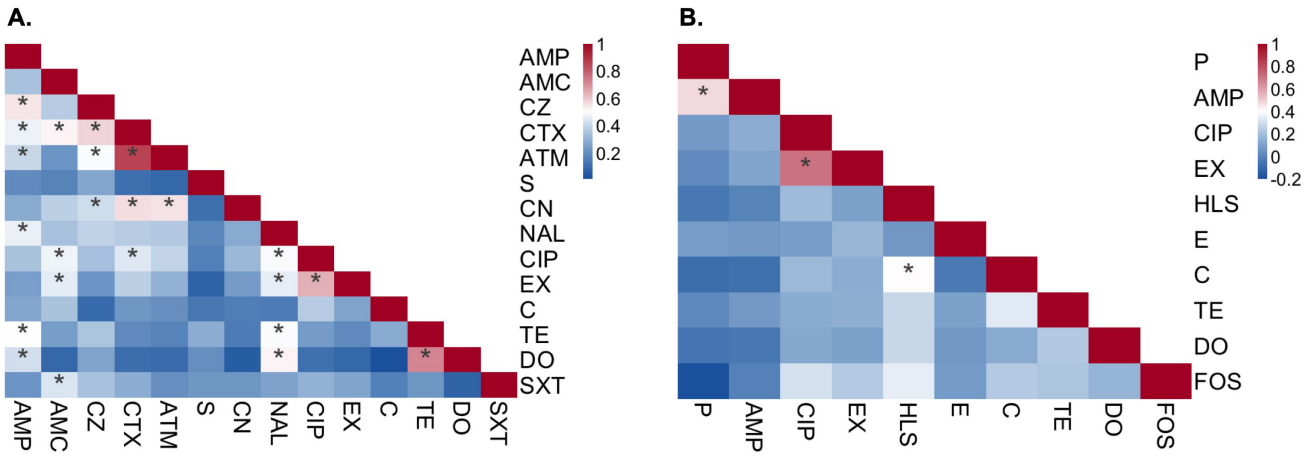

Fig. 2. Pearson correlation heatmaps based on disk diffusion results of *Escherichia coli* (A), *Enterococcus* spp. (B). The phi coefficient (r) ranges from +1 to −1, which indicates strong to weak correlation. Correlations were calculated using binary susceptibility data (1 = non-susceptible; 0 = susceptible). Statistical significance was determined after false discovery rate (FDR) correction (*FDR<0.05 and r≥0.4). P=penicillin; AMP=ampicillin; AMC=amoxicillin; CZ=cefazolin, CTX=cefotaxime; ATM=aztreonam; S=streptomycin; CN=gentamicin; NAL=nalidixic acid; CIP=ciprofloxacin; EX=enrofloxacin; C=chloramphenicol; TE=tetracycline; DO=doxycycline; SXT=sulfamethoxazole-trimethoprim; HLS=High-level streptomycin; E=erytromicin; FOS=fosfomycin.

Table 2. Antimicrobial susceptibility test of *E. coli* isolated from Indonesian companion animals.

E. coli (n=164)	Antibiotics[a] (%)															
	AMP	AMC	CZ	CTX	ATM	MEM	S	CN	NAL	CIP	EX	C	TE[b]	DO[c]	SXT	CT
Susceptible	60.4	92.7	69.5	87.2	90.2	100.0	37.8	87.2	81.1	90.9	92.7	92.1	78.0	86.0	92.1	100.0
Intermediate	3.0	3.7	17.1	1.8	1.2	0.0	40.9	1.8	6.1	6.1	4.9	0.0	6.1	5.5	1.2	-
Resistant	36.6	3.7	13.4	11.0	8.5	0.0	21.3	11.0	12.8	3.0	2.4	7.9	15.9	8.5	6.7	0.0
-Resistant in Cats (n=126)	38.9	4.8	14.3	11.1	8.7	0.0	21.4	12.7	13.5	3.2	2.4	9.5	17.5	10.3	7.9	0.0
-Resistant in Dogs (n=38)	28.9	0.0	10.5	10.5	7.9	0.0	21.1	5.3	10.5	2.6	2.6	2.6	10.5	2.6	2.6	0.0
-Resistant in Phylogroup A (n=23)	43.5	4.3	13.0	8.7	8.7	0.0	21.7	17.4	0.0	0.0	0.0	0.0	13.0	0.0	4.3	0.0
-Resistant in Phylogroup B1 (n=52)	23.1	0.0	13.5	13.5	7.7	0.0	11.5	11.5	11.5	5.8	5.8	7.7	7.7	1.9	5.8	0.0
-Resistant in Phylogroup B2 (n=73)	45.2	6.8	11.0	8.2	6.8	0.0	30.1	6.8	17.8	1.4	0.0	11.0	24.7	16.4	8.2	0.0
-Resistant in Phylogroup D (n=16)	31.3	0.0	25.0	18.8	18.8	0.0	12.5	18.8	12.5	6.3	6.3	6.3	6.3	6.3	6.3	0.0

[a] AMP – ampicillin, AMC – amoxicillin, CZ – cefazolin, CTX – cefotaxime, ATM – aztreonam, S – streptomycin, CN – gentamicin, NAL – nalidixic acid, CIP – ciprofloxacin, EX – enrofloxacin, C – chloramphenicol, TE – tetracycline, DO – doxycycline, SXT – sulfamethoxazole-trimethoprim, CT = colistin.
[b] Significant different between 1st Region and 3rd Region ($p<0.05$).
[c] Significant different between phylogroup B1 and B2 ($p<0.05$).

1st region than in the 3rd region, as mentioned above. MDR was detected in 44 of 164 (26.8%) *E. coli* isolates. The greatest frequency of MDR was found in group B2 (56.8%; 25/44), followed by B1 (22.7%; 10/44), A (11.4%; 5/44), and D (9.1%; 4/44). The antimicrobial susceptibility and MDR profiles of *E. coli* are summarised in Table 2 and Table 4.

The Phi correlation coefficient heatmap in Fig. 2A reveals the correlation among the tested isolates in response to their inhibitory activity. Most antibiotics in the same group showed a high-magnitude correlation (r>0.4). AMP was strongly associated with β-lactam agents as well as with tetracycline classes and NAL. A very strong positive correlation was observed between CTX and ATM (r=0.86, FDR<0.05). The presence of bla_{CTX-M} was detected in 14 of 18 (77.8%) CTX-resistant isolates.

Antimicrobial resistance in enterococci

Overall, 53 of 94 (56.4%) AMR enterococci isolates were identified from rectal swab samples. The highest resistance of enterococci was identified to TE (24.5%), followed by HLS (21.3%), E (21.3%), CIP (10.6%), EX (9.6%), P (7.4%), LZD (6.4%), AMP (5.3%), FOS (4.3%) and C (3.2%) (Table 3). In this study, none of the tested enterococci showed resistance to DO or VA. Interestingly, the proportion of intermediate level in the AST results for E, CIP, EX and FOS was significantly higher than the proportion of sensitive level ($p<0.01$).

Table 3. Antimicrobial susceptibility test of enterococci isolated from Indonesian companion animals.

Enterococci (n=94)	Antibiotics[a] (%)											
	P[b]	AMP[b]	HLS	E	CIP	EX[b]	C	TE	DO	FOS	VA	LZD
Susceptible	92.6	94.7	78.7	8.5	25.5	17.0	91.5	73.4	95.7	37.2	91.5	70.2
Intermediate	0.0	0.0	-	70.2	63.8	73.4	5.3	2.1	4.3	58.5	8.5	23.4
Resistant	7.4	5.3	21.3	21.3	10.6	9.6	3.2	24.5	0.0	4.3	0.0	6.4
-Resistant in Cats (n=72)	8.3	4.2	22.2	19.4	9.7	8.3	4.2	25.0	0.0	2.8	0.0	4.2
-Resistant in Dogs (n=22)	4.5	9.1	18.2	27.3	13.6	13.6	0.0	22.7	0.0	9.1	0.0	13.6
-Resistant in *E. faecalis* (n=75)	2.7	1.3	24.0	17.3	8.0	5.3	4.0	25.3	0.0	5.3	0.0	8.0
-Resistant in *E. faecium* (n=19)	26.3	21.1	10.5	36.8	21.1	26.3	0.0	21.1	0.0	0.0	0.0	0.0

[a] P – penicillin, AMP – ampicillin, HLS – High-level streptomycin, E – erytromicin, CIP – ciprofloxacin, EX – enrofloxacin, C – chloramphenicol, TE – tetracycline, DO – doxycycline, FOS – fosfomycin, VA – vancomycin, LZD – linezolid.
[b] Significant different between *E. faecalis* and *E. faecium* ($p<0.05$).

Comparison between the proportion rate of resistance among both enterococci showed that *E. faecium* was more resistant to P, AMP, E, CIP and EX than *E. faecalis*. Significant differences were highly identified in *E. faecium* against P, AMP and EX than in *E. faecalis*. The proportion of intermediate-level resistance in enterococci was also highly identified for E, CIP, EX and FOS. Furthermore, MDR was detected in 13 of 94 (13.8%) enterococci isolates; 10 of 13 (76.9%) MDR enterococci belonged to *E. faecalis*, whereas 3 of 13 (23.1%) belonged to *E. faecium* (Table 5). Although species-specific resistance profiles may differ between *E. faecalis* and *E. faecium*, co-resistance analysis was conducted at the genus level due to the limited number of *E. faecium* isolates. As a result, a significant positive correlation was observed for antibiotics in the same group, such as AMP-P and CIP-EX, whereas a positive correlation between different groups was observed for HLS and C (Fig. 2B).

Discussion

This study provides multiple clinic baseline data on AMR in companion animals from Indonesia. Our results revealed that the isolation rates for *E. coli* between cats and dogs were over 85%, whereas for enterococci they were about 50%. These findings are higher then from a previous report that revealed isolation rate of *E. coli* from rectal swab of healthy cats (29.4%) and dogs (28.03%) from Thailand (Buranasinsup et al. 2023). Another study from Japan showed that enterococci (*E. faecalis* and *E. faecium*) were isolated from 82.9% (358/432) and 83.9% (215/256) of dogs and cats, respectively, which were higher than our study (Furuya et al. 2022). The difference in isolation rate of *E. coli* and enterococci in the present study compared with previous reports may be attributable to differences in sampling strategy, host characteristics and laboratory protocols (Buranasinsup et al. 2023). The age of the studied companion animals influenced the isolation of resistant *E. coli*, which suggests further investigations are needed, such as variable analysis for prior use of antimicrobials or environmental exposure (Dzulkifli et al. 2025). Therefore, the potential influence of host-related factors on antimicrobial resistance patterns was not deeply explored in this study and should be considered when interpreting the findings.

In *E. coli* isolates, phylogroup analysis was conducted to predict the pathogenicity potential by distinguishing potential extraintestinal pathogenic *E. coli* (B2 and D) and commensal-associated strains (A and B1) (Doumith et al. 2012). Extraintestinal pathogenic *E. coli* (ExPEC) represent a group of *E. coli* that typically reside harmlessly in the gastrointestinal tract as commensal organisms. However, due to the presence of particular virulence factors, they can migrate beyond the intestine, colonize extraintestinal sites, and lead to serious conditions such as urinary tract infections (UTIs) and bacteremia (Bok et al. 2020). However, our analysis did not aim to represent the actual bacterial composition ratio between ExPEC- and commensal-associated strains. Nevertheless, our results revealed that more than half of MDR *E. coli* that were categorised as ExPEC possess the potential for public health implications (Shaker et al. 2023).

Resistance to AMP was the most frequent in *E. coli* isolates, and showed a significantly strong correlation with tetracyclines and NAL, which suggests potential co-selection or shared resistance determinants. In our study, resistance to first- and third-generation cephalosporins (CZ and CTX, respectively) in tested *E. coli* isolates was also strongly correlated with resistance to CN (aminoglycoside). Extended-spectrum β-lactamases (ESBLs) comprise a class of enzymes that can inactivate third-generation cephalosporins (e.g., CTX) and monobactams (e.g., ATM) through hydrolysis, while remaining susceptible to inhibition by clavulanic

Table 4. Multidrug resistance pattern of 44 *E. coli* isolated from Indonesian companion animals.

Amount of resistance	Pattern*	n
9	AMP+AMC+CZ+CTX+ATM+S+CN+C+TE	1
	AMP+CZ+CTX+ATM+S+CN+C+TE+STX	1
	AMP+CZ+CTX+ATM+S+CN+NAL+CIP+EX	1
	AMP+CZ+CTX+ATM+S+NAL+CIP+EX+C	1
8	AMP+AMC+CZ+CTX+ATM+S+CN+STX	1
	AMP+CZ+CTX+CN+NAL+CIP+EX+TE	1
	AMP+CZ+CTX+S+CN+NAL+C+TE	1
7	AMP+AMC+CZ+S+C+TE+STX	1
	AMP+CZ+CTX+ATM+TE+DO+STX	1
6	AMP+AMC+CZ+CTX+S+STX	1
	AMP+CZ+CTX+ATM+CN+NAL	1
	AMP+CZ+CTX+ATM+CN+TE	1
	AMP+CZ+CTX+ATM+TE+DO	1
5	AMP+S+NAL+TE+DO	7
	AMP+CZ+CTX+ATM+CN	4
	AMP+S+C+TE+DO	1
	AMP+S+CN+TE+STX	1
	AMP+S+NAL+CIP+DO	1
4	AMP+S+NAL+TE	3
	AMP+CZ+CTX+ATM	1
	AMP+CZ+S+CN	1
	AMP+CZ+S+STX	1
	AMP+NAL+CIP+EX	1
	AMP+AMC+S+CN	1
	AMP+S+TE+STX	1
	S+CN+C+TE	1
3	AMP+S+C	2
	AMP+CZ+C	1
	AMP+CZ+CTX	1
	AMP+AMC+C	1
	AMP+S+STX	1
	CN+NAL+STX	1

*AMP – ampicillin; AMC – amoxicillin; CZ – cefazolin, CTX – cefotaxime; ATM – aztreonam; S – streptomycin; CN – gentamicin; NAL – nalidixic acid; CIP – ciprofloxacin; EX – enrofloxacin; C – chloramphenicol; TE – tetracycline; DO – doxycycline; SXT – sulfamethoxazole-trimethoprim.

acid which was observed among collected CTX-resistant *E. coli* (Sukmawinata et al. 2019, Woerde et al. 2023). Moreover, our findings showed a significant association between CTX and CIP, which are critically important antimicrobials in both humans and animals. These findings are similar to with recent studies which also reported co-resistance of cephalosporin-resistant *E. coli* with aminoglycosides and quinolones from pets in Malaysia and Bangladesh (Dzulkifli et al. 2025, Rahman et al. 2025). Correlations identified between resistance phenotypes suggest potential co-occurrence of antimicrobial resistance traits within isolates. Nevertheless, these findings do not imply underlying genetic linkage or co-localization of resistance determinants and should be interpreted as preliminary associations that warrant further investigation using molecular approaches.

Most of the tested antibiotics in the same group showed significant correlation in their susceptibility profiles, but not for the aminoglycoside group (S and CN; Fig. 2A). Some studies reported that resistance to S is not automatically co-resistant to CN, which is consistent with our result (Marinho et al. 2016, Marchetti et al. 2021). This suggests that resistance

Table 5. Multidrug resistance pattern of 10 *E. faecalis* and 3 *E. faecium* isolated from Indonesian companion animals.

Amount of resistance	Pattern*	n
E. faecium		
7	P+AMP+CIP+EX+HLS+E+TE	1
6	P+AMP+CIP+EX+E+TE	1
4	P+AMP+EX+E	1
E. faecalis		
5	CIP+HLS+E+C+TE	1
4	CIP+HLS+E+FOS	1
4	LZD+CIP+EX+HLS	1
4	LZD+HLS+E+FOS	1
3	HLS+E+TE	1
3	HLS+C+TE	2
3	CIP+HLS+TE	1
3	HLS+E+TE	1
3	LZD+E+TE	1

* P – penicillin, AMP – ampicillin, CIP – ciprofloxacin, EX – enrofloxacin, C – chloramphenicol, TE – tetracycline, HLS – High-level streptomycin, E – erytromicin, FOS – fosfomycin.

to one aminoglycoside does not reliably predict susceptibility to the other in the present dataset.

The relatively high intermediate susceptibility rates observed in this study (intermediate susceptibility to E, CIP, EX and FOS) have also been noted in previous reports. The high proportion of intermediate susceptibility to E was reported from 58.2% (164/282) and 33.3% (13/51) of enterococci isolated from poultry and pasteurized milk, respectively, in Brazil (Fracalanzza et al. 2007), 37.9% of dogs from Italy (Stępień-Pyśniak et al. 2021), and 31.2% (30/96) of dogs from South Korea (Kwon et al. 2022). The high proportion of intermediate level for CIP (41.8%; 41/98) and EX (42.86%; 42/98) among enterococci isolates were also reported from chickens in Italy (Cagnoli et al. 2024). Additionally, 71.4% (10/14) of intermediate susceptibility to CIP from enterococci isolated from an animal hospital environment has been reported from Portugal. These results may reflect broader biological patterns rather than isolated methodological issues. *Enterococcus* species are widely recognized for their high resistance to quinolones, a feature that is often regarded as an inherent characteristic rather than merely an acquired trait (Kim et al. 2018). Although the specific underlying factors cannot be identified, isolates classified as intermediate may demonstrate different clinical responses, especially in cases of infection where exposure to antimicrobials at the infection site is inadequate (Rodloff et al. 2008). Given the presence of intermediate resistance, achieving the highest attainable antibiotic concentration at the infection site is essential to ensure therapeutic effectiveness (Kwon et al. 2022). Although intermediate susceptibility does not equate to clinical resistance, its prevalence may signal emerging selective pressure, highlighting the need for strengthened antimicrobial stewardship (Hombach et al. 2013).

Multidrug resistant *E. faecium* and *E. faecalis* have been reported among infections in hospitalized small companion animals, as reported in Southern Brazil (da Silva et al. 2024), where the proportion of MDR *E. faecium* was more frequent than *E. faecalis*. In contrast, our findings differed from those reported in Southern Brazil but were consistent with those from South Korea. Moon et al. (2023) similarly reported that MDR *E. faecalis* was more frequent than MDR *E. faecium* among companion animals in South Korea. Although an even lower proportion of MDR was shown by *E. faecium*, this species has been reported to be more resistant to antibiotics than *E. faecalis* (Kwon et al. 2022).

The occurrence of resistant *E. coli* and enterococci in companion animals has implications beyond individual clinical cases, such as treatment failure, long hospitalisation, and reduced therapeutic options (Tesin et al. 2023, Monteiro et al. 2025). Moreover, given the close interaction between companion animals and owners, resistant strains may be transmitted due to sharing the same living environment, strengthening the role of companion animals in the One Health context (Tóth et al. 2025). Environmental contamination via fecal shedding also further amplifies the ecological impact of AMR (Overgaauw et al. 2020).

The generated data provide important baseline information and contribute to raising awareness among veterinarians and animal owners of the prudent and responsible use of antimicrobials. Since genotypic

characterization was not conducted in this study, further investigations that incorporate genomic or molecular analyses are required to validate the present findings, elucidate the mechanisms and pathways through which AMR emerges and spreads, and determine the potential for transmission of resistant bacteria between animals and humans. Therefore, the conclusions of this study are limited to phenotypic resistance patterns and the relationship between antimicrobial susceptibility profiles in each indicator *E. coli* and enterococci. In addition to a substantial proportion of resistant isolates, the phi correlation analysis revealed structured co-occurrence patterns among non-susceptibility phenotypes. The observed network of associations underscores the importance of prudent antimicrobial use to prevent further consolidation of MDR phenotypes. These findings highlight the need to strengthen antimicrobial stewardship in companion animal practices, including more judicious antimicrobial prescribing and adherence to evidence-based treatment guidelines. Integrating companion animal data into national antimicrobial resistance surveillance systems within a One Health framework is recommended to enhance early detection and coordinated response strategies.

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Author Declarations

Ethics approval

This study was conducted with the approval of the Ethical Committee of IPB University (Approval number: 333/KEH/SKE/V/2025; 16 May 2025).

Use of generative artificial intelligence

No generative artificial intelligence tools were used in this study.

Conflict of interest

The authors declare that they have no conflicts of interest.

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