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Research Article

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Antimicrobial Resistance Patterns of *Escherichia coli* Strains in Pork at Traditional Markets in Gianyar Regency: Phenotypic and Genotypic Characterization

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ABSTRACT

Background: The microbiological safety of pork remains a critical public health concern, particularly in traditional retail markets where hygiene control and regulatory enforcement are limited. *Escherichia coli* indicates fecal contamination, causes foodborne illness, and harbors antimicrobial resistance (AMR).

Aims: This study determined the prevalence and antimicrobial resistance profiles of *Escherichia coli* in pork from Gianyar's traditional markets using phenotypic and molecular methods.

Methods: A cross-sectional study was conducted from January to June 2025 across ten traditional markets in seven sub-districts of Gianyar Regency. Nineteen fresh pork samples were collected aseptically and subjected to bacteriological analysis using Eosin Methylene Blue Agar, Gram staining, and IMViC biochemical testing. Antimicrobial susceptibility was assessed using the Kirby–Bauer disk diffusion method against ampicillin, tetracycline, chloramphenicol, and gentamicin. Molecular confirmation of *E. coli* was performed by polymerase chain reaction (PCR) targeting the *lacZ* gene. Data were analyzed descriptively and statistically using one-way ANOVA.

Results: *E. coli* was detected in 52.63% (10/19) of pork samples. Of these, 6 were genotypically confirmed. Antimicrobial susceptibility testing revealed high resistance to ampicillin and tetracycline, with intermediate susceptibility suggesting emerging resistance. Lower resistance levels to chloramphenicol and gentamicin indicate retained efficacy. The presence of multidrug-resistant isolates highlights the potential for foodborne dissemination of AMR.

Conclusion: In conclusion, pork sold in traditional markets in Gianyar Regency is frequently contaminated with *E. coli*, including strains exhibiting antimicrobial resistance.

INTRODUCTION

Pork is a widely consumed meat, but its safety is a concern due to its susceptibility to contamination by pathogenic *E. coli* (Kim et al., 2024). To address this, Indonesia's National Agency of Drug and Food Control (BPOM) established a policy under the 1966 HACCP system to reduce pathogens in meat and poultry products and prevent associated foodborne illnesses.

E. coli is a genetically diverse species comprising both benign and pathogenic serotypes (Desvaux et al., 2020). (Ahmed et al., 2011). For example, intestinal infections are often associated with serotype O157:H7 (EHEC). On the other hand, extra-intestinal infections are usually caused by a group of *E. coli* known as ExPEC (Frank et al., 2011; Mengistu & Mengesha, 2023). Critically, the spread of these diverse pathogenic strains is often

linked to antibiotic resistance, undermining therapeutic responses and threatening human, animal, and ecosystem health (Zheng et al., 2025).

Traditional markets serve as the socio-economic backbone of many Southeast Asian regions, providing access to fresh animal foods, generating livelihoods, and maintaining cultural identity through food. In Indonesia, particularly in Bali Province, pork is the most widely consumed animal product and is primarily traded through traditional retail markets. The presence of *E. coli* in pork, particularly in traditional markets, is strongly influenced by the level of sanitation, handling practices, and environmental conditions of the location (Thi Huyen Le et al., 2026). Recent research in Gianyar found that 72.7% of pork-based lawar samples were contaminated with *E. coli (Purnama et al., 2017)*.

Global trends show persistently high and increasing levels of resistance. The use of commercial antibiotics in animal feed can be a pathway for antibiotic residue contamination in pig meat and the environment (Chen et al., 2017; Udikovic-Kolic et al., 2014). The risk is exacerbated by the persistence of antibiotics in the environment and the processing of raw or undercooked pork in traditional foods, particularly in Bali. Consuming pork containing antibiotic-resistant E. coli can cause difficult-to-treat infections and increase the risk of the spread of antibiotic resistance in the community, resulting in *Multidrug resistance* (MDR) (Mila Meha et al., 2024).

This study employed an integrated chemical-biological approach to assess *E. coli* contamination and antibiotic resistance in pork from Gianyar's traditional markets. The objectives were to phenotypically and genotypically identify *E. coli* isolates and characterize their resistance patterns. The findings provide insights into farming practices and offer a scientific basis for targeted management and conservation strategies in pork production and traditional market environments.

MATERIALS AND METHODS

The Location of Study

This research was conducted for six months starting from January to June 2025 in traditional markets within the Gianyar Regency, Bali Province. Sample collection was conducted in traditional markets spread across 7 sub-districts in Gianyar Regency, namely Blahbatuh Village (1 market), Gianyar Village (1 market), Payangan Village (1 market), Sukawati Village (3 markets), Tampaksiring Village (2 markets), Tegalalang Village (1 market), and Ubud Village (1 market).

Samples Collection

A total of 19 samples were collected from 10 traditional markets, randomly selected based on the total number of pork vendors in each market. Fresh pork samples from each vendor were collected aseptically using sterile gloves, a *stainless steel* knife, and a sterile plastic container of 10 g, and then stored in a cooler box equipped with ice gel to maintain a temperature of 4°C. The samples were then transported to the Laboratory of Science of Universitas Dhyana Pura for further testing.

Research Procedure

Isolation and Identification of *E. coli*

E. coli isolation followed established protocols. Briefly, 10 g of pork was ground, homogenized in NaCl solution, and inoculated onto EMBA selective media (Oxoid, UK) at 37°C for 24 h. Grown colonies were purified on TSA media (Oxoid, UK). Presumptive *E. coli* were identified by Gram staining and confirmed via IMViC biochemical tests.

Antimicrobial Susceptibility Testing

Antibiotic resistance was tested using the Kirby-Bauer method. Confirmed *E. coli* isolates were suspended in 0.7 M NaCl to 0.5 McFarland standard and swabbed onto Mueller-Hinton Agar (MHA). Antibiotic discs tetracycline (30 µg), ampicillin (10 µg), gentamicin (30 µg), and chloramphenicol (30 µg) were placed 25 mm apart and incubated. To ensure the acquisition of representative data, antibiotic resistance testing for each isolate was carried out in three replicates.

DNA Extraction And Molecular Identification of *E. coli*

E. coli was identified by amplifying the DNA product with *lacZ* gene primers, followed by PCR analysis. The presence of *E. coli* in the sample was confirmed by targeting the *lacZ* gene through PCR amplification using primers (ZL-1675-F: TGAAAGCTGGCTACAGGAAGGCC and ZR-2548-R: CACCATG CCGTGGGTTT CAATATT) (Kumalasari et al., 2022). One loop of confirmed *E. coli* colony from EMBA medium was transferred to a 1.5 ml microcentrifuge tube filled with 200 µL of nuclease-free water (NFW). The suspension was then centrifuged at 15,000 rpm and 10°C for 5 minutes. The supernatant was discarded, and the resulting pellet was resuspended in 200 µL of NFW and vortexed until homogeneous. The mixture was then heated in a thermoblock at 95°C for 6 minutes. After heating, the sample was centrifuged

again under the same conditions (15,000 rpm, 10°C, 5 minutes). The clear supernatant containing the extracted DNA was finally pipetted and stored at -20°C until used for PCR amplification. PCR amplification targeted the *E. coli lacZ* gene (876 bp). A 12.5 µL reaction contained MyTaq™ HS Red Mix, primers, nuclease-free water, and DNA template. Cycling conditions were: initial denaturation at 94°C for 10 min; 30 cycles of 94°C for 60 s, 58°C for 65 s, and 72°C for 60 s; and final extension at 72°C for 5 min. Amplicons were visualized by electrophoresis on 2% agarose gel in 0.5× TBE buffer (Islam et al., 2025).

Data Analysis

The data obtained were analyzed descriptively to describe bacterial contamination in pork samples and bacterial resistance. One-Way ANOVA was used, followed by Duncan's test to examine the average differences in pork isolates' susceptibility to antibiotics using IBM SPSS V22 software.

RESULTS AND DISCUSSION

Phenotypic *E. coli* Isolates in Pork

A total of 19 pork samples collected from 20 vendors in 10 traditional markets in Gianyar Regency showed that 10 samples (52.63%) tested positive for *E. coli* after being cultured on EMBA media. However, IMVIC test results showed that of the three *E. coli* isolates successfully cultured from Gianyar's public markets, only 2 (10.5%) tested positive. Two positive isolates (10.5%) were from Sayan Ubud Market, one positive isolate (5.2%) from Tampaksiring Traditional Village Market, one positive isolate (5.2%) from Payangan Market, two positive isolates (10.5%) from Sukawati Traditional Market, and two positive isolates (10.5%) from Bulan Market. Overall, 10 samples tested positive by IMVIC, which will be further tested for antibiotic resistance (Table 1).

On EMBA media, presumptive *E. coli* grew as metallic green colonies (Figure 1A), indicating lactose/sucrose fermentation which lowers pH and causes dye precipitation. Ten isolates were biochemically confirmed as *E. coli* via the IMViC test, showing the characteristic pattern: indole (+), methyl red (+), Voges-Proskauer (−), and citrate (−). This profile differentiates *E. coli* from other enteric bacteria like *Enterobacter* based on carbohydrate metabolism differences. Animal-derived foods, particularly pork and its derivatives, can transmit foodborne infections due to their diverse microbial populations, posing serious health risks. Research links the consumption of *E. coli*-contaminated pork products to urinary tract infections, sepsis, and neonatal meningitis (Khan et al., 2017). Contamination data presented in the results table indicate an *E. coli* contamination rate of 52.63% in fresh pork samples from a traditional market in Gianyar Regency. Compared with previous research in Indonesia, particularly in Bali, over the past five years, these results are relatively consistent but demonstrate variations in prevalence influenced by market location and conditions. A similar study conducted at Kreneng Traditional Market in Denpasar revealed a 40% *E. coli* contamination rate in fresh pork, lower than the results of this study. Meanwhile, another study found a 67% *E. coli* contamination rate in the traditional pork-based dish "lawar barak" (red lawar) in Denpasar (Yulianto et al., 2019). Research in Badung Regency showed a 20% *E. coli* prevalence in broiler chicken, lower than pork, but still indicating a risk of contamination from animal products in traditional markets in Bali (Septryani Sidebang et al., 2021).

The variability of *E. coli* contamination observed in the samples is significantly influenced by sanitary conditions and differences among traditional markets within Gianyar Regency. Specifically, the environmental conditions of the markets and the application of hygiene and sanitation protocols in the traditional markets of Gianyar, Sayan Ubud, Tampaksiring, Sukawati, Batu Bulan, and Payangan warrant further attention and improvement. This recommendation is underscored by the high level of interpersonal contact between vendors and customers, the current lack of adequate sanitation measures, and the necessity for stricter enforcement of meat quality monitoring standards. Inadequate sanitation and hygiene practices among vendors and the market environment are the main factors contributing to the high *E. coli* contamination in fresh pork products (Magqupu et al., 2024).

This contamination can also be attributed to unhygienic traditional processing and the use of contaminated raw materials (Bonilla-Luque et al., 2023). Previous studies have found varying prevalence rates, suggesting that *E. coli* is more prevalent in areas central to the food supply chain, including pork (Blanco-Lizarazo & Sierra-Cadavid, 2023; Gonçalves et al., 2024; Saechue et al., 2024). Geographical differences, husbandry practices, and pig farm management systems are suspected to contribute to the prevalence variations (Holt et al., 2019). Regions with intensive or large-scale pig farms have the potential to increase *E.*

coli transmission among pig populations (Wen et al., 2024; Xin et al., 2022).

Antimicrobial Resistance Testing

The results of the resistance testing of E. *coli* isolates obtained from pork in traditional markets to several types of antibiotics. The antibiotics tested consisted of ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), and gentamicin (30 µg) are shown in Table 2.

E. coli isolates obtained from pork samples in traditional markets in Gianyar Regency in this study showed quite high levels of resistance. The percentage of *E. coli* isolates resistant to ampicillin, tetracycline, chloramphenicol, and gentamicin reached more than 50% (Table 2), as indicated by the average obtained from the statistical standard deviation of each antibiotic isolate. Ampicillin exhibited high resistance (47.4%; $p < 0.05$), indicating that β-lactam resistance is a major concern among *E. coli* isolates from traditional market pork. Tetracycline also showed significant resistance, with numerous intermediate isolates suggesting a progressive resistance trend. In contrast, chloramphenicol and gentamicin retained low resistance levels, indicating their continued therapeutic efficacy.

The prevalence of *E. coli* in fresh pork in traditional markets in Gianyar Regency showed a positive sample of 52.63%. Pork samples positive for *E. coli* are closely linked to the phenomenon of antibiotic resistance. The presence of antibiotic residues in raw pork may also be attributed to antibiotic usage practices on pig farms,

Table 1. *E. coli* contamination in pork from traditional markets in Gianyar Regency based on phenotype

No	Sample Sources	EMBA	IMVIC test results						IMVIC test description
			SIM			MR	VF	C	
			Sulfur	Indol	Motil				
1	Pork from Gianyar Market I (M1)	+	-	+	+	+	-	-	Positive
2	Pork from Gianyar Market 2 (M2)	+	-	+	+	+	-	-	Positive
3	Pork from Gianyar Market 3 (M3)	-	-	-	-	-	-	-	Negative
4	Pork from Blahbatuh Market I (M4)	-	-	-	-	-	-	-	Negative
5	Pork from Blahbatuh Market II (M5)	-	-	-	-	-	-	-	Negative
6	Pork from Sayan Ubud Market I (M6)	+	-	+	+	+	-	-	Positive
7	Pork from Sayan Ubud Market II (M7)	+	-	+	+	+	-	-	Positive
8	Pork from Tampaksiring Market I (M8)	+	-	+	+	+	-	-	Positive
9	Pork from Pejeng Market I (M9)	-	-	-	-	-	-	-	Negative
10	Pork from Tegalalang Market I (M10)	-	-	-	-	-	-	-	Negative
11	Pork from Tegalalang Market II (M11)	-	-	-	-	-	-	-	Negative
12	Pork from Sukawati Market I (M12)	+	-	+	+	+	-	-	Positive
13	Pork from Sukawati Market II (M13)	+	-	+	+	+	-	-	Positive
14	Pork from Singapadu Market I (M14)	-	-	-	-	-	-	-	Negative
15	Pork from Singapadu Market II (M15)	-	-	-	-	-	-	-	Negative
16	Pork from Bulan Market I (M16)	+	+	+	+	-	-	+	Positive
17	Pork from Bulan Market II (M17)	+	+	+	+	-	-	+	Positive
18	Pork from Payangan Market I (M18)	+	+	+	+	-	-	+	Positive
19	Pork from Payangan Market II (M19)	-	-	-	-	-	-	-	Negative
Total Number Positive Percentage (Persentage %)		10 (52,63%)							

especially along the pork supply chain operating within Gianyar Regency. To bridge this knowledge gap, further investigations are required, with particular emphasis on evaluating contamination levels, resistance profiles, and the associated resistance-encoding genes within the pig farming environment of Gianyar Regency, Bali. Widespread contamination allows resistant bacteria to spread through the food chain, increasing the risk of difficult-to-treat infections in humans and animals (Conceição et al., 2023; Lautan et al., 2025). Furthermore, uncontrolled antibiotic use in pig farming is a major factor driving the selection of resistant bacteria (Hmidi et al., 2025). The percentage of *E. coli* isolates resistant to ampicillin, tetracycline, chloramphenicol, and gentamicin reached more than 50%. This is evident from the average value obtained from the statistical calculation of the standard deviation of each antibiotic isolate. Similar research revealed that *Klebsiella spp*. *E. coli* isolated from broiler chickens in Shandong Province, China, was resistant to 98.9% of ampicillin, 80.0% of ciprofloxacin, 78.9% of tetracycline, and 92.2% of chloramphenicol (Li et al., 2022).

Several previous studies confirmed that *E. coli* isolates from pigs and fresh pork products exhibited significant resistance patterns to various antibiotics, including ampicillin, streptomycin, tetracycline, and gentamicin. For example, a study in Tabanan Regency by Nugraha et al. (2013) reported MDR *E. coli* isolates resistant to streptomycin, chloramphenicol, gentamicin, and sulfamethoxazole. Another study by (Ardiana et al., 2023) in Badung Regency, Bali, revealed that 100% of *E. coli* isolates from pig feces and barn waste showed resistance to penicillin and high levels of resistance to streptomycin (80%) and tetracycline (60%). Furthermore, a study by (Meha et al., 2024) showed that *E. coli* isolates from pork were multidrug resistant (MDR), resistant to 2 to 3 classes of antibiotics, including ampicillin and tetracycline.

Antimicrobial resistance (AMR) is caused by antimicrobial resistance genes (ARGs)) (Badescu et al., 2022). These genes can spread either through inheritance from parent to offspring (vertical gene transfer) or through gene exchange between unrelated bacteria (horizontal gene transfer) (Biondo, 2023; Urban-Chmiel et al., 2022). This horizontal transfer process can occur through several mechanisms, such as transformation, transduction, or conjugation with other bacteria in the environment. Generally, ARGs are found in mobile genetic elements (MGEs) such as integrons, transposons, plasmids, or phages, and act as additional components (accessories) in the bacterial genetic material (Olsen & Riber, 2025). A further impact of this condition is the emergence of multiresistant (MDR) bacteria, namely bacteria that are resistant to three or more types of antimicrobials. This poses a serious public health problem (Pérez-Rodríguez & Mercanoglu Taban, 2019;

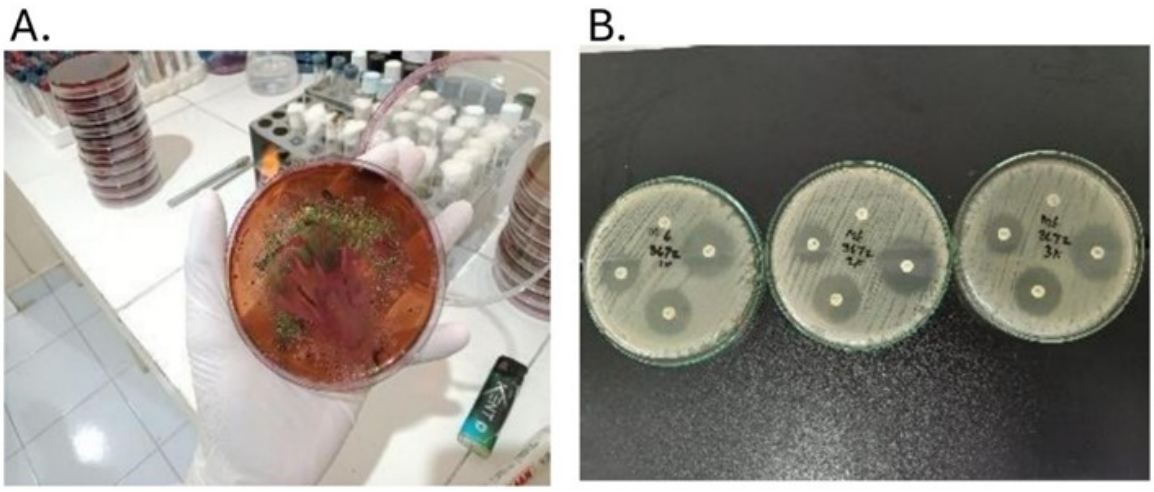

Figure 1. *E. coli* isolates in pork at traditional markets in Gianyar Regency. (A). *E. coli* in Eosin Methylene Blue Agar (EMBA) culture and (B). Antimicrobial resistance test of *E. coli* isolates.

Rafailidis & Kofteridis, 2022). Infections caused by multidrug-resistant (MDR) bacteria are difficult to treat because increased AMR leads to a high risk of treatment failure, disease relapse, prolonged hospitalization, and excessive patient deterioration (Gargiullo et al., 2019). In the context of this study, E. coli isolates from pork emphasize that multidrug-resistant (MDR) is a major concern because it can hinder the effectiveness of antibiotic therapy and increase the risk of resistance spreading to humans through the food chain. The presence of multidrug-resistant (MDR) *E. coli* in pigs has the potential to become a reservoir of resistance that can spread to other pathogenic bacteria, increasing the risk of

Table 2. Results of antimicrobial resistance tests of positive *E. coli* isolates

Sample Code	Ampicilin (AMP 10 µg)	Tetracyclin (TE 30 µg)	Chloramphenicol (C 30 µg)	Gentamycin (CN 30 µg)	Description
M1	0,00±0,00^{a,} *	7,77±0,70^{b,} *	9,89±5,63^{c,} *	18,28±0,89$^{d,\#}$	MDR
M2	0,00±0,00^{a,} *	6,66±0,16^{bc,} *	7.78±0,44^{a,} *	19,98±0,25$^{c,\#}$	MDR
M6	0,00±0,00^{a,} *	21,12±1,05$^{f,\#}$	23,36±0,30^{j,} *	18,15±0,67$^{d,\#}$	Tidak MDR
M7	0,00±0,00^{a,} *	9,78±0,07^{d,} *	23,33±0,42$^{j,\#}$	18,18±0,34$^{d,\#}$	Tidak MDR
M8	8,30±0,61^{a,} *	0,00±0,00^{a,} *	20,93±0,48$^{c,\#}$	17,05±0,27$^{c,\#}$	Tidak MDR
M12	12,53±0,14^{a,} *	18,82±0,39$^{c,\#}$	20,05±0,32$^{d,\#}$	18,21±9,90$^{d,\#}$	Tidak MDR
M13	0,00±0,00^{a,} *	6,65±0,28^{b,} *	8,68±0,25^{b,} *	17,73±0,36$^{c,\#}$	MDR
M16	0,00±0,00^{a,} *	20,98±0,09$^{f,\#}$	22,96±0,19$^{j,\#}$	6,67±0,03^{b,} *	Tidak MDR
M17	0,00±0,00^{a,} *	20,34±0,71$^{f,\#}$	21,95±0,26$^{f,\#}$	17,80±0,18$^{c,\#}$	Tidak MDR
M18	0,00±0,00^{a,} *	0,00±0,00^{a,} *	8,31±0,39^{b,} *	0,00±0,00^{a,} *	MDR

Note: Values are expressed as mean±standard deviation (n=3). Letters a, b, etc. indicate significant differences in values based on One Way ANOVA followed by Duncan's test ($p<0.05$). *: resistant; #: sensitive. MDR if resistant to ≥ 3 types of antibiotics.

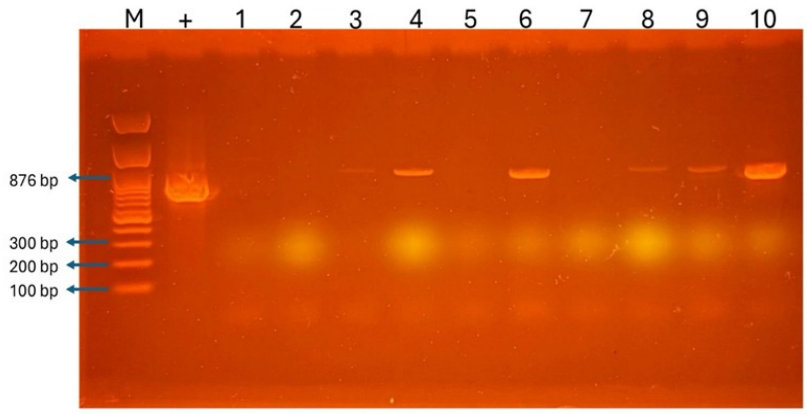

Figure 2. Results of PCR amplification of the *lacZ* gene from total genomic DNA isolated from positive *E. coli* samples. Lane M marker for DNA molecular weight, Lane + positive control *E. coli*, Lane 1-10 PCR amplification product (876 bp).

antibiotic therapy failure in humans and animals (Kallau et al., 2018).

Genotyping of *E. coli* isolates

Ten isolates were confirmed positive based on phenotypic and biochemical tests, followed by confirmatory testing based on PCR amplification results targeting the *lacZ* gene. Of the 10 isolates that tested positive on EMBA media and the IMViC test, only six isolates showed positive results targeting the *lacZ* gene (**Figure 2**). This study identified 6 of 10 identified *E. coli* isolates as encoding the *lacZ* gene based on genotypic identification. The *lacZ* gene is a gene found in *E. coli* bacteria that encodes the β-galactosidase enzyme (Hamed et al., 2020). This mini-enzyme breaks down lactose into glucose and galactose, which the bacteria can then use as an energy source (Chon et al., 2020; Király et al., 2025). The absence of bands in the other four *E. coli* isolates could be due to low DNA concentration, DNA degradation, or problems with the amplification process (PCR).

The presence of PCR inhibitors or genetic variation among the four isolates represents an alternative explanation that may have influenced the PCR amplification success rates observed in this study. Further investigation is required to incorporate a negative control into the PCR assay, thereby enabling the detection of potential contamination during the PCR reaction. Because the negative control contains no DNA template, its inclusion would support the inference that the failure of isolate samples to amplify in this PCR process may be attributable to contamination arising during DNA extraction. Additionally, the use of a negative control in future research would strengthen quality assurance by preventing false-positive outcomes from samples.

CONCLUSION

This study confirmed *E. coli* contamination in pork from Gianyar's traditional markets, including multidrug-resistant (MDR) strains harboring the *lacZ* gene. These findings underscore a public health risk, particularly if pork is consumed raw or undercooked. Future studies should focus on detecting ESBL-producing *E. coli* and characterizing resistance genes (*bla*TEM, *bla*SHV, *bla*CTX-M) to understand horizontal gene transfer and zoonotic transmission risks.

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AUTHORS CONTRIBUTION

N.W.A.W. contributed to manuscript drafting, manuscript review and editing, validation, and investigation. P.A.W., I.M.W.A.P., and I.G.W. contributed to conceptualization, formal analysis, and preparation of the original manuscript draft. P.E.S., M.K.J.K., and N.S.D.P. contributed to manuscript review and editing, validation, and formal analysis. All authors read and approved the final manuscript.

"The author declares that there is no conflict of interest with the parties involved in this research".

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