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Detection of *iss* virulence gene in Avian Pathogenic *Escherichia coli* multidrug resistance from quail cloacal swabs in traditional markets in Surabaya, Indonesia

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ABSTRACT

Quail is a source of animal protein that is widely consumed in Indonesia. Quail is susceptible to *Escherichia coli* bacterial infection, especially the Avian Pathogenic *Escherichia coli* (APEC) strain which can cause colibacillosis and is zoonotic. This infection is characterized by symptoms of pericarditis, perihepatitis, salpingitis, and colisepticemia. Antibiotic resistance in APEC is a serious challenge, including Multidrug Resistance (MDR), which reduces the effectiveness of treatment and increases the risk of pathogen spread. This study aimed to detect the *iss* virulence gene in APEC isolates from quail cloacal swabs in five areas of Surabaya. The identification results showed a prevalence of *E. coli* of 98% (138/140) with the highest antibiotic resistance levels in erythromycin (61%), streptomycin (37%), ciprofloxacin (35%), tetracycline (22%), and aztreonam (14%). The occurrence of MDR was found in 18% of isolates, with the highest resistance pattern ATM/CIP/S/E (32%). PCR test showed (20%) isolates have the *iss* virulence gene. The *iss* gene in APEC is known to increase the ability of bacteria to survive in blood serum, thus potentially affecting poultry health and causing zoonotic risks. This study revealed high levels of antibiotic resistance, MDR, and the presence of dangerous pathogen virulence genes in *E. coli* isolates from Surabaya traditional markets. The need for antibiotic surveillance and implementation of biosecurity according to the One Health concept to prevent the spread of resistant pathogenic bacteria and their impact on human, animal, and environmental health.

Introduction

Quail is an important source of animal protein and is widely consumed by the Indonesian people (Lokapirnasari *et al*., 2024). Quails are farmed in large numbers for food purposes including eggs and meat. The high animal protein in quail meat makes it the basic ingredient for making raw food feed (pet feed), in addition quail feces are used for animal feed and for making fertilizer (Vargas-Sánchez *et al*., 2019). Quails are included in the group of poultry that are susceptible to *Escherichia coli* bacterial infections, one of which is as a cause of colibacillosis (El-Ghany, 2019).

Symptoms of colibacillosis due to pathogenic *E. coli* in quail include pericarditis, perihepatitis, salpingitis, salpingoperitonitis, colisepticemia, and airsaculitis infection (Dziva and Stevens, 2008). Treatment management that is usually carried out involves the use of antibiotics to treat infections, but inappropriate or excessive use can cause antibiotic resistance and potentially cause residues that are harmful to public health and the environment (Widodo *et al*., 2022).

Antibiotic resistance is a serious problem that affects the health of poultry consumption and food safety. The main challenge in the world of health is the emergence of infections by pathogenic *E. coli* in poultry, especially Avian Pathogenic *Escherichia coli* (APEC) (Khairullah *et al*., 2024). APEC strains are one of the agents that cause dangerous diseases for quail because they contribute to antibiotic resistance to the cause of Multidrug Resistant (MDR). The occurrence of MDR is the occurrence of resistance to three or more classes of antibiotics (Ramandinianto *et al*., 2020; Effendi *et al*., 2021). According to Ma *et al*. (2023) in Egypt there has been MDR in quail in three classes of antibiotics, amphenicol (90%), aminoglycoside (80%), beta-lactam (40%). According to Nehru and Pranav (2024) that the occurrence of MDR in quail in India is in the cephalosporine (90%), beta-lactam (70%), and fluroquinolone (40%) groups. This incident has the potential to worsen the impact of infection, reduce the effectiveness of treatment, and increase the risk of spreading dangerous pathogens.

APEC is one of the agents that causes colibacillosis in poultry, especially in quail. *E. coli* pathogens in quail have many virulence factors, which play a role in the infection process and pathogenicity (Kalule *et al*., 2018). According to Ewers *et al*. (2007) virulence genes (*iro*N, *tra*T, *iuc*D, *cvi/cva*, *ibe*A, *gim*B, *tia*, *neu*C, *kps*MTII, *tsh*, *iss*, *sit*D, *chu*A, *fyu*A, *irp*2, *vat*, *mal*X, and *pic*) are present in APEC isolates. One of the APEC virulence genes is Increased Serum Survival (*iss*) (Kathayat *et al*., 2021). The *iss* gene contributes to bacterial resistance to serum complement activity by producing a protein that has a characteristic signal sequence in the outer membrane protein (OMP) and encodes a bacterial outer membrane lipoprotein, the protein produced helps bacteria survive in the host blood serum by inhibiting components of the complement system that normally kill bacteria (Biran *et al*., 2021).

The highest *iss* virulence gene in quail was 90.3% in septicemic and 64.3% in fecal isolate samples taken in Iran (Badouei *et al*., 2015). According to research conducted in Iran by Fasaei *et al*. (2019) the *iss* gene

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in APEC isolates was 68.2% and was found in 24.5% feces. According to Khusnan *et al*. (2021) *E. coli* isolates in quail in Indonesia had an *iss* gene of 61.9%.

Various markets in the Surabaya area, as one of the distribution and sales centers of quail, have the potential to become a place for the spread of pathogens and antibiotic resistance. The presence of APEC bacteria in quail has not been widely reported in Indonesia, so that in the isolation of *E. coli* identification, the occurrence of MDR and molecular virulence gene *iss* in poultry based on symptoms of colibacillosis disease caused by APEC. This study was conducted to determine the virulence gene *iss* in APEC quail in Indonesia.

Materials and methods

Ethical approval

Ethical approval for this study was obtained from the Animal Ethics Committee, Faculty of Veterinary Medicine, Wijaya Kusuma University Surabaya, Indonesia (Ethics Number: 170-KKE-2025).

Location and time of study

This study was conducted from November 2024 to December 2024, at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Wijaya Kusuma University, Surabaya. Polymerase Chain Reaction (PCR) tests were conducted at the Institute of Tropical Disease, Airlangga University.

Sample collection

The number of samples used was 140 quail cloacal swabs taken from various areas of Surabaya including Central Surabaya, South Surabaya, East Surabaya, West Surabaya, and North Surabaya. Fecal samples were taken from quail cloacal swabs using sterile cotton swabs (Onemed, Indonesia). Quail fecal swab samples were then inserted into enrichment media, namely, test tubes containing Buffer Pepton Water (BPW) (HiMedia) during transportation. All samples were transported using a thermobox at a temperature of 4°C (Putri *et al*., 2024).

Isolation and identification of E. coli

Isolation of *E. coli* bacteria using selective media Eosin methylene Blue Agar (EMBA) (HiMedia M317) and Mac Conkey Agar (MCA) (HiMedia MH081). EMB *E. coli* media has a morphology like metallic green, there is a black dot in the middle, while in MCA the morphological characteristics of *E. coli* colonies are small, round, separate, irregular, and pink. The bacterial isolation samples were then incubated at 37°C for 18-24 hours. Identification of the morphology of *E. coli* was carried out through Gram staining, then continued with biochemical tests using Triple Sugar Iron Agar (TSIA) (HiMedia M021), Simmons Citrate Agar (SCA) (HiMedia M099), and IMVIC media, such as Sulfide Indole Motility (SIM) (HiMedia M181), Methyl Red (MR), and Voges-Proskauer (VP) (Himedia GM070) (Wibisono *et al*., 2021; Yanestria *et al*., 2022).

Antibiotic sensitivity test

Antibiotic sensitivity testing was carried out using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) media (HiMedia M173) according to the Clinical Laboratory Standards Institute (CLSI, 2022) standards. The antibiotic paper disks used consisted of five groups, namely aztreonam 30 µg (Oxoid CT0264B) beta-lactam group, ciprofloxacin 5 µg (Oxoid CT0425B) fluoroquinolone group, tetracycline 30 µg tetracycline group (Oxoid CT0054B), streptomycin 10 µg (Oxoid CT0047B) aminoglycoside group, erythromycin 15 µg (Oxoid CT054B) macrolide group. Pure bacterial colonies obtained from EMBA media were taken as many as one to two colonies using a sterile loop, then placed in a physiological NaCl solution with a McFarland standard of 0.5 (1.5×10^8 CFU/ml). Culture was carried out using a sterile cotton swab that was scratched on the entire surface of the MHA media in a petri dish. The media was divided into five parts, and antibiotic paper disks were placed in each part according to the procedure (Musa *et al*., 2020). The MHA media containing antibiotics was then incubated at 37°C for 24 hours. Interpretation of the inhibition zone diameter results was carried out based on CLSI standards (CLSI, 2022; Waruwu *et al*., 2023; Widodo *et al*., 2023). An isolate is declared as MDR if the bacteria show resistance to three or more different classes of antibiotics (Khairullah *et al*., 2019; Putri *et al*., 2023; Wibisono *et al*., 2022).

Characteristics of the iss gene using PCR

DNA extraction was performed using the QIAamp® DNA Kit (QIAGEN, Germany). The forward primer used was AAGTCAAAGCAGGGGTTGCCCG, while the reverse primer was GATCGCCGACATTAAGACGCAG, with a target amplification length of 550 bp. The thermal cycler protocol included pre-denaturation at 94°C for 7 minutes, followed by denaturation at 94°C for 1 minute, annealing at 63°C for 30 seconds, and extension at 72°C for 30 seconds. The cycle was repeated 35 times, ending with a final extension at 72°C for 5 minutes. Before amplification using PCR, each primer was calibrated to determine the optimal annealing temperature. The amplification results (amplicons) were then visualized by electrophoresis using 2% agarose gel. The amplification results in the form of DNA were visualized by electrophoresis on a 1% agarose gel of 0.75g. The electrophoresis process was carried out on an electrophoresis machine at a pressure of 100 V, 400 mA, for 30 minutes. After electrophoresis, the DNA fragment bands can be observed with a UV transilluminator which aims to determine the length of the DNA. The size of the PCR DNA results is compared with the marker (ladder) to be further documented using a camera (Farizqi *et al*., 2023).

Data analysis

Data analysis using a descriptive approach without the use of software.

Results

Based on the results of the isolation and identification of *E. coli* bacteria in this study, 98% (138/140) were positive for *E. coli* and 2% (2/140) were negative in quail cloaca swabs from five areas in Surabaya (Table 1).

Table 1. Results of identification of *E. coli* bacteria from quail cloaca swabs at Surabaya traditional market.

Market location	Number of samples	*E. coli* (%)	
		Positive	Negative
Central Surabaya	20	100% (20/20)	0% (0/20)
East Surabaya	30	100% (30/30)	0% (0/30)
North Surabaya	30	100% (30/30)	0% (0/30)
South Surabaya	30	97% (29/30)	3% (1/30)
West Surabaya	30	97% (29/30)	3% (1/30)
Total	140	98% (138/140)	2% (2/140)

The results of the isolation and identification tests of *E. coli* bacteria originating from quail cloaca swabs from five areas in Surabaya showed that the high percentage of *E. coli* bacteria came from Central Surabaya at 100% (20/20), East, and North at 100% (30/30), followed by South and West at 97% (29/30).

Sampling was carried out using Buffered Pepton Water (BPW) enrichment media. The samples were then taken to the laboratory for testing

using a cool box at a temperature of 4°C. The initial isolation process was carried out on EMBA media, where the *E. coli* colonies were characterized by a metallic green morphology and a black dot in the middle (Figure 1A). Furthermore, purification isolation was carried out on MCA media in the second and third stages, with the characteristics of small, round, separate, irregular, and pink colonies (Figure 1B).

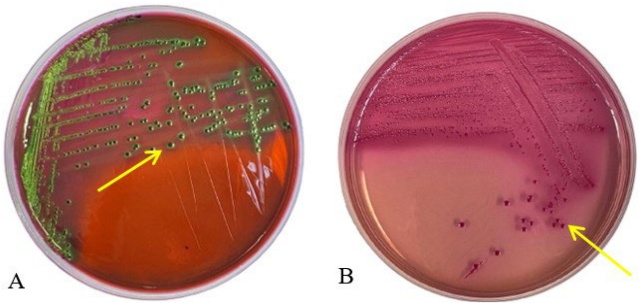

Figure 1. Colony of *E. coli* bacteria on EMBA media (A), Colony of *E. coli* bacteria on MCA media (B).

The results of antibiotic resistance tests of five different antibiotic groups obtained from quail cloacal swabs showed that the highest incidence occurred in the antibiotic Erythromycin with a percentage of 61% (84/138), then antibiotic resistance in streptomycin 37% (51/138), antibiotic ciprofloxacin 35% (49/138), antibiotic tetracycline 22% (33/138), while the antibiotic with the lowest incidence of resistance was aztreonam 14% (19/138) (Table 2).

The results of the MDR test on quail cloaca swabs from five areas in Surabaya showed an MDR incidence of 18% (25/138) (Figure 2). The highest percentage was in Central Surabaya 25% (5/20), second place was West Surabaya 21% (6/29), third place was North Surabaya 20% (6/30), followed by East Surabaya 16% (5/30), and the lowest MDR percentage was in South Surabaya 10% (3/29) (Table 3).

The occurrence of MDR in *E. coli* isolates with the highest to lowest resistance patterns including ATM/CIP/S/E there were 8 samples (8/25; 32%), consisting of PSP codes 24, PSP 28, PSS 22, PST 22, PST 28, PST 29, PST 30, and PSU 25. This was followed by CIP/S/E as many as 4 samples (4/25; 16%), consisting of PST 20, PSB 12, PSB 21, and PSU 21. Then the

Table 2. Identification of antibiotic resistance against *E. coli* bacteria.

Market location	Number of samples	Amount of resistance				
		ATM	CIP	TE	S	E
Central Surabaya	20	4/20 (20%)	8/20 (40%)	4/20 (20%)	8/20 (40%)	15/20 (75%)
East Surabaya	30	3/30 (10%)	7/30 (23%)	6/30 (20%)	11/30 (36%)	21/30 (70%)
North Surabaya	30	6/30 (20%)	6/30 (20%)	3/30 (10%)	12/30 (40%)	14/30 (46%)
South Surabaya	29	1/29 (3%)	16/29 (55%)	8/29 (27%)	10/29 (34%)	15/29 (51%)
West Surabaya	29	5/29 (17%)	12/29 (41%)	9/29 (31%)	10/29 (34%)	19/29 (65%)
Total	138	19/138 (14%)	49/138 (35%)	33/138 (22%)	51/138 (37%)	84/138 (61%)

Note: ATM: Aztreonam, CIP: Ciprofloxacin, TE: Teracycline, S: Streptomycin, and E: Erythromycin

CIP/TE/E resistance pattern with sample codes PSS 10, PSB 9, and PSB 10; ATM/CIP/TE/S/E sample codes PSP 29, PSS 21, and PSU 26; TE/S/E sample code PSP 27, PSB 11, and PSU 2, each resistance pattern contained 3 samples (3/25; 12%). ATM/TE/E resistance pattern contained 2 samples (2/25; 8%) consisting of sample codes PSU 23 and PSU 24. ATM/TE/S/E resistance pattern sample code PSP 25; CIP/TE/S/E sample code PSB 21, each resistance pattern contained 1 sample (1/25; 4%) (Table 4).

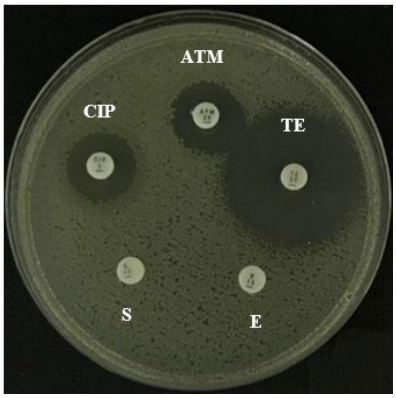

Figure 2. *E. coli* multidrug resistance. Description S: Streptomycin; TE: Tetracycline; E: Erythromycin; ATM: Aztreonam; CIP: Ciprofloxacin.

Table 3. Identification of MDR Antibiotics against *E. coli* Bacteria

Market location	Number of samples	MDR
Central Surabaya	20	5/20 (25%)
East Surabaya	30	5/30 (16%)
North Surabaya	30	6/30 (20%)
South Surabaya	29	3/29 (10%)
West Surabaya	29	6/29 (21%)
Total	138	25/138 (18%)

Table 4. MDR *E. coli* resistance patterns

Sample code	Antibiotic resistance					Resistance pattern
	ATM	CIP	TE	S	E	
PSP 24	R	R	S	R	R	ATM/CIP/S/E
PSP 25	R	S	R	R	R	ATM/TE/S/E
PSP 27	S	S	R	R	R	TE/S/E
PSP 28	R	R	S	R	R	ATM/CIP/S/E
PSP 29	R	R	R	R	R	ATM/CIP/TE/S/E
PSS 10	S	R	R	S	R	CIP/TE/E
PSS 21	R	R	R	R	R	ATM/CIP/TE/S/E
PSS 22	R	R	S	R	R	ATM/CIP/S/E
PST 20	S	R	S	R	R	CIP/S/E
PST 22	R	R	S	R	R	ATM/CIP/S/E
PST 28	R	R	S	R	R	ATM/CIP/S/E
PST 29	R	R	S	R	R	ATM/CIP/S/E
PST 30	R	R	S	R	R	ATM/CIP/S/E
PSB 9	S	R	R	S	R	CIP/TE/E
PSB 10	S	R	R	S	R	CIP/TE/E
PSB 11	S	S	R	R	R	TE/S/E
PSB 12	S	R	S	R	R	CIP/S/E
PSB 21	S	R	S	R	R	CIP/S/E
PSB 22	S	R	R	R	R	CIP/TE/S/E
PSU 2	S	S	R	R	R	TE/S/E
PSU 21	S	R	S	R	R	CIP/S/E
PSU 23	R	S	R	S	R	ATM/TE/E
PSU 24	R	S	R	S	R	ATM/TE/E
PSU 25	R	R	S	R	R	ATM/CIP/S/E
PSU 26	R	R	R	R	R	ATM/CIP/TE/S/E

Note: ATM: Aztreonam, CIP: Ciprofloxacin, TE: Teracycline, S: Streptomycin, E: Erythromycin, R: Resistance, S: Sensitive

Based on the results of this study, 5/25 *E. coli* isolates from quail cloaca swabs showed a positive band at 550 bp, so that 20% of *E. coli* isolates had the *iss* virulence gene in this study (Figure 3 and Figure 4).

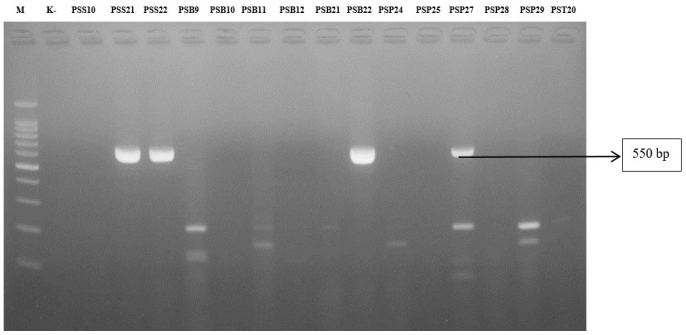

Figure 3. PCR results of *iss* gene of *E. coli* isolates positive at band 550 bp. Description; PSS (South Surabaya Market), PSB (West Surabaya Market), PSP (Central Surabaya Market), PST (East Surabaya Market), PSU (North Surabaya Market).

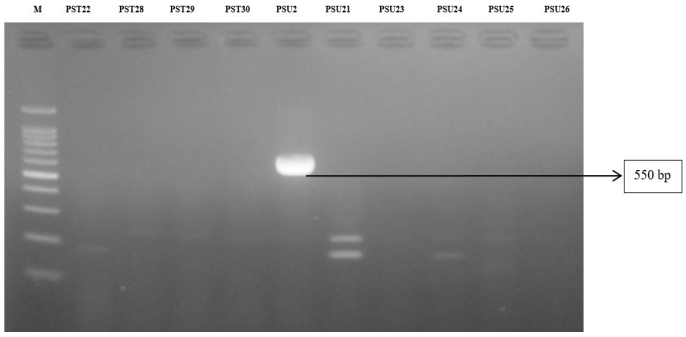

Figure 4. PCR results of *iss* gene of *E. coli* isolates positive at band 550 bp. Description; PSS (South Surabaya Market), PSB (West Surabaya Market), PSP (Central Surabaya Market), PST (East Surabaya Market), PSU (North Surabaya Market).

Discussion

E. coli resistance to antibiotics is a major problem in animal and public health. The mechanisms of *E. coli* resistance include mutations in genetic material or horizontal gene transfer, gene transfer through conjugation, transformation, or transduction, types of pathogenic strains, and the presence of certain virulence genes (Mancuso *et al*., 2021). This resistance causes decreased treatment effectiveness, increased mortality, and higher health care costs. Transmission of resistant bacteria can occur through water, waste, food, and direct contact (Gay *et al*., 2023). In line with research conducted by Geurtsen *et al*. (2022) *E. coli* has the ability to survive as a commensal organism as well as a cause of serious, potentially fatal infections in humans and animals. The ability of *E. coli* to have virulence factors that can cause serious infections (Widodo *et al*., 2024).

This study aims to detect the *iss* multidrug resistance virulence gene in APEC from quail cloacal swab samples in five areas of Surabaya. The results of the study identified *E. coli* bacteria in various areas in Surabaya using quail cloacal swab samples from Central, North, South, East, and West Surabaya. Showing a high percentage in Central Surabaya, East Surabaya, and North Surabaya is the highest result of 100% positive *E. coli*, while in South and West Surabaya the lowest incidence of *E. coli* bacteria was 97%. Based on the results of total isolation and identification from five markets in the Surabaya area, it showed that *E. coli* bacteria obtained from quail cloacal swabs were 98% (138/140). According to Ma *et al*. (2023) a study conducted in Egypt showed that quail samples taken from liver and gizzard swabs had the highest prevalence of *E. coli* (37.5%) and (25%). According to Al-Amin *et al*. (2022) in Bangladesh, *E. coli* was found in the quail cloaca fecal swab samples (82.22%). The results obtained from quail cloaca swabs in the Surabaya area, Indonesia showed a higher percentage than in Egypt and Bangladesh.

E. coli has become the center of attention for the health world and has caused public concern, because pathogenic *E. coli* can become resistant to antibiotics and then transmitted to humans through consumption of animal products (Pradika *et al*., 2019). Transmission of this infection can occur through direct contact with animals, exposure to animal waste, or consumption of contaminated food (Carvalho *et al*., 2020). *E. coli* is known to carry antimicrobial resistance genes and has the ability to carry out horizontal gene transfer between strains, even between different bacterial species (Khairullah *et al*., 2025). *E. coli*, especially APEC strains, are the main cause of colibacillosis, which is a major challenge in the poultry industry, causing economic losses, decreased productivity, and high morbidity and mortality rates (Newman *et al*., 2021). APEC strains are sub-strains of ExPEC that cause systemic infections in poultry, including quail, chicken, turkey, duck, and other birds, with significant impacts on productivity (Ovi *et al*., 2023; Ramatla *et al*., 2023; Khairullah *et al*., 2024).

The results of antibiotic resistance tests of five different antibiotic groups obtained from quail cloacal swabs showed the highest incidence occurred in erythromycin antibiotics with a percentage of 61% (84/138), followed by streptomycin antibiotic resistance of 37% (51/138), ciprofloxacin antibiotics 35% (49/138), tetracycline antibiotics 22% (33/138), and aztreonam antibiotics 14% (19/138). The high incidence of antibiotic resistance in this study indicates that there has been a high spread of antibiotic resistance in quail in the Surabaya area, Indonesia. Erythromycin antibiotic resistance in Surabaya taken from quail feces swab samples was (61%). According to Anggita *et al*. (2021) that *E. coli* isolates from quail cloacal swabs had high resistance to erythromycin antibiotics 90% in Yogyakarta. Erythromycin is a broad-spectrum macrolide antibiotic. The mechanism of erythromycin can inhibit the synthesis of bacterial ribosomal protein subunit 50S (Siibak *et al*., 2009). The results of the study showed that the incidence of antibiotic resistance to erythromycin in Surabaya was lower compared to Yogyakarta.

Streptomycin showed the second highest result at 37%. According to Anggita *et al*. (2021) *E. coli* in Yogyakarta as much as 20% have resistance to streptomycin antibiotics. According to research conducted by Farghal *et al*. (2017) that streptomycin antibiotics have 71.4% resistance in *E. coli* isolates taken from quail samples. Streptomycin is an aminoglycoside antibiotic that works by binding to the 30S ribosomal subunit in bacteria. Interferes with the protein synthesis process by causing incorrect reading of the mRNA genetic code, leading to defective protein synthesis (Demirci *et al*., 2013).

Ciprofloxacin showed the third highest result of 35%, because this antibiotic preparation is often used in the poultry industry for the therapy of *E. coli* infections in the urinary tract, digestive and respiratory systems, and is broad spectrum (Ivanova *et al*., 2017). Previous research in Egypt was higher, namely 57.1%, resistance to ciprofloxacin in quail (Farghal *et al*., 2017) According to Nehru and Pranav (2024) that the antibiotic ciprofloxacin in India showed resistance (30%). Ciprofloxacin is a broad-spectrum antibiotic and is effective against Gram-negative bacteria (Pseudomonas and Enterobacteriaceae) and Gram-positive bacteria (Ivanova *et al*., 2017). This antibiotic is broad spectrum with the process of resistance, namely the antibiotic ciprofloxacin binds to the DNA gyrase enzyme by breaking the single strand in DNA. Mutations in the gene encoding DNA gyrase cause the production of an active enzyme that cannot be bound by fluoroquinolones, thus causing resistance (van der Putten *et al*., 2019).

Tetracycline showed the fourth highest result of 22%. According to Farghal *et al*. (2017) that in Egypt tetracycline antibiotics have resistance of 71.4% in *E. coli* isolates taken from quail samples. Tetracycline works by binding to the 30S ribosomal subunit in bacteria, inhibiting the ribosome's ability to bind tRNA that carries amino acids to the A site of the ribosome during protein synthesis. This interferes with the formation of polypeptide chains necessary for bacterial growth and reproduction (Krawczyk *et al*., 2024).

Aztreonam showed the lowest results compared to other antibiotics in this study, which was 14%. According to Nehru and Pranav (2024) that aztreonam antibiotics in India showed resistance of (30%). Aztreonam is a monobactam antibiotic that works by binding to protein 3 (PBP-3) from

Gram-negative, causing lysis and death of bacteria (Rahmahani *et al*., 2020). The mechanism of aztreonam resistance is caused by bacteria that produce the enzyme Extended spectrum beta-lactamase (ESBL) which can inhibit penicillin, cephalosporin and aztreonam antibiotics (Saeed *et al*., 2023).

The occurrence of MDR has become one of the challenges of global problems related to the emergence of antibiotic resistance, which is worrying for public health (Khairullah *et al*., 2022). Antibiotics are said to be MDR if they are resistant to three antibiotics from different classes (Kaben *et al*., 2024). The occurrence of MDR in *E. coli* isolates was 18% obtained from quail cloacal swabs in the Surabaya market. The level of antibiotic use by farmers to increase livestock productivity, without referring to the correct antibiotic use standards, triggers the occurrence of MDR. In line with research in Zimbabwe where antibiotics were strongly associated with the level of resistance in *E. coli* isolates from cattle, pigs, and poultry (Chantziaras *et al*., 2014). The occurrence and spread of antibiotic resistance can also be caused by high demand in the poultry industry. The widespread occurrence of antibiotic resistance, especially MDR in pathogenic bacteria, can pose challenges in disease treatment therapy. In line with the one health concept, it is important to study the level of resistance in the environment, because it plays a role in the spread of bacteria to humans, animals and the environment.

Based on the results of the MDR test on *E. coli* isolates, the most common resistance patterns are as follows: ATM/CIP/S/E (32%), followed by CIP/S/E (16%), then the resistance patterns CIP/TE/E, ATM/CIP/TE/S/E, and TE/S/E, each with a resistance pattern of (12%). The resistance pattern ATM/TE/E (8%). The resistance patterns ATM/TE/S/E and CIP/TE/S/E each with a resistance pattern of (4%). The differences in resistance patterns that arise are due to differences in the combination of types of antibiotics given by farmers. This is because the use of various antibiotics, geographical differences and different poultry production systems cause differences in resistance patterns. Resistance patterns to various antibiotics can be influenced by the use of broad-spectrum antibiotics (Samuel *et al*., 2023).

The results of this study indicate that the MDR *E. coli* isolates were then continued by detecting the presence of the *iss* gene virulence gene. PCR tests showed that there were 5/25 with a percentage of 20% of *E. coli* bacterial isolates from quail cloacal swabs having the *iss* virulence gene. The *iss* virulence gene is a virulence gene found in APEC where the *iss* gene plays a role in increasing the ability of *E. coli* to survive in blood serum and avoid the body's immune system (El-Shenawy *et al*., 2023). These results are lower than those in the study conducted by Khusnan *et al*. (2021) that *E. coli* bacteria isolated from quail in Yogyakarta showed genotypic results of (61.9%) containing the *iss* gene, this isolate is a pathogenic strain that has the potential to act as a zoonotic agent.

Conclusion

The results of the study on quails found *E. coli* that had MDR properties in quail cloaca swabs in five markets in the Surabaya area of 18% and the *iss* virulence gene was found at 20%. It is important to increase public awareness of food safety originating from quails, as well as to increase farmers' understanding of colibacillosis disease caused by the APEC strain. The use of antibiotics that can trigger resistance and MDR requires serious attention and strict supervision from veterinarians.

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Conflict of interest

The authors have no conflict of interest to declare.

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