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Molecular detection of extended-spectrum β-lactamase-producing *Escherichia coli* in imported canaries (*Serinus canaria*) from Malaysia

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ABSTRACT

The increasing public interest in keeping canaries has driven the growth of international trade in ornamental birds in Indonesia, especially in East Java. Data from the East Java Animal, Fish, and Plant Quarantine Center shows a significant increase in imports of canaries (*Serinus canaria*) from Malaysia. The high volume of imports has the potential to be a route of entry and spread of antibiotic-resistant bacteria, one of which is *Escherichia coli*. The ability of *E. coli* to transfer resistance genes to other bacteria makes it an important reservoir in the dynamics of antimicrobial resistance in the environment. This study aimed to identify the presence of *E. coli* producing Extended-Spectrum Beta-Lactamase (ESBL) in imported canaries, as well as to analyze their antimicrobial resistance profiles. A total of 150 canary feces samples were taken aseptically at the Malang Animal Quarantine Installation. Isolation of *E. coli* was carried out using Eosin Methylene Blue Agar (EMBA) media and confirmed by the IMViC biochemical test. Antibiotic sensitivity test (amoxicillin, ceftazidime, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole) using the Kirby-Bauer method. Detection of the *bla*TEM gene in Multidrug Resistance (MDR) isolates was carried out by PCR. Of the 150 samples, 27 isolates (18%) were confirmed as *E. coli*, with 81.4% (22/27) showing an MDR pattern. The highest resistance was found to tetracycline (88.9%), amoxicillin (85.1%), and trimethoprim-sulfamethoxazole (70.4%). The *bla*TEM gene was detected in 59.1% (13/22) of the MDR isolates, indicating a plasmid-mediated beta-lactam resistance mechanism. Imported canaries have the potential to be a reservoir of ESBL-producing *E. coli* with a high prevalence of MDR. These findings highlight the need for strict supervision of bird imports and regulation of antibiotic use in farms to prevent the spread of antimicrobial resistance.

Introduction

East Java Province is one of the regions in Indonesia with a fairly high level of canary (*Serinus canaria*) imports from Malaysia. Based on data from the East Java Animal, Fish, and Plant Quarantine Center, in 2021 there were four imports with a total of 4,250 birds, which increased in 2022 to 14 imports with a total of 11,807 birds. Canaries, along with other pet birds from the Passeriformes and Psittaciformes orders, are known to be close to humans and play a role in social life (Sigirci *et al*., 2020). However, this interaction also increases the risk of zoonosis because birds can be reservoirs of enteric pathogens, including antibiotic-resistant bacteria (Ahmed *et al*., 2021). In cross-border trade, pet birds have the potential to be a medium for transmitting resistant bacteria or Antimicrobial Resistance (AMR) genes (Oteo *et al*., 2018). Previous studies have shown that pet birds are an important source of multidrug-resistant (MDR) bacteria, which are of concern to public health due to the potential spread of difficult-to-treat diseases (Sigirci *et al*., 2020; Mohamed *et al*., 2022).

The practice of using antibiotics without medical supervision in the poultry farming sector has contributed to the increase in cases of antibiotic resistance (Varriale *et al*., 2020). One of the important pathogens often found in birds is *Escherichia coli*, a Gram-negative bacterium known to cause various diseases in humans, including gastroenteritis, bacteremia, and urinary tract infections (Ong *et al*., 2020). In birds, *E. coli* is the main agent of diseases such as aerosaculitis, polyserositis, septicemia, and other extraintestinal infections (Varriale *et al*., 2020).

Colonization of *E. coli* in the digestive tract of birds from the order Passeriformes can cause sepsis and death, and has the potential to be a source of transmission to humans (Widodo *et al*., 2023). Poultry has been reported as a reservoir of resistant *E. coli* that can cause zoonotic infections (Aklilu *et al*., 2022). This resistance is not only limited to pathogenic strains, but is also found in commensal *E. coli*, which acquires its resistance genes through horizontal gene transfer mechanisms (Prayudi *et al*., 2023). The ability of *E. coli* to transfer resistance genes to other bacteria makes it one of the main reservoirs in the spread of antibiotic resistance in the environment (Harijani *et al*., 2020).

Antibiotic resistance occurs due to genetic changes in bacteria that cause insensitivity to antimicrobial drugs (Khairullah *et al*., 2022). One of the main mechanisms of the spread of this resistance is through horizontal gene transfer, especially mediated by plasmids (Yunita *et al*., 2020). Plasmid-mediated beta-lactamase enzymes were first identified in *E. coli* (Farizqi *et al*., 2023). Research by Sigirci *et al.* (2020) in Turkey showed that most *E. coli* isolates from pet bird shops showed high levels of resistance to tetracycline (84%), sulfamethoxazole-trimethoprim (46%), streptomycin (34%), and kanamycin (25%). Meanwhile, a study in Kampung Melayu, Malaysia, reported that all *E. coli* isolates from wild birds and chickens showed MDR characteristics to all antibiotics tested (Mohamed *et al*., 2022). One form of resistance that is of concern is the production of Extended Spectrum Beta-Lactamase (ESBL), an enzyme encoded by plasmids in Gram-negative bacteria, especially from the *Enterobacteriaceae* family. This enzyme is able to hydrolyze penicillin antibiotics, third-generation cephalosporins (such as ceftazidime, ceftriaxone, and cefotaxime), and monobactams (aztreonam), thereby reducing the effectiveness of an-

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tibiotic therapy (Widodo *et al*., 2020; Widodo *et al*., 2022).

ESBL-producing bacteria are now widely found as nosocomial pathogens and causes of community infections (Riwu *et al*., 2022). Genetic mutations that increase beta-lactamase activity enhance the ability of bacteria to hydrolyze new generation antibiotics (Faridah *et al*., 2023). The main genes encoding ESBL such as bla_{CTX-M}, bla_{TEM}, and bla_{SHV} are commonly found in pathogenic bacteria from poultry (Wibisono *et al*., 2020a). Transmission can occur through medical devices, livestock products, or environments exposed to domestic animals and poultry (Ye *et al*., 2018). Among these genes, bla_{TEM} is the most frequently reported globally from human, animal, and environmental isolates (Alcock *et al*., 2020), and has also been identified in nosocomial pathogens, companion animals, food products, and poultry farming environments (Castanheira *et al*., 2021).

Globally, ESBL-producing *E. coli* isolates have been successfully identified from various animal sources, including poultry, wild birds, food animals, and pets (Yılmaz and Dolar, 2017). However, studies that specifically evaluate the characteristics of ESBL-producing *E. coli* in the context of bird import traffic are still limited. The increasing frequency of bird imports, especially from Malaysia, is an important basis for conducting studies on the detection and identification of ESBL-producing *E. coli* in birds traded across countries. This study aimed to provide an overview of the potential of imported canaries as a reservoir for the spread of ESBL-producing *E. coli*, which can have an impact on public health and environmental biosecurity.

Materials and methods

Place and time of research

This research was conducted from October 2023 to December 2023. Sampling was carried out at the Animal Quarantine Installation, Malang City (Figure 1), while the bacterial isolation process and antibiotic sensitivity testing were carried out at the Animal, Fish, and Plant Quarantine Center (BKHIT) Laboratory of East Java Province.

Figure 1. Location of sampling of imported canaries at the Animal Quarantine Installation, Malang City, Indonesia

Sample size

A total of 150 fecal swab samples from imported canaries from Malaysia were collected based on fresh feces criteria. Sampling was carried out aseptically using a sterile swab stick (Oxoid, Basingstoke, UK) placed in a tube containing 2.5% Buffered Peptone Water (BPW). The samples were then stored in a cooler box and immediately taken to the laboratory for further analysis.

Isolation and identification

Fecal swabs (n = 150) were inoculated into Eosin Methylene Blue Agar (EMBA) media in streak and incubated at 37°C for 24 hours. Colonies with typical *E. coli* morphology (Figure 2) were selected and identified conventionally according to the method described by Yilmaz and Guvensen (2016).

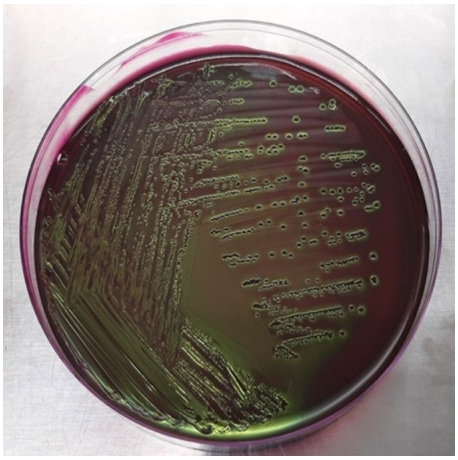

Figure 2. Presumptive colonies of *E. coli* on EMBA media, characterized by a distinctive dark greenish metallic color

Antibiotic sensitivity test

Antibiotic sensitivity testing was carried out using the disk diffusion method (Kirby-Bauer disk diffusion test) on Mueller Hinton Agar (MHA) media, which produced qualitative interpretations in the form of sensitive, intermediate, and resistant categories (Mustika *et al*., 2024).

Bacterial culture

Bacterial colonies from EMBA media were inoculated into test tubes containing 8 ml of physiological NaCl. The suspension was shaken using a vortex until it reached a turbidity equivalent to the McFarland standard of 0.5 (≈ 1.5×10^8 CFU/ml).

Resistance test

A total of 1–2 colonies of *E. coli* were inoculated into a physiological NaCl solution that had been adjusted for turbidity. A total of 0.2 ml of the suspension was spread evenly onto the surface of the MHA medium, left for 15 minutes, then the antibiotic disc was placed on the medium and pressed slightly. The medium was incubated at 37°C for 24 hours to read the inhibition zone.

PCR test for detection of bla_{CTX-M} gene

E. coli isolates showing MDR characteristics were further analyzed for detection of the bla_{CTX-M} gene using the PCR method.

DNA preparation

Several colony loops from one isolate were placed in an Eppendorf tube containing 300 µl of TE solution (10 mM Tris, pH 8; 10 mM EDTA). The suspension was shaken using a vortex, then boiling lysis was performed at 98°C for 10 minutes using ThermoStat™. After that, the sample was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was stored at −20°C until used as a PCR template (Ahmed *et al*., 2021).

Amplification procedure

Detection was carried out using specific primers: TEM-F (5′-ATG AGT ATT CAA CAT TTC CG-3′) and TEM-R (5′-CTG ACA GTT ACC AAT GCT TA-3′), which target an 867 bp amplicon. As a positive control, an isolate that is known to contain the bla_{TEM} gene was used, while a negative control (K–) used nuclease-free water. The PCR reaction was carried out with a 100 bp DNA ladder marker (Promega) as a marker for band size. PCR reactions were prepared with a total volume of 20 µl, consisting of 12.5 µl GoTaq Green Master Mix, 1 µl each of bla_{CTX-M} forward and reverse primers, 0.5 µl DNase-free water, and 5 µl DNA template. The amplification reaction was run on a thermal cycler with the following protocol: pre-denaturation at 95°C for 1 min; 40 cycles of denaturation (95°C, 1 min.), annealing (55°C, 1 min.), and extension (72°C, 1 min); ending with a final extension at 72°C for 2 min. (Kurniawan *et al*., 2025).

Interpretation of amplification products

A total of 1.2 grams of agarose powder was dissolved in 60 ml of 1× TBE buffer solution and 9 µl of SYBR® Safe DNA gel stain was added. After being poured into the gel chamber and left to harden, 10 µl of PCR sample was loaded into the well, along with a 100 bp DNA marker (6 µl). Electrophoresis was run at 100 volts, 250 mA for 35 minutes. The results of DNA band visualization were observed using a UV transilluminator and compared with a DNA ladder to confirm the band size (Khairullah *et al*., 2023).

Results

The isolation results showed that 19.3% (29 out of 150) samples contained presumptive *E. coli* based on typical colony growth on EMBA media.

Colonies suspected of being *E. coli* on EMBA media were then subjected to Gram staining. Microscopic observation at 1000x magnification showed a short rod-shaped morphology (coccobacilli) with red staining, indicating that the bacteria were Gram-negative. The results of microscopic observations are shown in Figure 3.

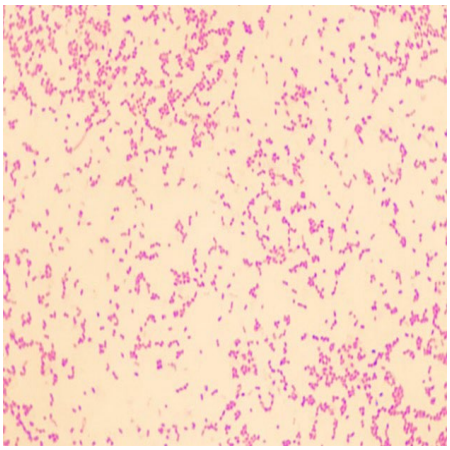

Figure 3. The results of microscopic examination of *E. coli* isolates with Gram staining at 1000× magnification, showed a short rod morphology (coccobacilli) in red which indicated Gram-negative bacteria

Confirmation of *E. coli* bacteria was carried out through a series of IMViC biochemical tests, including the Indole, Methyl Red (MR), Voges-Proskauer (VP), and Citrate tests. In the Indole test, the isolate was inoculated into SIM media and incubated for 24 hours at 37°C. Positive results were indicated by the formation of a red ring on the surface of the media after the addition of Kovacs reagent, indicating the ability of bacteria to break down the amino acid tryptophan into indole, including the water-insoluble para-aminobenzaldehyde compound. Motility was also confirmed by the appearance of turbidity along the puncture path, indicating the motile nature of the bacteria. The Methyl Red (MR) test produced a red color after the addition of MR reagent, indicating the production of strong acid and stable glucose fermentation results, which are typical characteristics of *E. coli*. Conversely, the Voges-Proskauer (VP) test showed negative results in the isolate, indicated by the absence of a color change to brownish red after the addition of α-naphthol reagent and 40% KOH. The Citrate test also showed negative results, indicated by the absence of a color change in the Simmons Citrate Agar media from green to blue, indicating that the isolate could not utilize citrate as the main carbon source. Overall, the IMViC pattern (+ + – –) shown by the isolate supports presumptive identification as *E. coli*. Of the 150 samples tested, 27 samples (18%) showed positive results for *E. coli* based on the IMViC test. Visualization of the test results can be seen in Figure 4.

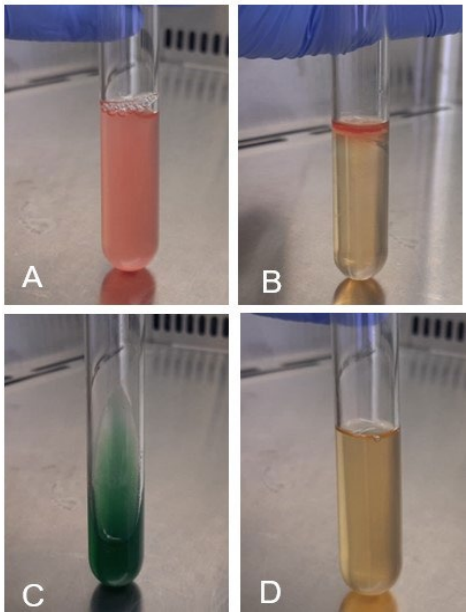

Figure 4. The results of the IMViC biochemical test on *E. coli* isolates were positive: (A) Indole test showed a red ring on the surface of the media (+), (B) Methyl Red test showed a color change to red (+), (C) Voges-Proskauer test showed no color change to reddish brown (–), (D) Citrate test without a green color change on the media (–)

Based on the results of isolation and identification of 150 samples of rectal swabs of imported canary feces from Malaysia, 27 isolates were confirmed positive for *E. coli* through the IMViC test. All isolates were then tested for their sensitivity to several types of antibiotics using Mueller-Hinton Agar (MHA) media. Details of the isolation and identification results are presented in Table 1.

A total of 29 isolates showed typical colony growth on EMBA media, which were then further tested using the IMViC biochemical test series. Of the 29 presumptive isolates, 27 isolates showed positive results in the Indole, motility, and Methyl Red tests, and negative results in the Citrate and Voges-Proskauer tests. This biochemical reaction pattern (+ + - -) in-

Table 1. Results of antibiotic sensitivity tests on *E. coli* isolates (n = 27)

Number of samples	CAZ		TE		AML		SXT		CIP	
	R	%	R	%	R	%	R	%	R	%
27	0	0	24	88.90%	23	85.10%	19	70.40%	9	33.30%

Note: CAZ = Ceftazidime, AML = Amoxicillin, CIP = Ciprofloxacin, TE= Tetracycline, SXT = Trimethoprim-sulfamethoxazole, R = Resistant

dicates that the isolates are in accordance with the typical characteristics of *E. coli*, so that 27 isolates were confirmed as *E. coli* and continued to the antibiotic sensitivity test stage.

Antibiotic sensitivity test on 27 *E. coli* isolates was conducted using the Disk Diffusion Test method on Mueller-Hinton Agar media. The results showed that the highest level of resistance was found to Tetracycline antibiotics, which was 88.9% (24/27). Furthermore, the isolates also showed high levels of resistance to Amoxicillin at 85.1% (23/27), and Trimethoprim-sulfamethoxazole at 70.4% (19/27). Meanwhile, resistance to Ciprofloxacin was recorded at 33.3% (9/27). Interestingly, no isolates were found to be resistant to Ceftazidime (0%), indicating the effectiveness of this antibiotic against the tested isolates. Details of the sensitivity test results are presented in Table 1.

The results of antibiotic sensitivity tests showed variations in resistance levels among the 27 *E. coli* isolates tested. A total of 1 isolate (3.7%) showed resistance to one class of antibiotics, while 2 isolates (7.4%) were resistant to two classes of antibiotics. The majority of isolates, namely 22 isolates (81.4%), were classified as MDR because they showed resistance to three to four classes of antibiotics. A total of 2 isolates (7.4%) did not show resistance to the antibiotics tested. Details of the antibiotic resistance profile are presented in Table 2.

Table 2. Antibiotic resistance profile of *E. coli* isolates based on the number of antibiotic classes rejected (n = 27).

Amount of antibiotics	Resistance profile	Number of resistant isolates (n=27)	Percentage (%)
0	Not Resistant	2	7.40%
1	CIP	1	3.70%
2	TE-SXT	1	7.40%
	AML-TE	1	
3	AML-CIP-TE	4	29.60%
	AML-TE-SXT	4	
4	AML-CIP-TE-SXT	14	51.80%

Note: AML = Amoxicillin, CIP = Ciprofloxacin, TE = Tetracycline, SXT = Trimethoprim-sulfamethoxazole

Based on the antibiotic resistance profile of 27 *E. coli* isolates tested, 22 isolates (81.4%) showed a MDR pattern, which is a condition where bacteria show resistance to three or more different antibiotic classes. The sensitivity test in this study used five types of antibiotics representing several classes, namely beta-lactam (amoxicillin and ceftazidime), fluoroquinolone (ciprofloxacin), tetracycline (tetracycline), and sulfonamide (trimethoprim-sulfamethoxazole). The high prevalence of MDR found indicates a significant potential threat of antimicrobial resistance and is important to monitor regularly. Details of the MDR profile are shown in Figure 5.

The results of the sensitivity test showed that 81.4% (22/27) of *E. coli* isolates showed a MDR pattern, which is resistance to three or more antibiotic classes. The most dominant MDR pattern was a combination of resistance to AML-TE-SXT which was found in 51.9% (14/27) of isolates. Meanwhile, the combinations of AML–CIP–TE and AML–CIP–TE–SXT were each found in 14.8% (4/27) of isolates. Details of the MDR pattern are shown in Table 3.

A total of 22 *E. coli* isolates previously identified as MDR from canary feces samples were further tested to detect the presence of the bla_{TEM} gene using the Polymerase Chain Reaction (PCR) method. Electrophoresis results showed that 13 of the 22 isolates (59.1%) produced a DNA band measuring 867 bp, indicating the presence of the bla_{TEM} gene. This shows that more than half of the MDR isolates carry genes that play a role in the ESBL mechanism, which contributes to resistance to beta-lactam antibiotics. Details of the visualization of the bla_{TEM} gene detection results are shown in Figure 6.

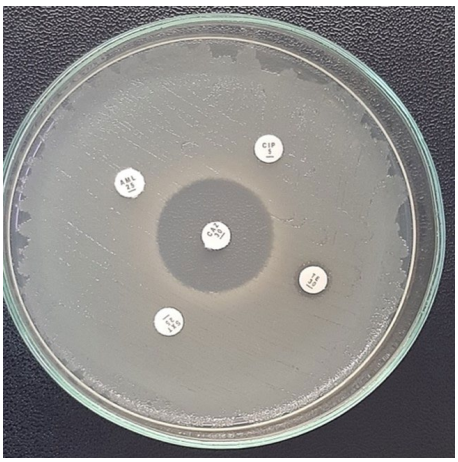

Figure 5. The results of antibiotic sensitivity tests on *Escherichia coli* isolates using the disk diffusion method (Kirby-Bauer) on Mueller-Hinton Agar media. Five antibiotic discs were used, namely Amoxicillin (AML), Ciprofloxacin (CIP), Ceftazidime (CAZ), Tetracycline (TE), and Trimethoprim-sulfamethoxazole (SXT). The diameter of the inhibition zone around the disc indicates the variation in the level of sensitivity of the isolate to each antibiotic.

Table 3. MDR patterns in *E. coli* isolates from imported canary feces (n = 27).

Sample type	Resistance profile	Number of isolates	MDR Isolate	Percentage (%)
Feces	AML-TE-SXT		14	51,9%
Bird	AML-CIP-TE	27	4	14,8%
Imported canary	AML-CIP-TE-SXT		4	14,8%
Total		27	22	81,4%

Note: AML = Amoxicillin, CIP = Ciprofloxacin, TE= Tetracycline, SXT = Trimethoprim-sulfamethoxazole

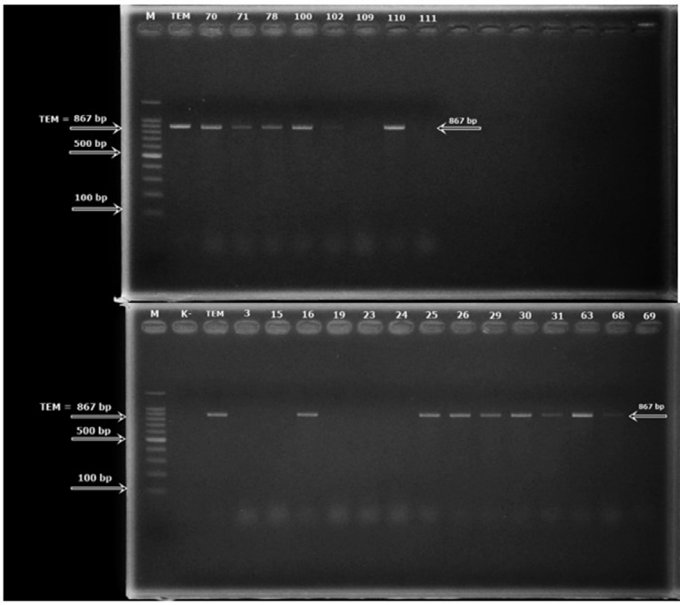

Figure 6. Results of *bla*TEM gene detection encoding ESBL in MDR *E. coli* isolates using the PCR method. (A) Visualization of DNA bands from isolates numbered 70 to 111. (B) Visualization of DNA bands from isolates numbered 3 to 69. The positive band is seen at a size of 867 bp, according to the target of *bla*TEM gene amplification. Description: M = DNA marker (100 bp ladder); K^- = Negative control (nuclease-free water); TEM = Positive control (*E. coli* carrying the *bla*TEM gene); the numbers indicate the isolate code number.

Discussion

Based on the results of isolation and identification carried out on imported canary bird feces swabs from Malaysia at the Malang City Animal Quarantine Installation, as well as sensitivity tests carried out at the East Java Animal, Fish, and Plant Quarantine Center Laboratory, it was found that out of 150 samples tested using EMBA media, 29 samples (19.3%) showed a strong suspicion of containing *E. coli* bacteria. This bac-

teria is a normal flora commonly found in the digestive tract of humans, animals, and birds. Although commensal, some strains of *E. coli* have pathogenic properties and have the potential to cause various infections, including gastroenteritis, urinary tract infections, and systemic infections (Nowaczek *et al*., 2021).

Gram staining examination of the isolate showed that *E. coli* has a short rod morphology and appeared red when observed under a microscope at 1000× magnification (Agustin *et al*., 2024). This red color is a characteristic of Gram-negative bacteria that are unable to retain crystal violet dye during the fixation and decolorization process. This is due to the structure of the *E. coli* cell wall which consists of a thin layer of peptidoglycan and an outer membrane rich in lipoproteins and lipopolysaccharides. This structure makes the cell wall more permeable to solvents, so that the primary dye is easily dissolved and replaced by the contrast dye safranin. Therefore, *E. coli* appears red after the Gram staining process (Rahmahani *et al*., 2020).

Confirmation of the presence of *E. coli* was carried out through the IMViC biochemical test, which is a series of differential tests to identify members of the *Enterobacteriaceae* family, including *E. coli*. The IMViC test consists of Indole, Methyl Red (MR), Voges–Proskauer (VP), and Citrate tests (Riwu *et al*., 2024). The indole test results showed a positive reaction, indicated by the formation of a red ring in the upper layer of the media after the addition of Kovac's reagent. Another characteristic is the turbidity resembling an inverted pine tree on the needle puncture path, indicating the motility of *E. coli* in semi-solid media (Yanestria *et al*., 2022). This positive reaction is caused by the activity of the tryptophanase enzyme which hydrolyzes the amino acid tryptophan into indole and pyruvic acid. The indole formed then reacts with Kovac's reagent to produce a red ring as a positive indicator (Agustin *et al*., 2025).

The Methyl Red test also showed positive results, indicated by a change in the color of the media from yellow to red after the addition of the pH indicator Methyl Red. This color change is caused by the production of strong acid from glucose fermentation by *E. coli*, which lowers the pH of the media to ≤4.4 (Bren *et al*., 2016; Rombouts *et al*., 2020). In contrast, the VP test gave negative results indicated by the absence of a pink color after the addition of α-naphthol and 40% KOH, but instead turned brownish yellow. This indicates that *E. coli* does not produce acetoin as the end product of glucose fermentation (Ahadini *et al*., 2025).

The results of the citrate test using Simmons Citrate Agar (SCA) media showed a negative reaction, indicated by the persistence of the green color on the media after 24 hours of incubation at 37°C (Ariyanti *et al*., 2025). The absence of color change indicates that the *E. coli* isolate is unable to utilize citrate as the sole carbon source, in accordance with the typical characteristics of the species (Zlatkov and Uhlin, 2019). Based on the results of the biochemical test, 27 out of 150 isolates (18%) from imported canary feces were confirmed as *E. coli*. Furthermore, antibiotic sensitivity testing was carried out using the Kirby–Bauer diffusion method using Mueller-Hinton Agar (MHA) media. The bacterial suspension was standardized using McFarland 0.5, and the media was incubated at 37°C for ±24 hours. The inhibition zone was measured with a caliper, then compared with the interpretation standards of the Clinical and Laboratory Standards Institute (CLSI, 2020).

The results of the sensitivity test showed that 88.9% (24/27) of *E. coli* isolates showed resistance to tetracycline. In addition, 85.1% (23/27) of the isolates were resistant to amoxicillin, 70.3% (19/27) to trimethoprim-sulfamethoxazole, and 33.3% (9/27) to ciprofloxacin. No isolates were found to be resistant to ceftazidime. Based on the resistance pattern, 1 isolate (3.7%) showed resistance to one class of antibiotics, 2 isolates (7.4%) to two classes, and the majority, namely 22 isolates (81.4%), showed resistance to three to four classes of antibiotics. These findings indicate a high prevalence of MDR *E. coli*, which is a concern in the context of public health and biosecurity.

Antibiotic resistance is a major challenge in global public health (Ramandinianto *et al*., 2020). Organisms that have developed resistance can spread rapidly in the environment and population, increasing the risk of infections that are difficult to cure and require more complex treatment (Khairullah *et al*., 2019). Healthy birds generally only contain a small population of *E. coli* in their digestive tract (Yilmaz and Guvensen, 2016). However, in this study it was found that 88.9% (24/27) of *E. coli* isolates showed high resistance to tetracycline. This high level of resistance is most likely due to the widespread use of tetracycline in veterinary practice, including in birds and poultry, as well as the routine use of other antibiotics such as trimethoprim-sulfamethoxazole (Hunter *et al*., 2010).

Research by Sigirci *et al*. (2020) also reported a similar resistance pattern, where most *E. coli* isolates from a bird shop in Turkey showed resistance to tetracycline (84%), sulfamethoxazole/trimethoprim (46%), streptomycin (34%), and kanamycin (25%). Tetracycline belongs to a group of bacteriostatic antibiotics that work by inhibiting bacterial protein synthesis by binding to the 30S and 50S ribosomal subunits (Suhendi *et al*., 2024). The mechanism of resistance to tetracycline generally involves the presence of resistance genes on extrachromosomal plasmids, which are able to synthesize ribosomal protective proteins or pump out antibiotics from the cell (Afnani *et al*., 2022).

Amoxicillin, a broad-spectrum beta-lactam antibiotic that is bactericidal, also showed a high level of resistance in *E. coli* isolates in this study, namely 85.2% (23/27). This antibiotic works by binding to penicillin-binding proteins (PBPs), especially PBP-1A, which plays a role in the synthesis and cross-linking of peptidoglycan in the bacterial cell wall. This binding inhibits the activity of the transpeptidase enzyme, thereby disrupting cell wall formation and resulting in bacterial cell lysis (Moses *et al*., 2024). In addition, as many as 70.4% (19/27) of isolates showed resistance to the combination of trimethoprim-sulfamethoxazole antibiotics. This combination is synergistic in inhibiting folic acid biosynthesis, namely by trimethoprim inhibiting the enzyme dihydrofolate reductase and sulfamethoxazole inhibiting the enzyme dihydropteroate synthetase (Agumah *et al*., 2025). However, uncontrolled use can leave residues in animal products and accelerate the selection of resistant bacterial strains. Trimethoprim residues in animal foods can also cause negative impacts in humans, such as nausea, skin rashes, and the emergence of new antibiotic resistance (Ugbo *et al*., 2024).

Resistant organisms can spread rapidly, increasing the risk of treatment failure and mortality from difficult-to-treat infections (Kholik *et al*., 2024). In this study, 33.3% (9/27) of *E. coli* isolates showed resistance to ciprofloxacin, a quinolone antibiotic that is often used as the therapy of choice. Resistance to ciprofloxacin is generally caused by three main mechanisms, namely mutations in target enzymes (DNA gyrase and topoisomerase IV), decreased membrane permeability to the drug, and the presence of plasmid resistance genes such as qnr (Putri *et al*., 2024).

The sensitivity test results also showed that 81.4% (22/27) of *E. coli* isolates were MDR, with resistance to three to four classes of antibiotics. The dominant MDR pattern included a combination of resistance to amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole; and a combination that also involved ciprofloxacin. These findings are in line with a report from Malaysia showing that 44.4% of *E. coli* isolates from wild birds and 100% of isolates from chickens showed resistance to all antibiotics tested (Mohamed *et al*., 2022).

Beta-lactam antibiotics, such as amoxicillin, are often used as first-line treatment for *Enterobacteriaceae* infections (Ghenea *et al*., 2022). However, uncontrolled antibiotic use in the poultry farming sector has contributed to increasing levels of resistance (Varriale *et al*., 2020). *E. coli* can act as a reservoir for the spread of antimicrobial resistance, potentially transferring from animals to humans or vice versa, through food, the environment, or direct contact (Sigirci *et al*., 2020).

Genetic changes that occur through mutations, plasmid acquisition, transposons, and integrons can cause persistent resistance and are vertically inherited between bacterial generations (Islam *et al*., 2023). In this study, of the 22 MDR *E. coli* isolates tested by PCR, 59.1% (13/22) were detected to carry the bla_{TEM} gene. All isolates also showed resistance to

amoxicillin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole.

The bla_{TEM} gene is one of the most common ESBL encoding genes found in Gram-negative bacteria (Alcock *et al*., 2020). The ESBL enzyme encoded by this gene is capable of hydrolyzing beta-lactam antibiotics including third-generation cephalosporins, causing clinical therapy failure (Ghenea *et al*., 2022). The bla_{TEM} gene has been detected in pathogens from various sources, such as pets, the environment, food products, and poultry farms (Castanheira *et al*., 2021), and is reported to have the highest prevalence in isolates originating from poultry (Wibisono *et al*., 2020b).

The World Health Organization (WHO) estimates that antibiotic resistance causes more than 700,000 deaths each year worldwide (Zarei-Baygi and Smith, 2021). International bird trade can accelerate the spread of resistant bacteria and Antimicrobial Resistance (AMR) genes during transportation (Oteo *et al*., 2018). Therefore, strict biosecurity implementation in Animal Quarantine Installations and the surrounding environment is essential to reduce the risk of AMR spread (Ansharieta *et al*., 2021). The presence of ESBL-producing *E. coli* in pet birds is a real threat to public health. Previous studies have also shown that wild and pet birds can play a significant role in the spread of resistant bacteria (Yılmaz and Dolar, 2017).

Conclusion

This study revealed that imported canaries from Malaysia entering East Java have the potential to become a reservoir of *E. coli* producing ESBL. Of the 150 fecal samples examined, 27 isolates (18%) were confirmed as *E. coli*, with 22 isolates (81.4%) showing a MDR pattern, especially against tetracycline antibiotics (88.9%), amoxicillin (85.1%), and trimethoprim-sulfamethoxazole (70.4%). Molecular detection showed that the bla_{TEM} gene, which encodes the ESBL enzyme, was identified in 59.1% of MDR isolates. These findings confirm that imported ornamental birds have the potential to become a route of entry and spread of antimicrobial-resistant bacteria across national borders, so strict supervision and risk-based policy interventions are needed to prevent threats to public health.

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Conflict of interest

The authors have no conflict of interest to declare.

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