IJTID. Volume 12 No 3 September-December 2024 e-ISSN: 2356-0991; p-ISSN:2085-1103
Sinta 2 (Decree No: 105/E/KPT/2022) Available online at https://e-journal.unair.ac.id/IJTID

Original Article

IJTID
(INDONESIAN JOURNAL OF TROPICAL AND INFECTIOUS DISEASE)
Scientific Journal of Tropical and Infectious Disease

Antibiotic-Resistant Genes and Polymorphisms of *bla$_{TEM1}$* gene in Multidrug-resistant *Escherichia coli* from Chicken Eggs and Cloacal Swabs in Sleman, Yogyakarta: The Impact on Public Health

Niken Irfa Nastiti [1*], **Widya Asmara** [2], **Khrisdiana Putri** [2]

[1]Tropical Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

OPEN ACCESS

ARTICLE INFO

Received: October 20, 2023
Accepted: November 18, 2024
Published: December 30, 2024
Available online: December 30, 2024

*) Corresponding author:
E-mail: irhamniken@gmail.com

Keywords:
Escherichia coli
Multi drug-resistance
Resistance gene
Polymorphism
bla$_{TEM1}$

Abstract

Antimicrobial resistance in pathogenic bacteria is a serious problem in public health. Antibiotic-resistant pathogens are the cause of many deaths. *Escherichia coli* (*E. coli)* is one of the bacteria that experienced multi-drug resistance (MDR). Infection of *Escherichia coli* in humans occurs through transmission of fecal-oral. This study**,** conducted at the Veterinary Public Health Laboratory of Gadjah Mada University, aimed to assess MDR *E. coli* prevalence in 200 chicken egg samples sourced from poultry farms and supermarkets, alongside 63 cloacal swab samples from broiler poultry in Sleman, Yogyakarta. The study focused on detecting resistance genes including *tetA, aadA1, aph(3)IIa, and bla$_{TEM}$1*, also analyzing polymorphisms in the *bla$_{TEM1}$* gene associated with antibiotic resistance. Identification technique of *E. coli* positivity refers to the Indonesian National Standard (SNI) 2897:2008, then *E. coli* identification was performed using the Analytical Profile Index (API) Test 20E Kit. Antibiotic sensitivity was determined by the Kirby Bauer method. Detection of antibiotic resistance genes in *E. coli* were determine using Polymerase Chain Reaction (PCR) method. Sequencing and analysis of polymorphism and phylogenetic were performed only in *bla$_{TEM1.}$* There were 12 samples identified as having *E. coli* (1 from chicken eggs and 11 from cloacal swabs), resistance percentages were highest for erythromycin (100%), ampicillin (91.7%), ciprofloxacin (91.7%), sulfamethoxazole (83.3%), streptomycin (83.3%) gentamicin (75%), tetracycline (41.7%), and chloramphenicol (25%). respectively. All of 12 *E. coli* samples were bacteria with MDR. Resistant genes were prevalent, notably *bla$_{TEM1}$* and *aadA1* (100% each), with *aph(3)IIa* and *tetA* genes also detected in 58.3% of samples each. Sequencing of the *bla$_{TEM1}$* gene revealed polymorphisms in isolate A8. However, these did not alter its antibiotic resistance phenotype. Sequences of *E. coli* isolates showed similarities to strains from Vietnam, China, and India, countries with high antibiotic consumption, particularly ampicillin.

Cite this as: Nastiti, N.I, Asmara, W., Putri, K. (2024). Antibiotic-Resistant Genes and Polymorphisms of *bla$_{TEM1}$* gene in Multidrug-resistant *Escherichia coli* from Chicken Eggs and Cloacal Swabs in Sleman, Yogyakarta: The Impact on Public Health . *Indonesian Journal of Tropical and Infectious Disease*, 12(3) : 208–225. https://doi.org/10.20473/ijtid.v12i3.49885

INTRODUCTION

Antimicrobial resistance (AMR) in bacteria, especially pathogenic bacteria, is a serious problem in public health, both human and veterinary. Antimicrobial resistance is one of the threats to human health, which can cause death. Mortality caused by infection with AMR pathogens was estimated at 4.95 million deaths worldwide in 2019, with three infectious diseases dominating the cause of death, which are associated with AMR pathogens: thoracic, infection of the lower respiratory tract, bloodstream infections, and intra-abdominal infections. Pathogens with antimicrobial resistance that have caused more than 250,000 deaths include *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. *Escherichia coli* alone is the pathogen that causes many deaths due to AMR[1].

Escherichia coli is a normal bacterium found in the digestive tract of humans and animals. However, pathogenic *E. coli* can infect humans and cause gastrointestinal disorders. The transmission route is generally, through contamination of food or beverages consumed by humans (food borne diseases). Therefore, *E. coli* is used as an indicator to indicate contamination and health risks in food[2]. In addition, *E. coli* may be associated with extraintestinal disorders such as urinary tract infections, which is one of the nosocomial bacterial diseases[3].

Serious diarrhea may occur as a result of food-borne pathogens caused by harmful strains of *E. coli*. One of the bacteria that causes food poisoning is pathogenic *E. coli*, which can be found in a wide range of countries, including developing ones like Indonesia. The latest data obtained from Japan shows that 3,000 elementary school students experienced food poisoning, and one of the causes is the contamination of pathogenic *E. coli in* food[4]. A study conducted by Djaja, Puteri, and Wispriyono [5], in the canteen of one of the universities in Jakarta, also showed the presence of *E. coli* contamination in food (42%).

Food contamination by pathogenic *E. coli* may occur during any of the steps in the farm-to-table continuum, coming from a neighbouring environment polluted by *E. coli*. This is certainly a problem that needs to be considered, especially if the pathogen *E. coli* that contaminates is MDR *E. coli*[6]. Previous research showed that there was *E. coli* with MDR that produced extended-spectrum beta-lactamase (ESBL) in beef in Surabaya traditional market, where the bla_{TEM} and bla_{CTX-M} genes are genes encoding ESBL in *E. coli* and are often found in food of animal origin[7]. The other important resistance genes obtained in *E. coli* are *tetA, aadA1, and aph(3)IIa, that are* resistance gene for tetracycline, streptomycin and aminoglycoside respectively. *Escherichia coli* bacteria can be found in animal products due to contamination from animal faeces[8].

Escherichia coli from livestock faeces have the potential to contaminate livestock products and the surrounding environment if hygiene in animal husbandry and the management of livestock waste disposal systems are poor. *E. coli* can contaminate livestock products such as meat, chicken eggs, and milk, which the products are the sources of protein that are mostly consumed by the Indonesian people, especially chicken eggs. The consumption of chicken eggs in Indonesia consistently rises annually, with egg consumption reaching 20.02 kg/capita per year in 2022[9].

Some food processing and serving in Indonesian society still needs attention to improve in cleanliness and hygiene. People

sometimes do not wash their hands after managing raw chicken eggs or other animal products, then managing the food being served, is one of the factors of *E. coli* contamination in food. Based on research conducted by Lee *et al*[10], the bacteria found in eggshells are *E. coli* and become a food borne outbreak pathogen that is often found compared to Salmonella or other pathogenic bacteria. These bacteria can come from chicken feces, and then contaminate chicken eggs through the pores of the eggs. This should be a concern especially if these bacteria have developed antibiotic resistance and carry antibiotic-resistant genes that could potentially be transmitted to other pathogens. Therefore, this study aimed is to determine the profile of antibiotic resistance genes, which are *tetA*, *aadA1*, *aph(3)IIa*, and *bla*$_{TEM1}$ in *E. coli* isolated from chicken eggs and cloacal swabs from several farms in Sleman, Yogyakarta which have the potential to be transmitted to humans.

MATERIALS AND METHODS

Materials

This study used 200 chicken eggs collected from four layer farms and three supermarkets, along with 63 cloacal swabs obtained from three broiler farms and three-layer farms, as samples.

The materials drug needed in the study are antibiotic disks ampicillin, chloramphenicol, sulfamethoxazole, tetracycline, ciprofloxacin, erythromycin, aminoglycoside antibiotics (streptomycin and gentamicin). As mention in the previous study, streptomycin and gentamicin are aminoglycoside drugs[11].

The other materials needed in this research are *E. coli* isolates, Macfarland 0.5, Brain Heart Infuse (BHI) medium (Thermo Fisher Scientific, USA), Chromocult Coliform Agar (CCA) medium (Sigma-Aldrich, USA), Eosin Methylene Blue Agar (EMBA) medium (Sigma-Aldrich, USA), MacConkey Agar (MCA) medium (Thermo Fisher Scientific, USA), Mueller Hinton agar (MHA) medium (Thermo Fisher Scientific, USA), Nutrient Agar (NA) medium (Thermo Fisher Scientific, USA), NaCl 0.9% (Himedia, USA), aluminum foil, plastic bag, cotton swab, label paper, sterile swab, tissue, Analytical Profile Index (API) Test 20E Kit (Biomerieux, USA, Catalog No. 20100), 70% alcohol (Onemed, Indonesia), absolute alcohol (Sigma-Aldrich, USA), Tris/Borate/EDTA (TBE) buffer (Sigma-Aldrich, USA), QIAamp® DNA Mini Kit (QIAGEN, Germany, Catalog No. 51104), and primers for antibiotic-resistant gene detection, Gotaq® Green Master Mix (Promega Corporation, Hollow Road-Madison, USA, Catalog No. M7112), agarose powder (Sigma-Aldrich, USA), and FluoroVue™ Nucleic Acid Gel Stain (SMOBIO Technology Inc., Taiwan).

The various equipment used are an autoclave, incubator, micropipette, bunsen, petri dish, cover glass, erlenmeyer, Eppendorf tube, beaker glass, object glass, measuring cup, hot plate equipped with a magnetic stirrer, incubator, test tube rack, test tube, scale, centrifuge (DLAB D2012 plus High-Speed Mini Centrifuge), refrigerator (Polytron), Polymerase Chain Reaction (PCR) machine (Swift™ MiniPro® Thermal Cycler), electrophoresis gel tank (Fisherbrand™ Horizontal Electrophoresis System), gel documentation (SMOBIO B-Box Blue Light LED epi-illuminator, AC 100 – 240V, 50/60Hz), scale (Precisa 205 A), vortex (Gemmy VM-300), and Sequencer Machine.

Methods

Identification of *Escherichia coli*

Identification technique of *E. coli* positivity from chicken eggs and cloacal

swabs used the procedure from Indonesian National Standard (SNI) 2897:2008[12]. *Escherichia coli* morphology of the samples were identified on selective media EMBA, MCA, and CCA, followed by biochemical tests using API Test 20E Kit (Biomerieux, USA, Catalog No. 20100) that can for testing β-galactosidase enzyme hydrolysis activity to the substrate o–nitrophenyl–b-D-galactopyranoside (ONP-G), decarboxylation of the amino acid arginine by arginine decarboxylase that also known as arginine dihydrolase (ADH), decarboxylation of the amino acid lysine by lysine decarboxylase (LDC), decarboxylation of the amino acid ornithine (ODC), citrate utilization (CIT), production of hydrogen sulfide (H_2S), urease (URE), tryptophan deaminase (TDA), Indole test (IND), Voges-Proskauer test (VP), production of the enzyme gelatinase (GEL), fermentation of glucose (GLU), mannose (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY), and arabinose (ARA), for clarification that the microbials are exactly *E. coli*.

Antibiotic Susceptibility Test and Multidrug-resistant Classification

Escherichia coli isolates are rejuvenated on BHI medium and then incubated for 24 hours. Afterwards, *Escherichia coli* isolate is suspended by inoculating 10 ml of sterile saline (0.9% NaCl). Then, the bacterial suspension was homogenized by vortex and compared its turbidity to a MacFarland 0.5 standard solution. Antibiotic susceptibility test was performed using the disk diffusion method (Kirby-Bauer)[13] on MHA and incubated at 37 °C for 16 – 18 hours. Antibiotics used were ampicillin (10 µg), chloramphenicol (30 µg), sulfamethoxazole (23.75 µg), tetracycline (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), streptomycin (10 µg), gentamicin (10 µg). The size of the inhibition zone diameter is used to determine antibiotic sensitivity into three results statuses: sensitive, intermediate, and resistance, which varies for each antibiotic according to the Clinical and Laboratory Standards Institute guidelines[14].

Multiple antibiotic resistance (MAR) indication was also performed in this study to assess the risk level of *Escherichia coli* isolates. If the MAR index is greater than 0.2, considered high risk, that means the isolate is multi-drug resistant. The MAR index is determined using the following formula[15].

$$MAR\ Index = \frac{Total\ of\ resistant\ antibiotic\ in\ every\ isolate}{Total\ antibiotics\ tested} \quad (1)$$

Detection of Antibiotic Resistance Genes in *Escherichia coli*

Bacterial genomic DNA was extracted using the QIAamp® DNA Mini Kit (Catalog No. 51104) as follows: One milliliter of *E. coli* stock was cultured in 5 ml of BHI medium at 37^0 C overnight. The culture was harvested by transferring 1 ml fresh culture into a 1.5 ml Eppendorf micro centrifuge tube, followed by centrifugation at 7500 rpm for 5 minutes to get the pellet of the bacterial cells. The supernatant was discarded, and this process was repeated for each fresh culture batch. Then, add Buffer ATL (supplied with QIAamp DNA mini kit) a total volume of 180 µl to the bacterial pellet. Add 20 µl proteinase K, mix by vortexing, and incubate at 56°C (10 – 30 min). Vortex occasionally during incubation to disperse the sample, and briefly centrifuge the 1.5 ml microcentrifuge be to remove droplets from the inside of the lid.

Mixture the sample add with 200 µl of Buffer AL for 15 seconds in a pulsing vortex, and then place it in an incubator at 70 °C for 10 minutes. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove

droplets from the lid. Add 200 µl of ethanol (96-100%) to the sample and pulse-vortex for 15 seconds to mix. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove droplets from the lid. Afterwards, gradually pour the mixture onto the QIAamp mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap and centrifuge for 1 minute at 8000 rpm. Place the QIAamp Mini Spin Column into the clean 2 ml collection tube provided and discard the tube containing the filtrate. Carefully open the end of the QIAamp Mini spin column and add 500 µl of Buffer AW1 without wetting the rim. Close the cap and centrifuge for 1 minute at 8000 rpm. After that, transfer the QIAamp mini spin column to a 2 ml collection tube (provided) and eliminate the collection tube that holds the filtrate. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at maximum speed (14,000 rpm) for 3 minutes. Place the QIAamp mini spin column into a new 2 ml collection tube (not included) and discard the old collection tube containing the filtrate. Centrifuge for 1 minute at maximum speed. Place the QIAamp mini spin column into a clean 1.5 mL micro centrifuge tube (not included) and discard the collection tube containing the filtrate. Carefully add 200 µL of Buffer AE to the QIAamp Mini spin column. Incubate for 1 min at room temperature and centrifuge for 1 min at 8000 rpm[16]. Removed QIAamp Mini spin column and the DNA solution in 1.5 ml microcentrifuge tube was kept at minus 20°C.

Polymerase Chain Reaction is conducted using specific primers, to detect antibiotic resistance genes in *Escherichia coli*. There are four types of resistant genes to the drugs tested in this study, i.e to tetracycline (*tetA*), to streptomycin (*aadA1*), to aminoglycoside (*aph(3)IIa*), and to β-lactamase (bla_{TEM1}. The primers used were *tetA*[17], *aadA1*[18], *aph(3)11a*[19] and bla_{TEM1}[20] (**Table 1)**. Briefly, reactions were performed in a total volume of 30 µL, using Promega Green Gotaq Master-mix (Catalog No. M7112). PCR optimization conditions, denaturation started at 95°C for 5 min followed by the 35 cycles with denaturation (95°C for 1 min), annealing (55°C for 1 min), extension (72°C for 1 min), and after 35 cycles, add an extension (72°C for 2 min). PCR results were visualized using gel electrophoresis and documented with UV light or gel documentation system[19].

Sequencing and Analysis of Phylogenetic and Polymorphism of bla_{TEM1} gene

Sequencing analysis was performed only in bla_{TEM1} gene, as understandable that the gene is one of the responsible genes in extended-spectrum beta-lactamase (ESBL) *E.coli*[21]. The writer used the sequencing service from PT. Genetika Science Indonesia for bla_{TEM1} gene sequencing analysis.

Phylogenetic and polymorphism analyses are performed using MEGA X software, where bla_{TEM1} gene sequences are compared with nine control bla_{TEM1} genes obtained from gene banks: Malaysia (NZ PKNA01000132), Singapore (NZ CP102064), Thailand (NZ RKKJ01000503), Vietnam (AP027949), India (MT174046 and KP724850), China (NG 050209. 1), Norway (NG 050185), and Hamburg (NG 050185).

RESULT AND DISCUSSION

Result

Identification of *E. coli*

Based on a pilot study conducted on 200 chicken eggs and 63 cloacal swabs in Sleman, Yogyakarta, 15 isolates (4 samples from chicken eggs and 11 samples from cloacal swabs) were suspected as

Table 1. Antibiotic-resistant Gene Primers

Resistance Gene	Primer	Nucleotide Sequence 5' – 3'	Product size (bp)	Annealing Temperature (^{0} C)	Reference Source
tetA	F	GGTTCACTCGAACGACGTCA	577	57	[17]
	R	CTGTCCGACAAGTTGCATGA			
aadA1	F	TATCAGAGGTAGTTGGCGTCAT	793	58	[18]
	R	GTTCCATAGCGTTAAGGTTTCATT			
aph(3)11a	F	TCTGAAACATGGCAAAGGTAG	484	55	[19]
	R	AGCCGTTTCTGTAATGAAGGA			
bla$_{TEM-1}$	F	CATTTCCGTGTCGCCCTTAT	582	55	[20]
	R	TCCATAGTTGCCTGACTCCC			

Escherichia coli based on the result of morphological characteristics on selective media (mentioned in method).

The macroscopic characteristics were determined on EMBA media, the colonies that grew were metallic green in colour which identified them as *E. coli*. Furthermore, the isolates were also cultured on CCA media, and the colonies that grew were violet/dark blue, which means they were positive for *E. coli*. Also, MCA was used and the positive *E. coli* colonies formed are in pink colour (Table 2).

Table 2. Morphological Identification of *E. coli* on Selective Media

No	Isolate code	Source	Selective Media		
			EMBA*	CCA**	MCA***
1	KP7	egg	green metallic	violet	pink
2	KP3	egg	green metallic	violet	pink
3	KP2	egg	green metallic	dark blue	pink
4	KP8	egg	green metallic	dark blue	pink
5	B6Ka	cloacal swab	green metallic	dark blue	pink
6	A7	cloacal swab	green metallic	dark blue	pink
7	C3	cloacal swab	green metallic	dark blue	pink
8	C2	cloacal swab	green metallic	dark blue	Pink
9	A1Ka	cloacal swab	green metallic	violet	Pink
10	A8	cloacal swab	green metallic	dark blue	Pink
11	B11	cloacal swab	green metallic	dark blue	Pink
12	B10	cloacal swab	green metallic	dark blue	Pink
13	C9	cloacal swab	green metallic	dark blue	Pink
14	A3	cloacal swab	green metallic	dark blue	pink
15	C4	cloacal swab	green metallic	dark blue	Pink

*__EMBA test:__ positive *E. coli* = green metallic; negative *E. coli* = brown/pink

**__CCA est:__ positive *E. coli* = violet/dark blue; negative *E. coli* = colorless

***__MCA test:__ positive *E. coli* = pink; negative *E. coli* = colorless

Despite that, after doing API 20E test there were only 12 isolates, which are 1 isolate (KP7) from chicken egg and 11 isolates from cloacal swabs, that were *E. coli* positive. The result of API 20E test was presented in Table 3**.**

Table 3. API 20E Test result

No	Isolate Code	Source	API 20E Test Result
1	KP7	Egg	*Escherichia coli*
2	KP3	Egg	*Hafnia alvei*
3	KP2	Egg	*Enterobacter cloacae*
4	KP8	Egg	*Enterobacter cloacae*
5	B6Ka	cloacal swab	*Escherichia coli*
6	A7	cloacal swab	*Escherichia coli*
7	C3	cloacal swab	*Escherichia coli*
8	C2	cloacal swab	*Escherichia coli*
9	A1Ka	cloacal swab	*Escherichia coli*
10	A8	cloacal swab	*Escherichia coli*
11	B11	cloacal swab	*Escherichia coli*
12	B10	cloacal swab	*Escherichia coli*
13	C9	cloacal swab	*Escherichia coli*
14	A3	cloacal swab	*Escherichia coli*
15	C4	cloacal swab	*Escherichia coli*

Antibiotic Susceptibility Test and Multidrug-resistant Classification

Susceptibility analysis of *E. coli* isolates obtained from one chicken egg (KP7) and 11 cloacal swab samples showed the highest resistance to the antibiotics erythromycin (100%), followed by ampicillin and ciprofloxacin (91.7%); then sulfamethoxazole, streptomycin, gentamicin, tetracycline, and chloramphenicol (in percentage order as follows: 83.3%; 83.3%; 75%; 41.7%; and 25% respectively. Multidrug-resistant *E. coli* analysis was performed on isolates acquired from chicken egg and cloacal swabs, and all isolates (12 or 100%) were categorized as MDR *E. coli*. All *E. coli* isolate from egg and cloacal swab samples were categorized as multi-drug-resistant based on the MAR index result, also they showed resistance to three or more classes of antibiotics tested.

Multidrug-resistant *E. coli* in this study were resistant to 5-6 classes of antibiotic tested on average, and there were even three isolates derived from cloacal swab samples (B6Ka, A8, and C4) experiencing resistance to all antibiotics tested. Moreover, the MAR index was also determined to analyze the risk level of *E. coli* isolates, and the MAR index value of 12 *E. coli* in this study was more than 0.2, which means that the all of *E. coli* isolates are at high risk when infecting humans or animals, because they experience multiple resistance to antibiotics (Table. 4).

The intermediate category was also observed in few samples (A7 & KP7). Antibiotics with intermediate status in A7 and KP7 are chloramphenicol and streptomycin respectively. It means that bacteria can be eliminated in body compartments that easily reached by the drug, while with the same dose antibiotic may not effectively treat the bacteria if they are infecting other body organs. Furthermore, in some cases of infectious bacterial infections, higher doses are required for treatment. The intermediate result is considered sensitive in the antibiogram, because in the antibiotic susceptibility test showed a zone of inhibition[22].

Table 4. Detail of MDR *E. coli* obtained from Chicken Egg and Cloacal swabs

Sample code	Resistance to**								MAR Index	MDR***
	AMP*	C*	SMX	TE*	CIP*	E*	Aminoglycoside drugs			
							S*	GM*		
	10 µg	30 µg	23.75 µg	30 µg	5 µg	15 µg	10 µg	10 µg		
KP7	R	S	S	S	S	R	I	S	0.25	+
B6Ka	R	R	R	R	R	R	R	R	1.00	+
A7	R	I	R	S	R	R	R	R	0.88	+
C3	R	S	R	S	R	R	R	R	0.75	+
C2	R	S	R	S	R	R	R	R	0.75	+
A1Ka	R	S	R	S	R	R	R	R	0.75	+
A8	R	R	R	R	R	R	R	R	1.00	+
B11	R	S	R	R	R	R	S	S	0.63	+
B10	S	S	R	R	R	R	R	S	0.63	+
C9	R	S	S	S	R	R	R	R	0.63	+
A3	R	S	R	S	R	R	R	R	0.75	+
C4	R	R	R	R	R	R	R	R	1.00	+
% of R	**91.7%**	**25%**	**83.3%**	**41.7%**	**91.7%**	**100%**	**83.3%**	**75%**		**100%**

*** AMP:** ampicillin, **C:** chloramphenicol, **SMX**: sulfamethoxazole, **TE:** tetracycline, **CIP:** ciprofloxacin, **S:** streptomycin , **E:** erythromycin,
GM : gentamicin.
** Antibiotic susceptibility status; **R**: resistance**,** S**:** susceptibility, **I**: intermediate (calculate as sensitive**)**
******* Multidrug-resistance: **negative MDR (-)** if score less than 0.2 ; **positive MDR** (+) if score more than 0.2**.**

Detection of Antibiotic Resistance Genes in *Escherichia coli*

Detection of resistant genes was also carried out to see the presence of resistant genes supporting antibiotic resistance in *E. coli* isolated from cloacal swabs and chicken eggs (Figure 1). Four types of resistant genes tested in this study, were *tetA*, *aadA1*, *aph(3)IIa*, and bla_{TEM1}. The results (Figure 2) performing DNA extraction and PCR with specific resistant gene primers showed that all *E. coli* isolates from both chicken egg and cloacal swabs had *aadA1* and bla_{TEM1} resistant genes (100%), while *tetA* and *aph(3)IIa* are found in 7 isolates (58.3%).

The presence of resistant genes in *E. coli* isolates supports and matches the phenotype profile of antibiotic resistance in these isolates, whereas ampicillin (β-lactamase) and streptomycin are common antibiotics resistant to *E. coli* in this experiment, according to their antibiotic susceptibility test. However, there are differences in the molecular analysis of resistant genes for these two classes of antibiotics. The resistant genes *aadA1* and bla_{TEM1} are found in all *E. coli* isolates (Table 5), but the antibiotic susceptibility results (Table 4) showed two isolates, one is still susceptible to ampicillin (isolate B10) but has the resistant gene to ampicillin and the other one is susceptible to streptomycin (isolate B11) but has the resistant gene to streptomycin. This allows those two *E. coli* isolates to be resistant if ampicillin and streptomycin are overused because resistant genes that support resistance to those two antibiotics are discovered in their genome.[23]

Table 5. PCR Result of Resistance Gene in *E. coli*

No	Gene Resistance	*E. coli* Isolate*											Percentage of positive gene resistance (%)
		B10	B11	C9	B6Ka	C4	A1Ka	C3	A8	A3	A7	KP7	C2	
1	*tetA*	-	+	-	-	+	-	-	+	+	+	+	+	58.3
2	*aadA1*	+	+	+	+	+	+	+	+	+	+	+	+	100
3	*aph(3)IIa*	+	+	-	+	-	-	-	-	+	+	+	+	58.3
4	bla_{TEM1}	+	+	+	+	+	+	+	+	+	+	+	+	100

***Positivity of gene resistance**: positive (+); negative (-)

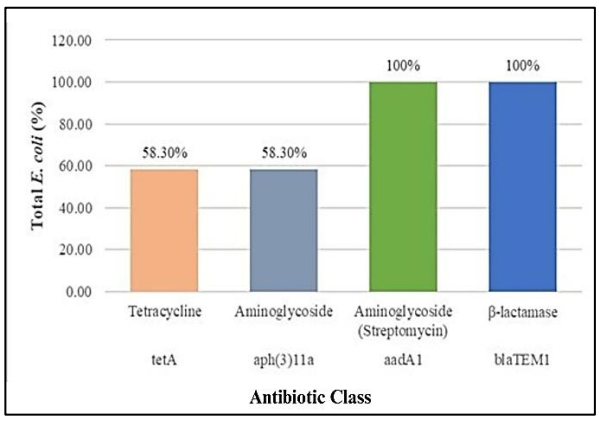

Figure 1. Percentage Prevalence of Antibiotic Resistance Genes in *E. coli* from chicken egg and cloacal swabs

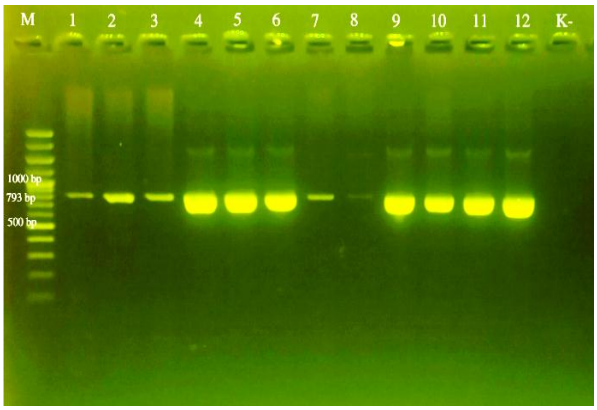

Figure 2. Electrophoresis of bla_{TEM1} resistant gene PCR positives results, with a band size is 793 bp. Lane: M : Marker (100 bp); 1: B10; 2: B11; 3: C9; 4: B6Ka; 5: C4; 6: A1Ka; 7: C3; 8: A8; 9: A3; 10: A7; 11: KP7; 12: C2; K- : negative control.

Sequencing and Analysis of Phylogenetic and Polymorphism of *bla*$_{TEM1}$ Gene

Sequencing was performed in this study, only on the *bla*$_{TEM1}$-resistant gene. The sequencing results showed genomic profiles in the twelve samples of *E. coli* that were resistant to β-lactamase class antibiotics. After editing and alignment using MEGA X software, then BLAST on the NCBI website https://blast.ncbi.nlm.nih.gov/Blast.cgi), it is known that 100% of *E. coli* in this research are *E. coli* resistant to β-lactamase class antibiotics.

The alignment process was performed to examine the presence of genetic variation in each gene sequence of *Escherichia coli* isolates. Alignment is done by comparing the gene sequences of all isolates with nine *bla*$_{TEM1}$ genes of *E. coli* obtained from gene banks as mentioned in the method.

Construction of phylogenetic trees performed in this study (Figure 3) showed that the twelve *E. coli* obtained had close similarities with *Escherichia coli* from Vietnam, India, China, Norway, and Hamburg (branch length 0.0000 - 0.0038), while *E. coli* from Singapore, Malaysia, and Thailand have distant similarities with the twelve *E. coli* in the study (branch length 1.7954 and 2.1368).

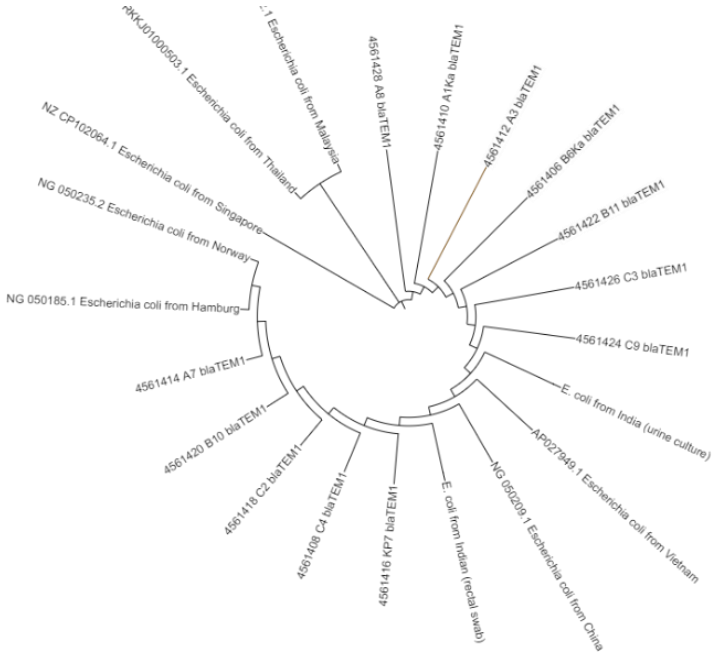

Figure 3. Phylogenetic tree of twelve *Escherichia coli* isolates (obtained from chicken egg and cloacal swabs). The phylogenetic tree was reconstructed using the neighbour-joining tree algorithm.

The alignment results show that there is one *E. coli* (A8) has a different nucleotide base from the other isolates. The nucleotide base in isolate A8 has genetic variations in the nucleotide 137th of the sequence. The codon formed in other *E. coli* is AGT (AGU: encodes serine amino acid), while in isolate A8 the codon formed is AAT (AAU), there is a substitution of guanine to adenine, so that the coded amino acid at those sites changes to asparagine. However, the difference in nucleotide bases in the isolates A8 did not affect the beta-lactamase class antibiotic resistance phenotype, which in this study the antibiotic used was ampicillin.

Discussion

Antibiotic resistance, especially in pathogenic bacteria, poses a serious threat to public health. *Escherichia coli* is one of the important bacteria that is a concern in human health, as well as veterinary. *Escherichia coli* bacteria are known to have developed resistance to many antibiotics, such as colistin, erythromycin, streptomycin, ciprofloxacin, sulfamethoxazole, and gentamicin[13]. This research was conducted to determine the antibiotic resistance profile and resistant genes in *E. coli* bacteria obtained from chicken eggs and cloacal swabs from broiler farms, as well as chicken eggs obtained from farms and supermarkets in the Sleman area, Yogyakarta, that potentially be transmitted to humans. It is known that *E. coli* when contaminating food, water, or the environment[7] and then infecting humans can cause several diseases such as intestinal (diarrhoea) and extraintestinal (urinary tract infection/bladder infection, sepsis, neonatal meningitis in humans and animals)[24].

The antibiotic susceptibility test profile of the twelve *E. coli* obtained in this study is shown in the result. Erythromycin is the highest antibiotic that has been resistant to *E. coli,* followed by ampicillin and ciprofloxacin.

Antibiotics are commonly used to treat *E. coli* infections in humans including ciprofloxacin (first-line therapy), penicillin (ampicillin), and sulfamethoxazole[25]. Ciprofloxacin is the best antibiotic in the treatment of *E. coli,* but it has experienced resistance[26].

Ciprofloxacin resistance is widely occurring, especially in broiler farms. An experiment by Kiiti *et al*.[27] showed that *E. coli* in broiler and layer chickens have the highest resistance to ampicillin (100%), sulfamethoxazole (89.2%), ciprofloxacin (68.6%), chloramphenicol (53.9%), ceftriaxone (46.6%), ertapenem (30.4%), and gentamicin (10.3%). Antibiotic resistance in *E. coli* is also clinically prevalent. Previous study showed antibiotics that have developed resistance and are primarily used to treat *E. coli* infection are ciprofloxacin[28], cefazolin, amoxicillin, cefuroxime, ceftriaxone, ceftazidime, gentamicin, and sulfonamide[24].

Multidrug-resistant *E. coli* in this study was discovered 100%, from 12 isolates of *E. coli*, only 1 isolate (KP7) has the lowest MAR index (0,25). Even tough, this isolate only resistant to two antibiotics out of 8 antibiotics tested, but its MAR result was more than 0,2 that means this isolate has a high risk as multidrug-resistant bacteria. *Escherichia coli* is one of the bacteria that present multidrug-resistance, this can impact human or animal *E. coli* infection treatment. Previous studies showed that *E. coli* obtained from pig farm and barbeque beef have MDR percentages of 57.3% and 87%[13,29] whereas the percentage of MDR *E. coli* from urine samples of urinary tract infection (UTIs) patients is 97.5%[30].

Antibiotic resistance and MDR that occur in *E. coli* or other bacteria are caused by the factor of unwise or excessive use of antibiotics[23]. Data from the WHO Regional Office for South-East Asia showed that the antibiotic use in South East Asia countries was high, and there was a misused of antibiotic in some countries[31]. The irrational use of antibiotics still occurs in many regions in Indonesia, recent research conducted by Hanifa[4], with the subject of a study was a health facility in the Loa Janan area, East Kalimantan. This result study showed that 27 cases (33.75%) of the antibiotics usage included in the category of irrational

antibiotic administration with details: short duration of consumption (12.50%), using less effective antibiotics (8.75%), and using antibiotics without an indication (12.50%). Another study conducted by Sholih, Muhtadi, and Saidah[32], also showed irrational use of antibiotics in Bandung regional hospitals, with drug utilization (DU) values reaching 90% for penicillin, cephalosporin, quinolone, and macrolide antibiotics.

Based on data obtained by Nguyen *et al*,[33] ampicillin usage, especially in Vietnam, is very high. Based on those data, antibiotics that are often purchased in rural Vietnam are extended-spectrum penicillins (amoxicillin and ampicillin), and first-generation cephalosporins (cefalexin). Similarly, in China and India, consumption of extended-spectrum penicillin antibiotics is also high. The use of extended-spectrum penicillin antibiotics (amoxicillin and ampicillin) in India almost reached 3 billion of antibiotic usage during the period 2000 – 2010[25], while in China extended-spectrum penicillin became the most frequently utilized antibiotic at about 21.21% of total antibiotic consumption[34]. The consumption of ampicillin in Indonesia itself, especially in the human health sector, reached 28.0 defined daily doses (DDD)/100 patient per days[35].

Livestock products such as meat, eggs, and chicken can be a medium for transmitting antibiotic-resistant pathogenic bacteria if these products are contaminated by them[36]. This can occur due to a lack of hygiene during product processing or farm location cleanliness[38]. Bacteria in the farm environment can also experience antibiotic resistance by the presence of resistant genes obtained from genetic transfer, or gene mutations that occur[38]. The resistance genes obtained in this study showed that *E. coli* samples from egg and cloacal swabs carry *tetA, aadA1, aph(3)IIa and* bla_{TEM1} genes , with the two highest (100%) resistance genes are *aadA1* and bla_{TEM1} while the two lower (58.3%) resistance genes are *tetA* and *aph(3)IIa.*

β-lactamase resistant genes (such as bla_{TEM1}, bla_{CTXM}, bla_{OXA}) are widely found in *E. coli*, where these genes can cause it extended-spectrum β-lactamase (ESBL)[39]. The percentage of β-lactamase resistant genes found in previous studies ranged from 42.86% to 98.2%[40]. In the other research arranged by Deku *et al.*[41], isolating *E. coli* from various clinical samples, such as urine, blood, sputum, and vaginal swabs also obtained ESBL genes, which the majority (83.9%) of ESBL *E. coli* had bla_{TEM1} gene.

Resistant genes discovered in *E. coli* isolates in this experiment also support the results of antibiotic susceptibility tests of these isolates. This also shows that one factor contributing to the occurrence of antibiotic-drug-resistant in pathogens is antibiotic-resistant genes. In a study carried out by Aworh *et al.*[42], testing the susceptibility of *E. coli* obtained from faeces samples of farm workers, chicken faeces, farm waste, and water around the farm, the highest resistance occurred to tetracycline, ampicillin, sulfamethoxazole/ trimethoprim, streptomycin, nalidixic acid, and gentamicin respectively. The resistance that occurs is supported by the discovery of resistant genes to the aminoglycoside class of antibiotics (streptomycin and gentamicin), and six types of β-lactamase resistant genes.

β-lactamase resistant genes are an important concern because the presence of these genes in bacteria can lead to the production of ESBL, that result in bacteria resistant to β-lactamase class antibiotics, and need a higher doses of antibiotics to treat infections from ESBL bacteria. The bacteria produce ESBL widely found in hospital facilities, and bacteria that are often produce

ESBLs are *E. coli* and *Klebsiella pneumonia*, that can cause of nosocomial infections.[21]

Resistant genes found in pathogenic bacteria are obtained from a horizontal genetic transfer process from other bacteria found in the surrounding environment[43]. Thus, resistant genes in *E. coli* obtained in this research are likely can be transferred to other pathogenic bacteria also. Antibiotic-resistant genes (ARGs) are transferred across bacteria by horizontal gene transfer, which is mediated by mobile genetic elements (MGE), such as integrons located on transposons and plasmids[44]. Based on the latest research [45,46], the bla_{TEM1} gene is known to be found in the plasmid of *Escherichia coli* bacteria, so it has the potential to be transferred horizontally to other pathogenic bacteria.

Escherichia coli carrying antibiotic-resistant genes can pollute the environment, and then the resistant genes can be transferred to other pathogenic bacteria, which can cause problems in public health. This can be happened because *E. coli* acts as a donor and as a recipient of resistance genes[47]. Thus, the detection of antibiotic-resistant genes in bacteria is important in the livestock, agriculture, or aquatic sectors that have the potential to transmit antibiotic-resistant bacteria through food (foodborne disease). The result study of Wintersdorff *et al.*[48] also found that MGE can spread to the environment. This result study is expected to be a reference for policymakers to give more attention to the use of antibiotics in every sector, which can increase antibiotic-resistant bacteria.

The β-lactamase TEM1 gene (bla_{TEM1}) sequenced in this study was carried out to ascertain the genomic profile of the isolated *E. coli* and the potential for gene changes in the sequence of DNA pair bases. The BLAST process on the NCBI website was carried out on the twelve samples, it was found that all *E. coli* isolates had close similarity with *E. coli* strains from Vietnam, India, China, Norway, and Hamburg in Gene-Bank.

Phylogenetic analysis was conducted to examine the relatedness of the twelve *E. coli* samples, which were aligned with nine *E. coli* bla_{TEM1} controls, e.g. *E. coli* from Malaysia, Singapore, Vietnam, Thailand, China, India, Norway, and Hamburg. Neighbour-joining tree reconstruction was used in the phylogenetic analysis. The twelve *E. coli* samples in this study have very close similarities with *E. coli* bla_{TEM1} strains from Vietnam, India, and China. These three countries are the countries with the three highest sales and consumption of FDC (fixed dose combination) antibiotics, and ampicillin β is the most widely sold antibiotic without a license from the FDA (Food and Drug Administration) when compared to Indonesia which is ranked 30th.[49] Moreover, Vietnam, China, and India are countries that use most antibiotics in food-producing animals, including Indonesia.[50]

There was a difference in the nucleotide sequence of A8 isolate compared with the other 11 isolates. The substitution of nitrogen base occurred in the 137th nucleotide of the sequence of A8 isolate causing a different amino acid in its sequence, so the genetic code serine amino acid change to be genetic code for asparagine amino acid, while the other isolates have serine as their amino acid. Changes in amino acids of isolate A8 did not affect the results of β-lactamase class antibiotic resistance phenotypes tested (ampicillin), where isolate A8 is still resistant to ampicillin antibiotics with no inhibition zone formed. Serine amino acid in the bla_{TEM1} gene is one of the amino acid that support β-lactam antibiotic degradation by producing serine-β-

lactamases (SBLs) enzyme. SBLs utilize a catalytic serine residue to initiate a nucleophilic attack on the β-lactam carbonyl, similar to the mechanism of serine-dependent protease.[51]

However, asparagine (Asn) has a similar function as serine, which can hydrolyze β-lactam antibiotics, also affect catalytic mechanism of β-lactam antibiotics[52]. Asparagine is critical for penicillin and cephalosporin hydrolysis, which can lead to resistance against these antibiotics[53]. Thus, the polymorphism of amino acid serine to asparagine does not affect the β-lactam antibiotic resistance phenotype of *E. coli* isolates.

Human mobilization can also affect the similarity of *E. coli* strains in Indonesia with other countries, especially with China and Vietnam, both are countries that have high mobilization to Indonesia or vice versa. Although human mobility from Indonesia to Malaysia, Singapore, and Thailand is also high, *E. coli* bla_{TEM1} strains from the three countries have a distant similarity with *E. coli* in Indonesia. This happens because the consumption of ampicillin antibiotics in public from those three countries is lower than in Indonesia.[49]

STRENGTH AND LIMITATION

The strength of the study obtained gave molecular information about resistant genes in *E. coli* collected from egg and swab cloacal of broiler chicken in Yogyakarta, Indonesia, and polymorphism of bla_{TEM1} gene. The limitation of the study was sample collection only done in supermarkets and farms and only from livestock. Samples collection needs to be expanded in human and environmental samples.

CONCLUSIONS

This study obtained 12 *Escherichia coli* isolates that are resistant to antibiotics tested with the highest percentage are erythromycin (100%), follow by ciprofloxacin (91.7%), ampicillin (91.7%), sulfamethoxazole (83.3%), streptomycin (83.3%), gentamicin (75%), tetracycline (41.7%), and chloramphenicol (25%). Resistant genes were also found in the samples obtained, with the highest percentage of *aadA1* and bla_{TEM1} genes (100% each), followed by *tetA* and *aph(3)IIa* genes (58.3% each). It is important to pay attention to this result study because a the resistant gene bla_{TEM1} that causes ESBL *E. coli* which has an important influence on human health, was found in 100% *E.coli* in this study and all of 12 *E. coli* samples were bacteria with MDR. One of the *E. coli* samples has a polymorphism in its gene, causing changes in the amino acid sequences, which is serine change with asparagine. The polymorphism did not affect the phenotype of *E. coli* resistance to beta-lactamase class antibiotics tested (ampicillin). This is because both amino acids have the same function, which can hydrolyze beta-lactamase antibiotics. Phylogenetic analysis showed that all the *E. coli* samples have close similarity with *E. coli* strains from China, Vietnam, and India. This happened because it was influenced by several factors, including high human mobility from the three countries to Indonesia. In addition, these three countries are known to have the highest consumption of ampicillin antibiotics (beta-lactam class) in the human or animal health sector. Detection of resistant genes and analysis of gene sequences in pathogens is crucial, to provide information on AMR data, which in Indonesia itself is still very limited. Therefore, this study is expected to provide

information on antibiotic-resistant genes in bacteria that could potentially become human pathogens from other sectors outside the health sector, which will help paramedics recognize the possibility of AMR transmission from the environment to the human health sector.

ACKNOWLEDGEMENT

The authors thank the Medicine Faculty and Veterinary Medicine Faculty of Universitas Gadjah Mada, and the Excellent Scholarship from the Indonesian Ministry of Education, for valuable support throughout this research.

FUNDING

This research was not supported by other grants.

CONFLICT OF INTEREST

The authors emphasize that they have no conflict of interest.

AUTHOR CONTRIBUTION

Experimental design: NIN. Materials preparation: NIN. Research implementation: NIN. Research supervision: WA, KP. Manuscript writing: NIN. Manuscript editing: NIN.

REFERENCES

1. Mulchandani R., Wang Y., Gilbert M., Van Boeckel T.P. Global trends in antimicrobial use in food-producing animals: 2020 to 2030. PLOS Glob Public Health. 2023. 3(2): 1-11.
2. Ansari, S., Nepal H.P., Gautam R., Shrestha S., Neopane P., Gurung G., and Cjapagain M.L. Community acquired multi-drug resistant clinical isolates of *Escherichia coli* in a tertiary care center of Nepal. Antimicrobial Resistance & Infection Control. 2015. 4:15.
3. Zhou Y., Zhou Z., Zheng L., Gong Z., Li Y., Jin Y., Huang Y., and Chi M. Urinary Tract Infection and Treatment Options. International Journal of Molecular Sciences. 2023. 24. 1 – 33.
4. Hanifa, D. N. C. Rasionalitas Penggunaan Antibiotik Pada Pasien Rawat Jalan Di Puskesmas Loa Janan Tahun 2020. Borneo Student Research (BSR). 2021. 3(1): 1002–1010.
5. Djaja, I. M., Puteri, A. R., & Wispriyono, B. Escherichia coli's contaminated food from Faculty Canteen in University X, Jakarta. Journal of Pure and Applied Microbiology. 2018. 12(2): 223–227.
6. Daneshmand, T.N., Friedrich M.N.D., Gachter M., Montealerge M.C., Nhiwatiwa T., Mosler H.J., and Julian T.R. Escherichia coli Contamination across Multiple Environmental Compartments (Soil, Hands, Drinking Water, and Handwashing Water) in Urban Harare: Correlations and Risk Factors. Amrican Journal of Tropical Medicine and Hygiene. 2018. 17-0521. 803 - 813.
7. Wardhana, D. K., Safitri D.A., Annisa S., Harijani N., Estoepangestie A.T.S., Maghfiroh L. Prevalence of Extended-Spectrum Beta-Lactamases in producing Escherichia coli in beef sold in traditional markets in Surabaya, Indonesia. Biodiversitas. 2021. 22(5). pp. 2789–2793. doi:

10.13057/biodiv/d220542.

8. Qiu, X., Zhou, G. , and Wang, H. Nanoscale zero-valent iron inhibits the horizontal gene transfer of antibiotic resistance genes in chicken manure compost. Journal of Hazardous Materials. 2022. p. 126883.
9. Kementerian Pertanian. Outlook Komoditas Peternakan Telur Ayam Ras Petelur. Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal. 2021. ISSN 1907 – 1507.
10. Lee, M., Seo D.J., Jeon S.B., Ok H.E., Jung H., Choi C., dan Chun H.Y. Detection of foodborne pathogens and mycotoxins in eggs and chicken feeds from farms to retail markets. Korean Journal for Food Science of Animal Resources. 2016. 36(4) : 463–468.
11. Wellner S.M., Alobaidallah M.S.A., Fei X., Herrero-Fresno A., Olsen J.E. Genome-wide identification of fitness-genes in aminoglycoside-resistant Escherichia coli during antibiotic stress. Sci Rep. 2024.14(1):4163.
12. Pengujian Cemaran Mikroba dalam Daging, Telur, dan Susu, serta Hasil Olahannya. 2008. Bogor: Bandar Standardisasi Indonesia.
13. Kallau, N. H. G., Wibawan I.W.T., Lukman D.W., Sudarwanto M.B. Detection of multi-drug resistant (MDR) *Escherichia coli* and *tet* gene prevalence at a pig farm in Kupang, Indonesia. Journal of Advanced Veterinary and Animal Research. 2018. 5(4): 388–396.
14. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing 31st Edition. West Valley Road. USA. 2021. 1 – 352.
15. Mthembu T.P., Zishiri O.T., El Zowalaty M.E. Molecular Detection of Multidrug-Resistant *Salmonella* Isolated from Livestock Production Systems in South Africa. *Infect Drug Resist*. 2019. 12: 3537 – 3548.
16. Qiagen. Qiamp® DNA Mini and Blood Mini Handbook. USA. 2016. 1 -72.
17. Adeyanju, G.T. and Ishola, O. *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *SpringerPlus*. 2014. 3(1): 139.
18. Algammal A.M., El-Tarabili R.M., Abd El-Ghany W.A., Almanzalawi E.A., Alqahtani T.M., Ghabban H., Al-Otaibi A.S., Alatfeehy N.M., Abosleima N.M., Hetta H.F., Badawy G.A. Resistance profiles, virulence and antimicrobial resistance genes of XDR *S. Enteritidis* and *S. Typhimurium*. AMB Express. 2023.13(1):110.
19. Adesiji, Y. O., Deekshit, V. K. and Karunasagar, I. . Antimicrobial-resistant genes associated with Salmonella spp. isolated from human, poultry, and seafood sources. Food Science & Nutrition. 2014. 2(4): 436–442.
20. Islam, M. S., Sobur M.A., Rahman S., Ballah F.M., Levy S., Siddique M.P., Rahman M., Kafi M.A., Rahman M.T. Detection of *blaTEM*, *blaCTX-M*, *blaCMY*, and *blaSHV* Genes Among Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Isolated from Migratory Birds Travelling to Bangladesh. Microbial Ecology. 2022. 83(4): 942–950.

21. Karlowsky, J. A., Lob S.H., DeRyke C.A., Siddiqui F., Young K., Motyl M.R., Sahm D.F. Prevalence of ESBL non-CRE *Escherichia coli* and *Klebsiella pneumoniae* among clinical isolates collected by the SMART global surveillance program from 2015 to 2019. International Journal of Antimicrobial Agents. 2022. 59(3): 106535.
22. Kahlmeter G. EUCAST proposes to change the definition and usefulness of the susceptibility category 'Intermediate'. Clinical Microbiology and Infection. 2017. 23(12): 894 – 895.
23. Jian Z., Zeng L., Xu T., Sun S., Yan S., Yang L., Huang Y., Jia J., Dou T. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. Journal of Basic Microbiology. 2021. 61: 1049 - 1070.
24. Wu, D., Ding Y., Yao K., Gao W., Wang Y. Antimicrobial Resistance Analysis of Clinical *Escherichia coli* Isolates in Neonatal Ward. Frontiers in Pediatrics. 2021. 9(1): 1–7
25. Laxminarayan R. dan Chaudhury R.R. Antibiotic Resistance in India: Opportunities for Action. PLoS Med. 2016. 13(3): 1 – 7.
26. Rahman, Md Masudur., Husna A., Elshabrawy H.A., Alam J., Runa N.Y.,Badruzzaman A.T.M., Banu N.A., Al Mamun M., Paul B., Das S., Rahman Md.M., Mahbub-E-Elahi A.T.M. Khairalla A.S., dan Ashour H.M. Isolation and molecular characterization of multidrug-resistant *Escherichia coli* from chicken meat. Scientific Reports. 2020. 10(1). pp. 1–11.
27. Kiiti, R. W., Komba E.V., Msoffe P.L., Mshana S.E., Rweyemamu M., dan Matte M.I.N. Antimicrobial Resistance Profiles of Escherichia coli Isolated from Broiler and Layer Chickens in Arusha and Mwanza, Tanzania. International Journal of Microbiology. 2021. doi: 10.1155/2021/6759046.
28. Neyestani Z., Khademi F., Teimourpour R., Amani M., and Arzanlou M. Prevalence and mechanisms of ciprofloxacin resistance in Escherichia coli isolated from hospitalized patients, healthy carriers, and wastewaters in Iran. BMC Microbiol. 2023. 23(1):191 – 202.
29. Datok, D. W., Ishaleku D., Tsaku P.A., Agya E.O., dan Adoga M.P. Multidrug resistance to commonly prescribed antibiotics in escherichia coli isolated from barbecued beef (Suya) sold in a Nigerian city. Pan African Medical Journal. 2021. 39. doi: 10.11604/pamj.2021.39.50.25502.
30. Ramírez-Castillo, F. Y., Flores A.C.M., Gonzalez F.J.A., Diaz F.M., Harel J., dan Barrera A.L.G. An evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: Cross-sectional study. Annals of Clinical Microbiology and Antimicrobials. 2018. 17(1): 1–13.
31. Holloway K. Antibiotic use in South East Asia and policies to promote appropriate use: reports from country situational analyses. BMJ. 2017. 358:j2291.
32. Sholih, M. G., Muhtadi, A. dan Saidah, S. Rasionalitas Penggunaan Antibiotik di Salah Satu Rumah Sakit Umum di Bandung Tahun

2010. 2015. Indonesian J Clin Pharm. 4(1): 64–70.

33. Nguyen N.V., Do N.T.T., Nguyen C.T.K., Tran T.K., Ho P.D., Nguyen H.H., Vu H.T.L., Wertheim H.F.L., Van Doorn H.R., dan Lewycka S. Community-level consumption of antibiotics according to the AWaRe (Access, Watch, Reserve) classification in rural Vietnam. JAC Antimicrob Res. 2020. 1(1): 1-8.
34. Song Y., Han Z., Song K., dan Zhen T.. Antibiotic Consumption Trends in China: Evidence From Six-Year Surveillance Sales Records in Shandong Province. Front. Pharmacol. 2020. 11:491.
35. Limato, R., Lazarus G., Dernison P., Mudia M., Alamanda M., Nelwan E.J., Sinto R., Karuniawati A. Lancet Reg Health. 2022. 2(1): 1 – 23.
36. Abreu R., Lemsaddek T.S., Cunha E., Tavares L., and Oliveira M. Antimicrobial Drug Resistance in Poultry Production: Current Status and Innovative Strategies for Bacterial Control. Microorganisms. 2023. 11(4): 1 -35.
37. Qian J., Wu Z., Zhu Y., Liu C. One Health: a holistic approach for food safety in livestock. Science in One Health. 2022. 1(1) : 1 – 10.
38. Scicchitano D., Babbi G., Palladino G., Turroni S., Mekonnen Y.T., Laczny C., Wilmes P., Leekitcharoenphon P., Castagnetti A., D'Amico F., Brigidi P., Savojardo C., Manfreda G., Martelli P., De Cesare A., Aarestrup M.C., and Rampelli S. Routes of dispersion of antibiotic resistance genes from the poultry farm system. Sci Total Environ. 2024. 1(1) : 1 – 12.
39. Islam, M. S., Sobur M.A., Rahman S., Ballah F.M., Levy S., Siddique M.P., Rahman M., Kafi M.A., Rahman M.T. Detection of *blaTEM*, *blaCTX-M*, *blaCMY*, and *blaSHV* Genes Among Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Isolated from Migratory Birds Travelling to Bangladesh. Microb Ecol. 2022. 83(4): 942–950.
40. Zhao, X., Yang J., Ju Z., Chang W., dan Sun S. Molecular Characterization of Antimicrobial Resistance in *Escherichia coli* from Rabbit Farms in Tai'an, China. BioMed Res Int. 2018. 1(1). doi: 10.1155/2018/8607647.
41. Deku, J. G., Duedu K.O., Ativi E., Kpene G.E., dan Feglo P.K. Occurrence and distribution of extended-spectrum β-lactamase in clinical Escherichia coli isolates at Ho Teaching Hospital in Ghana. Ghana Med J. 2021. 55(4): 298–307.
42. Aworh, M. K., Kwaga J.K.P, Hendriksen R.S., Okolocha E.C., dan Siddhartha Thakur. Genetic relatedness of multidrug- resistant Escherichia coli isolated from humans, chickens and poultry environments. Antimicrob Res Infect Control. 2021.10(1) : 1–13
43. Mecik M., Hubeny M.B., Paukszto L., Mazdiarz M., Wolak I., Harnisz M., and Korzeniewska E. Poultry manure-derived microorganism as a revoir and source of antibiotic resistance genes transferred to soil autochthonous microorganisms. J Environ Manag. 2023. 348 (2023) 119303.
44. Qiu, X., Zhou, G. dan Wang, H. Nanoscale zero-valent iron inhibits the horizontal gene transfer of antibiotic resistance genes in chicken manure compost. J Hazard Mater.

2022. p. 126883.
45. Ramírez-Bayard, I. E. Mejia F., Medina-Sanchez J.R., Cornejo-Reyes H., Castillo M., Querol-Audi J., Martinez-Torres A.O. Prevalence of Plasmid-Associated Tetracycline Resistance Genes in Multidrug-Resistant Escherichia coli Strains Isolated from Environmental, Animal and Human Samples in Panama. Antibiotics. 2023. 12(2): 280.
46. Wang, J., Stephan R., Zurfluh K., Hachler H., dan Fanning S. Characterization of the genetic environment of blaESBL genes, integrons and toxin-antitoxin systems identified on large transferrable plasmids in multi-drug resistant Escherichia coli. Frontiers in Microbiology. 2015. 5(1). pp. 1–8.
47. Poirel L., Madec J.Y., Lupo A., Schink A.K., Kieffer N., Nordmann P., and Schwarz S. Antimicrobial resistance in *Escherichia coli*. Microbiol Spectrum. 2017. 6(4) : ARBA-0026-2017.
48. Von Wintersdorff, C. J. H., Penders J., van Niekerk J.M., Mils N.D., Majumder S., van Alphen L.B., Savelkoul P.H.M., dan Wolffs P.F.G. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Frontiers in Microbiology. 2016. 7(1): 1–10.
49. Bortone, B., Jackson C., Hsia Y., Bielicki J., Magrini N., and Sharland M. High global consumption of potentially inappropriate fixed dose combination antibiotics: Analysis of data from 75 countries. PLOS ONE. 2020. 16(1): 1 – 11.
50. Mulchandani R., Wang Y., Gilbert M., Van Boeckel T.P. Global trends in antimicrobial use in food-producing animals: 2020 to 2030. PLOS Glob Public Health. 2023. 3(2): 1-11.
51. Bahr, G., González L.J., Vila A.J. Metallo-β-lactamases in the Age of Multidrug Resistance: From Structure and Mechanism to Evolution, Dissemination, and Inhibitor Design. Chem Rev. 2021 .121(13):7957-8094.
52. Skalweit MJ, Li M, Taracila MA. Effect of asparagine substitutions in the YXN loop of a class C β-lactamase of Acinetobacter baumannii on substrate and inhibitor kinetics. Antimicrob Agents Chemother. 2015. 59(3): 1472-1477.
53. Judge, A., Hu, L., Sankaran, B. et al. Mapping the determinants of catalysis and substrate specificity of the antibiotic resistance enzyme CTX-M β-lactamase. Commun Biol.2023.6(35): 1 – 11.