BIODIVERSITAS
Volume 25, Number 3, March 2024
Pages: 1246-1253

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250341

Molecular detection of *hly*F gene on multidrug resistance of avian pathogenic *Escherichia coli* isolated from ducks on wet markets of Surabaya, Indonesia

IRFAN ALIAS KENDEK[1], MARIANA FEBRILIANTI RESILINDA PUTRI[1], FRESHINTA JELLIA WIBISONO[2], MUSTOFA HELMI EFFENDI[3,♥], WIWIEK TYASNINGSIH[4], EMMANUEL NNABUIKE UGBO[5], NNABUIFE BERNARD AGUMAH[5]

[1]Master Program in Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C FKH-UNAIR, Surabaya 60115, East Java, Indonesia

[2]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma. Jl. Dukuh Kupang XXV No. 54, Surabaya 60225, East Java, Indonesia

[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C FKH-UNAIR, Surabaya 60115, East Java, Indonesia. Tel./fax.: +62-31-5992785, ♥email: mhelmieffendi@gmail.com

[4]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Raya Mulyorejo, Surabaya 60115, East Java, Indonesia

[5]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, P M B 053, Abakaliki Ebonyi State, Nigeria

Manuscript received: 11 February 2023. Revision accepted: 28 March 2024.

Abstract. *Kendek IA, Putri MFR, Wibisono FJ, Effendi MH, Tyasningsih W, Ugbo EN, Agumah NB. 2024. Molecular detection of hly*F *gene on multidrug resistance of avian pathogenic* Escherichia coli *isolated from ducks on wet markets of Surabaya, Indonesia. Biodiversitas 25: 1246-1252.* Avian Pathogenic *Escherichia coli* (APEC) is a strain of pathogen that can cause colibacillosis in poultry, including ducks worldwide. This disease can be influenced by various virulence genes, one of which is the *hly*F gene which can cause systemic disorders and potentially become zoonotic in poultry. This study aimed to detect the *hly*F virulence gene from the multidrug resistance (MDR) properties of *Escherichia coli* originating from 158 duck cloacal swab samples from seven live markets in Surabaya. Samples were isolated and identified using Eosin Methylene Blue Agar (EMBA) and MacConkey Agar (MCA) media, Gram staining, and then continued with biochemical tests of Triple Sugar Iron Agar (TSIA), Simmons Citrate Agar (SCA), Sulfide Indole Motility (SIM), Methyl red (MR) and Voges-Proskauer (VP). Samples were tested for MDR properties and continued with confirmation of the *hly*F virulence gene using PCR. Based on the results of this study, it showed that 85% (134/158) were positive for *Escherichia coli*. *Escherichia coli* bacteria experienced resistance to the antibiotic erythromycin 96% (129/134), ciprofloxacin 16% (22/134), antibiotics gentamicin 15% (20/134), aztreonam 4.4% (6/134) and chloramphenicol 3% (5/58). The MDR test results in this study were 15% (20/134), while the PCR test results for the *hly*F gene were 60% (12/20). Therefore, *Escherichia coli* was found to have the *hly*F virulence gene, an MDR in *Escherichia coli* bacteria at the Surabaya live market. Furthermore, APEC strains with the *hly*F gene, an MDR, potentially affect public health.

Keywords: C*olibacillosis*, *hly*F gene, multidrug resistance, public health, virulence gene

INTRODUCTION

Escherichia coli bacteria are Gram-negative bacteria that are normal flora in the digestive tract and are pathogenic and capable of attacking animals and humans (Na et al. 2019; Sora et al. 2021; Wibisono et al. 2021; Islam et al. 2023). Based on the level of pathogenesis, *Escherichia coli* bacteria are classified into three groups, namely Extraintestinal Pathogenic *Escherichia coli* (ExPEC), intestinal pathogenic *Escherichia coli* (InPEC) and commensal strains (Ovi et al. 2023). ExPEC strains are a group of *Escherichia coli* that infect extraintestinal tissues, such as the urinary tract, respiratory tract, and yolk sac infections (Akanbi et al. 2022; Hu et al. 2022). One of the ExPEC substrains is Avian Pathogenic *Escherichia coli* (APEC) which causes systemic disorders that can result in colibacillosis in poultry, high morbidity, high mortality, production losses, and the risk of transmission through food to humans (Logue et al. 2017; Maciel et al. 2017; Sarowska et al. 2019; Kathayat et al. 2021; Aberkane et al. 2023). Avian Pathogenic *Escherichia coli* (APEC) strains cause a wide range of local and systemic infections in poultry, including chickens, turkeys, ducks, and other bird species (Papouskova et al. 2020; Kathayat et al. 2021). Lesions caused by APEC are characterized by sacculitis, peritonitis, pericarditis, salpingitis, synovitis, osteomyelitis, omphalitis, septicemia, and others (Azam et al. 2020; Ievy et al. 2022). APEC strains have the potential to be zoonotic this is because general virulence factors of APEC have been found in humans, and there is a relationship between APEC, UPEC, and NMEC (Oliveira et al. 2019; Xu et al. 2019).

APEC strains can carry various virulence genes related to the pathogenesis of colibacillosis, including adhesion, invasion, iron acquisition system, siderophores, and toxins (Saha et al. 2020). APEC virulence factors are influenced

by several genes contained in the plasmid (Ramaditya et al. 2019; Bakhshi et al. 2020). Avian Pathogenic *Escherichia coli* virulence factors vary, such as iss, tsh, iroN, episomal/ chromosomal ompT, iutA, cvaC, *hly*F, iucD, papG allele (II/III), iroN and papC (Kathayat et al. 2021; Ovi et al. 2023). The *hly*F gene encodes various types of toxins, *hly*F, *hly*A, *hly*E (avian hemolysin), tong (vacuolation toxin autotransporter), cdtB, cdtS (cytolethal swelling factor), stx2f (shiga toxin variant), pic (serine protease autotransporter), espC (serine protease), ace4/35 (acetylcholine esterase), sat (porter toxin auto trans) and toxins that facilitate biofilm formation, agglutination, vacuolization induction, and outer membrane vesicle formation (Murase et al. 2015; Velhner et al. 2018; Kathayat et al. 2021). These vesicles are bacterial virulence factors that cause pathological changes in infected hosts (Murase et al. 2015).

Measures to prevent APEC infection can be carried out by vaccination with respiratory agents, reducing stress in chickens, predisposing factors, and treatment with antibiotics (Adrenalin et al. 2020). The use of antibiotics in animal production has a major impact on public health, increasing the incidence of antibiotic and multidrug resistance (Saha et al. 2020; Effendi et al. 2021; Yousef et al. 2023). Resistance events can be transmitted to other animals and humans through direct contact, production of animal products, and indirectly through the environment (Vidovic and Vidovic 2020; Tyasningsih et al. 2022; Faridah et al. 2023). Resistance to more than three types of antibiotics from different groups is called multidrug resistance (MDR). Antibiotic resistance can occur through mechanisms of transfer of resistance genes between various bacteria vertically or horizontally (transformation, conjugation, and transduction) (Amer et al. 2020; Dameanti et al. 2023).

Moreover, APEC bacteria in ducks has not yet been widely reported in Indonesia. Hence, in isolating the bacteria, the incidence of multidrug resistance and the molecular gene encoding *hly*F in poultry are based on symptoms of colibacillosis caused by Avian Pathogenic *Escherichia coli* (APEC). Therefore, it is necessary to carry out this research to determine the *hly*F virulence gene in APEC in Indonesia. The finding of the virulence factor is to increase public awareness of the safety of food originating from ducks and among breeders of the colibacillosis disease caused by the APEC strain.

MATERIALS AND METHODS

Ethical approval

The research commission for animal ethics approval was obtained from the Faculty of Veterinary Medicine, Wijaya Kusuma University, Surabaya, Indonesia (ethics number: 139-KKE-2023).

Sample collection

The number of samples used was 158 Mojosari duck cloacal swabs in wet markets in Surabaya, including Wonokromo Market, Pucang, Pacar Keling, Benowo, Keputran, Pabean, and Krampung. Samples were taken from sterile cotton swabs of duck cloaca (Onemed, Indonesia). All samples were transported into test tubes containing Buffer Peptone Water (BPW) (HiMedia). All samples were transported using a thermobox at a temperature of 4°C (Wibisono et al. 2020; Yanestria et al. 2022).

Isolation and identification of *Escherichia coli*

Escherichia coli bacteria were isolated using Eosin Methylene Blue Agar (EMBA) (HiMedia M317) and MacConkey Agar (MCA) (HiMedia MH081) media: incubated at 37°C for 18-24 hours. Morphological identification of *Escherichia coli* using Gram staining then continued with the biochemical test Triple Sugar Iron Agar (TSIA) (HiMedia M021): Simmons Citrate Agar (SCA) (HiMedia M099), IMVIC media, such as Sulfide Indole Motility (SIM) (HiMedia M181): Methyl red (MR): Voges-Proskauer (VP) (Merck; 105712) (Wibisono et al. 2020; Effendi et al. 2021; Yanestria et al. 2022).

Antibiotic sensitivity test

Antibiotic Sensitivity Tests were done using the Kiebery-Beur disk diffusion method isolated on Mueller Hinton Agar (MHA) media (HiMedia M173) and adjusted to the standards of the Clinical Laboratory Standards Institute (CLSI 2021). The antibiotic disk paper uses five classes of antibiotics, including Aztreonam 30 µg (Oxoid CT0264B), Chloramphenicol 30 µg (Oxoid CT0013B), Gentamicin 10 µg (Oxoid CT0026B), Ciprofloxacin 5 µg (Oxoid CT0425B) and Erythromycin 15 µg (Oxoid CT054B). One to two colonies were taken from the MCA medium using a sterile tube, then placed in physiological NaCl with Mc Farland standard 0.5 (1.5x108 CFU/mL). The next process was to carry out a culture using a sterile cotton swab and smear it on Mueller Hinton Agar (MHA) media over the entire surface of the petri dish. The media was divided into five parts, and then the disc was placed on the MHA media (Musa et al. 2020) and incubated at 37°C for 24 hours. Interpretation of the results of the diameter of the inhibition zone is adjusted based on CLSI (CLSI 2020; Effendi et al. 2021; Yanestria et al. 2022). Isolates are declared multidrug-resistant (MDR), where bacteria are resistant to three or more different classes of antibiotics (Kissinga et al. 2018; Effendi et al. 2022; Putri et al. 2023).

Characteristics of the *hly*F Gene using Polymerase Chain Reaction (PCR)

DNA extraction using QIAamp® DNA kit (QIAGEN, Germany). The forward primer used is GGCGATTTAGGC ATTCCGATACTC, while the reverse primer is ACGGGG TCGCTAGTTAAGGAG with a target of 599 bp. Thermal cycler conditions with predenaturation parameters at 94°C for seven minutes, denaturation at 94°C for one minute, annealing at 56°C for 30 seconds, extension at 72°C for 30 seconds, cycle repeated 35 times, and final extension at 72°C for five minutes. Each primer is calibrated first to determine the appropriate annealing temperature and amplification using PCR. After that, the amplicons were visualized by electrophoresis using 2% agarose gel (Ramaditya et al. 2019).

RESULTS AND DISCUSSION

Based on the results of the isolation and identification of *Escherichia coli* bacteria in this study, 85% (134/158) were positive for *Escherichia coli*, and 15% (24/158) were negative in duck cloacal swabs from seven wet markets in Surabaya.

The results of isolation and identification tests for *Escherichia coli* bacteria originating from duck cloacal swabs from seven wet markets in Surabaya showed that the highest percentage of *Escherichia coli* bacteria came from the Wonokromo wet market at 100% (30/30).

Sampling uses enrichment media, namely Buffered Peptone Water (BPW), then the samples were taken to the laboratory for testing using a cool box at a temperature of 4ºC. The first isolation used Eosin Methylene Blue Agar (EMBA) media, with the colony morphology of *Escherichia coli* bacteria being metallic green and having a black dot in the middle. The second and third purification isolations used Mac Conkey Agar (MCA) media with the morphology of small round, separate, irregular colonies, and pink.

The results of antibiotic sensitivity tests for five different classes of antibiotics obtained from duck cloacal swabs showed that the highest incidence occurred in the antibiotic Erythromycin with a percentage of 96% (129/134), then antibiotic resistance in ciprofloxacin was 16% (22/134), the antibiotic gentamicin was 15% (20/134). In comparison, the antibiotics with the lowest incidence of resistance were aztreonam and chloramphenicol at 4.4% (6/134). MDR test results on duck cloacal swabs from seven live markets in Surabaya showed an incidence of 15% (20/134). The highest percentage was in the Wonokromo market at 23% (7/30), followed by the Pabean market at 18.7% (3/16), Pucang market at 16.6% (2/12), and Keputran at 16.6% (4/24). The Kapas Krampung was 11.7% (2/17), the Kelling market at 6.2% (1/16), and the Benowo market at 5.2% (1/19).

The resistance pattern of Escherichia coli from duck cloca swabs at the Surabaya live market shows that the incidence of Multidrug Resistance (MDR) is in (Table 3).

Based on the results of this study, 12/20 *Escherichia coli* isolates from duck cloacal swabs showed a positive band at 667 bp, so 60% of multidrug-resistant *Escherichia coli* isolates had the *hly*F virulence gene in this study.

Table 1. Isolation and identification of *Escherichia coli* from duck cloacal swabs at Wet markets, Surabaya

Wet market	Sample size	*Escherichia coli* (%)	
		Positive	Negative
Pabean	20	80% (16/20)	20% (4/20)
Wonokromo	30	100% (30/30)	0% (0/30)
Pucang	20	60% (12/20)	40% (8/20)
Kapas Krampung	21	81% (17/21)	19% (4/21)
Keputran	26	92% (24/26)	8% (2/26)
Pacar Keling	21	76% (16/21)	24% (5/21)
Benowo	20	95% (19/20)	5% (1/20)
Total	**158**	**85% (134/158)**	**15% (24/158)**

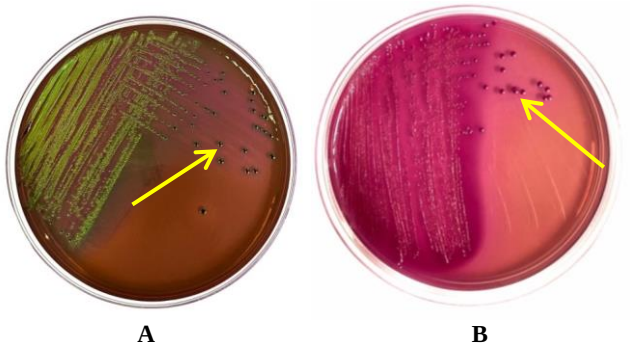

Figure 1. A. Colonies of *Escherichia coli* bacteria on Eosin Methylene Blue Agar (EMBA) media, B. Colonies of *Escherichia coli* bacteria on Mac Conkey Agar (MCA) media

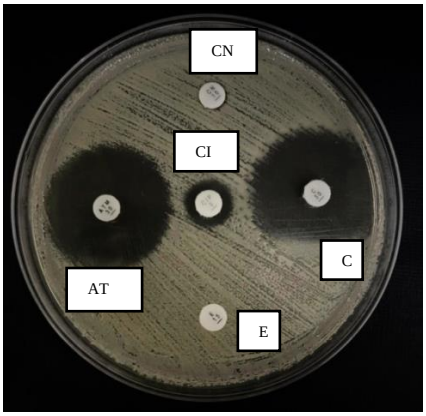

Figure 2. Multidrug resistance of *Escherichia coli*. Note: CN: Gentamicin; C: Chloramphenicol; E: Eritromisin; ATM: Aztreonam; CIP: Ciprofloxacin

Table 2. Profile of antibiotic resistance on *Escherichia coli*

Wet Market Surabaya	Sample size	Antibiotic-Resistant (%)					MDR
		ATM	CIP	CN	C	E	
Pabean	16	0% (0/16)	37% (6/16)	37% (6/16)	6% (1/16)	81% (13/16)	18,7% (3/16)
Wonokromo	30	3% (1/30)	27% (8/30)	3% (1/30)	7% (2/30)	97% (29/30)	23% (7/30)
Pucang	12	8% (1/12)	17% (2/12)	17% (2/12)	8% (1/12)	100% (12/12)	16,6% (2/12)
Kapas Krampung	17	6% (1/17)	12% (2/17)	12% (2/17)	0% (0/17)	94% (16/17)	11.7% (2/17)
Keputran	24	4% (1/24)	8% (2/24)	13% (3/24)	4% (1/24)	100% (24/24)	16,6% (4/24)
Kelling	16	6% (1/16)	0% (0/16)	25% (4/16)	0% (0/16)	100% (16/16)	6.2% (1/16)
Benowo	19	5% (1/19)	11% (2/19)	11% (2/19)	5.2% (1/19)	100% (19/19)	5.2% (1/19)
Total	134	4.4% (6/134)	16% (22/134)	15% (20/134)	4.4% (6/134)	96% (129/134)	15% (20/134)

Note: ATM: Aztreonam, CIP: Ciprofloxacin, CN: Gentamisin, C: Chloramfenicol, E: Eritromisin and MDR: Multidrug resistant

Table 3. Resistance pattern of Multidrug-resistant *Escherichia coli*

Sample code	Antibiotics					Resistance pattern
	ATM	CIP	CN	C	E	
PKE14	R	R	R	S	R	ATM/CIP/CN/E
PAW9	R	R	S	R	R	ATM/CIP/C/E
PPC7	S	R	R	R	R	CIP/CN/C/E
PAB19	S	R	R	R	R	CIP/CN/C/E
PKR18	R	R	S	S	R	ATM/CIP/E
PKP9	R	S	R	S	R	ATM/CN/E
PAB20	R	R	R	S	R	CIP/CN/E
PAW23	S	R	R	S	R	CIP/CN/E
PAW26	S	R	R	S	R	CIP/CN/E
PAW28	S	R	R	R	R	CIP/CN/C/E
PAW29	S	R	R	S	R	CIP/CN/E
PAW30	S	R	R	S	R	CIP/CN/E
PAB18	S	R	R	S	R	CIP/CN/E
PPC14	S	R	R	S	R	CIP/CN/E
PKR12	S	R	R	S	R	CIP/CN/E
PKP11	S	R	R	S	R	CIP/CN/E
PKP23	S	R	R	S	R	CIP/CN/E
PBW14	S	R	R	S	R	CIP/CN/E
PAW2	S	R	S	R	R	CIP/C/E
PKP16	S	S	R	R	R	CN/C/E

Note: ATM: Aztreonam, CIP: Ciprofloxacin, CN: Gentamicin, C: Chloramphenicol, E: Eritromisin, R: Resistant, S: Sensitive

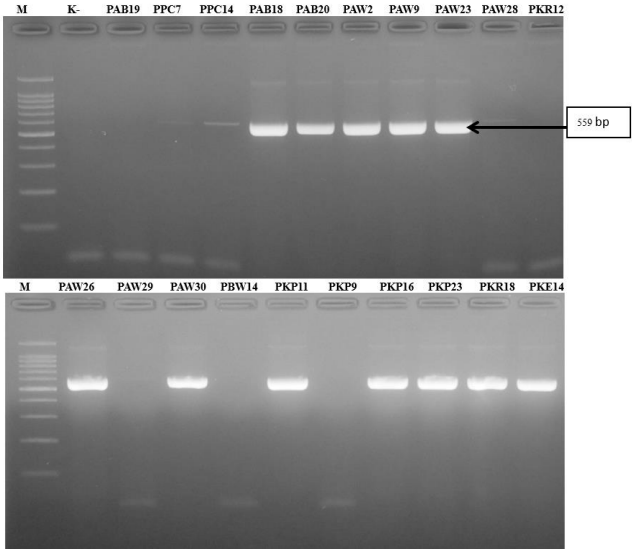

Figure 4. PCR results for the *hly*F gene of *Escherichia coli* isolates were positive in the 599 bp band. Sample codes that were positive for the *hly*F gene included PAB18, PAB20, PAW2, PAW9, PAW23, PAW26, PAW30, PKP11, PKP16, PKP23, PKR18, and PKE14, while those that were negative were PAB19, PPC7, PPC14, PAW28, PKR12, PAW29, PBW14, and PKP9

Discussion

Ducks originating from live markets in Indonesia are still sold traditionally, exacerbated by the lack of biosecurity and sanitation implemented by workers in the markets (Effendi et al. 2022). The ducks can be infected by bacteria, both pathogenic and non-pathogenic, including *Pasteurella multocida*, *Salmonella enterica*, and *Escherichia coli* (Wei et al. 2013; Soman et al. 2014; Li et al. 2016; Abd El-Ghany, 2023). *Escherichia coli* bacteria cause colibacilosis disease by APEC strains (Newman et al. 2021; Ievy et al. 2022). Colibacillosis in the poultry world is a significant challenge, resulting in production problems, economic losses, and mortality in poultry (Afayibo et al. 2022; Kika et al. 2023). Colibacillosis in livestock and live markets is due to poor sanitation, hygiene, and environment (Saha et al. 2020; Ievy et al. 2022).

The isolation and identification results showed that the *Escherichia coli* bacteria obtained from duck cloaca swabs in seven live markets in Surabaya was 85% (134/158). This result is lower than in Tanzania at 91% of duck cloacal swabs (Kissinga et al. 2018) but higher than previous research in Zimbabwe at 41% (12/29) (Dube and Mbanga 2018); in Egypt at 16.0% (40/120) (Darwish et al. 2015); China 15.6% (Li et al. 2023) and 32% (32/100) of samples from duck cloacal swabs in Surabaya traditional markets (Prayudi et al. 2023). Isolation on EMBA media for identification of *Escherichia coli* bacteria with metallic green colored colonies marked by a black dot in the center while isolation on MCA media with a small round, separate, irregular, and pink colony morphology (Kissinga et al. 2018; Na et al. 2019; Effendi et al. 2021; Wibisono et al. 2021). The results of bacterial staining showed Gram-negative bacteria, characterized by short rod-shaped, red, and uniform bacterial cell morphology (Yanestria et al. 2022; Prayudi et al. 2023). The biochemical test results at TSIA showed Acid/Acid, positive gas, and negative H2S because *Escherichia coli* can ferment glucose, sucrose, and lactose. The citrate test results will remain green in SCA media because bacteria cannot ferment citrate as carbon (Jiang et al. 2021). The results of indole were positive, and H2S were negative which showed motility in the SIM media. The Methyl-Red (MR) test on the sample tested showed a positive result, which appeared red after adding the MR reagent, while the Voges-Praskeur (VP) test showed a negative result after adding 10% KOH and α-naphthol, there was no color change or appeared yellow (Vivijs et al. 2014; Rahmahani et al. 2020).

The test results for the incidence of resistance to the antibiotic Erythromycin showed 96% (129/134) with a high incidence of resistance. Unwise use of antibiotics in the poultry industry can increase the incidence of MDR against *Escherichia coli* bacteria (Wall 2019; Wibisono et al. 2021; Akanbi et al. 2022; Sarker et al. 2022; Zhang et al. 2023). The results of this study are lower than those in Bangladesh (97.2%) and Nigeria (100%) (Akanbi et al. 2022; Ievy et al. 2022). The incidence of Ciprofloxacin antibiotic resistance against *Escherichia coli* bacteria was 16% (22/134). Ciprofloxacin is a second-generation fluoroquinolone antibiotic that is most often used in the treatment of poultry industry caused by *Escherichia coli* bacterial infections (Wibisono et al. 2021). Previous research states that all breeds of poultry are 85% in Northern Nigeria (Akanbi et al. 2022), in ducks it is 88% in Zimbabwe (Dube and Mbanga 2018), in ducks it is 69% in Bangladesh and Nepal (Singh et al. 2012) and ducks in Egypt amounted to 26.32% (Abd El-Samie et al. 2019).

Ducks are relatively resistant to certain diseases (Eid et al. 2019). Many factors increase ducks' susceptibility to bacterial infections, such as poor management and sanitation, malnutrition, overcrowding, and environmental stress (Eid et al. 2019). The results of gentamicin antibiotic resistance in this study were 15% (20/134). The antibiotic gentamicin is a class of aminoglycosides commonly used in the poultry industry. This result is lower than previous research on ducks in China of 22.7% (10/44) (Yassin et al. 2017). The incidence of aztreonam resistance in this study showed 6.8% (3/44) of samples obtained from ducks in China (Yassin et al. 2017). The resistance test results for the antibiotic chloramphenicol were 4% (6/134) from duck cloacal swabs at the Surabaya live market. According to Yassin et al. (2017) the incidence of resistance of *Escherichia coli* bacteria to the antibiotic chloramphenicol is 25% (11/44). This result is also lower than research in Egypt amounted to 25.79% (Abd El-Samie et al. 2019)

Moreover, antibiotics have become an option to reduce the death rate due to colibacillosis in poultry. However, excessive and inappropriate use of antibiotics has triggered the emergence of antibiotic resistance and MDR, resulting in therapeutic failure and economic losses for farmers (Azam et al. 2020; Jeong et al. 2021). This study shows a 15% (20/134) experienced MDR, which is lower than Jeong et al. (2021) of 65.5% from ducks in China. The incidence of multidrug resistance is increasing worldwide due to the spread of genes located on genetic elements in the form of plasmids and the combination of genes with resistance genes, which produce bacteria resistant to all classes of antibiotics (Allocati et al. 2013; Hardiati et al. 2021). This study's results were lower than Prayudi et al. (2023) who experienced resistance to more than 3-4 (31.25%) types of antibiotics originating from Surabaya traditional markets. Previous research showed that the incidence of MDR in ducks was 100% from five different types of antibiotics (Yassin et al. 2017). This shows that the problem of antibiotic resistance has a huge impact on public health from feces being scattered in the market environment (Manyi-Loh et al. 2018; Januari et al. 2019). Additionally, the incidence of antibiotic resistance in ducks in West Bengal, India, includes ciprofloxacin at 31.91% (15/47), chloramphenicol at 2.1% (1/47), and gentamicin at 8.5% (4/47) (Banerjee and Acharyya 2021).

This study revealed differences in MDR resistance patterns arise due to differences in the combination of types of antibiotics used by breeders. This follows Hardiati et al. (2021), who stated that resistance patterns show diversity due to various antibiotics used, geographical differences, and different poultry production systems causing differences in resistance patterns. Furthermore, broad-spectrum antibiotics can influence resistance patterns to shared antibiotics (Samuel et al. 2023).

Infectious diseases can attack poultry, one of which is ducks caused by avian pathogenic *Escherichia coli* (APEC) and is considered the main cause of health problems in poultry farms worldwide (Abreu et al. 2023; Putri et al. 2023). APEC infection causes symptoms systemic, such as airsacculitis, pericarditis, perihepatitis, septicemia, enteritis, granuloma, sinusitis, omphalitis, peritonitis, and swollen head syndrome in poultry (Azam et al. 2020; Wang et al. 2023). Several genes in the plasmid cause virulence factors in APEC strains, such as cvaC, tsh, sitA, iutA, ompT, etsABCD, eitABC, *hly*F, and iroN. Pathogenic isolates have genes with a high frequency of around 85% compared to commensal bacteria, which is around 25% (Kathayat et al. 2021).

The PCR test showed that 12/20 with a percentage of 60% of *Escherichia coli* isolates from duck cloacal swabs had the *hly*F virulence gene. This result is lower than previous research regarding the incidence of APEC caused by *hly*F virulence of 80% in Korea (Jeong et al. 2021). Moreover, reports regarding APEC containing the *hly*F virulence gene in ducks are still rare. There are reports regarding APEC regarding other poultry, such as chickens, which have the *hly*F gene at 83.33% in Bangladesh (Hossain et al. 2021) and Nepal at 100% (Subedi et al. 2018). The *hly*F virulence gene shows an incidence of 99% in Qatar (Johar et al. 2021), and in Indonesia, *hly*F is 100% in free-range chickens (Ramaditya et al. 2019). The *hly*F gene is found in APEC, which becomes a toxin, causing cells to undergo lysis and damage, inducing host cell vacuolization, colonization, motility, biofilm formation, agglutination, formation of outer membrane vesicles, further contributing to bacterial virulence including cytolethal distending toxin (CDT) and cytolysin A factors (ClyA) (Murase et al. 2015; Kathayat et al. 2021; Sgariglia et al. 2019).

In conclusion, *Escherichia coli* with Multidrug Resistance (MDR) properties were found in duck cloacal swabs at seven live markets in Surabaya at 15%, and the *hly*F virulence gene was found at 60%. There is public awareness of the safety of food originating from ducks and among breeders of the colibacillosis disease caused by the APEC strain. Furthermore, the use of antibiotics that trigger resistance and the incidence of MDR is a concern and requires veterinary supervision.

ACKNOWLEDGEMENTS

The International Research Consortium, *Lembaga Penelitian dan Pengabdian Masyarakat*, Universitas Airlangga, Surabaya, Indonesia Year 2024, partly supported this study.

REFERENCES

Abd El-Ghany WA. 2023. An overview on riemerellosis: A worldwide emerging disease of ducks. Bulg J Vet Med 26 (3): 309-324. DOI: 10.15547/bjvm.2021-0071.

Abd El-Samie LK, Abd-Elmoaty AM, Ibrahim NA, Darwish WS. 2019. Prevalence and molecular characterization of Avian Pathogenic *E. coli* in ducks. Adv Anim Vet Sci 7 (2): 163-168. DOI: 10.17582/journal.aavs/2019/7.s2.163.168.

Aberkane C, Messaï A, Messaï CR, Boussaada T. 2023. Antimicrobial resistance pattern of avian pathogenic *Escherichia coli* with detection of extended-spectrum β-lactamase-producing isolates in broilers in east Algeria. Vet World 16 (3): 449-454. DOI: 10.14202/vetworld.2023.449-454.

Abreu R, Semedo-Lemsaddek T, Cunha E, Tavares L, Oliveira M. 2023. Antimicrobial drug resistance in poultry production: Current status and innovative strategies for bacterial control. Microorganisms 11: 953. DOI: 10.3390/microorganisms11040953.

Adrenalin SL, Imanjati LN, Fauziah I, Prakasita VC, Widyarini S, Wahyuni AET. 2020. Virulence characteristic of avian pathogenic *Escherichia coli* (APEC) isolates. J Sain Vet 38 (1): 55-60. DOI: 10.22146/jsv.46494.

Afayibo DJA, Zhu H, Zhang B, Yao L, Abdelgawad HA, Tian M, Qi J, Liu Y, Wang S. 2022. Isolation, molecular characterization, and antibiotic resistance of avian pathogenic *Escherichia coli* in Eastern China. Vet Sci 9: 319. DOI: 10.3390/vetsci9070319.

Akanbi OB, Olorunshola ID, Osilojo P, Ademola E, Agada G, Aiyedun J, Odita CI, Ola-Fadunsin SD. 2022. *Escherichia coli* Infections, and Antimicrobial Resistance in Poultry Flocks, in North Central Nigeria. Media Kedokteran Hewan 33 (3): 188-207. DOI: 10.20473/mkh.v33i3.2022.188-207.

Allocati N, Masulli M, Alexeyev MF, Di Ilio C. 2013. *Escherichia coli* in Europe: An overview. Intl J Environ Res Public Health 10: 6235-6254. DOI: 10.3390/ijerph10126235.

Amer MM, Mekky HM, Fedawy HS, El-Shemy A, Bosila MA, Elbayoumi KM. 2020. Molecular identification, genotyping of virulence-associated genes, and pathogenicity of cellulitis-derived *Escherichia coli*. Vet World 13 (12): 2703-2712. DOI: 10.14202/vetworld.2020.2703-2712.

Azam M, Mohsin M, Johnson TJ, Smith EA, Johnson A, Umair M, Saleemi MK, Sajjad-ur-Rahman. 2020. Genomic landscape of multidrug resistant avian pathogenic *Escherichia coli* recovered from broilers. Vet Microbiol 20: 108766. DOI: 10.1016/j.vetmic.2020.108766.

Bakhshi M, Zandi H, Bafghi MF, Astani A, Ranjbar VR, Vakili M. 2020. A survey for phylogenetic relationship; presence of virulence genes and antibiotic resistance patterns of avian pathogenic and uropathogenic *Escherichia coli* isolated from poultry and humans in Yazd, Iran. Gene Rep 20: 100725. DOI: 10.1016/j.genrep.2020.100725.

Banerjee A, Acharyya S. 2021. Prevalence of antibiotic resistance genes in Indian ducks: A Comparative study between Deshi and Khaki campbell ducks. Exploratory Anim Med Res 11 (1): 30-37. DOI: 10.52635/EAMR/11.1.30-37.

CLSI [Clinical and Laboratory Standards Institute]. 2020. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Dameanti FNAP, Yanestria SM, Widodo A, Effendi MH, Plumeriastuti H, Tyasningsih W, Sutrisno R, Akramsyah MA. 2023. Incidence of *Escherichia coli* producing Extended-spectrum beta-lactamase (ESBL) in wastewater of dairy farms in East Java, Indonesia. Biodiversitas 24: 1143-1150. DOI: 10.13057/biodiv/d240254.

Darwish WS, Eldin WFS, Eldesoky KI. 2015. Prevalence, molecular characterization and antibiotic susceptibility of *Escherichia coli* isolated from duck meat and giblets. J Food Saf 35 (3): 410-415. DOI: 10.1111/jfs.12189.

Dube N, Mbanga J. 2018. Molecular characterization and antibiotic resistance patterns of avian fecal *Escherichia coli* from turkeys, geese, and ducks. Vet World 11: 859-867. DOI: 10.14202/vetworld.2018.859-867.

Effendi MH, Faridah HD, Wibisono FM, Wibisono FJ, Fatimah, Nisa N, UGBO EN. 2022. Detection of virulence factor encoding genes on *Escherichia coli* isolated from broiler chicken in Blitar District, Indonesia. Biodiversitas 23: 3429-3436. DOI: 10.13057/biodiv/d230716.

Effendi MH, Tyasningsih W, Yurianti YA, Rahmahani J, Harijani N, Plumeriastuti H. 2021. Presence of multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) of *Escherichia coli* isolated from cloacal swabs of broilers in several wet markets in Surabaya, Indonesia. Biodiversitas 22 (1): 304-310. DOI: 10.13057/biodiv/d220137.

Eid HM, Algammal AM, Elfeil WK, Youssef FM, Harb SM, Abd-Allah EM. 2019. Prevalence, molecular typing, and antimicrobial resistance of bacterial pathogens isolated from ducks. Vet World 12 (5): 677-683. DOI: 10.14202/vetworld.2019.677-683.

Faridah HD, Wibisono FM, Wibisono FJ, Nisa N, Fatimah F, Effendi MH, Ugbo EN, Khairullah AR, Kurniawan SC, Silaen OSM. 2023. Prevalence of the blaCTX-M and blaTEM genes among extended-spectrum beta lactamase–producing *Escherichia coli* isolated from broiler chickens in Indonesia. J Vet Res 67: 179-186. DOI: 10.2478/jvetres-2023-0025.

Hardiati A, Safika S, Wibawan IWT, Indrawati A, Pasaribu FH. 2021. Isolation and detection of antibiotics resistance genes of *Escherichia coli* from broiler farms in Sukabumi, Indonesia. J Adv Vet Anim Res 8 (1): 84-90. DOI: 10.5455/javar.2021.h489.

Hossain FE, Islam S, Islam MA, Islam S, Ahmed F. 2021. Detection of virulence genes of APEC (avian pathogenic *Escherichia coli*) isolated from poultry in Noakhali, Bangladesh. Biores Commun 7 (1): 967-972. DOI: 10.3329/brc.v7i1.54253.

Hu J, Afayibo DJA, Zhang B, Zhu H, Yao L, Guo W, Wang X, Wang Z, Wang D, Peng H, Tian M, Qi J, Wang S. 2022. Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic *Escherichia coli* (APEC). Front Microbiol 13: 1049391. DOI: 10.3389/fmicb.2022.1049391.

Ievy S, Hoque MN, Islam MS, Sobur MA, Ballah FM, Rahman MS, Rahman MB, Hassan J, Khan MFR, Rahman MT. 2022. Genomic characteristics, virulence, and antimicrobial resistance in avian pathogenic *Escherichia coli* MTR_BAU02 strain isolated from layer farm in Bangladesh. J Glob Antimicrob Resist 30: 155-162. DOI: 10.1016/j.jgar.2022.06.001.

Islam MS, Hossain MJ, Sobur MA, Punom SA, Rahman, AMMT, Rahman MT 2023. A systematic review on the occurrence of antimicrobial-resistant *Escherichia coli* in poultry and poultry environments in Bangladesh between 2010 and 2021. BioMed Res Intl 2023: 2425564. DOI: 10.1155/2023/2425564.

Januari C, Sudarwanto MB, Purnawarman T. 2019. Antibiotic resistance in *Escherichia coli* isolated from chicken meat of traditional markets in the city of Bogor. J Vet 20: 125-131. DOI: 10.19087/jveteriner.2019.20.1.125.

Jeong J, Lee JY, Kang MS, Lee HJ, Kang SIl, Lee OM, Kwon YK, Kim JH. 2021. Comparative characteristics and zoonotic potential of avian pathogenic *Escherichia coli* (APEC) isolates from chicken and duck in South Korea. Microorganisms 9 (5): 946. DOI: 10.3390/microorganisms9050946.

Jiang F, Huang X, Barbieri NL, Logue CM, Nolan LK, Li G. 2021. Citrate utilization under anaerobic environment in *Escherichia coli* is under direct control of Fnr and indirect control of ArcA and Fnr via CitA-CitB system. Environ Microbiol 23 (3): 1496-1509. DOI: 10.1111/1462-2920.15357.

Johar A, Al-Thani N, Al-Hadidi SH, Dlissi E, Mahmoud MH, Eltai NO. 2021. Antibiotic resistance and virulence gene patterns associated with avian pathogenic *Escherichia coli* (APEC) from broiler chickens in Qatar. Antibiotics 10 (5): 564. DOI: 10.3390/antibiotics10050564.

Kathayat D, Lokesh D, Ranjit S, Rajashekara G. 2021. Avian pathogenic *Escherichia coli* (APEC): An overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens 10 (4): 467. DOI: 10.3390/pathogens10040467.

Kika TS, Cocoli S, Pelić DL, Puvača N, Lika E, Pelić M. 2023. Colibacillosis in modern poultry production. J Agron Technol Eng Manag 6 (6): 975-987. DOI: 10.55817/yzfa3391.

Kissinga HD, Mwombeki F, Said K, Katakweba AAS, Nonga HE, Muhairwa AP. 2018. Antibiotic susceptibilities of indicator bacteria *Escherichia coli* and *Enterococci* spp. isolated from ducks in Morogoro Municipality, Tanzania. BMC Res Notes 11: 87. DOI: 10.1186/s13104-018-3201-4.

Li G, Li X, Hu J, Pan Y, Ma Z, Zhang L, Xiong W, Zeng D, Zeng Z. 2023. Molecular epidemiology and transmission of rmtB-positive *Escherichia coli* among ducks and environment. Poul Sci 102: 102579. DOI: 10.1016/j.psj.2023.102579.

Li R, Li N, Zhang J, Wang Y, Liu J, Cai Y, Chai T, Wei L. 2016. Expression of immune-related genes of ducks infected with avian pathogenic *Escherichia coli* (APEC). Front Microbiol 7: 637. DOI: 10.3389/fmicb.2016.00637.

Logue CM, Wannemuehler Y, Nicholson BA, Doetkott C, Barbieri NL, Nolan LK. 2017. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. Front Microbiol 8: 283. DOI: 10.3389/fmicb.2017.00283.

Maciel JF, Matter LB, Trindade MM, Camillo G, Lovato M, Botton SDA, de Vargas AC. 2017. Virulence factors and antimicrobial susceptibility profile of extraintestinal *Escherichia coli* isolated from an avian colisepticemia outbreak. Microb Pathog 103: 119-122. DOI: 10.1016/j.micpath.2016.12.020

Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. Molecules 23: 795. DOI: 10.3390/molecules23040795.

Murase K, Martin P, Porcheron G, Houle S, Helloin E, Pénary M, Nougayrède JP, Dozois CM, Hayashi T, Oswald E. 2015. *Hly*F produced by extraintestinal pathogenic *Escherichia coli* is a virulence factor that regulates outer membrane vesicle biogenesis. J Infec Dis 213: 856-865. DOI: 10.1093/infdis/jiv506.

Musa L, Proietti PC, Branciari R, Menchetti L, Bellucci S, Ranucci D, Marenzoni ML, Franciosin MP. 2020. Antimicrobial susceptibility of *Escherichia coli* and ESBL-producing *Escherichia coli* diffusion in conventional, organic and antibiotic-free meat chickens at slaughter. Animals 10: 1215. DOI: 10.3390/Ani10071215.

Na SH, Moon DC, Choi MJ, Oh SJ, Jung DY, Sung EJ, Kang HY, Hyun BH, Lim SK. 2019. Antimicrobial resistance and molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from ducks in South Korea. Foodborne Pathog Dis 16 (12): 799-806. DOI: 10.1089/fpd.2019.2644.

Newman DM, Barbieri NL, de Oliveira AL, Willis D, Nolan LK, Logue CM. 2021. Characterizing avian pathogenic *Escherichia coli* (APEC) from colibacillosis cases, 2018. PeerJ 9: 11025. DOI: 10.7717/peerj.11025.

Oliveira ES, Cardozo MV, Borzi MM, Borges CA, Guastalli EAL, Ávila FA. 2019. Hig*hly* pathogenic and multidrug resistant avian pathogenic *Escherichia coli* in free-range chickens from Brazil. Braz J Poultry Sci 21 (1): 1-8. DOI: 10.1590/1806-9061-2018-0876.

Ovi F, Zhang L, Nabors H, Jia L, Adhikari P. 2023. A compilation of virulence-associated genes that are frequently reported in avian pathogenic *Escherichia coli* (APEC) compared to other *Escherichia coli*. J Appl Microbiol 134 (3): 1-20. DOI: 10.1093/jambio/lxad014.

Papouskova A, Masarikova M, Valcek A, Senk D, Cejkova D, Jahodarova E, Cizek A. 2020. Genomic analysis of *Escherichia coli* strains isolated from diseased chicken in the Czech Republic. BMC Vet Res 16: 189. DOI: 10.1186/s12917-020-02407-2.

Prayudi SKA, Effendi MH, Lukiswanto BS, Az Zah-Ra RL, Benjamin MI, Kurniawan SC, Khairullah AR, Silaen OSM, Lisnanti EF, Baihaqi ZA, Widodo A, Riwu KHP. 2023. Detection of genes on *Escherichia coli* producing extended spectrum β-lactamase isolated from the small intestine of ducks in traditional markets Surabaya City, Indonesia. J Adv Vet Res 13 (8): 1600-1608.

Putri MFR, Kendek IA, Wibisono FJ, Effendi MH, Rahardjo D, Tyasningsih W, Ugbo EN. 2023. Molecular detection of iron gene on multidrug resistant avian fecal *Escherichia coli* isolated from broiler on traditional markets, Surabaya, Indonesia. Biodiversitas 24 (12): 6454-6460. DOI: 10.13057/biodiv/d241207.

Rahmahani J, Salamah, Mufasirin, Tyasningsih W, Effendi MH. 2020. Antimicrobial resistance profile of *Escherichia coli* from cloacal swab of domestic chicken in Surabaya traditional market. Biochem Cell Arch 20 (1): 2993-2997. DOI: 10.35124/bca.2020.20.S1.2993.

Ramaditya NA, Besung INK, Mahardika IGNK. 2019. Detection and sequencing genes Iron, Iuta, and *Hly*f in avian pathogenic *Escherichia coli*. Bul Vet Udayana 11 (2): 229-238. DOI: 10.24843/bulvet.2019.v11.i02.p16.

Saha O, Hoque MN, Islam OK, Rahaman MM, Sultana M, Hossain MA. 2020. Multidrug-resistant avian pathogenic *Escherichia coli* strains and association of their virulence genes in Bangladesh. Microorganisms 8: 1135. DOI: 10.3390/microorganisms8081135.

Samuel M, Wabwire TF, Tumwine G, Waiswa P. 2023. Antimicrobial usage by small-scale commercial poultry farmers in Mid-Western District of Masindi Uganda: Patterns, public health implications, and antimicrobial resistance of *Escherichia coli*. Vet Med Intl 2023: 6644271. DOI: 10.1155/2023/6644271.

Sarker MS, Kabir ME, Mahua FA, Ahmed A, Ripon RK, Silva V, Poeta P, Ahad A. 2022. Antibiotic-resistant *Escherichia coli* isolated from duck cloacal and tap water samples at live bird markets in Bangladesh. Poultry Sci J 11: 19-27. DOI: 10.22069/psj.2022.20028.1789.

Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, Choroszy-Krol I. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: Recent reports. Gut Pathog 11: 10. DOI: 10.1186/s13099-019-0290-0.

Sgariglia E, Mandolini NA, Napoleoni M, Medici L, Fraticelli R, Conquista M, Gianfelici P, Staffolani M, Fisichella S, Capuccella M, Sargenti M, Perugini G. 2019. Antibiotic resistance pattern and virulence genes in avian pathogenic *Escherichia coli* (APEC) from different breeding systems. Vet Ital 55 (1): 27-33. DOI: 10.12834/VetIt.1617.8701.1.

Singh A, Khan MSR., Saha S, Hassan J, Roy U. 2012. Isolation and detection of antibiotic sensitivity pattern of *Escherichia coli* from ducks in Bangladesh and Nepal. Microbes Health 1 (1): 6-8. DOI: 10.3329/mh.v1i1.13704.

Soman M, Nair SR, Mini M, Mani BK, Joseph S, Department. 2014. Isolation and polymerase chain reaction-based identification of *Riemerella anatipestifer* from ducks in Kerala, India. Vet World 7 (9): 765-769. DOI: 10.14202/vetworld.2014.765-769.

Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A. 2021. Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. Pathogens 10: 1355. DOI: 10.3390/pathogens10111355.

Subedi M, Bhattarai RK, Devkota B, Phuyal S, Luitel H. 2018. Correction: Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* (APEC) from broiler chickens in Chitwan, Nepal. BMC Vet Res 14: 113. DOI: 10.1186/s12917-018-1442-z.

Tyasningsih W, Ramandinianto SC, Ansharieta R, Witaningrum AM, Permatasari DA, Wardhana DK, Effendi MH, Ugbo EN. 2022. Prevalence and antibiotic resistance of *Staphylococcus aureus* and *Escherichia coli* isolated from raw milk in East Java, Indonesia. Vet World 15 (8): 2021-2028. DOI: 10.14202/vetworld.2022.2021-2028.

Velhner M, Suvajdžić L, Todorović D, Milanov D, Kozoderović G. 2018. Avian pathogenic *Escherichia coli:* Diagnosis, virulence and prevention. Arch Vet Med 11: 21-31. DOI: 10.46784/e-avm.v11i2.23.

Vidovic N, Vidovic S. 2020. Antimicrobial resistance and food animals: Influence of livestock environment on the emergence and dissemination of antimicrobial resistance. Antibiotics 9: 52. DOI: 10.3390/antibiotics9020052.

Vivijs B, Moons P, Aertsen A, Michiels CW. 2014. Acetoin synthesis acquisition favors *Escherichia coli* growth at low pH. Appl Environ Microbiol 80 (19): 6054-6061. DOI: 10.1128/AEM.01711-14.

Wall S. 2019. Prevention of antibiotic resistance–an epidemiological scoping review to identify research categories and knowledge gaps. Glob Health Action 12: 1756191. DOI: 10.1080/16549716.2020.1756191.

Wang Z, Xu S, Zheng X, Zheng X, Liu M, Guo G, Yu Y, Han X, Liu Y, Wang K, Zhang W. 2023. Identification of subunits for novel universal vaccines against three predominant serogroups and the emerging O145 among avian pathogenic *Escherichia coli* by Pan-RV pipeline. Appl Environ Microbiol 89: e0106122. DOI: 10.1128/aem.01061-22.

Wei B, Cha SY, Kang M, Park IJ, Moon OK, Park CK, Jang HK. 2013. Development and application of a multiplex PCR assay for rapid detection of 4 major bacterial pathogens in ducks. Poultry Sci 92 (5): 1164-1170. DOI: 10.3382/ps.2012-02823.

Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. 2020. CTX gene of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* on broilers in Blitar, Indonesia. Syst Rev Pharm 11: 396-403. DOI: 10.31838/srp.2020.7.59.

Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. 2021. Molecular identification of CTX gene of extended spectrum betalactamases (ESBL) producing *Escherichia coli* on layer chicken in blitar, indonesia. J Anim Plant Sci 31 (4): 954-959. DOI: 10.36899/JAPS.2021.4.0289.

Xu X, Sun Q, Zhao L. 2019. Virulence factors and antibiotic resistance of avian pathogenic *Escherichia coli* in eastern China. J Vet Res (Poland) 63 (3): 317-320. DOI: 10.2478/jvetres-2019-0056.

Yanestria SM, Dameanti FNAEP, Musayannah BG, Pratama JWA, Witaningrum AM, Effendi, MH, Ugbo EN. 2022. Antibiotic resistance pattern of Extended-Spectrum β-Lactamase (ESBL) producing *Escherichia coli* isolated from broiler farm environment in Pasuruan district, Indonesia. Biodiversitas 23: 4460-4465. DOI: 10.13057/biodiv/d230911.

Yassin AK, Gong J, Kelly P, Lu G, Guardabassi L, Wei L, Han X, Qiu H, Price S, Cheng D, Wang C. 2017. Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. PLoS One 12: e0185326. DOI: 10.1371/journal.pone.0185326.

Yousef HMY, Hashad ME, Osman KM, Alatfeehy NM, Hassan WMM, Elebeedy LA, Salem HM, Shami A, Al-Saeed FA, El-Saadony MT, El-Tarabily KA, Marouf S. 2023. Surveillance of *Escherichia coli* in different types of chicken and duck hatcheries: one health outlook. Poultry Sci 102 (12): 103108. DOI: 10.1016/j.psj.2023.103108.

Zhang S, Shu Y, Wang Y, Zhong Z, Wang M, Jia R, Chen S, Liu M, Zhu D, Zhao X, Wu Y, Yang Q, Huang J, Ou X, Mao S, Gao Q, Sun D, Tian B, Cheng A. 2023. High rate of multidrug resistance and integrons in *Escherichia coli* isolates from diseased ducks in select regions of China. Poultry Sci 102: 102956. DOI: 10.1016/j.psj.2023.102956.