











Submitted: 11/10/2023

Accepted: 14/02/2024

Published: 31/03/2024

Antimicrobial resistance patterns and genes of *Campylobacter jejuni* isolated from chickens in Pasuruan, Indonesia

Sheila Marty Yanestria¹ , Mustofa Helmi Effendi^{2*} , Wiwiek Tyasningsih³ , Ikechukwu Benjamin Moses⁴ , Aswin Rafif Khairullah⁵ , Shendy Canadya Kurniawan⁶ , Fidi Nur Aini Eka Puji Dameanti⁷ , Rosmita Ikaratri⁸ , Junianto Wika Adi Pratama⁹ , Miarsono Sigit¹⁰ , Abdullah Hasib¹¹ , and Otto Sahat Martua Silaen¹² 

¹Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

²Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

³Division of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

⁴Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria

⁵Research Center for Veterinary Science, National Research and Innovation Agency (BRIN), Bogor, Indonesia

⁶Master Program of Animal Sciences, Department of Animal Sciences, Specialisation in Molecule, Cell and Organ Functioning, Wageningen University and Research, Wageningen, The Netherlands

⁷Microbiology and Immunology Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia

⁸Bacteriology Laboratory, Balai Besar Veteriner Wates, Yogyakarta, Indonesia

⁹Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

¹⁰Division of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia

¹¹School of Agriculture and Food Sustainability, The University of Queensland, Gatton, Australia

¹²Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Abstract

Background: Poultry is one of the most prominent sources of *Campylobacter jejuni*, which is also a major means of transmission to people. *Campylobacter jejuni* contamination in chicken meat comes from chicken feces because it naturally exists in the intestines of chickens.

Aim: The purpose of this study is to identify the antibiotic resistance patterns and genes of *C. jejuni*, which was found in chickens in Pasuruan, Indonesia.

Methods: The samples used in this study were 200 contents of the small intestine of broiler chickens from 40 farms in Pasuruan Regency. The enriched sample was streaked on the selective media of modified charcoal cefoperazone deoxycholate agar containing the CCDA selective supplement. Antimicrobial susceptibility test utilizing the Kirby-Bauer diffusion test method in accordance with Clinical and Laboratory Standards Institute standards. The polymerase chain reaction (PCR) method was used to detect the (*hipO*), which encodes the *C. jejuni* strain, fluoroquinolone resistance (*gyrA*), beta-lactam resistance (*bla*_{OXA-61}), and tetracycline resistance (*tetO*) genes.

Results: The findings revealed a 14% (28/200) prevalence of *C. jejuni* in the small intestine of broiler chickens. These isolates showed high resistance to enrofloxacin (92.9%). All isolates (100%) were susceptible to amoxicillin-clavulanate. The PCR results showed all *C. jejuni* isolates (100%) detected the *gyrA* gene, 96.4% detected the *bla*_{OXA-61} gene, and 50% detected the *tetO* gene.

Conclusion: The findings of antimicrobial resistance at a high level from the small intestine of broiler chickens illustrate the potential threat to human health. To lessen the effects now and in the future, coordinated and suitable action is needed, as well as steps to guarantee the poultry industry's economic survival and public health insurance.

Keywords: *Campylobacter jejuni*, Antimicrobial resistance, Gene, Chickens, Indonesia.

Introduction

The poultry sector is the main source of animal protein that supplies human food in the world. The advantage of poultry meat is that the price is lower

when compared to other sources of animal protein, but the animal protein content is higher (Choi *et al.*, 2023). Poultry in Indonesia is growing rapidly in line with the higher demand for commodities (Syahlani *et al.*, 2022).

*Corresponding Author: Mustofa Helmi Effendi. Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. Email: mhelmieffendi@gmail.com

Articles published in Open Veterinary Journal are licensed under a Creative Commons Attribution-NonCommercial 4.0 International License



The purchasing power of the Indonesian people is comparable to the price of chicken meat so that animal protein nutrition can be fulfilled at all socioeconomic levels (Faridah *et al.*, 2023).

The problem, poultry is one of the most prominent sources of *Campylobacter jejuni*, which is also a major means of transmission to people (Thomrongsuwannakij *et al.*, 2017). Systems for managing the risk of campylobacteriosis currently exist at the national and international levels, but it is still not possible to provide consumers with chicken that is free of campylobacter (Facciola *et al.*, 2017). Data have been published that the incidence of foodborne illness is related to food handlers (Narvaez-Bravo *et al.*, 2017). Sources of infection in humans are mostly caused by consuming chicken carcasses contaminated with *C. jejuni* which is cooked with imperfect heating (El-Saadony *et al.*, 2023). *Campylobacter jejuni* contamination in chicken meat comes from chicken feces because it naturally exists in the intestines of chickens (Wangroongsarb *et al.*, 2021).

Campylobacter jejuni is an enteric bacterium that is pathogenic to humans (Thomrongsuwannakij *et al.*, 2017). Currently, *C. jejuni* is a zoonotic agent that is quite important for industrialized and developing countries (Kaakoush *et al.*, 2015). This bacterium has been an emerging bacteria in the last decade since many species that are resistant to antibiotics were discovered (Facciola *et al.*, 2017). Epidemiology of *C. jejuni* infection does not only occur in developing countries but also in developed countries (Sison *et al.*, 2014). In Indonesia, the toddlers with diarrhea who visited hospitals and health centers, the bacteria that caused the most diarrhea was *C. jejuni* at 70.8% (Budaiilmawan *et al.*, 2022).

Antibiotics are frequently used in medicine to treat illnesses, prevent them (via prophylaxis and metaphylaxis), and promote growth (Manyi-Loh *et al.*, 2018). According to estimates, the livestock industry uses two-thirds of the antimicrobials produced globally (Hosain *et al.*, 2021). In a 2021 assessment of antimicrobial drugs used in animals, it was found that 26% of the 160 nations examined in 2019 continued to utilize antibiotics as growth promoters in animal products (Muurinen *et al.*, 2021). Antimicrobial resistance in enteric bacteria is a result of the overuse and improper use of antibiotics in chickens (Efendi *et al.*, 2022). Antimicrobial resistance in *Campylobacter* strains has been frequently isolated, this causes infections that are difficult to treat and can be transmitted quickly through food of animal origin so that the impact on human health is even wider (Gharbi *et al.*, 2022).

The increasing ability of bacteria to become resistant to antibiotics commonly used in clinical practice makes information regarding antimicrobial resistance in *C. jejuni* important for considering alternative therapies and implementing safety strategies. In Indonesia, information regarding antimicrobial resistance in *C. jejuni* is still very limited, so this research was carried

out which aims to detect antimicrobial resistance patterns and genes of *C. jejuni* isolated from chickens in Pasuruan, Indonesia.

Materials and Methods

Sample collection

Sampling was carried out from July to September 2022. The samples used in this study were 200 contents of the small intestine of broiler chickens from 40 farms in Pasuruan Regency. Samples come from 3 sub-districts in Pasuruan Regency, namely Kejayan, Grati and Lekok. The contents of the chicken's small intestine are put into a sterile plastic that has been prepared to prevent microbial contamination from the environment. The sample is then taken using a cool box to the laboratory for analysis.

Culture on enrichment media

The chicken intestine content in the amount of 1 g was put into a bottle containing 10 ml of Nutrient Broth No.2 (Oxoid CM0067, England) which had been added with 5% lyzed sheep blood, Preston supplements (Oxoid SR0117, England) and FBP (sodium pyruvate, sodium metabisulfite, and ferrous sulfate) then incubated for 24 hours at 42°C under microaerophilic conditions (5% O₂, 85% N₂, and 10% CO₂). Microaerophilic conditions were obtained by placing the bacterial culture into a 2.5l anaerobic jar (Oxoid, England) and adding a 2.5l Gas Generating Kit CampyGen sachet (Oxoid CN0025, England).

Isolation and identification of *Campylobacter sp*

The enriched sample was streaked on the selective media of modified charcoal cefoperazone deoxycholate (mCCDA) (Oxoid CM0739, England) containing the CCDA selective supplement (Oxoid SR 155E, England), then kept in an atmosphere of microaerophilia for 24 hours at 42°C. Identification of *Campylobacter sp* was then carried out by Gram staining, oxidase test, and catalase test.

Antibiotic sensitivity test

Antimicrobial susceptibility test (AST) utilizing the Kirby–Bauer Diffusion Test method in accordance with Clinical and Laboratory Standards Institute (CLSI) standards. On Mueller–Hinton agar (Oxoid CM 0337b, England) plates, antimicrobial resistance testing was performed on all *C. jejuni* isolates, supplemented with 5% defibrinated sheep blood, containing ciprofloxacin 5 µg, enrofloxacin 5 µg, erythromycin 15 µg, tetracycline 30 µg, streptomycin 10 µg, gentamicin 10 µg, ampicillin 10 µg, and dan amoxicillin-clavulanate 30 µg antibiotic disks. The media were incubated under microaerophilic conditions for 24 hours at 42°C. The inhibition zone was measured, and the CLSI table was used as a reference to assess the bacterial susceptibility to antibiotics.

Detection of *C. jejuni* strain (*hipO* gene) dan antimicrobial resistance genes (*gyrA*, *bla*_{OXA-61}, and *tetO*)

The polymerase chain reaction (PCR) method was used to detect the *hipO*, *gyrA*, *bla*_{OXA-61}, and *tetO* genes, *hipO* is a gene encoding *C. jejuni* strain, while

Table 1. Primer sequences, target genes, amplicon sizes, and cycling conditions.

Target gene	Primer sequences	Size (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Ref
				Secondary denaturation	Annealing	Extension		
<i>hipO</i>	F-ACTTCTTTATTGCTTGCTGC	323	95°C	95°C	59°C	72°C	72°C	(Gharbi et al., 2022)
	R-GCCACAACAAGTAAAGAAGC		0.5 minute	0.5 minute	0.5 minute	0.5 minute	7 minutes	
<i>gyrA</i>	F-GAAGAATTTATATGCTATG	235	95°C	95°C	53°C	72°C	72°C	(Chatur et al., 2014)
	R-TCAGTATAACGCATCGCAGC		5 minutes	50 seconds	30 seconds	1 minute	7 minutes	
<i>bla_{OXA-61}</i>	F-AGAGTATAATACAAGCG	372	95°C	95°C	54°C	72°C	72°C	(Obeng et al., 2012)
	R-TAGTGAGTTGTCAAAGCC		5 minutes	50 seconds	30 seconds	1 minute	7 minutes	
<i>tetO</i>	F-GGCGTTTGTATTATGTGCG	559	95°C	95°C	49°C	72°C	72°C	(Gibreel et al., 2004)
	R-ATGGAACAACCCGACAGAAAGC		1 minute	1 minute	1 minute	1 minute	7 minutes	

gyrA is a gene encoding fluoroquinolone resistance, *bla_{OXA-61}* encodes beta-lactam resistance, *tetO* encodes beta-lactam resistance. The QIAamp DNA Mini Kit (Qiagen, USA) was used to extract DNA according to the manufacturer's instructions. The master mix formulation for PCR amplification consisted of 5 µl template DNA, 1 µl each primer (Integrated DNA Technologies, Iowa), 0.5 µl Nuclease Free Water, and 12.5 µl PCR master mix (Promega, USA) containing Taq dNTPs, DNA polymerase, reaction buffer, and MgCl₂. The final volume of the reaction mixture is 20 µl. The PCR primers, supplied by Integrated DNA Technologies (Iowa), are listed in Table 1. The PCR reagent mixture is then loaded into the BioProducts Select Cyclor II thermal cycler. Positive control *C. jejuni* strain using ATCC 33560 (Microbiologics, Minnesota).

The amplification results of the PCR products were carried out by electrophoresis in 1.5% agarose gel (Invitrogen, USA) which had been added with RedSafe Nucleid Acid Staining Solution gel dye (Intron, South Korea). To gauge the size of the DNA produced by the PCR, marker 100 was further placed into the agarose gel's wells, afterward the electrophoresis was carried out for 30 minutes at a constant 100 volts. The gel was taken out and put under a UV lamp for inspection after stopping the electrophoresis.

Ethical approval

Animal ethics approval was obtained via the ethical clearance committee of the Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya, Indonesia (Ethics no: 86-KKE / 2022).

Results

Based on the outcomes of the biochemical tests and the results of the isolation and identification using morphology, from a total of 200 samples of the contents of the small intestine of broiler chickens, 32 samples were positive for *Campylobacter sp* (16%). *Campylobacter sp* colonies growing on mCCDA agar media are grayish-white, round, convex, smooth, and shiny (Fig. 1). *Campylobacter sp* bacteria appear red with Gram hucker staining, have a spiral shape, and wavy rods, including Gram-negative, active oxidase, and active catalase. The PCR results showed that of the 32 *Campylobacter sp* isolates, there were 14% (28/200) *C. jejuni* strains, which was indicated by the presence of a PCR product of 323 bp, as shown in (Fig. 2).

The results of the AST for *C. jejuni* showed that there was high resistance to enrofloxacin (92.9%), ciprofloxacin (89.3%), and ampicillin (75%). To a lesser extent, *C. jejuni* is resistant to tetracyclines (50%). Very low levels of resistance were seen for the antibiotics streptomycin (7.1%), gentamycin (3.6%), and erythromycin (3.6%), whereas all isolates (100%) were susceptible to amoxicillin-clavulanate (Table 2). Of 28 *C. jejuni* isolates, 50% (*n* = 14) were multidrug resistant (MDR). The frequency of resistance profiles

to 3 drug classes was the highest at 46.4%, and the remaining resistance to 4 drug classes was 3.6%. In this research, *C. jejuni* showed 7 different antibiotic resistance patterns (Table 3). The combination CIP–ENR–TE–AMP is the most frequently occurring pattern (39.3%).

A total of 28 *C. jejuni* isolates were tested using PCR to detect *gyrA*, *bla*_{OXA-61}, and *tetO* genes (Table 2). Of the 28 isolates, all (100%) detected the *gyrA* gene as indicated by the presence of 235 bp PCR product (Fig. 3), 96.4% detected the *bla*_{OXA-61} gene as indicated by the presence of 372 bp PCR product (Fig. 4), and 50% detected the *tetO* gene as indicated by the presence of 559 bp PCR product (Fig. 5).

Discussion

The results showed that the prevalence of *C. jejuni* was 14% from a total of 200 samples taken from chicken in Pasuruan, Indonesia. In comparison to earlier research on chicken carcasses in Indonesia by Budiaimiawan et al. (2022), which found that the prevalence of *C. jejuni* was 23.5%, this study's prevalence of the bacteria was lower. These results were similar to the studies from China, with the prevalence of *C. jejuni* of 10.8% from broiler cloacal swabs in China (Yang et al., 2023). Comparing the findings of research conducted in Thailand to the prevalence in this study, it was determined to be lower. According to Wangroongsarb et al. (2021), 33.5% of chicken samples from Thailand included *Campylobacter sp.* In other countries, the prevalence rates vary, which is undoubtedly influenced

by variations in sampling designs and testing techniques.

Of the 32 isolates of *Campylobacter sp.*, 28 isolates were *C. jejuni* species. These findings support earlier research studies that indicated *C. jejuni* was the predominant species isolated from chickens because it is in fact the species most frequently seen in the digestive tract of poultry (Sierra-Arguello et al., 2018). Infection in poultry mostly via the oral-fecal route or via vertical transmission from the broodstock (Facciola et al., 2017). Cross-contamination is generally passed from generation to generation of the same poultry and it is indeed very uncommon for cross-contamination to be passed from the environment to animals (Stella et al., 2017).

In this study, the contents of the small intestine of chickens have been shown to carry *C. jejuni* bacteria. It is conceivable that chicken could serve as a human campylobacteriosis reservoir. It is known that poultry is the main food source from which humans contract *Campylobacter sp.* High levels of *Campylobacter sp.* in broiler chicks are the primary cause. Therefore, *Campylobacter sp.* is frequently found in poultry farms, the environment, and water supplies, including soil, dust, air, and building surfaces. When people improperly prepare raw poultry in home kitchens, there is a risk of exposure because of the presence of *Campylobacter sp.* in poultry. The majority of incidents include handling raw chicken, consuming undercooked or raw poultry, or cross-contamination between raw and cooked meals (García-Sánchez et al., 2020).

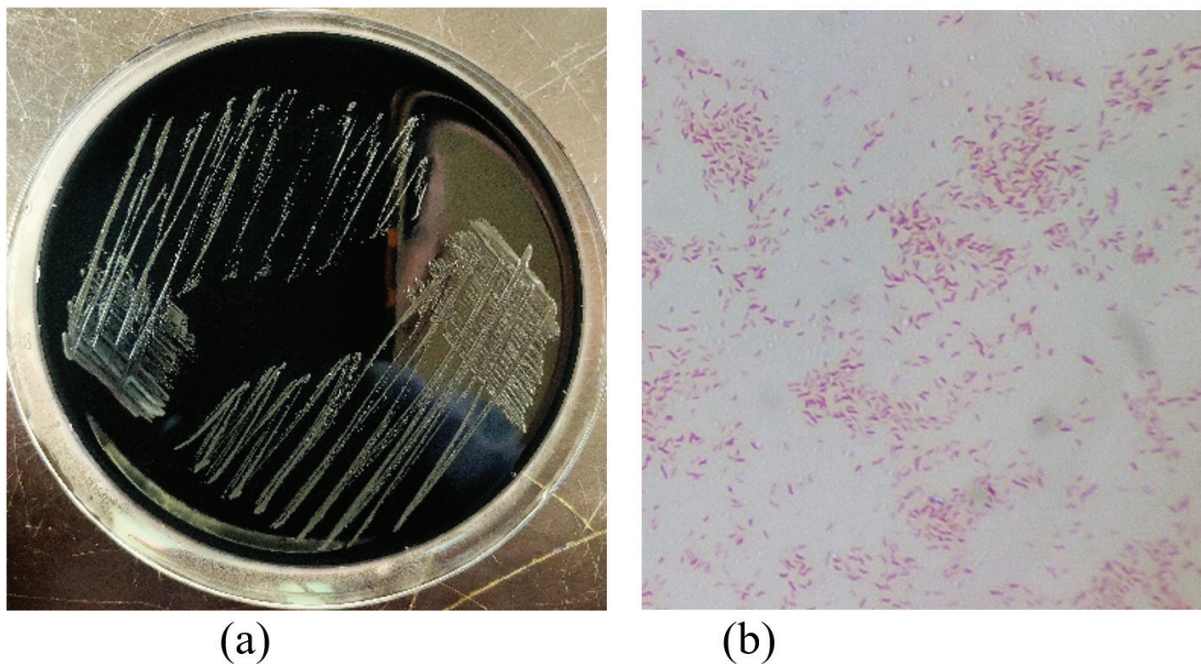


Fig. 1. (a) *Campylobacter sp.* on mCCDA media; (b) Microscopically *C. jejuni* (1,000x).

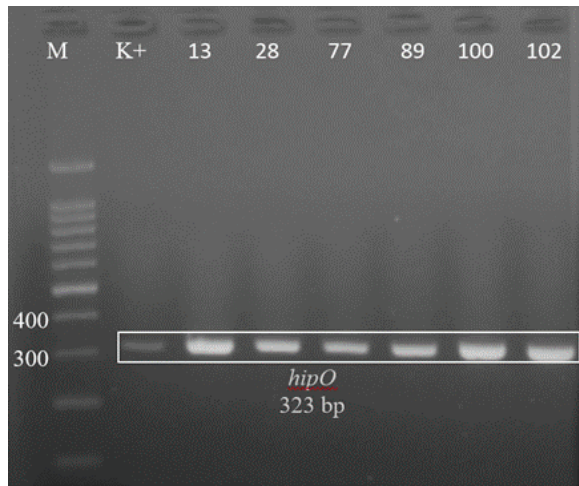


Fig. 2. Representative gel of *C. jejuni* strains gene (*hipO*). Note: Lanes: M, 100-bp marker; K+, positive control.

Antimicrobial sensitivity test results showed that *C. jejuni* isolates had the highest level of resistance to enrofloxacin (92.9%), ciprofloxacin (89.3%), and ampicillin (75%), followed by lower resistance to tetracyclin (50%). The combination “CIP–ENR–TE–AMP” is the most common pattern (39.3%). Due to the extensive usage of these antibiotics in Indonesia’s poultry farming industry, there is little doubt that enrofloxacin, ampicillin, and tetracyclin resistance is high (Efendi *et al.*, 2022). Around 60% of Indonesian poultry farmers use enrofloxacin, with tetracycline and ampicillin following closely behind (Zong *et al.*, 2022). In fact, research has linked increased resistance to *C. jejuni* isolated from humans and poultry to the usage of fluoroquinolones in poultry (Gharbi *et al.*, 2022). The high incidence of bacterial resistance to ampicillin isolated from poultry in Pasuruan due to the high use of ampicillin has been shown by previous research in the same area, but on *Escherichia coli* bacteria from the

Table 2. Antimicrobial resistance profiles and genes of *C. jejuni* isolated from chickens.

Drug Class	Drug	Resistance Zone of Inhibition (mm)	<i>C.jejuni</i> n = 28 (%)	PCR n = 28 (%)		
				<i>gyrA</i>	<i>bla</i> _{OXA-61}	<i>tetO</i>
Fluoroquinolone	Ciprofloxacin (CIP)	≤15	25 (89.3)	28 (100)	–	–
	Enrofloxacin (ENR)	≤24	26 (92.9)	–	–	–
Macrolide	Erythromycin (E)	≤13	1 (3.6)	–	–	–
Aminoglycoside	Streptomycin (S)	≤1	2 (7.1)	–	–	–
	Gentamycin (CN)	≤12	1 (3.6)	–	–	–
Tetracycline	Tetracycline (TE)	≤11	14 (50)	–	–	14 (50)
Beta-Lactam	Ampicillin (AMP)	≤13	21 (75)	–	27 (96.4)	–
	Amoxicillin-clavulanate (AMC)	≤13	0 (0)	–	–	–

Table 3. Antimicrobial resistance patterns of *C. jejuni* isolated from chickens.

Antimicrobial resistance patterns	No. of drug class	<i>C. jejuni</i> n = 28 (%)
CIP–ENR–E–S–CN–TE–AMP	5	1 (3.6)
CIP–ENR–TE–AMP	3	11 (39.3)
CIP–TE–AMP	3	1 (3.6)
ENR–S–AMP	3	1 (3.6)
ENR–TE	2	1 (3.6)
CIP–ENR–AMP	2	7 (25)
CIP–ENR	1	5 (3.6)

(CIP): Ciprofloxacin; (ENR): Enrofloxacin; (E): Erythromycin; (S): Streptomycin; (CN): Gentamycin; (TE): Tetracycline; (AMP): Ampicillin.

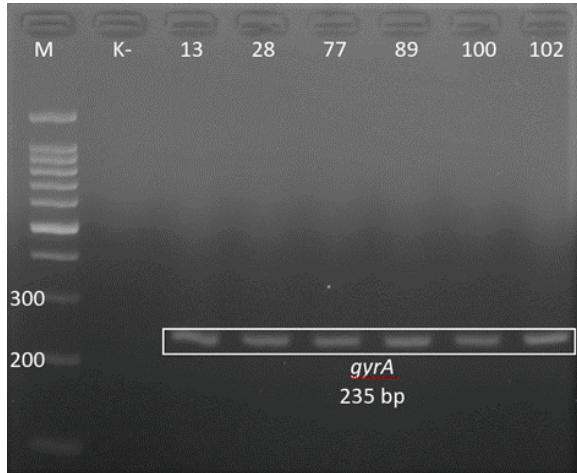


Fig. 3. Representative gel of fluoroquinolone resistance gene (*gyrA*) from *C. jejuni*. Note: Lanes: M, 100-bp marker; K-, negative control.

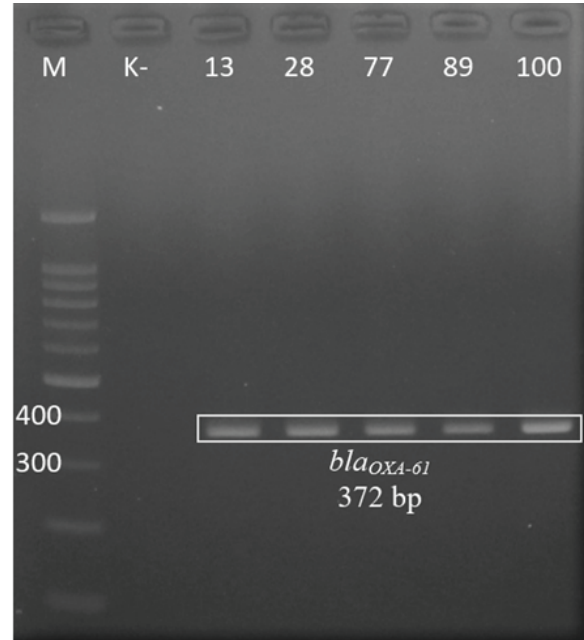


Fig. 4. Representative gel of beta-lactam resistance gene (*bla_{OXA-61}*) from *C. jejuni*. Note: Lanes: M, 100-bp marker; K-, negative control.

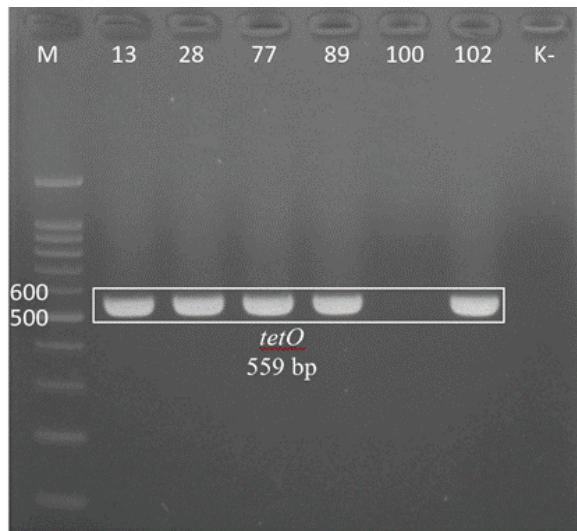


Fig. 5. Representative gel of tetracycline resistance gene (*tetO*) from *C. jejuni*. Note: Lanes: M, 100-bp marker; K-, negative control.

poultry environment (Yanestria *et al.*, 2022). The low resistance to streptomycin (7.1%), gentamicin (3.6%), and erythromycin (3.6%) may be due to infrequent use in poultry because the price of these antibiotics is relatively expensive (Gharbi *et al.*, 2022).

Of 28 *C. jejuni* isolates, 50% ($n = 14$) were MDR. This case is lower than MDR cases in other countries, Tunisia (100%) (Gharbi *et al.*, 2022) and China (88.6%) (Yang *et al.*, 2023). In Indonesia, the ban on the use of antibiotic growth promoters in the livestock sector became effective in January 2018, referring to Minister of Agriculture Regulation No. 14/2017; however, the impact of antibiotic resistance has already

occurred and continues to exist today (Untari *et al.*, 2021), because antibiotic resistance bacteria (ARB) and antibiotic resistant genes (ARGs) from destroyed ARB have spread in the environment. The proliferation of ARBs and ARGs is made worse by the improper use of antibiotics in both humans and animals. Humans and animals excrete ARBs and ARGs through urine and feces into the environment (Yanestria *et al.*, 2022).

All *C. jejuni* isolates (100%) had the *gyrA* gene detected. The study's findings are comparable to those of studies carried out by other researchers. Research by Sierra-Arguello *et al.* (2018) showed that 100% of *C. jejuni* from chicken farms detected the *gyrA* gene. The majority of the *C. jejuni* isolates in this study were genotypically and phenotypically resistant to the fluoroquinolone antibiotic class, indicating a close relationship between the two (Šoprek *et al.*, 2022). This supports the hypothesis that particular point mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene lead to fluoroquinolone resistance in *C. jejuni* (Thomrongsuwannakij *et al.*, 2017). In reality, DNA *gyrAse* and the structurally related topoisomerase IV are two intracellular enzymatic targets of fluoroquinolones, but this is different in the bacteria *C. jejuni* (Iovine, 2013). DNA *gyrAse* is encoded by *gyrA* and *gyrB* (Jaktaji and Mohiti, 2010). Numerous investigations have demonstrated that *C. jejuni* lacks the *parC* and *parE* genes, ruling out their involvement in the development of fluoroquinolone resistance (Shakir *et al.*, 2021).

DNA *gyrAse* in *C. jejuni* is encoded by *gyrA* and *gyrB*, but mutations in the QRDR gene *gyrA* are the only mechanism causing resistance to fluoroquinolones because mutations in *gyrB* are silent mutations (Han *et al.*, 2012). The *gyrB* gene is not involved in the fluoroquinolone mechanism in *C. jejuni* as shown in several previous studies (Yang *et al.*, 2023).

In this study, all *C. jejuni* isolates had the *gyrA* resistance gene, but phenotypically there were three isolates that were still sensitive to ciprofloxacin and two isolates that were still sensitive to enrofloxacin. This could be due to the presence or absence of *cmeABC* overexpression. According to several researches, ciprofloxacin resistance was connected with the overexpression of *CmeABC* (Grinnage-Pulley *et al.*, 2015). Strains with overexpressing *CmeABC* show high levels of resistance to ciprofloxacin (Wieczorek and Osek, 2013). However, further research is needed to prove it.

The *bla*_{OXA-61} gene was detected in almost all isolates (27/28). Most *C. jejuni* are capable of producing beta-lactamase enzymes which can inactivate beta-lactam molecules by hydrolyzing structural (Bush and Bradford, 2020). In this study, all *C. jejuni* isolates were phenotypically resistant to ampicillin, genotypically also having the *bla*_{OXA-61} gene. This is in line with earlier research that found a substantial association between the phenotype of antibiotic resistance and the genotype coding for antibiotic resistance in the isolates evaluated for antimicrobial resistance (Wieczorek and Osek, 2013). The presence of *bla*_{OXA-61} is strongly correlated with beta-lactam resistance, but six isolates that are ampicillin susceptible also have this gene. This result is supported by another report which also showed that 59% of ampicillin susceptible isolates carried the *bla*_{OXA-61} gene (Zeng *et al.*, 2014). This demonstrates that ampicillin-susceptible isolates have weak *bla*_{OXA-61} gene expression and produce less beta-lactamase than resistant isolates (Schreyer *et al.*, 2022). The presence of *C. jejuni* which remains susceptible to ampicillin despite carrying the *bla*_{OXA-61} gene, could also have something to do with the *CmeABC* efflux pump because *CmeABC* also contributes to beta-lactamase resistance (Gharbi *et al.*, 2022).

The genotypically discovered *tetO* gene, which encodes tetracycline resistance, was present in all 12 *C. jejuni* isolates that were phenotypically resistant to the antibiotic. This result is the same as several previous studies which showed that 100% of tetracycline-resistant *C. jejuni* had the *tetO* gene (Sharifi *et al.*, 2021). This demonstrates a relationship between the *tetO* gene and phenotypic tetracycline resistance (Zhang and Zhang, 2019).

The most significant known mechanism of tetracycline resistance in *C. jejuni* is mediated by the protein *tetO*, which protects the 16S rRNA ribosome site (Pérez-Boto *et al.*, 2014). Usually, resistance to tetracycline antibiotics occurs due to the expression

of various types of tet genes, namely *tetA*, *tetB*, *tetC*, *tetE*, *tetG*, and *tetO* which are found in plasmids and chromosomes of various Gram-positive and Gram-negative organisms (Almeida *et al.*, 2021). However, only *tetO* was reported to be highly prevalent in *Campylobacter* species (Bai *et al.*, 2021). Another study, Yadav *et al.* (2020), has proven by detecting the *tetO*, *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, and *tetG* genes in 43 *C. jejuni*, the result is that only *tetO* was detected in 74.41% of isolates.

The high level of resistance of *C. jejuni* isolates in this study may be caused by the unchecked use of antibiotics as growth promoters and in animal care without a prescription. Because fluoroquinolone antibiotics are the preferred treatment for campylobacteriosis in humans, the underlying issue for which antibiotic use as a treatment is limited, the significant resistance rate found in this study is concerning (Portes *et al.*, 2023). Consequently, it is essential to control the use of antibiotics in both humans and animals (Rahman *et al.*, 2022). The broad use of alternative medications made from medicinal plants is also urgently needed (Abd El-Hamid *et al.*, 2019).

Farmers are obligated to adhere to and maintain a food safety management system based on Hazard Analysis and Critical Control Point (HACCP) principles (Awuchi, 2023). HACCP training enhances knowledge and hygiene practices in poultry handling and poultry carcass handling and avoids the inappropriate use of antibiotics that have an impact on antimicrobial resistance (Chowdhury *et al.*, 2021).

Conclusion

These findings indicate that the bacteria *C. jejuni*, which has antimicrobial resistance properties and contributes to the spread of antimicrobial resistance bacteria to the environment and poses a risk to human health, can be transmitted through chicken. Antimicrobial resistance is a widespread issue that needs to be addressed using a “One Health” strategy. To lessen the effects now and in the future, coordinated and suitable action is needed, as well as steps to guarantee the poultry industry’s financial stability and public health insurance.

Acknowledgment

The authors are thankful to the Faculty of Veterinary Medicine, Airlangga University, and Balai Besar Veteriner Wates for providing the necessary facilities for the study.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

This article was supported in part by the Postgraduate Research-Doctoral Dissertation Research Funding from Center for Higher Education Funding (Balai Pembiayaan Pendidikan Tinggi) and Indonesia Endowment Funds for Education (Lembaga Pengelola Dana Pendidikan), with grant number

from KEPUTUSAN KEPALA PUSAT LAYANAN PENDIDIKAN Nomor: 1306/J5.2.3/BPL.06/10/2021. BPI ID Number: 202101121565; 202209091803.

Data availability

All data supporting the findings of this study are available within the manuscript and no additional data sources are required.

Author's contributions

Conceptualization and design: SMY and FNAEPD; acquisition of data: AH and OSMS; formal analysis and interpretation of data: WT, JWAP, and RI; writing-original draft preparation: ARK and SCK; writing-review and editing: IBM, MS, and MHE. All authors have read and agreed to the published version of the manuscript.

References

- Abd El-Hamid, M.I.A., El-Sayed, M.E., Aisha, R.A., Abdallah, H.M., Marwa, I.A. and El-mowalid, G.A. 2019. Marjoram extract downregulates the expression of *Pasteurella multocida* adhesion, colonization and toxin genes: a potential mechanism for its antimicrobial activity. *Comp. Immunol. Microbiol. Infect. Dis.* 62(1), 101–108.
- Almeida, A.R., Tacão, M., Soares, J., Domingues, I. and Henriques, I. 2021. Tetracycline-resistant bacteria selected from water and zebrafish after antibiotic exposure. *Int. J. Environ. Res. Public Health* 18(6), 3218.
- Awuchi, C.G. 2023. HACCP, quality, and food safety management in food and agricultural systems. *Cogent Food Agric.* 9(1), 1.
- Bai, J., Chen, Z., Luo, K., Zeng, F., Qu, X., Zhang, H., Chen, K., Lin, Q., He, H., Liao, M. and Zhang, J. 2021. Highly prevalent multidrug-resistant *Campylobacter spp.* isolated from a yellow-feathered broiler slaughterhouse in South China. *Front. Microbiol.* 12(1), 682741.
- Budialmiawan, L., Parwati, I., Rita, C. and Lokida, D. 2022. Campylobacteriosis in an outbreak of foodborne disease at Bantar Gadung Village Palabuhan Ratu. *Indones. J. Clinical Pathol. Med. Laboratory* 28(2), 209–214.
- Bush, K. and Bradford, P.A. 2020. Epidemiology of β -Lactamase-producing pathogens. *Clin. Microbiol. Rev.* 33(2), e00047–19.
- Chatur, Y.A., Brahmabhatt, M.N., Modi, S. and Nayak, J.B. 2014. Fluoroquinolone resistance and detection of Topoisomerase Gne mutation in *Campylobacter jejuni* isolated from animal and human sources. *Int. J. Curr. Microbiol. Appl. Sci.* 3(6), 773–783.
- Choi, J., Kong, B., Bowker, B.C., Zhuang, H. and Kim, W.K. 2023. Nutritional strategies to improve meat quality and composition in the challenging conditions of broiler production: a review. *Animals* 13(8), 1386.
- Chowdhury, S., Ghosh, S., Aleem, M.A., Parveen, S., Islam, M.A., Rashid, M.M., Akhtar, Z. and Chowdhury, F. 2021. Antibiotic usage and resistance in food animal production: what have we learned from Bangladesh? *Antibiotics (Basel)* 10(9), 1032.
- Efendi, R., Sudarnika, E., Wibawan, I.W.T. and Purnawarman, T. 2022. An assessment of knowledge and attitude toward antibiotic misuse by small-scale broiler farmers in Bogor, West Java, Indonesia. *Vet. World.* 15(3), 707–713.
- El-Saadony, M.T., Saad, A.M., Yang, T., Salem, H.M., Korma, S.A., Ahmed, A.E., Mosa, W.F.A., Abd El-Mageed, T.A., Selim, S., Al Jaouni, S.K., Zaghloul, R.A., Abd El-Hack, M.E., El-Tarabily, K.A. and Ibrahim, S.A. 2023. Avian campylobacteriosis, prevalence, sources, hazards, antibiotic resistance, poultry meat contamination, and control measures: a comprehensive review. *Poult. Sci.* 102(9), 102786.
- Facciola, A., Riso, R., Avventuroso, E., Visalli, G., Delia, S.A. and Laganà, P. 2017. Campylobacter: from microbiology to prevention. *J. Prev. Med. Hyg.* 58(2), E79–E92.
- Faridah, H.D., Wibisono, F.M., Wibisono, F.J., Nisa, N., Fatimah, F., Effendi, M.H., Ugbo, E.N., Khairullah, A.R., Kurniawan, S.C. and Silaen, O.S.M. 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(1), 179–186.
- García-Sánchez, L., Melero, B., Diez, A.M., Jaime, I., Canepa, A. and Rovira, J. 2020. Genotyping, virulence genes and antimicrobial resistance of *Campylobacter spp.* isolated during two seasonal periods in Spanish poultry farms. *Prev. Vet. Med.* 176(1), 104935.
- Gharbi, M., Béjaoui, A., Hamda, C.B., Ghedira, K., Ghram, A. and Maaroufi, A. 2022. Distribution of virulence and antibiotic resistance genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler chickens in Tunisia. *J. Microbiol. Immunol. Infect.* 55(6 Pt 2), 1273–1282.
- Gibreel, A., Sköld, O. and Taylor, D.E. 2004. Characterization of plasmid-mediated aphA-3 kanamycin resistance in *Campylobacter jejuni*. *Microb. Drug Resist.* 10(2), 98–105.
- Grinnage-Pulley, T. and Zhang, Q. 2015. Genetic basis and functional consequences of differential expression of the CmeABC Efflux pump in *Campylobacter jejuni* isolates. *PLoS One* 10(7), e0131534.
- Han, J., Wang, Y., Sahin, O., Shen, Z., Guo, B., Shen, J. and Zhang, Q. 2012. A fluoroquinolone resistance associated mutation in gyrA Affects DNA supercoiling in *Campylobacter jejuni*. *Front. Cell. Infect. Microbiol.* 2(1), 21.
- Hosain, M.Z., Kabir, S.M.L. and Kamal, M.M. 2021. Antimicrobial uses for livestock production in developing countries. *Vet. World* 14(1), 210–221.
- Iovine, N.M. 2013. Resistance mechanisms in *Campylobacter jejuni*. *Virulence* 4(3), 230–240.

- Jaktaji, R.P. and Mohiti, E. 2010. Study of mutations in the DNA gyrase *gyrA* gene of *Escherichia coli*. Iran J. Pharm. Res. 9(1), 43–48.
- Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. and Man, S.M. 2015. Global epidemiology of campylobacter infection. Clin. Microbiol. Rev. 28(3), 687–720.
- Manyi-Loh, C., Mamphweli, S., Meyer, E. and Okoh, A. 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules 23(4), 795.
- Muurinen, J., Richert, J., Wickware, C.L., Richert, B. and Johnson, T.A. 2021. Swine growth promotion with antibiotics or alternatives can increase antibiotic resistance gene mobility potential. Sci. Rep. 11(1), 5485.
- Narvaez-Bravo, C., Taboada, E.N., Mutschall, S.K. and Aslam, M. 2017. Epidemiology of antimicrobial resistant *Campylobacter spp.* isolated from retail meats in Canada. Int. J. Food Microbiol. 253(1), 43–47.
- Obeng, A.S., Rickard, H., Sexton, M., Pang, Y., Peng, H. and Barton, M. 2012. Antimicrobial susceptibilities and resistance genes in *Campylobacter* strains isolated from poultry and pigs in Australia. J. Appl. Microbiol. 113(2), 294–307.
- Pérez-Boto, D., Herrera-León, S., García-Peña, F.J., Abad-Moreno, J.C. and Echeita M.A. 2014. Molecular mechanisms of quinolone, macrolide, and tetracycline resistance among *Campylobacter* isolates from initial stages of broiler production. Avian Pathol 43(2), 176–182.
- Portes, A.B., Panzenhagen, P., Dos Santos, A.M.P. and Junior, C.A.C. 2023. Antibiotic resistance in campylobacter: a systematic review of South American isolates. Antibiotics (Basel) 12(3), 548.
- Rahman, M.M., Tumpa, M.A.A., Zehravi, M., Sarker, M.T., Yamin, M., Islam, M.R., Harun-Or-Rashid, M., Ahmed, M., Ramproshad, S., Mondal, B., Dey, A., Damiri, F., Berrada, M., Rahman, M.H. and Cavalu, S. 2022. An overview of antimicrobial stewardship optimization: the use of antibiotics in humans and animals to prevent resistance. Antibiotics (Basel) 11(5), 667.
- Schreyer, M.E., Olivero, C.R., Rossler, E., Soto, L.P., Frizzo, L.S., Zimmermann, J.A., Signorini, M.L. and Virginia, Z.M. 2022. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *C. coli* identified in a slaughterhouse in Argentina. Curr. Res. Food Sci. 5(1), 590–597.
- Shakir, Z.M., Alhatami, A.O., Khudhair, Y.I. and Abdulwahab, H.M. 2021. Antibiotic resistance profile and multiple antibiotic resistance index of campylobacter species isolated from poultry. Arch. Razi. Inst. 76(6), 1677–1686.
- Sharifi, S., Bakhshi, B. and Najjar-peerayeh, S. 2021. Significant contribution of the CmeABC efflux pump in high-level resistance to ciprofloxacin and tetracycline in *Campylobacter jejuni* and *Campylobacter coli* clinical isolates. Ann. Clin. Microbiol. Antimicrob. 20(1), 1–9.
- Sierra-Arguello, Y.M., Furian, T.Q., Perdoncini, G., Moraes, H.L.S., Salle, C.T.P., Rodrigues, L.B., dos Santos, L.R., Gomes, M.J.P. and do Nascimento, V.P. 2018. Fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli* from poultry and human samples assessed by pcr-restriction fragment length polymorphism assay. PLoS One 13(7), e0199974.
- Sison, F.B., Chaisowong, W., Alter, T., Tiwananthagorn, S., Pichpol, D., Lampang, K.N., Baumann, M.P.O. and Gözl, G. 2014. Loads and antimicrobial resistance of *Campylobacter spp.* on fresh chicken meat in Nueva Ecija, Philippines. Poult. Sci. 93(5), 1270–1273.
- Šoprek, S., Duvnjak, S., Kompes, G., Jurinović, L. and Andrašević, A.T. 2022. Resistome analysis of *Campylobacter jejuni* strains isolated from human stool and primary sterile samples in Croatia. Microorganisms 10(7), 1410.
- Stella, S., Soncini, G., Ziino, G., Panebianco, A., Pedonese, F., Nuvoloni, R., Di Giannatale, E., Colavita, G., Alberghini, L. and Giaccone, V. 2017. Prevalence and quantification of thermophilic *Campylobacter spp.* in Italian retail poultry meat: analysis of influencing factors. Food Microbiol. 62(1), 232–238.
- Syahliani, S.P., Haryadi, F.T., Setyawan, A.A., Mayasari, I., Dewi, N.M.A.K. and Qui, N.H. 2022. Key driver of repurchase intention in the poultry farming input market in Indonesia. Trop. Anim. Sci. J. 45(4), 490–498.
- Thomrongsuwannakij, T., Blackall, P.J., Chansiripornchai, N. 2017. A study on *Campylobacter jejuni* and *Campylobacter coli* through commercial broiler production chains in Thailand: antimicrobial resistance, the characterization of DNA gyrase subunit a mutation, and genetic diversity by Flagellin a gene restriction fragment length polymorphism. Avian Dis. 61(2), 186–197.
- Untari, T., Herawati, O., Anggita, M., Asmara, W., Wahyuni, A.E.T.H. and Wibowo, M.H. 2021. The effect of antibiotic growth promoters (AGP) on antibiotic resistance and the digestive system of broiler chicken in Sleman, Yogyakarta. BIO Web. Conf. 33(1), 04005.
- Wangroongsarb, P., Cheunban, N., Jittaprasatsin, C., Kamthlang, T., Saipradit, N., Chaichana, P., Pulsrikarn, C., Parnmen, S. and Sripichai, O. 2021. Prevalence and antimicrobial susceptibility of *Campylobacter* isolated from retail chickens in Thailand. Int. J. Food Microbiol. 339(1), 109017.
- Wieczorek, K. and Osek, J. 2013. Antimicrobial resistance mechanisms among *Campylobacter*. Biomed. Res. Int. 2013(1), 340605.

- Yadav, R. and Maherchandani, S. 2020. Antibiotic resistance profiling of *Campylobacter jejuni* isolated from poultry in India. *Int. J. Livest. Res.* 10(3), 141–151.
- Yanestria, S.M., Dameanti, F.N.A.E.P., Musayannah, B.G., Pratama, J.W.A., Witaningrum, A.M., Effendi, M.H. and Ugbo, E.N. 2022. Antibiotic resistance pattern of extended-spectrum β -lactamase (ESBL) producing *Escherichia coli* isolated from broiler farm environment in Pasuruan district, Indonesia. *Biodiversitas* 23(9), 4460–4465.
- Yang, H., Li, Y., Zhang, Y., Dong, B., Duan, B., Guo, L., Wang, T., Lv, X., Zheng, M., Cui, X. and Bai, R. 2023. Prevalence, drug resistance spectrum and virulence gene analysis of *Campylobacter jejuni* in broiler farms in central Shanxi, China. *Poult. Sci.* 102(3), 102419.
- Zeng, X., Brown, S., Gillespie, B. and Lin, J. 2014. A single nucleotide in the promoter region modulates the expression of the β -lactamase OXA-61 in *Campylobacter jejuni*. *J. Antimicrob. Chemother.* 69(5), 1215–1223.
- Zhang, Y. and Zhang, Q. 2019. Relationship between tetracycline antibiotic susceptibility and genotype in oral cavity *Lactobacilli* clinical isolates. *Antimicrob. Resist. Infect. Control.* 8(1), 27.
- Zong, S., Xu, D., Zheng, X., Zaeim, D., Wang, P., Han, J. and Qu, D. 2022. The diversity in antimicrobial resistance of MDR Enterobacteriaceae among Chinese broiler and laying farms and two mcr-1 positive plasmids revealed their resistance-transmission risk. *Front. Microbiol.* 13(1), 912652.