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Identification of *Campylobacter jejuni* from Chicken Carcasses and Characterization of Their Antibiotic Resistance Using Molecular Approach

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ABSTRACT

Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are foodborne pathogenic bacteria that cause campylobacteriosis, an acute gastroenteritis in humans. The most important transmission source to humans is contaminated animal products, such as chicken carcasses. Recently, there has been a growing concern about emerging antibiotic-resistant pathogens, including *Campylobacter*. Improper use of antibiotics in livestock can increase the risk of antibiotic resistance and the transmission of resistant bacterial strains to humans. This study aims to identify the species of *Campylobacter* spp. isolated from chicken carcasses and detected their antibiotic resistance genes using MinION sequencing in *C. jejuni* isolates. The disc diffusion method was also used to evaluate their resistance to macrolide, tetracycline, and fluoroquinolone antibiotics. The study results show that *C. jejuni* was identified in 64% of the 25 isolates, while *C. coli* was identified in 36% of the isolates. Molecular testing on *C. jejuni* isolates revealed that macrolide resistance genes were absent, specifically the *23S rRNA* and the *erm*B genes. The *tet*O gene encoding for tetracycline resistance was detected in 62.5% of the isolates, and all 16 isolates (100%) were found to have the *gyr*A gene. In *C. jejuni* isolates, the *cme*ABC gene, which functions as a multidrug efflux pump, was also detected. The antibiotic resistance of *C. jejuni* isolates based on the disc diffusion method indicated high resistance to fluoroquinolone antibiotics, followed by tetracycline antibiotics.

Keywords: antibiotic resistance; Campylobacter spp.; C. jejuni; chicken carcasses; MinION sequencing

INTRODUCTION

Two *Campylobacter* species that cause gastrointestinal infections ranging from mild to bloody diarrhea in humans are the foodborne pathogen *Campylobacter jejuni* and, to a lesser extent, *Campylobacter coli* (Yoon *et al.*, 2017). Data in the European Union indicate that *C. jejuni* is the most prevalent zoonotic pathogen, accounting for more than 90% of infection cases; whereas, cases caused by *C. coli* are less frequent (Yoon *et al*., 2017). Based on the WHO data, the frequency of *Campylobacter* spp. gastroenteritis cases in developed countries vary from 4.4 to 9.3 per 1,000 population per year (Shen *et al*., 2018). *Campylobacter* infection in humans is primarily through the intake of contaminated animal products, among which chicken carcass is the major source (García-Sánchez *et al*., 2018). To ensure that food products from poultry have a contamination level of ≤ 1,000 CFU/g, the European Union has implemented standards (Stella *et al*., 2021; García-Sánchez *et al*., 2018). On the other hand, Indonesia does not have a definite limit for *Campylobacter* contamination in the food. Such a situation signals the need for contamination control at the food production stage to ensure the safety of the population and to allow scientists the freedom to implement a supporting food safety policy.

Antibiotics are very important in stopping infections in humans and animals. However, this practice also contributes to the development of antimicrobial resistance (AMR). As a result, various global regulations, including those in Indonesia, have been implemented to restrict their use in livestock. A 2017 survey conducted by the Center for Indonesian Veterinary Analytical Studies reported that prior to the implementation of the ban, the most commonly used antibiotics in poultry for treatment, prevention, and productivity enhancement were, in order of prevalence, fluoroquinolones, tetracyclines, and a combination of tetracyclines and macrolides. Resistance in zoonotic bacteria such as *Campylobacter* originating from poultry is of particular concern due to its potential transmission to humans through the food chain, thereby posing direct threats to public health and food safety (Yang *et al*., 2023; Magnusson *et al*., 2021).

According to 2022 AMR surveillance data from the European Union, analyzed by the European Food Safety Authority (EFSA) in collaboration with the European Centre for Disease Prevention and Control (ECDC), both *C. jejuni* and *C. coli* from human and animal sources exhibit the highest fluoroquinolone resistance. In 2017, the WHO classified *Campylobacter* spp. as a high-priority pathogen for fluoroquinolone resistance. Outbreaks of antibiotic-resistant *C. jejuni* infections originating from domestic animals were reported by Watkins *et al*. (2021) in the United States between 2016 and 2020, with 168 cases. The presence of antibiotic resistance genes in *Campylobacter* that infect humans, such as erythromycin in the macrolide group, tetracycline, and ciprofloxacin in the fluoroquinolone group, which are often used in treatment, has also been widely reported (Awad *et al*., 2023; Quino *et al*., 2022; Wanja *et al*., 2023). Resistant bacteria occur due to repeated exposure to antibiotics, which causes bacteria to modify their genetic material either spontaneously or through transmission between bacteria, causing the antibiotics not to work optimally (Quino *et al*., 2022; Wanja *et al*., 2023). This mechanism involves modifying the antibiotic's site of action by removing it from the cell or inactivating it with enzymes (Shen *et al*., 2018; Awad *et al*., 2023). Antibiotic resistance in *Campylobacter* includes fluoroquinolone resistance caused by mutated *gyrA* genes, as well as macrolide resistance due to mutations in the *23S rRNA* gene and methylation of the *ermB* gene, while tetracycline resistance involves the *tetO* gene. The naturally occurring mechanism in *Campylobacter* that causes and can increase resistance is the *cmeABC* gene with a multidrug efflux pump mechanism (Shen *et al*., 2018; Awad *et al*., 2023; Quino *et al*., 2022; Wanja *et al*., 2023).

In farms with structured systems, resistant bacterial strains in livestock can retain their resistance properties due to repeated exposure to antibiotics. This situation increases the reservoir of resistance genes in the environment and heightens the risk of their transmission to humans (Magnusson *et al*., 2021). Therefore, characterizing and monitoring genetic changes are necessary steps to trace the source, understand the processes, and assess the spread capacity of antimicrobial resistance, especially for those bacteria that can be transferred from animals to humans, like *Campylobacter* (Frazao *et al*., 2021).

In Indonesia, there are limited reports on species identification and AMR profiles of *Campylobacter* spp. One emerging molecular approach increasingly used in foodborne pathogen and antimicrobial resistance surveillance is whole genome sequencing (WGS) (Frazao *et al*., 2021). MinION nanopore sequencing is a WGS platform that utilizes protein-based nanopores embedded in an electrically resistant polymer membrane, functioning as biosensors (Wang *et al*., 2021). One of the advantages of the method is the capability to thoroughly uncover the resistance genes of the entire bacterial genome by sequencing DNA of up to one million base pairs, the simultaneous handling of multiple samples and an easy movement of data to the bioinformatics pipelines.

This study aimed to identify *C. jejuni* and *C. coli* species from *Campylobacter* spp. isolates obtained from chicken carcasses using MinION sequencing; to detect macrolide, tetracycline, and fluoroquinolone resistance genes in *C. jejuni* isolates using MinION sequencing; and to evaluate the antibiotic resistance profiles of *C. jejuni* against macrolides, tetracyclines, and fluoroquinolones using the disk diffusion method.

MATERIALS AND METHODS

Bacterial Isolates

This research has used 25 *Campylobacter* spp. isolates that were cultured from 50 positive chicken carcass samples collected from NKV-certified poultry slaughterhouses in Bogor, West Java, Indonesia. These samples were collected in 2023 by the National Veterinary Public Health Laboratory (BPMSPH) as part of the monitoring and surveillance program. The number of isolates was determined using an exploratory and descriptive approach, focusing on the comprehensive characterisation of all viable and available isolates, rather than employing statistical sampling methods.

Culture Preparation of *Campylobacter* spp. Isolates

To confirm the viability of the isolates, re-culturing was performed by inoculating them into Bolton Broth (Oxoid, CM0983) and incubating at 42 °C for 24–48 hours under microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2) using an anaerobic jar (Oxoid) and Campygen sachet (Oxoid, CM0025). Grown cultures were then streaked onto non-selective Blood Agar (BA) plates (Oxoid, CM0055B) supplemented with 5% sheep blood and incubated at 42 °C for 24–48 hours. The resulting colonies were used for further molecular identification and antibiotic resistance testing.

DNA Extraction

DNA was extracted using the DNeasy PowerWater Kit (Qiagen, Germany) following the manufacturer's protocol, with a modification in the initial lysis step. DNA extraction and purification were performed by transferring a loopful of a pure *Campylobacter* colony from BA into a microtube containing 1 mL sterile phosphate-buffered saline (PBS; Himedia). The suspension was centrifuged at 13,000 rpm for 5 minutes, the supernatant discarded, and the pellet resuspended in 200 µL of sterile PBS. This step was repeated. Then, 200 µL of Fast Lysis Buffer (Qiagen DNeasy Power Water Kit) was added to the pellet, and the tube was incubated in a thermoshaker (Eppendorf) at 100 °C and 800 rpm for 10 minutes, followed by 2 minutes at room temperature. The supernatant containing DNA was transferred to a new 2 mL microtube. DNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific).

Nanopore MinION Sequencing

DNA library preparation and sequencing were performed according to the official protocol of the Rapid

Sequencing gDNA–Barcoding Kit (SQK-RBK110.96), as outlined in the Oxford Nanopore Technologies documentation (2023). Approximately 50 ng of each DNA extract was transferred into a LoBind tube and adjusted to 9 µL with nuclease-free water. After gentle mixing, 1 µL of barcode (RB01-96) was added. The mixture was centrifuged, incubated at 30 °C for 2 minutes, and then at 80 °C for another 2 minutes. Barcoded samples were pooled, and the total volume was recorded. A 1:1 volume of AMPure XP Beads (AXP or SPRI) was added and incubated at room temperature for 5 minutes, and the tubes were placed on a magnetic rack to separate the beads. The supernatant was discarded, and the pellet was washed twice with 1.5 mL of 80% ethanol. The pellet was then eluted with 15 µL of elution buffer (EB) and incubated at room temperature for 10 minutes. The beads were removed on the magnetic rack, and the clear eluate containing the DNA library (15 µL) was transferred to a clean 1.5 mL LoBind tube. An 11 µL aliquot was mixed with 1 µL of Rapid Adapter F (RAP F), gently mixed, and incubated at room temperature for 5 minutes. The DNA library was stored on a plate cooler until ready for sequencing.

The sequencing was performed using a MinION Flow Cell (Oxford Nanopore Technologies). The flow cell cover was opened, and the priming port was exposed. A total of 200 µL of fluid was withdrawn from the flow cell, followed by adding 800 µL priming mix (Flush Tether and Flush Buffer) into the priming port and left for 5 minutes. After gently opening the sample port, an additional 200 µL of priming mix was added. A 75 µL aliquot of the prepared library mix (containing 37.5 µL Sequencing Buffer II, 25.5 µL Loading Beads II, and 12 µL DNA library) was slowly added dropwise to the sample port. The ports and flow cell were sealed, and sequencing was initiated using the MinKNOW software on a computer connected to the MinION device. Sequencing was performed for 72 hours. The raw sequencing data in FASTQ format were used for subsequent bioinformatics analyses.

Species Identification of *Campylobacter* spp. Using MinION Nanopore Sequencing

Species identification protocol in this study followed the workflow provided by Oxford Nanopore Technologies (ONT) for real-time sequencing-based taxonomic classification, as described in the EPI2ME documentation and previous studies (Kim *et al*., 2016; ONT, 2023; Tyler *et al*., 2023). The identification was conducted by uploading raw sequence files (FASTQ format) generated from the MinION platform (Oxford Nanopore Technologies) to the EPI2ME cloud platform. The initial taxonomic classification was performed using the "What's In My Pot?" (WIMP) workflow, which automatically maps each read to reference genomes in the NCBI RefSeq database and assigns taxonomy based on the NCBI taxonomy tree (Taxonomy IDs).

The selection criteria of species were based on read dominance classified under a single species. An isolate was defined as *Campylobacter jejuni* (TaxID: 197) or *Campylobacter coli* (TaxID: 195) when more than 50% of its reads aligned to the reference genome of that species. This approach was implemented in earlier research for the recognition of species from uniform single isolates (Kim *et al*., 2016; Tyler *et al*., 2023). The WIMP workflow also served as the procedural base of the next step in the analysis process for the identification of antimicrobial resistance genes**.**

Detection of Antibiotic Resistance Genes in *Campylobacter jejuni*

The procedure for identifying antibiotic resistance coding genes refers to the standard EPI2ME application protocol issued by Oxford Nanopore Technologies. The "Fastq Antimicrobial Resistance" workflow in the EPI2ME application will be used to detect resistance-coding genes (ONT, 2023; Maguire *et al*., 2021). Raw data in FASTQ format, generated from the sequencing process and identified as reads, will be directly compared with reference data without any assembly process.

Quality control procedures first examined the reads for length and integrity. The next step, taxonomic assignment, was performed using the WIMP (What's In My Pot?) algorithm, which combines centrifuge-based classification with taxonomic templates retrieved from the NCBI RefSeq archive to verify strain identity. Resistance element identification was then performed by aligning the reads with references in the Comprehensive Antibiotic Resistance Database (CARD). CARD encodes extensive annotations of antibiotic resistance genes and their biochemical mechanisms, and read mapping to these references thus produces an integrated report of potential resistance determinants present in each isolate. (Maguire *et al*., 2021).

Antibiotic Susceptibility Testing by Kirby-Bauer Disc Diffusion Method

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion procedure, as detailed in CLSI M45, the protocol for fastidious or rarely isolated species, as specified by CLSI in 2015. A bacterial suspension equivalent to 0.5 McFarland standard was prepared by mixing *Campylobacter* spp. Cultures were measured in 5 mL of sterile water using a nephelometer (Thermo Fisher Scientific). The culture suspension with concentration 0.5 McFarland was spread evenly onto Mueller-Hinton Agar (MHA; Oxoid, CM0337) supplemented with 5% sheep blood using a sterile swab. Antibiotic discs used included erythromycin (15 µg; Oxoid, CT0020B), tetracycline (30 µg; Oxoid, CT0054B), and ciprofloxacin (5 µg; Oxoid, CT0425B). The plates were incubated under microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2) in an anaerobic jar with Campygen sachets (Oxoid, CM0025) at 42 °C for 48 hours. *Staphylococcus aureus* ATCC 25923 (Thermo Scientific) was the quality control strain (CLSI, 2015). The diameter of the inhibition zones was measured using a digital caliper (Mitutoyo), and susceptibility was interpreted according to CLSI guidelines (Table 1).

RESULTS

Identified *Campylobacter* Species

The species classification results obtained using the EPI2ME application are presented in Table 2, showing the number of reads classified at the genus and species levels. Classification was based on the dominant proportion of reads aligned with the reference genomes of *C. jejuni* or *C. coli*. Out of a total of 25 *Campylobacter* spp. isolates, 16 isolates (64%) were identified as *Campylobacter jejuni*, and nine (36%) as *Campylobacter coli*.

Antibiotic Resistance Genes in *C. jejuni* Isolates

Table 3 shows the presence of antibiotic-resistance genes in the 16 *C. jejuni* isolates. Analysis using the EPI2ME platform did not detect the macrolide resistance genes *23S rRNA* or *erm*B in any of the *C. jejuni* isolates. All 16 isolates (100%) harbored the *cme*ABC multidrug efflux gene. The tetracycline resistance gene *tet*O was detected in 10 isolates (62.5%), while the fluoroquinolone resistance gene *gyr*A was present in all isolates (100%).

Antibiotic Resistance Phenotypes in *C. jejuni* Isolates

The results of antibiotic resistance testing using the disc diffusion method on C. *jejuni* (n = 16) showed that all isolates (100%) were susceptible to macrolide antibiotics, nine isolates (56.25%) were resistant and seven isolates (43.75%) were susceptible to tetracycline antibiotics. In comparison, 14 isolates (87.50%) were resistant and two isolates (12.50%) were susceptible to fluoroquinolone antibiotics (Table 4).

DISCUSSION

Campylobacter is known to survive in various fresh food products, including chicken carcasses (Montero *et al*., 2024). *Campylobacter jejuni* is the most common species and constitutes the dominant proportion of *Campylobacter* spp. isolated from chicken carcasses (Schreyer *et al*., 2022), although the prevalence and distribution of *C. jejuni* and *C. coli* in chicken carcasses may vary depending on several factors such as geographical region, season, sampling method, poultry age, and the specific part of the carcass analyzed (Andritsos *et al*., 2023; Schreyer *et al*., 2022). *C. jejuni*

Table 1. Interpretation criteria for inhibition zone diameters in *Campylobacter* species

Antibiotic class	Antibiotic agent	Disc concentration	Inhibition zone diameter criteria (mm)		
			Susceptible	Intermediate	Resistance
Macrolides	Erythromycin	15 µg	≥ 16	13-15	≤ 12
Tetracyclines	Tetracycline	30 µg	≥ 26	23- 25	≤ 22
Fluoroquinolones	Ciprofloxacin	5 µg	≥ 24	21- 23	≤ 20

Table 2. Species identification results of *Campylobacter* spp. isolates

Isolate code	Barcode code	Number of reads assigned to *Campylobacter* species		
		Campylobacter genus *(Reads)*	Classified *(Reads)*	Species
2101	65	14.460	13.499	C. *coli*
2102	66	8.200	7.763	C. *coli*
2104	67	21.929	20.656	C. *jejuni*
2106	68	22.984	22.391	C. *coli*
2112	69	23.984	22.316	C. *jejuni*
2115	70	24.789	23.073	C. *jejuni*
2547	71	38.389	36.277	C. *jejuni*
2549	72	18.263	17.110	C. *jejuni*
2747	73	7.709	7.522	C. *jejuni*
3912	74	88.235	85.390	C. *jejuni*
3913	75	8.800	8.244	C. *jejuni*
3914	76	12.602	11.847	C. *jejuni*
4684	77	4,744	4.506	C. *coli*
4689	78	3.899	3.775	C. *jejuni*
4691	79	13.002	12.674	C. *jejuni*
195	80	6.889	6.232	C. *jejuni*
196	81	21.364	20.823	C. *coli*
197	82	6.195	5.921	C. *coli*
198	83	17.058	16.421	C. *jejuni*
199	84	33.439	30.655	C. *coli*
201	85	17.810	15.707	C. *jejuni*
2898	86	15.470	14.624	C. *jejuni*
4686	87	6.642	6.323	C. *coli*
4688	88	2.115	2.011	C. *coli*
4900	89	7.157	6.785	C. *jejuni*

Table 3. Detection of antibiotic resistance genes and efflux pump genes in *Campylobacter jejuni*

Isolate code	Resistance genes				Efflux pump genes		
	Macrolides		Tetracyclines	Fluoroquinolones			
	23S rRNA	*erm*B	*tet*O	*gyr*A	*cme*A	*cme*B	*cme*C
2104	-	-	-	+	+	+	+
2112	-	-	+	+	+	+	+
2115	-	-	+	+	+	+	+
2547	-	-	-	+	+	+	+
2549	-	-	+	+	+	+	+
2747	-	-	+	+	+	+	+
3912	-	-	+	+	+	+	+
3913	-	-	-	+	+	+	+
3914	-	-	-	+	+	+	+
4689	-	-	-	+	+	+	+
4691	-	-	+	+	+	+	+
195	-	-	+	+	+	+	+
198	-	-	+	+	+	+	+
201	-	-	-	+	+	+	+
2898	-	-	+	+	+	+	+
4900	-	-	+	+	+	+	+

Note: (-) non detected; (+) detected

Table 4. Antibiotic resistance interpretation of *Campylobacter jejuni* isolates from chicken carcasses based on the disc diffusion method

Isolate code	Inhibition zone diameter and interpretation of results by antibiotic class					
	Macrolides		Tetracyclines		Fluoroquinolones	
	IZ ± σ (mm)	I	IZ ± σ (mm)	I	IZ ± σ (mm)	I
2104	29.00 ± 0.46	S	58.00 ± 0.00	S	10.04 ± 0.00	R
2112	39.78 ± 0.46	S	9.73 ± 0.26	R	11.52 ± 0.27	R
2115	57.31 ± 0.02	S	11.44 ± 0.30	R	10.68 ± 0.17	R
2547	35.44 ± 0.11	S	38.08 ± 0.12	S	≤ 6.00 ± 0.00	R
2549	28.50 ± 0.10	S	≤ 6.00 ± 0.00	R	≤ 6.00 ± 0.00	R
2747	33.18 ± 0.23	S	7.23 ± 0.25	R	≤ 6.00 ± 0.00	R
3912	32.66 ± 0.38	S	≤ 6.00 ± 0.00	R	≤ 6.00 ± 0.00	R
3913	43.24 ± 0.20	S	54.23 ± 0.38	S	9.48 ± 0.16	R
3914	46.85 ± 0.29	S	56.92 ± 0.04	S	9.16 ± 0.31	R
4689	42.38 ± 0.11	S	12.81 ± 0.04	R	10.77 ± 0.08	R
4691	23.11 ± 0.18	S	15.58 ± 0.46	R	12.21 ± 0.31	R
195	37.82 ± 0.75	S	≤ 6.00 ± 0.00	R	≤ 6.00 ± 0.00	R
198	19.93 ± 0.00	S	≤ 6.00 ± 0.00	R	≤ 6.00 ± 0.00	R
201	43.49 ± 0.79	S	49.01 ± 0.07	S	45.22 ± 0.62	S
2898	58.00 ± 0.00	S	58.00 ± 0.00	S	≤ 6.00 ± 0.00	R
4900	18.87 ± 0.00	S	26.62 ± 0.00	S	28.94 ± 0.00	S

Note: IZ ± σ = mean inhibition zone diameter ± standard deviation; I = interpretation; R = resistant; S = susceptible.

has commonly been reported from poultry-associated *Campylobacter* isolates in countries such as Korea (Kim *et al*., 2019), China (Yang *et al*., 2023), Poland (Woźniak-Biel *et al*., 2018), and Brazil (Je *et al*., 2023). Based on meta-analysis data by Je *et al.* (2023), it was found that *C. jejuni* is more common in poultry products than *C. coli*, and caused a foodborne outbreak in Seoul, South Korea, in 2017. Consumption of undercooked poultry containing *C. jejuni* has also caused cases in the Netherlands (Je *et al*., 2023). Identification of *C. jejuni* was carried out by Yanestria *et al*. (2024) in Indonesia, who identified 31 *C. jejuni* from 50 positive *Campylobacter* isolates from broiler chickens in Pasuruan Regency, East Java. In the poultry production chain, there are critical points that need to be taken to prevent *Campylobacter* contamination, starting from farming, transportation, slaughter and gutting processes, to consumption (Skarp *et al*., 2016). The presence of antibiotic-resistant strains can affect treatment success and make isolates more resistant to environmental stressors such as low temperatures, disinfectants, and refrigeration. This can lead to increased colonization if optimal sanitation is not performed (García-Sánchez *et al*., 2018).

The maximum limit for *Campylobacter* contamination is expected to reduce the incidence of campylobacteriosis infections. The European Union has set a maximum limit to eliminate the risk of Campylobacter, by setting 1,000 CFU/g as the maximum limit of contamination that occurs in chicken carcasses after cooling, with European Commission Regulation

No. 2017/1495 (Stella *et al*., 2021; García-Sánchez *et al*., 2018). Indonesia has not yet issued regulations that specifically set maximum limits for *Campylobacter* contamination, thus creating challenges in food safety evaluation at the national level.

Antibiotic resistance remains a pressing public health issue despite almost universal bans on antibiotic use. In Indonesia, the ban was implemented in 2018, and in the United States and Europe, it was implemented in 2006, long before the ban in Indonesia (Magnusson *et al*., 2021; Widiyanti *et al*., 2019). This is due to the ability of bacteria to maintain their resistance in their offspring and spread it to other bacteria, even after they are no longer exposed to antibiotics. This is true for *Campylobacter*. Natural resistance mechanisms, such as efflux pumps, play a role in maintaining resistant strains for long periods, which can persist in the environment and in their hosts. Travel and movement of people between countries, imports of animal products, and cross-contamination can also spread resistant bacteria (Whitehouse *et al*., 2018). The spread of resistance traits that occurs through specific defense mechanisms, such as vertical mechanisms to offspring or horizontally through genetic transfer mechanisms such as conjugation, transformation, or transduction, is how bacteria survive and develop (Whitehouse *et al*., 2018; Shen *et al*., 2018).

Macrolide resistance in *Campylobacter* can occur through target mutations in the 23S *rRNA* gene and modification via methylation by the *ermB* gene (Whitehouse *et al*., 2018; Shen *et al*., 2018; Bravo *et al*., 2021). Ribosome protection by the ribosome protection protein encoded by the *tetO* gene is the main mechanism of tetracycline resistance in *Campylobacter* (Whitehouse *et al*., 2018). The *cmeABC* gene in *Campylobacter* species is one of the natural mechanisms that makes *Campylobacter* intrinsically resistant to various antibiotics and can synergize with structurally unrelated antibiotics (Whitehouse *et al*., 2018; García-Sánchez *et al*., 2018; Awad *et al*., 2023), including fluoroquinolones, macrolides, and tetracyclines (Shen *et al*., 2018; Bravo *et al*., 2021). DNA gyrase target modification and *cmeABC* multidrug efflux pump action are two mechanisms of quinolone resistance that have been identified in *Campylobacter* (Whitehouse *et al*., 2018). Among these, modification of the quinolone target site in the quinolone resistance-determining region (QRDR) of *gyr*A is the primary mechanism of fluoroquinolone resistance in *Campylobacter* (Whitehouse *et al*., 2018). The *cme*ABC efflux pump may also synergize with *gyr*A mutations to produce high-level fluoroquinolone resistance (Urban-Chmiel *et al*., 2022).

The disc diffusion antibiotic susceptibility testing showed that no *C. jejuni* isolates were resistant to macrolides (Table 4). This aligns with genotypic findings where neither the *23S rRNA* nor the *erm*B genes were detected (Table 3).

For tetracyclines, disc diffusion tests revealed that seven isolates were susceptible and nine were resistant (Table 4). Isolates 2104, 2547, 3913, 3914, and 201, which were phenotypically susceptible, also lacked the *tet*O gene. However, two susceptible isolates (2898 and 4900) carried the *tet*O gene. Similar findings were reported by Woźniak-Biel *et al.* (2018) and Awad *et al*. (2023), who observed *tet*O-positive *C. jejuni* isolates that were phenotypically susceptible to tetracycline. These findings suggest that the *tet*O gene may be inactive or not expressed in some *Campylobacter* isolates (Woźniak-Biel *et al*., 2018). Isolates 2112, 2115, 2549, 2747, 3912, 4691, 195, and 198 exhibited resistance in phenotypic testing and harbored the *tet*O gene, indicating genotypic-phenotypic concordance. However, isolate 4689, phenotypically resistant, did not harbor the *tet*O gene. A similar observation was made by Awad *et al*. (2023) and Quino *et al*. (2022), who reported phenotypically resistant isolates lacking *tet*O. Quino *et al*. (2022) attributed this resistance to the presence of the *cme*ABC gene, which may independently confer tetracycline resistance. Awad *et al*. (2023) also proposed that resistance in *tet*O-negative but phenotypically resistant isolates may be associated with efflux pump mechanisms. The presence of *cme*ABC in isolate 4689 explains its phenotypic resistance.

For fluoroquinolones, disc diffusion analysis showed that two of the 16 *C. jejuni* isolates (201 and 4900) were susceptible (Table 4). Although these two isolates were phenotypically susceptible, they carried the *gyr*A gene. This result is consistent with findings by Schreyer *et al.* (2022), who reported *C. jejuni* isolates that carried *gyr*A but remained susceptible to enrofloxacin. Whitehouse *et al.* (2018) demonstrated that a Thr86Ile mutation in *gyr*A was present in 53 phenotypically resistant isolates, while this mutation was absent in 61 susceptible isolates, although other mutations were occasionally observed. Urban-Chmiel *et al*. (2022) explained that the interaction between *gyr*A mutations and inactivation of the *cme*ABC gene may increase fluoroquinolone susceptibility. Similarly, Shen *et al.* (2018) observed that the inactivation of *cme*ABC in fluoroquinolone-resistant isolates harboring specific *gyr*A mutations could revert them to susceptibility.

This study observes the need for resistance surveillance and implementing integrated control strategies throughout the poultry production chain regarding *Campylobacter jejuni* as a foodborne pathogen in chicken carcasses by explaining the relationship between molecular analysis of antibiotic resistance and its phenotypic characteristics. Surveillance and control can be done to prevent the spread of *Campylobacter* in farms by implementing good sanitation and biosecurity processes, limiting the number of birds in each pen, and using probiotics instead of drugs (Skarp *et al*., 2016; García-Sánchez *et al*., 2018). Tightening sanitation is also needed during the transportation stage and the slaughter process (Skarp *et al*., 2016). According to García-Sánchez *et al.* (2018), the method used to reduce contamination in products is by using disinfectants according to the rules in the process. Additionally, cooling after the process helps reduce the *Campylobacter* population. This finding aligns with research conducted by Stella *et al*. (2021), who reported that the post-slaughter cooling process showed an average reduction in the proportion of *Campylobacter*-contaminated carcasses by 0.3 log CFU/g. Overall, controlling

Campylobacter and its resistance in the poultry food production chain in Indonesia requires an integrated One Health strategy.

CONCLUSION

Campylobacter spp. isolated from chicken carcasses consisted of *C. jejuni* (64%) and *C. coli* (36%). The *gyrA* and *tetO* genes were molecularly identified. This is consistent with the phenotypic resistance pattern to fluoroquinolones and tetracyclines. Continuous antimicrobial resistance surveillance requires greater attention, which can be achieved by improving farm-level biosecurity and *Campylobacter* control through a One Health approach. The public health and food safety threats posed by antibiotic-resistant *C. jejuni* are urgent and must be addressed immediately.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

We declare that generative AI and AI-assisted technologies were used for language refinement during the preparation of this work. After using the service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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