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Phenotypic and Genotypic Antimicrobial Resistance Profile of *Salmonella* spp. Isolated from Kampung Chicken Carcasses

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ABSTRACT

Kampung chicken meats have been widely consumed in Indonesia as well as broiler chicken. However, the extensive rearing allowed multidrug-resistant (MDR) bacteria exposure to kampung chicken, including through horizontal gene transfer. This study aimed to observe the correlation between the phenotypic and genotypic antimicrobial resistance profile of *Salmonella* spp. isolated from kampung chicken carcasses. Phenotypic antimicrobial resistance was evaluated by Kirby-Bauer's disk diffusion method, while the detection of drug resistance genes in seventeen isolates of *Salmonella* was carried out by PCR. All (17/17) isolates were susceptible to ampicillin and chloramphenicol. Most isolates of *Salmonella* were resistant to erythromycin (82%; 14/17), while the decreased susceptibility (intermediate category) most occurred in oxytetracycline (82%; 14/17). *Salmonella* Typhimurium showed a resistance pattern to more antimicrobial groups than *S.* Newport and *S.* Weltevreden. Several antimicrobial resistance genes (*bla*TEM, *tet*G, *cml*A, *gyr*A) were present in all (17/17) isolates of *Salmonella* spp. Resistance to antimicrobial agents and the presence of resistance genes were not always related. This study could provide beneficial information regarding the transmission of antimicrobial resistance among *Salmonella* spp. from kampung chickens.

1. Introduction

Chicken meat is an animal-based food commodity widely consumed in Indonesia. High interest in organic food products gradually increased the kampung chicken meat consumption. According to the Directorate General of Livestock and Animal Health Services (2020), consumption of kampung chicken meat per capita in 2019 was 0.782 kg, increasing by 7.1 percent from the consumption in 2018 (0.730 kg) and 24.9 percent compared to the consumption in 2016 (0.626 kg). Kampung chickens were generally reared extensively without the use of synthetic chemicals. Therefore, the kampung chicken meat was believed to be healthier (Miranda *et al.* 2008).

Conversely, most kampung chickens were reared in poor environmental sanitation, thereby increasing the risk of contamination by foodborne pathogens, including *Salmonella* spp. (Ulupi *et al.* 2013). *Salmonella* infection could cause salmonellosis, which interferes with the gastrointestinal tract, and even lead to death (Sartika *et al.* 2016). Nontyphoidal *Salmonella* (NTS) infections worldwide were estimated at 61.8 to 131.6 million cases, with an average of 155,000 deaths yearly (Majowicz *et al.* 2010). Based on a report from EFSA and ECDPC (2018), the phenomenon of foodborne outbreaks due to salmonellosis in 2013–2017 was caused mainly by chicken meat and its derivative products.

In most cases, salmonellosis was a self-limiting disease. Nevertheless, it also often caused various severe symptoms in infants, elderly, and immunocompromised patients, so antibiotic treatments were urgently needed, such as β-lactam, tetracycline, phenicol, aminoglycoside, quinolone, and macrolide (Gordon 2008; Frye and Jackson 2013). Unfortunately, antimicrobial resistance was growing in many foodborne pathogens. Swartz (2002) reported that animal-origin food consumption was the main source of antibiotic-resistant *Salmonella*.

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The use of the same antibiotics by humans and animals was a factor that caused resistant bacteria to move from animal-based products to humans (Nghiem *et al.* 2017).

Unlike broiler chicken, no growth promotor (e.g., antibiotic) was added to feeding kampung chicken. However, extensive rearing allowed resistant microbial exposure to kampung chicken due to its antimicrobial use in its surrounding environment (Lipsitch and Samore 2002). Multidrug-resistant (MDR) was a term for isolates of microorganisms that showed resistance to three or more different antimicrobial groups (Chuanchuen and Padungtod 2009). MDR *Salmonella* spp. has been found in kampung chickens from Nigeria (Ojo *et al.* 2012), India (Samanta *et al.* 2014), China (Zhao *et al.* 2016), Pakistan (Kamboh *et al.* 2018), and Malaysia (Jajere *et al.* 2020). It was frequently associated with increased mortality, longer duration of hospitalization, and high medical cost due to therapeutic failure (Jajere 2019).

Until then, studies on antimicrobial resistance (AMR) in *Salmonella* isolated from kampung chicken were rarely found, especially in Indonesia. Melati (2022) had previously studied the isolation and identification of *Salmonella* spp. from kampung chicken carcasses marketed in Bogor, Indonesia. Further research to characterize these isolates was crucial to study the prevalence of AMR and resistance patterns of *Salmonella* to commonly used antimicrobial agents so that foodborne outbreaks related to AMR issues could be monitored and anticipated.

The main mechanism underlying the transmission of AMR in *Salmonella* was horizontal gene transfer from other microorganisms in its surrounding environment (Wigley 2014). The presence of AMR genes in *Salmonella* could be analyzed by polymerase chain reaction (PCR). PCR was a nucleic acid-based rapid test to amplify DNA and detect a specific gene target of an organism. PCR was recently developed as an efficient and less time-consuming alternative method for molecular identification and characterization of *Salmonella* in food materials with good sensitivity and specificity according to primer sequence (Moraes *et al.* 2016). Therefore, this study aimed to identify and observe the correlation between phenotypic antimicrobial resistance profile and the presence of resistance genes in *Salmonella* spp. isolated from kampung chicken carcasses.

2. Materials and Methods

2.1. Materials

A total of 17 strains of *Salmonella* spp. isolated from kampung chicken carcasses in previous research (Melati 2022) were investigated in this study, consisting of *S.* Typhimurium (n = 3), *S.* Newport (n = 4), and *S.* Weltevreden (n = 10). *Escherichia coli* ATCC 25922 was used as a control for the antimicrobial susceptibility test.

2.2. Antimicrobial Susceptibility Test

Kirby-Bauer's disk diffusion method was performed to evaluate the phenotypic AMR of *Salmonella* spp. based on the Clinical and Laboratory Standards Institute guidelines as described by Hardiati *et al.* (2021). Strain-cultured on slant agar was enriched in 10 ml of buffered peptone water (Oxoid) at 35°C for 18 h. The bacterial suspension was diluted with sterile phosphate buffer until it reached the same turbidity level as a 0.5 McFarland standard (HiMedia Laboratories), equivalent to 1.5 × 10^8 CFU/ml. Supplementary Figure 1 the culture was inoculated using a sterile cotton swab and streaked evenly on the Mueller-Hinton agar (Sisco Research Laboratories) plate. Antimicrobial agents used in this study were ampicillin (AMP) 10 µg, tetracycline (TE) 30 µg, oxytetracycline (OT) 30 µg, gentamicin (CN) 10 µg, chloramphenicol (CHL) 30 µg, nalidixic acid (NA) 30 µg, ciprofloxacin (CIP) 5 µg, and erythromycin (E) 15 µg. Each antimicrobial disk was placed on the agar surface, then agar was incubated at 35°C for 24 h. The test was carried out three times of repetition. Antimicrobial inhibition zone diameter was interpreted into three categories, including susceptible, intermediate, and resistant (Supplementary Table 1) (Fouad 2011; CLSI 2020).

2.3. Bacterial DNA Extraction

Presto™ Mini gDNA Bacteria Kit (Geneaid) was used to isolate DNA from *Salmonella* culture according to the protocol procedure. Strain-enriched on broth medium (BPW) was transferred to a 2 ml

microcentrifuge tube. The culture was centrifuged for 1 min at 10,000 rpm to separate the bacterial cell. The cell pellet was suspended in 180 µL of GT buffer and 20 µL of proteinase K, then incubated at 60°C for 10 mins. It was mixed with 200 µL of GB buffer and incubated at 70°C for 10 mins. For RNA removal, 5 µL of RNAse A 50 mg/ml (Geneaid) was added, and the lysate was incubated at room temperature for 5 mins. After that, it was mixed immediately with 200 µL of absolute ethanol. The mixture was transferred to the GD column and centrifuged for 2 mins for DNA binding. The GD column was placed in a new collection tube, then 400 µL of W1 buffer was added and centrifuged. An amount of 600 µL of wash buffer was added, then it was centrifuged for 30 s and centrifuged again for 3 mins to dry the column matrix. Lastly, the purified DNA was eluted by adding 50 µL of pre-heated elution buffer, letting it stand for 3 mins, and centrifuging for 30 s.

2.4. Detection of Antimicrobial Resistance Genes

Genomic DNA of *Salmonella* was analyzed by PCR to detect the presence of genes encoding resistance to ampicillin (*bla*TEM), tetracycline and/or oxytetracycline (*tet*G), gentamicin (*aad*B), chloramphenicol (*cml*A), nalidixic acid and/or ciprofloxacin (*gyr*A), and erythromycin (*erm*B). The PCR was carried out in a total reaction volume of 25 µL, containing 12.5 µL of GoTaq® Green Master Mix (Promega), 1.5 µL of each primer (10 µM), 3 µL of DNA template, and 6.5 µL of nuclease-free water. PCR (Applied Biosystem Thermal Cycler 2720) conditions were set for an initial denaturation at 95°C for 3 mins followed by cycles of denaturation (95°C for 30 s), annealing (specific temperature for 1 min), and extension (72°C for 1 min) followed by a final extension at 72°C for 7 mins. Annealing temperature (Ta) and the number of cycles for each pair of primers are shown in Table 1. The PCR products were separated by electrophoresis (Bio-Rad) on 2% agarose gel at 90 V for 45 mins. The gel was stained in ethidium bromide (BioBasic Canada Inc.) and visualized under UV light by using gel documentation (Bio-Rad) (Wulan *et al.* 2021).

3. Results

Figure 1 illustrates the prevalence of AMR in *Salmonella* isolated from kampung chicken carcasses. Most isolates of *Salmonella* were resistant to erythromycin, with a prevalence of 82% (14/17). Resistance to tetracycline, oxytetracycline, nalidixic acid, and ciprofloxacin was found in 18% (3/17) of isolates. On the other hand, all (17/17) isolates of *Salmonella* were susceptible to ampicillin and chloramphenicol. A high percentage of susceptibility (82%; 14/17) was still seen in tetracycline, gentamicin, and nalidixic acid. The decreased susceptibility (intermediate category) of *Salmonella* to some antimicrobial agents should also be concerned, including oxytetracycline (82%; 14/17), ciprofloxacin (53%; 9/17), and gentamicin (18%; 3/17). All (3/3) isolates of *S.* Typhimurium showed a resistance pattern to tetracycline, quinolone, and macrolide (TE-OT-NA-CIP-E), so they were categorized as MDR. All (4/4) isolates of *S.* Newport and 7/10 isolates of *S.* Weltevreden were resistant to one antimicrobial agent (Table 2).

The presence of antimicrobial resistance gene in *Salmonella* is shown in Figures 2–7. Several antimicrobial resistance genes (*bla*TEM, *tet*G, *cml*A, *gyr*A) were identified in all (17/17) isolates of *Salmonella* spp. from kampung chicken carcasses, *erm*B was only found in 2/17 isolates, and *aad*B was detected in no isolate (0/17) (Table 2).

It was hypothesized that resistant or intermediately resistant isolates (phenotype) would be associated with the presence of resistance genes (genotype), and vice versa. A perfect correlation (100%) was only seen in oxytetracycline because the resistance gene was detected in all resistant or intermediately resistant isolates. A good correlation between phenotype and genotype was also shown by gentamicin (82%) and ciprofloxacin (71%). Meanwhile, a low percentage of correlation occurred in tetracycline (18%), nalidixic acid (18%), and erythromycin (12%). Ampicillin and chloramphenicol indicated no correlation between phenotype and genotype of AMR because the resistance genes were present in all susceptible isolates (Table 2).

Table 1. Primers used for the detection of antimicrobial resistance genes in this study

Gene target	Primer sequence (5'-3')	Product size	Ta and cycles	Accession No.
*bla*TEM[1]	(F) ATGAGTATTCAACATTTCCG (R) GACAGTTACCAATGCTTAATCA	866 bp	50°C; 35×	MZ666126
*tet*G[2]	(F) CCGGTCTTATGGGTGCTCTA (R) CCAGAAGAACGAAGCCAGTC	604 bp	50°C; 30×	AF071555
*aad*B[3]	(F) CTAGCTGCGGCAGATGAGC (R) CTCAGCCGCCTCTGGGCA	300 bp	58°C; 35×	OK209938
*cml*A[4]	(F) CGCCACGGTGTTGTTGTTAT (R) GCGACCTGCGTAAATGTCAC	364 bp	56°C; 30×	AF078527
*gyr*A[1]	(F) AAATCTGCCCGTGTCGTTGGT (R) GCCATACCTACTGCGATACC	344 bp	58°C; 30×	AE006468
*erm*B[5]	(F) GAAAAGGTACTCAACCAAATA (R) GTAACGGTACTTAAATTGTTTAC	638 bp	48°C; 40×	CP065569

[1]Eguale *et al.* (2017), [2]Walker *et al.* (2001), [3]Chuanchuen and Padungtod (2009), [4]Chen *et al.* (2004), [5]Song *et al.* (2004)

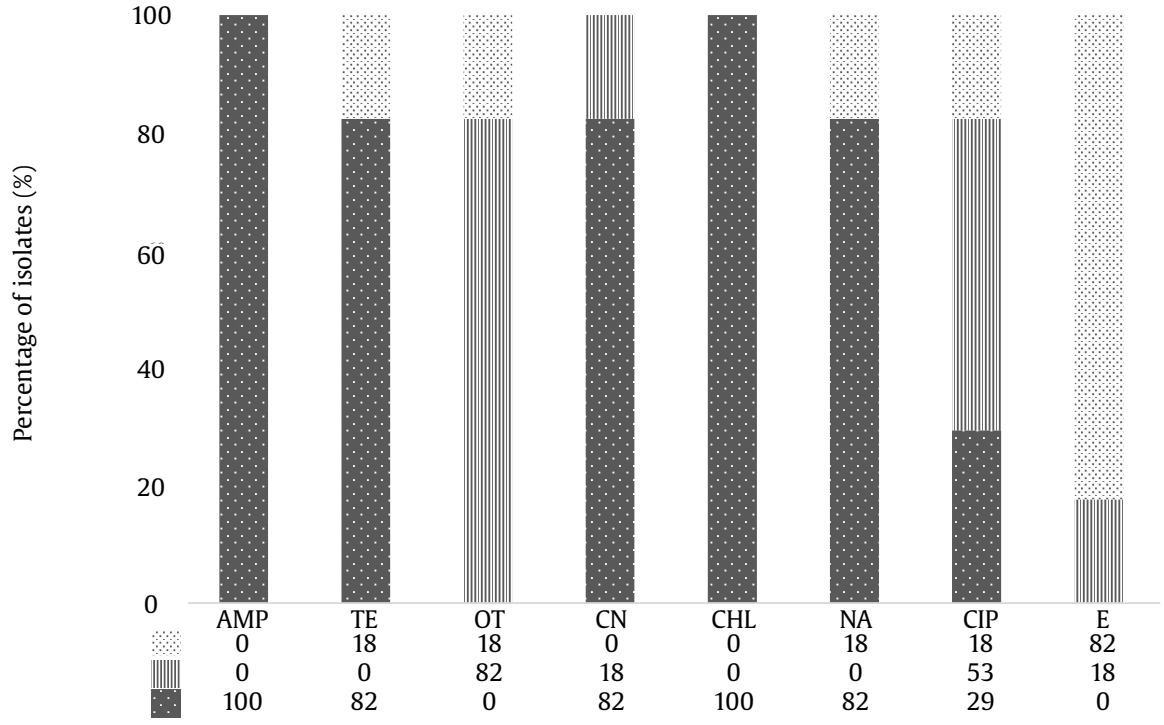

Figure 1. Antibiogram profile of *Salmonella* spp. (n = 17) isolated from kampung chicken carcasses marketed at Bogor, Indonesia. AMP: ampicillin, TE: tetracycline, OT: oxytetracycline, CN: gentamicin, CHL: chloramphenicol, NA: nalidixic acid, CIP: ciprofloxacin, E: erythromycin

Table 2. Correlation between phenotype and genotype of AMR in *Salmonella* spp. (n = 17) isolated from kampung chicken carcasses

Isolate	AMP			TE			OT			CN			CHL			NA			NA			NA		
	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]
KCID1.2[a]	S	+	No	R	+	Yes	R	+	Yes	I	-	No	S	+	No	R	+	Yes	R	+	Yes	R	-	No
KCID1.3[a]	S	+	No	R	+	Yes	R	+	Yes	I	-	No	S	+	No	R	+	Yes	R	+	Yes	R	-	No
KCID1.4[a]	S	+	No	R	+	Yes	R	+	Yes	I	-	No	S	+	No	R	+	Yes	R	+	Yes	R	-	No
KCID2.2[b]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	S	+	No	R	-	No
KCID2.3[b]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	S	+	No	R	+	Yes
KCID2.4[b]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	S	+	No	R	-	No
KCID2.5[b]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	S	+	No	R	-	No

Table 2. Continued

Isolate	AMP			TE			OT			CN			CHL			NA			NA			NA		
	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]	P	G	C[1]
KCID4.2[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	R	-	No
KCID4.3[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	R	-	No
KCID4.4[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	I	-	No
KCID4.5[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	R	+	Yes
KCID6.2[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	R	-	No
KCID6.3[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	R	-	No
KCID6.4[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	R	-	No
KCID7.2[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	S	+	No	I	-	No
KCID7.3[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	R	-	No
KCID7.5[c]	S	+	No	S	+	No	I	+	Yes	S	-	Yes	S	+	No	S	+	No	I	+	Yes	I	-	No
Total[2]	0	17		3	17		17	17		3	0		0	17		3	17		12	17		17	2	
Correlation	0%			18%			100%			82%			0%			18%			71%			12%		

[1]Hypothesis: phenotypic resistant/intermediate correlated with genotypic positive and phenotypic susceptible correlated with genotypic negative, [2]Total of resistant/intermediate isolates (phenotype) or a total of positive-gene isolates (genotype), [a]*S.* Typhimurium, [b]*S.* Newport, [c]*S.* Weltevreden, AMP: ampicillin, TE: tetracycline, OT: oxytetracycline, CN: gentamicin, CHL: chloramphenicol, NA: nalidixic acid, CIP: ciprofloxacin, E: erythromycin, P: phenotype, G: genotype, C: correlation, S: susceptible, I: intermediate, R: resistant, +: gene target detected, -: no gene target detected

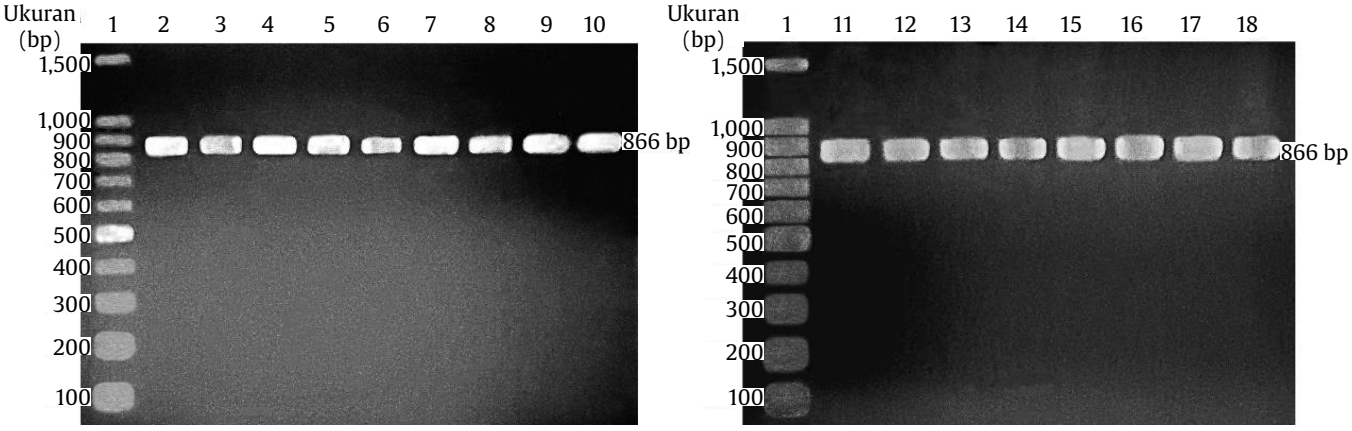

Figure 2. Amplification of *bla*TEM (866 bp), β-lactam (AMP) resistance gene. Lane 1: 100 bp DNA ladder, lane 2–18: *Salmonella* isolates

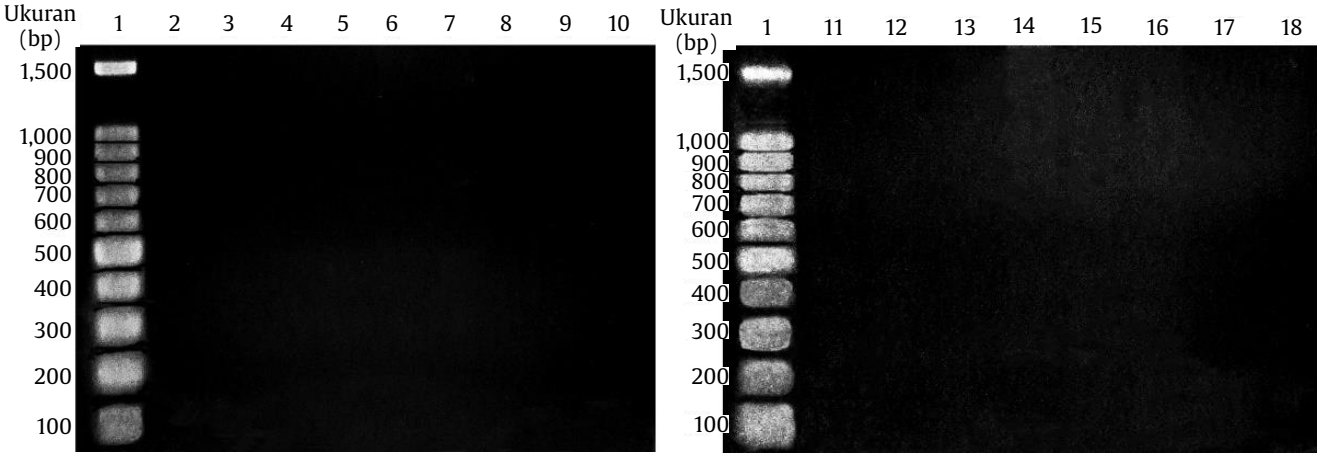

Figure 3. Amplification of *aad*B (300 bp), aminoglycoside (CN) resistance gene. Lane 1: DNA ladder, lane 2–18: *Salmonella* isolates (no gene detected)

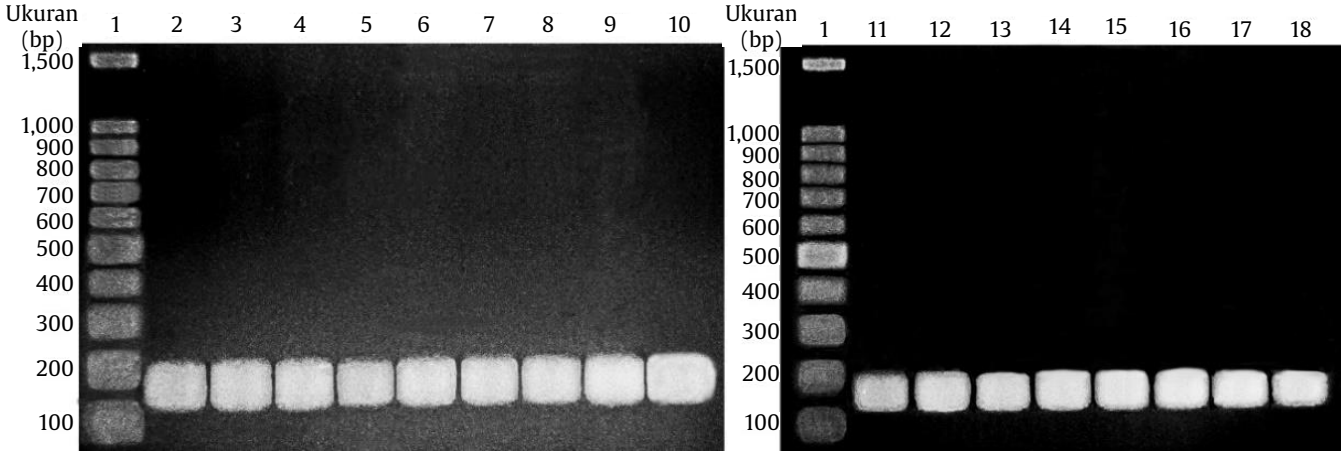

Figure 4. Amplification of *tet*G, tetracycline (TET/OT) resistance gene. Lane 1: 100 bp DNA ladder, lane 2–18: *Salmonella* isolates

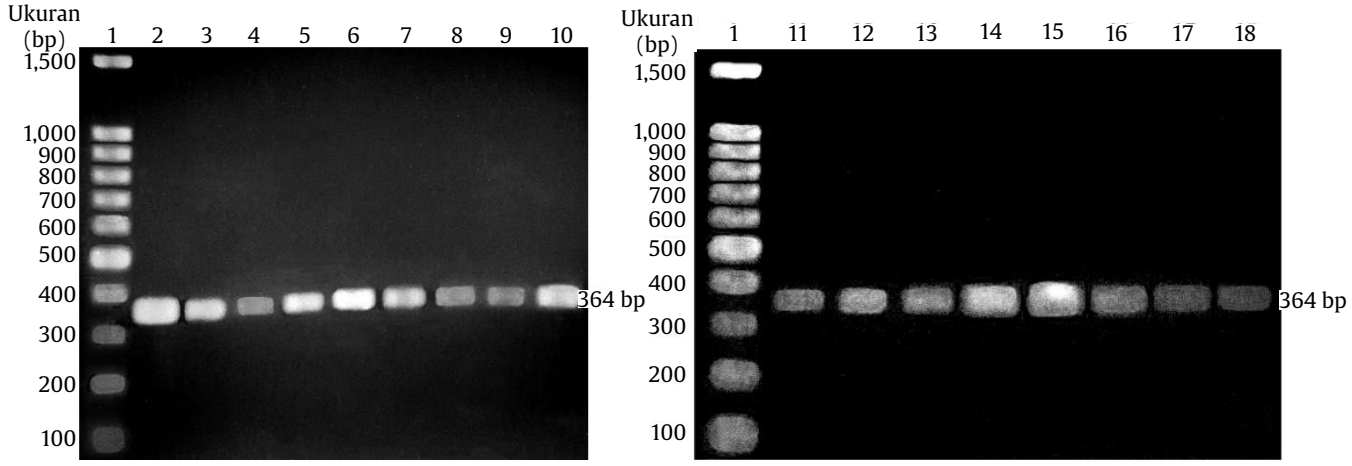

Figure 5. Amplification of *cml*A (364 bp), chloramphenicol (CHL) resistance gene. Lane 1: 100 bp DNA ladder, lane 2–18: *Salmonella* isolates

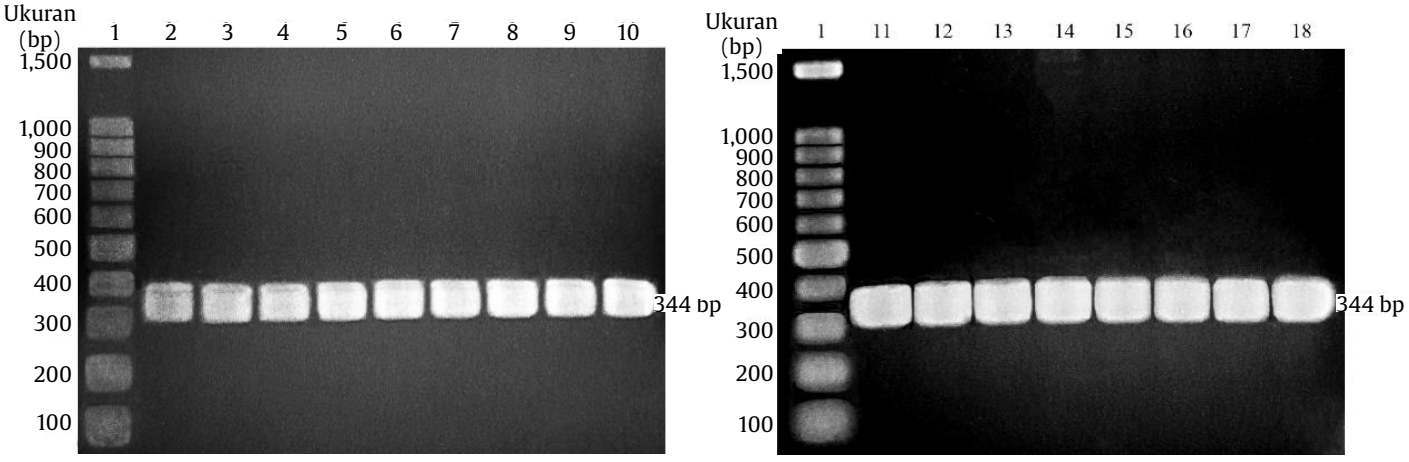

Figure 6. Amplification of *gyr*A (344 bp), quinolone (NA/CIP) resistance gene. Lane 1: 100 bp DNA ladder, lane 2–18: *Salmonella* isolates

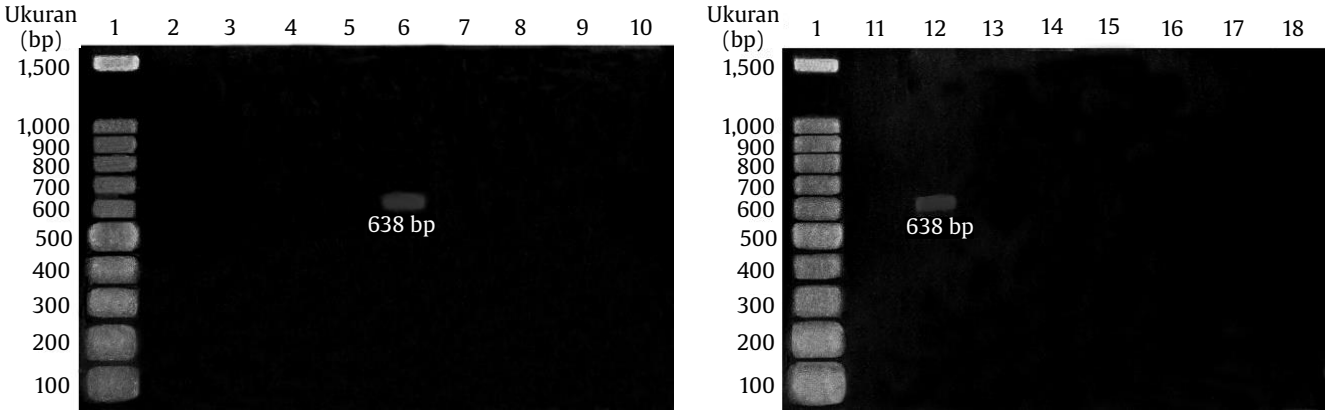

Figure 7. Amplification of *erm*B (638 bp), erythromycin (E) resistance gene. Lane 1: 100 bp DNA ladder, lane 2–18: *Salmonella* isolates

4. Discussion

The prevalence of AMR in *Salmonella* isolated from kampung chicken carcasses was not much different from previous studies in other countries (Samanta *et al.* 2014; Bhuvaneswa *et al.* 2015; Ghoddusi *et al.* 2015; Kamboh *et al.* 2018; Jajere *et al.* 2020). A similar study on *Salmonella* spp. from kampung chickens around Surabaya, Indonesia reported higher resistance to tetracycline, nalidixic acid, and chloramphenicol (Yulistiani *et al.* 2019). If compared with *Salmonella* isolated from broiler chicken carcasses at the same location of the study (Chanson 2022), the overall prevalence of AMR in this study was much lower. It was in accordance with a previous study in Malaysia (Jajere *et al.* 2020).

The higher percentage of AMR in broiler chickens was due to the high frequency of antimicrobial misuses to promote animal growth and prevent pathogenic infection in poultry farms, especially β-lactam, aminoglycoside, and quinolone (Kamboh *et al.* 2018). However, this study revealed a notable prevalence of AMR in *Salmonella* spp. isolated from kampung chickens that were fed without antimicrobial agents. The AMR in kampung chicken might be associated with continuous exposure to resistant *Salmonella* from the surrounding environment (Ojo *et al.* 2012). Poor waste management of commercial farms and slaughterhouses has contributed to environmental pollution and contamination of resistant microorganisms. Antimicrobial use could increase the potential of resistant bacterial colonization in animals not treated by antibiotics but shared in the same environment (Okoli 2006).

Most *Salmonella* were resistant to erythromycin due to a natural resistance always expressed in the species regardless of previous exposure. The lipopolysaccharide layer in the cell membrane of Gram-negative could reduce its permeability to macrolide (Blair *et al.* 2014). The high prevalence of decreased susceptibility to oxytetracycline and ciprofloxacin might reflect the use of these antimicrobial agents at the sample collection sites. A report from the Center for Indonesian Veterinary Analytical Studies (2017) mentioned that oxytetracycline was used in 35% of poultry farms in Indonesia.

It was also deduced that *Salmonella* spp. from kampung chicken carcasses could be a source of AMR transmission through horizontal gene transfer. The presence of resistance genes in this study was generally higher compared to a previous study on Salmonella isolated from broiler chicken carcasses at Bogor, Indonesia (Chanson 2022), except for *aad*B. Plasmid-mediated transmission of resistance genes was the most common mechanism for *Salmonella* to acquire genetic material from other species by conjugation (Reygaert 2018). Extensive rearing allowed kampung chickens to forage on the ground in poor sanitary conditions. Livestock waste-containing resistant organisms would create a bunch of resistance genes that could be transferred to other pathogens (Katakweba *et al.* 2012). It might be responsible for the higher presence of AMR genes in kampung chickens compared to broiler chickens reared in a more hygienic environment.

The presence of *bla*TEM gene was indicated by an 866-bp amplicon (Figure 2). Drug inactivation by β-lactamase (*bla*) was the most common mechanism

of ampicillin resistance in *Salmonella*. It hydrolyzed and prevented a β-lactam ring from binding to the drug target, a penicillin-binding protein (Frye and Jackson 2013). The enzymes included TEM, SHV, and CTX-M. The β-lactamase gene detected in most (77%) isolates of *Salmonella* was *bla*TEM (Eguale *et al.* 2017). Aminoglycoside resistance in *Salmonella* was also due to the enzymatic inactivation of the drug. The aad gene encoded a nucleotidyltransferase that conferred resistance to gentamicin by adenylation (Tîrziu *et al.* 2015). The absence of *aad*B in the isolates that were intermediately resistant to gentamicin (Figure 3) indicated that they might carry the other resistance genes that were not investigated in this study, e.g., *aad*A, *aac*C2, aph(3)-IIa, and aac(3)-IVa (Chen *et al.* 2004).

Resistance to the tetracycline group mainly occurred due to the active drug efflux by *tet*. The *tet*G gene was primarily found in *S.* Typhimurium DT104 and U302 (Randall *et al.* 2004). Amplification of *tet*G in this study resulted in a product size of less than 200 bp (Figure 4). It was presumably due to genetic mutation in bacterial chromosomes that modified metabolic pathways due to a certain environmental stimulus, such as nutrition deficiency, ultraviolet radiation, and chemical exposure (Reygaert 2018). The presence of *cml*A gene was indicated by a 364-bp amplicon (Figure 5). The mechanisms underlying chloramphenicol resistance in Gram-negative were the enzymatic inactivation of the drug and the active drug efflux (Frye and Jackson 2013). The *cml*A had a homology similar to the tetracycline resistance protein, later known as a transmembrane polypeptide that confered nonenzymatic resistance by efflux pump (Bissonnette *et al.* 1991).

The presence of *gyr*A gene was indicated by a 344-bp amplicon (Figure 6). Quinolone resistance was caused mainly by mutation in one or more genes that encoded the drug targets (DNA gyrase and topoisomerase). The mutation usually occurred in the quinolone resistance determining region (QRDR) that was conserved within the enzymes. Resistance of Gram-negative to nalidixic acid and ciprofloxacin was generally associated with amino acid substitution in Serin-83 of *gyr*A (Redgrave *et al.* 2014). The presence of ermB gene was indicated by a 638-bp amplicon (Figure 7). The commonly observed mechanism of macrolide resistance was the modification of the drug target (23S rRNA) by erythromycin ribosome methylase (erm) gene (Frye and Jackson 2013). Erythromycin resistance in Gram-negative was an example of bacterial intrinsic resistance to limit the uptake of antibiotics due to a reduced outer membrane permeability (Blair *et al.* 2014).

This study clarified that the phenotype and genotype of antimicrobial resistance in *Salmonella* spp. were not always related to each other. A previous study also declared no correlation between antibiotic resistance and the AMR gene expression in most isolates of *Salmonella* spp. (Nghiem *et al.* 2017). For example, the *bla*TEM gene expression was 0.5-fold in AMP-susceptible isolate and *cml*A was expressed (0.3-fold) in CHL-susceptible isolate. Vélez *et al.* (2017) reported that the *bla*TEM gene had a low correlation with ampicillin resistance, but the presence of *aad*B quietly correlated with gentamicin resistance.

Gentamicin and erythromycin resistance genes were not detected in resistant or intermediately resistant isolates, probably due to the other resistance mechanisms that had been discussed before. For other antimicrobial agents, the resistance genes were present in susceptible isolates because these genes might be silent and expressed at a clinically significant level when exposed to the drug (Reygaert 2018). Environmental factors, e.g., nutrition, temperature, light, and chemical exposure, significantly impacted gene expression and affected the emergence of phenotype (Ralston and Shaw 2008).

There was also a possibility that not only one gene was responsible for resistance or decreased susceptibility to an antimicrobial agent. A previous study on *Salmonella* spp. reported that isolates containing both *bla*TEM and *bla*PSE-1 genes required a higher minimum inhibitory concentration (MIC) of ampicillin compared to isolates containing only one gene (Chuanchuen and Padungtod 2009). It indicated a synergy among AMR genes for encoding resistance to an antibiotic. Cross-resistance is usually a combination of several mechanisms, such as permeability changes of the outer membrane, enzymatic inactivation, and modification of drug targets (Nghiem *et al.* 2017).

Martineau *et al.* (2000) studied that the use of gradient agar plates with increasing antimicrobial concentration proved to be more efficient in selecting resistant bacterial cells that were not identified in the antimicrobial susceptibility test.

It indicated that strains of *Salmonella* which were susceptible but contained AMR genes had the potential to develop resistance under the selective pressure of antimicrobial use.

Phenotypic and genotypic antimicrobial resistance were widely distributed in *Salmonella* spp. isolated from kampung chicken carcasses, including MDR *Salmonella* Typhimurium. The extensive rearing in poor environmental sanitation might accelerate the transmission of AMR through a horizontal gene transfer. A routine monitoring of the food chains and proper use of antimicrobial agents in poultry farms were essential steps to control the AMR issues. A future study will be needed to understand the mechanisms dominantly responsible for the development of AMR in *Salmonella* spp.

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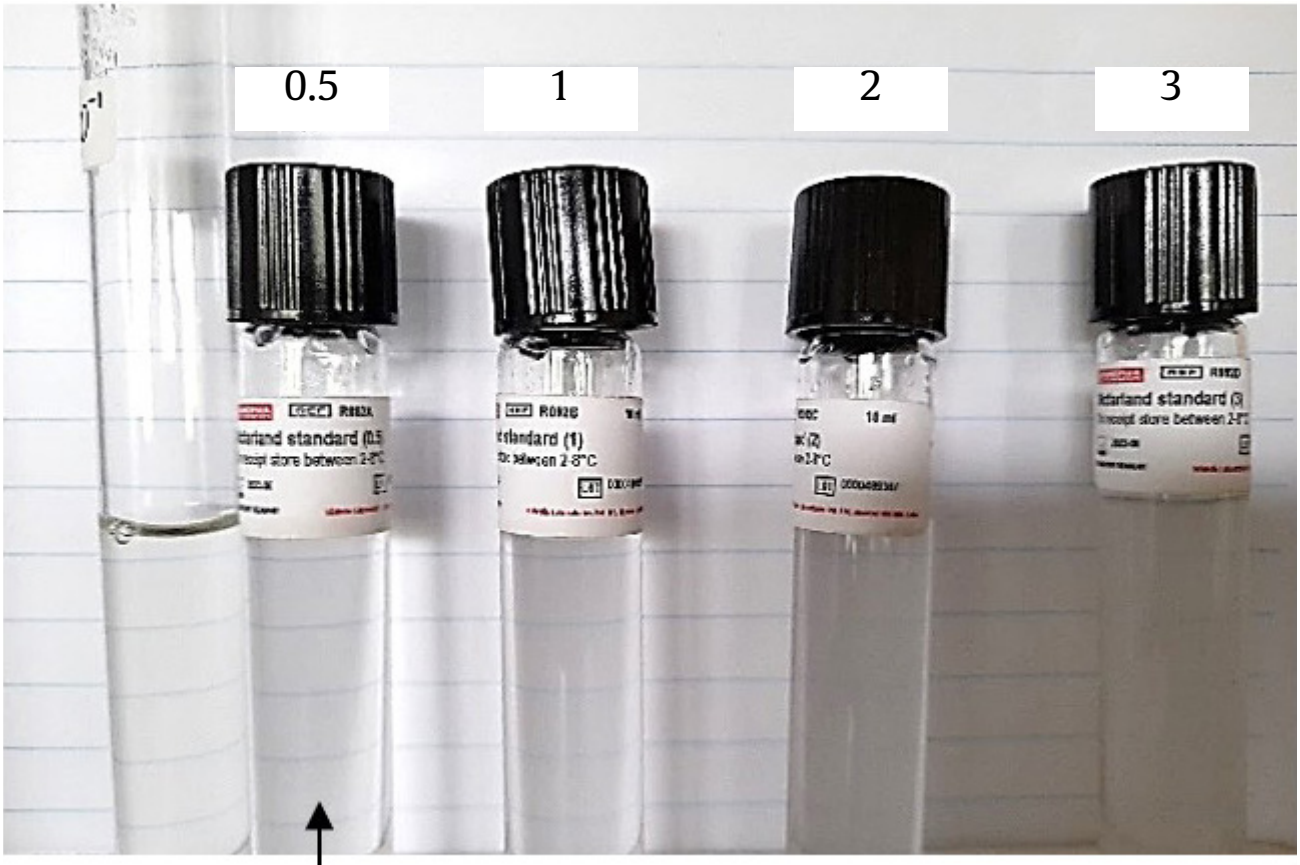
Supplementary Materials



Supplementary Figure 1. Turbidity of bacterial suspension compared with McFarland standard set

Supplementary Table 1. Interpretive category for antimicrobial inhibition zone against *Salmonella*

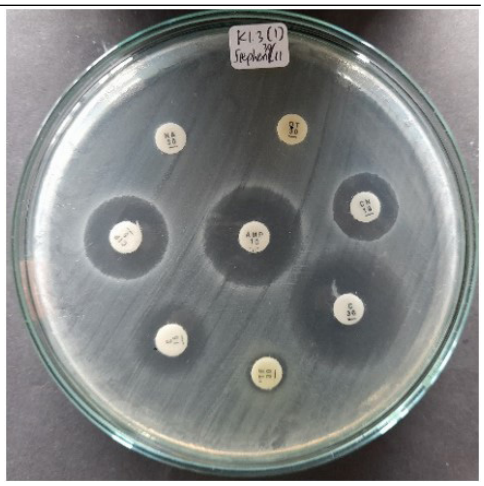
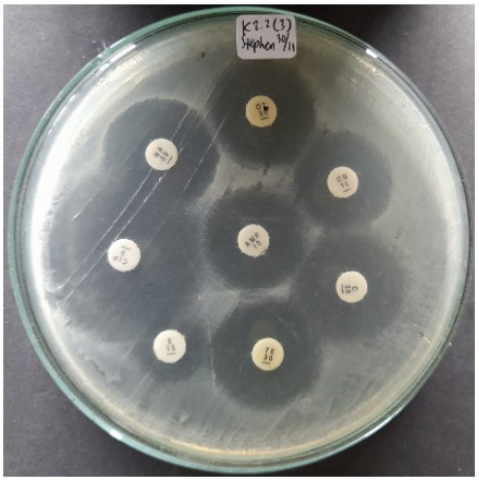
Group	Antibiotic	Disk content (µg)	Inhibition zone diameter (mm)		
			S	I	R
β-lactam	Ampicillin[a]	10	≥17	14–16	≤13
Tetracycline	Tetracycline[a]	30	≥15	12–14	≤11
	Oxytetracycline[b]	30	≥26	16–25	≤15
Aminoglycoside	Gentamicin[a]	10	≥15	13–14	≤12
Phenicol	Chloramphenicol[a]	30	≥18	13–17	≤12
Quinolone	Nalidixic acid[a]	30	≥19	14–18	≤13
	Ciprofloxacin[a]	5	≥31	21–30	≤20
Macrolide	Erythromycin[b]	15	≥23	14–22	≤13

[a]CLSI (2020), [b]Fouad (2011)

Supplementary Table 2. Confirmation of bacterial suspension concentration by colony count method to confirm McFarland testing equivalency

Serovar	Isolate	Concentration (CFU/ml)	Remark
Typhimurium	KCID1.2	5.0×10^8	The turbidity level was relatively similar to a 0.5 McFarland standard
Newport	KCID2.2	2.6×10^8	
Weltevreden	KCID4.2	3.6×10^8	

Supplementary Table 3. Documentation of antimicrobial susceptibility test of *Salmonella* spp.

Serovar	Isolate	Documentation
Typhimurium	KCID1.3	
Newport	KCID2.2	
Weltevreden	KCID6.4