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Comparison of antibiotic resistance pattern among Enteropathogenic bacteria isolated from broiler and backyard chicken meat

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ABSTRAK

Penelitian ini bertujuan untuk membandingkan pola resistensi antibiotik isolat *Enterobacteriaceae* dari daging broiler dan daging ayam kampung di Surabaya, Indonesia yang diisolasi pada tahun 2016-2017. Metode Kirby-Bauer difusi disk digunakan untuk menentukan resistensi isolat terhadap tetrasiklin (TE), gentamisin (CN), cefoxitin (FOX), sulfametoksazol-trimetoprim (SXT), asam nalidiksat (NA), dan kloramfenikol (C). Isolat daging broiler dan daging ayam kampung resisten terhadap keenam antibiotik yang diuji. Secara keseluruhan, isolat daging broiler yang resisten terhadap TE, CN, FOX, SXT, NA, C adalah 57,76% lebih tinggi dibandingkan isolat daging ayam kampung. Lebih dari 50% dari seluruh (304) isolat daging broiler resisten terhadap TE dan NA, sedangkan lebih dari 50% dari seluruh (310) isolat daging ayam kampung hanya resisten terhadap TE. Pada kedua jenis daging ayam, strain resisten ditemukan pada isolat *Salmonella* spp., *Escherichia coli*, *Shigella* spp., *Citrobacter* spp., *Klebsiella* spp., *Yersinia* spp., *Proteus* spp., *Enterobacter* spp., *Serratia* spp., *Edwardsiella* spp. Strain resisten antibiotik isolat daging broiler secara signifikan lebih tinggi ($P < 0{,}05$) dibandingkan isolat daging ayam kampung, kecuali *Edwardsiella* spp. Secara keseluruhan, isolat *Enterobacteriaceae* yang resisten multi-obat ditemukan lebih tinggi pada daging broiler dibandingkan daging ayam kampung. Daging ayam broiler dan daging ayam kampung berpotensi sebagai reservoir *Enterobacteriaceae* yang resisten multi-obat dan dapat mengancam kesehatan masyarakat.

Kata kunci: resistensi antibiotik, Enterobacteriaceae, broiler, ayam kampung, daging ayam

ABSTRACT

This study aimed to compare the antibiotic resistance patterns among original *Enterobacteriaceae* isolates from broiler and backyard chicken meats in Surabaya, Indonesia, isolated in 2016-2017. The

Kirby–Bauer disk diffusion method was used to determine the resistance of these isolates against tetracycline (TE), gentamicin (CN), cefoxitin (FOX), sulfamethoxazole-trimethoprim (SXT), nalidixic acid (NA), and chloramphenicol (C). Both broiler and backyard chicken meat isolates were resistant to the six antibiotics tested. Overall, broiler meat isolates which resistant to TE, CN, FOX, SXT, NA, C were 57.76% higher than backyard chicken meat isolates. More than 50% of broiler meat isolates (304 samples) were resistant to TE and NA, whereas backyard chicken meat isolates (310 samples) were only resistant to TE. The resistant strains found in both meat isolates were *Salmonella* spp., *Escherichia coli*, *Shigella* spp., *Citrobacter* spp., *Klebsiella* spp., *Yersinia* spp., *Proteus* spp., *Enterobacter* spp., *Serratia* spp., and *Edwardsiella* spp. Resistant strains of broiler meat isolates were significantly higher (P<0.05) than backyard chicken meat isolates, except *Edwardsiella* spp. Overall, multidrug-resistant *Enterobacteriaceae* was found to be higher in broiler meat isolates than in backyard chicken meat isolates. Broiler and backyard chicken meats are potential reservoirs of multidrug-resistant *Enterobacteriaceae* which threat to public health.

Keywords: antibiotic resistance, Enterobacteriaceae, broiler, backyard, chicken meat

INTRODUCTION

The use of antibiotics in animals has contributed to the increase in antibiotic resistance globally (FAO, 2015). This is is related to high morbidity and mortality because it can cause treatment failure for human infections (Akova, 2016; Friedman *et al*., 2017). Antibiotic-resistant bacteria may represent a reservoir of resistance genes that can be transferred to pathogenic or commensal bacteria in the human digestive tract (Alvarez-Fernandez *et al.,* 2013). The transfer of resistant bacteria from animals to humans via food products has been widely reported; therefore, resistant bacteria possess a significant threat to public health (Thorsteinsdottir *et al*., 2010; Asai *et al*., 2014). Multidrug-resistant (MDR) bacteria are commonly found in poultry, and poultry products have been recognized as a source of MDR bacteria that are multiresistant to human antibiotics (Jiang *et al*., 2011; Dierikx *et al*., 2012; Johnson *et al*., 2012; Mellata, 2013).

Enterobacteriaceae is a group of gram-negative enteropathogenic bacteria (48 genera and 219 species) that lives in human and animal intestines and plays an important role in intestinal disease (Baylis *et al*., 2011). *Enterobacteriaceae* can cause severe infections, and some important members of this family are becoming increasingly resistant to currently available antimicrobials (Delgado-Valverde *et al*., 2013). During evisceration after slaughtering, resistant bacteria from the animal intestine can contaminate chicken carcasses; therefore, chicken meat is often contaminated with resistant bacteria (Rasschaert *et al.*, 2007; Amir *et al*., 2017).

Meats from broilers and backyard chicken are the main sources of animal protein in Indonesia because they are consumed by people of all religions. The intensive use of antimicrobial agents in poultry production means that food derived from animals often contains bacteria resistant to several antimicrobial agents (Mehdi *et al*., 2018). The 2014 EFSA/ECDC EU summary report on antibiotic resistance in zoonotic bacterial diseases showed an increase in the prevalence of MDR *Salmonella infantis* (more than 70% in broiler meat), MDR *Escherichia coli* (55% in broiler meat), and MDR *Staphylococcus aureus* (26.5% in animal-based food) in Europe (EFSA and ECDC, 2016).

In Indonesia, broiler chickens are farmed intensively. Before the Antibiotic Growth-Promoters (AGP) ban in poultry, antibiotics are added to feed to treat, control, and prevent disease; promote growth; and improve feed efficiency (Hughes and Heritage, 2004). On the contrary, backyard chickens are native chickens farmed extensively and allowed to scavenge food scraps without additional feeding with antibiotics. Therefore, backyard chickens have less chances of developing antibiotic resistance than commercial broilers. Since January 1st, 2018 Indonesia has banned on the use of antibiotics as growth promoters in animal feed.

The bacteria used in this study were isolated from broiler and backyard chicken meat sold in traditional markets in Surabaya in 2016 - 2017, before the AGP ban in poultry. The environment is an important component for the transmission of resistant bacteria and in the emergence of resistant pathogens (Bengtsson-Palme *et al*., 2017). Resistance genes are horizontally transferred from environmental to pathogenic bacteria (von Wintersdorff *et al*., 2016). The traditional markets in Surabaya, classified as wet market where slaughtered chickens are manually processed, and freshly slaughtered chicken meat are sold in open air at room temperature with high humidity. All of these have the potential to pose a risk of chicken meat contamination by resistant bacteria.

The difference in feeding management in broiler and backyard chicken farms, and traditional market environment in Indonesia may lead to different types of antibiotic resistance for each product. Therefore, it is important to observe the existence of antibiotic resistance *Enterobacteriaceae* in both broiler and backyard chicken meats.

The purpose of this study was to compare antibiotic resistance patterns among original *Enterobacteriaceae* isolate isolates from broiler and backyard chicken meats that are sold in Surabaya, Indonesia, isolated in 2016-2017.

MATERIALS AND METHODS

Origin of *Enterobacteriaceae* isolates

This study used 614 *Enterobacteriaceae* isolates consist of 304 broiler chicken meat isolates (70 *Salmonella* spp., 76 *Escherichia coli*, 28 *Shigella* spp., 31 *Citrobacter* spp., 19 *Klebsiella* spp., 23 *Yersinia* spp., 38 *Proteus* spp., 5 *Enterobacter* spp., 8 *Serratia* spp., and 6 *Edwardsiella* spp.) and 310 backyard chicken meat isolates (77 *Salmonella* spp., 81 *Escherichia coli*, 12 *Shigella* spp., 33 *Citrobacter* spp., 25 *Klebsiella* spp., 24 *Yersinia* spp., 24 *Proteus* spp., 15 *Enterobacter* spp., 12 *Serratia* spp., and 7 *Edwardsiella* spp.). These bacteria were isolated from the chicken meat thigh sold at the traditional markets in Surabaya, Indonesia in 2016 – 2017.

Isolation of *Enterobacteriaceae* genera except *Salmonella* spp. were carried out according to Morello *et al*. (2002), and for *Salmonella* according to ISO 6579:2002. From each sample (25 gram of chicken meat) were chopped aseptically and homogenized with 225 ml of 0.1% Buffered Pepton Water (BPW, Oxoid), and incubated at 37°C for 24 h. A loop full pre-enrichment broth was streaked on MacConkey agar (MCA, Oxoid) plates and incubated at 37°C for 24 h. Especially for *Salmonella* spp., one ml of BPW pre-enrichment step transferred to 10 ml Selenite Cystine Broth (SCB, Oxoid) for selective enrichment, and incubated at 37°C for 24 h. A loopful of inoculum from selective enrichment broth was streaked onto Xylose-Lysine Deoxycholate (XLD) agar plate and incubated at 37°C for 24 h. For confirmation, single colonies with specific criteria on the MCA surface (for *Enterobacteriaceae* genera) and on the XLD agar surface (for *Salmonella* spp.) were based on microscopical examination of Gram stained smears, biochemical tests as` TSI (Triple Sugar Iron) test and IMViC test (Indole production, Methyl Red, Voges-Proskauer and Simmon's citrate utilization) (Morello *et al*., 2002). The isolates were stored at -80 ºC in LB broth (Difco, Becton Dickinson) with 30% (v/v) glycerol. Prior to use, the isolates were cultured in Tryptone Soya Broth (Oxoid, UK) for regeneration.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed for the 304 broiler chicken meat isolates and 310 backyard chicken meat isolates by using the Kirby–Bauer disk diffusion method following guidelines of Clinical Laboratory Standard Institute (CLSI, 2016). The antibiotic disks used in this study were tetracycline (TE), gentamicin (CN), cefoxitin (FOX), sulfamethoxazole-trimethoprim (SXT), nalidixic acid (NA), and chloramphenicol (C) (Oxoid, UK). Fresh cultures were inoculated into LB broth and incubated until they reached a turbidity of 0.5 by using the McFarland standard. Mueller-Hinton agar plates were swabbed with these cultures, and antibiotic disks (Oxoid, UK) were placed onto the inoculated plates in a sterile environment. The plates were incubated at 37 °C for 18 to 20 h. The inhibition zones (in millimeters) around the antimicrobial agent disks were measured using a precision caliper (Absolute, Mitutoyo, Japan) and were scored as sensitive, intermediate, or susceptibility and resistant. Isolates exhibiting resistance to at least two of the antimicrobial agents tested were considered multiresistant strains (NARMS, 2009).

Statistical analysis

The data obtained were expressed in both absolute values and in percentages. Microsoft Office Excel 2010 was used to determine the percentages and to perform calculations. Antibiotic resistance patterns were compared between *Enterobacteriaceae* isolates from broiler and backyard chicken meats by using a chi-squared test at $P < 0.05$ (two tailed) and the Statistical Software Package for Social Sciences version 23 (2015) with Yates' correction for continuity. Differences were considered significant if the probabilities were less than 0.05.

RESULTS AND DISCUSSION

Antimicrobial Susceptibility

The antimicrobial susceptibility testing of all *Enterobacteriaceae* isolates by the Kirby-Bauer disk diffusion method indicated different levels of sensitivity against the six antibiotics tested (Table 1). Figure 1 shows that the 304 broiler chickens meat isolates were resistant to TE (69.08%), CN (15.46%), FOX (10.20%), SXT (46.05%), NA (61.84%), and C (24.67%). A total of 310 backyard chicken meat isolates were resistant to TE (59.03%), CN (4.84%), FOX (8.07%), SXT (26.13%), NA (32.58%), and C (10.65%). Figure 1 also indicates that resistant strains were significantly higher in broiler chicken meat isolates than backyard chicken meat isolates.

Overall, it was found that *Enterobacteriaceae* isolates from broiler and backyard chicken meats were highly resistant to TE, followed by NA, SXT, and C. On the contrary, isolates from broiler and backyard chicken meats were less resistant to FOX (in broiler chicken) and CN (in backyard chicken) (Figure 1). The high resistance to TE of the meat isolates in this study can be explained by the extensive and relatively prolonged use of TE on poultry farms as a prophylaxis, as an antibiotic growth promoter and as therapy/ treatment or diseases. TE are not included in the list of allowable feed additives in Indonesia, but due to its low price and easy access, it is widely used as feed additives (Murdiati *et al*., 1991). This indicates the difficulty of monitoring the use of antibiotics as a feed additive in Indonesia. Some studies reported the use of TE as a feed additive in Indonesia causes TE residues in chicken meat (Marliana *et al*., 2015; Werdiningsih *et al*., 2013). Antibiotic residues in chicken meat can inhibit sensitive bacteria growth, consequently resistant strains become more dominant (Muaz *et al*., 2018). The main mechanisms responsible for bacterial resistance to TE include an active efflux systems, ribosomal protection and enzymatic inactivation (Speer *et al*., 1992). Rossa *et al.* (2013) demonstrated high resistance to TE in *Enterobacteriaceae* among the conventional poultry studied in Brazil, including *E. coli*. Similar data have also been obtained from other countries (Akbar *et al*., 2014; Alvarez-Fernandez *et al*., 2013; Jiang *et al*., 2011; Miranda *et al*., 2008; Miles *et al*., 2006; Ojo *et al*., 2012; Wu *et al*., 2014).

Resistance Patterns

The resistance patterns among genera of

Table 1. In vitro Sensitivity Testing of all *Enterobacteriaceae* Strains Isolated from Broiler and Backyard Chicken Meats against the Six Antibiotics Tested

Antibiotics	Breakpoints (CLSI, 2016) S/I/R (mm)	*Enterobacteriaceae* Isolates(n = 614)					
		Broiler Chicken Meat (n = 304)			Backyard Chicken Meat (n = 310)		
		S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Tetracycline	≥15/12–14/ ≤11	65 (21.38)	29 (9.54)	210 (69.08)	100 (32.26)	27 (8.71)	183 (59.03)
Gentamicin	≥15/13–14/ ≤ 12	240 (78.95)	17 (5.59)	47 (15.46)	289 (93.22)	6 (1.94)	15 (4.84)
Cefoxitin	≥18/15–17/ ≤ 14	264 (86.84)	9 (2.96)	31 (10.20)	272 (87.74)	13 (4.19)	25 (8.07)
Sulfamethoxazole –trimethoprim	≥16/11–15/ ≤ 10	159 (52.30)	5 (1.65)	140 (46.05)	225 (72.58)	4 (1.29)	81 (26.13)
Nalidixic acid	≥19/14–18/ ≤ 13	104 (34.21)	12 (3.95)	188 (61.84)	173 (55.81)	36 (11.61)	101 (32.58)
Chloramphenicol	≥18/13–17/ ≤ 12	207 (68.09)	22 (7.24)	75 (24.67)	252 (81.29)	25 (8.06)	33 (10.65)

* Value is the number (percentage) of isolates; n = number of isolates tested
R = resistant; I = intermediate; S = sensitive

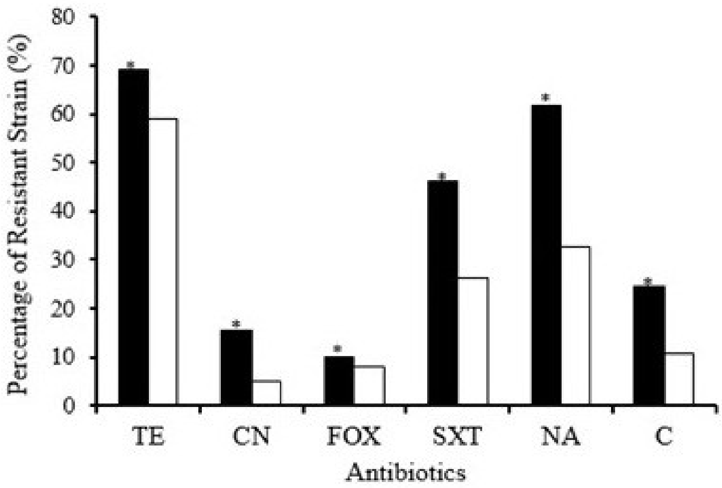

Figure 1. Percentage of *Enterobacteriaceae* Strains from Broiler and Backyard Chicken Meat Isolates that were Resistant to Each Antibiotic Tested. The symbol represent broiler chicken meat(■); backyard chicken meat(□); tetracycline (TE); gentamicin (CN); cefoxitin (FOX); sulfamethoxazole-trimethoprim (SXT) ; nalidixic acid (NA) ; chloramphenicol (C). *P values of < 0.05, Chi-squared test were considered significantly different.

Enterobacteriaceae (Table 2) indicate that *Salmonella* spp., *E. coli* spp., *Shigella* spp., *Citrobacter* spp., *Klebsiella* spp., *Yersinia* spp., and *Proteus* spp. from broiler chicken meat isolates were resistant to the six antibiotics tested (TE, CN, FOX, SXT, NA, and C). *Enterobacter* spp. and *Serratia* spp. from broiler chicken isolates were resistant to TE, FOX, SXT, NA, and C but not CN. *Edwardsiella* spp. showed resistance to TE, CN, SXT, NA, and C but not FOX. Table 2 confirms that the resistance patterns of *Salmonella* spp., *E. coli*, *Shigella* spp., *Citrobacter* spp., *Klebsiella* spp., *Yersinia* spp., *Proteus* spp., and *Serratia* spp. from backyard chicken meat isolates were resistant to the six antibiotics tested (TE, CN, FOX, SXT, NA, and C). *Enterobacter* spp. and *Edwardsiella* spp. were resistant to TE, FOX, SXT, NA, and C but not CN.

This study indicates that several genera of *Enterobacteriaceae* isolates from broiler and backyard chicken meats including *Salmonella* spp., *E. coli*, *Shigella* spp., *Citrobacter* spp., *Klebsiella* spp., *Yersinia* spp., *Proteus* spp., *Enterobacter* spp., *Serratia* spp., and *Edwardsiella* spp. could be reservoirs of antibiotic resistance. These results agree with data from several previous studies. Miranda *et al*. (2008) also reported that several strains of *Enterobacteriaceae* such as *Serratia* spp., *Enterobacter* spp., *Klebsiella* spp., *Yersinia* spp., *Hafnia* spp., and *Escherichia* spp. from chicken and turkey meat isolates were resistant to ampicillin, C, doxycycline, ciprofloxacin, sulfisoxazole, and CN. Over the last few years, several studies have also reported the resistance to antibiotics of several *Enterobacteriaceae* genera isolated from poultry, such as *E. coli*, *Salmonella*, *Klebsiella* and *Yersinia* (Akhtar *et al.,* 2016; Alvarez-Fenandez *et al*., 2013; Schwaiger *et al*., 2012; Shoaib *et a*l., 2016; Dallal *et al*., 2010; Tekiner and Ozpinar, 2016).

Enterobacteriaceae is a major pathogenic microorganism that is found in the intestinal tract and has been the cause of food-borne diseases worldwide (Al-Mutairi, 2011). The continuous use of antimicrobials as therapeutic agents, prophylactic agents, and growth promoters will create selective pressures that will eventually lead to the emergence of resistant bacterial strains (Smith *et al*., 2002; Braoudaki and Hilton, 2004). Antibiotic administration routes are significantly influenced by levels of antibiotic resistance in gut microbiota (Zang *et al*., 2013). Oral administration of antibiotic such as AGP increase antibiotic resistance *Enterobacteriaceae* in chicken intestinal. A systematic review by Simoneit *et al.* (2015), showed oral administration of antimicrobials increases antimicrobial resistance *E. coli* from chicken. Resistant bacteria

Table 2. The Resistance Patterns among the Genera of *Enterobacteriaceae* from Broiler and Backyard Chicken Meat Isolates to the Six Antibiotics Tested

Enterobacteriaceae Genera	Isolates Origin	n	No. of Resistant Strains (%)					
			TE	CN	FOX	SXT	NA	C
Salmonella spp.	Broiler	70	45 (64.29)	10 (14.29)	8 (11.43)	25 (35.71)	42 (60.00)	12 (17.14)
	Backyard	77	54 (70.13)	1 (1.30)	5 (6.490)	16 (20.78)	34 (44.15)	8 (10.39)
E. coli	Broiler	76	57 (75.00)	16 (21.05)	2 (2.63)	43 (56.58)	49 (64.47)	17 (22.37)
	Backyard	81	56 (69.14)	5 (6.17)	2 (2.47)	24 (29.63)	21 (25.93)	6 (7.41)
Shigella spp.	Broiler	28	15 (53.57)	3 (10.71)	3 (10.71)	12 (42.86)	20 (71.43)	7 (25.00)
	Backyard	12	7 (58.33)	2 (16.67)	0 (0.00)	4 (33.33)	6 (50.00)	1 (8.33)
Citrobacter spp.	Broiler	31	21 (67.74)	2 (6.45)	3 (9.68)	10 (32.26)	16 (51.61)	10 (32.26)
	Backyard	33	19 (57.58)	3 (9.09)	2 (6.06)	9 (27.27)	9 (27.27)	4 (12.12)
Klebsiella spp.	Broiler	19	13 (68.42)	4 (21.05)	1 (5.26)	8 (42.11)	10 (52.63)	4 (21.05)
	Backyard	25	9 (36.00)	0 (0.00)	2 (8.00)	4 (16.00)	2 (8.00)	2 (8.00)
Yersinia spp.	Broiler	23	18 (78.26)	4 (17.39)	5 (21.74)	14 (60.87)	17 (73.91)	7 (30.43)
	Backyard	24	10 (41.67)	0 (0.00)	3 (12.50)	5 (20.83)	7 (29.17)	2 (8.33)
Proteus spp.	Broiler	38	29 (76.32)	7 (18.42)	5 (10.53)	20 (52.63)	24 (63.16)	11 (28.95)
	Backyard	24	15 (62.50)	3 (12.50)	4 (16.67)	9 (37.5)	11 (45.83)	6 (25.00)
Enterobacter spp.	Broiler	5	3 (60.00)	0 (0.00)	1 (20.00)	3 (60.00)	2 (40.00)	2 (40.00)
	Backyard	15	6 (40.00)	0 (0.00)	2 (13.33)	4 (26.67)	2 (13.33)	1 (6.67)
Serratia spp.	Broiler	8	6 (75.00)	0 (0.00)	4 (50.00)	3 (37.50)	5 (62.50)	3 (37.50)
	Backyard	12	4 (3.33)	1 (8.33)	3 (25.00)	5 (41.67)	7 (58.33)	3 (25.00)
Edwardsiella spp.	Broiler	6	3 (50.00)	1 (16.67)	0 (0.00)	2 (33.33)	3 (50.00)	2 (33.33)
	Backyard	7	3 (42.86)	0 (0.00)	2 (28.57)	1 (14.29)	2 (28.57)	0 (0.00)
Total	Broiler	304	210 (69.08)	47 (15.46)	31(10.20)	140 (46.05)	188 (61.84)	75 (24.67)
	Backyard	310	183 (59.03)	15 (4.84)	25 (8.07)	81 (26.13)	101 (32.58)	33 (10.65)

Value is the number (percentage) of isolates; n = number of isolates tested; TE: tetracycline; CN: gentamicin; FOX: cefoxitin; SXT: sulfamethoxazole–trimethoprim;NA: nalidixic acid; C: chloramphenicol

from intestinal contents can contaminate chicken carcasses at various stages of the chicken slaughtering process (Amir *et al.* ,2017).

Antibiotic Resistance Strains

Table 3 shows that the percentage of resistant strains for each genus of *Enterobacteriaceae* isolates were high for both broiler and backyard chicken meats. The resistant strains from broiler chicken meat isolates and backyard chicken meat isolates ranged from 50.00% to 100.00% and 53.33% to 91.67%, respectively. Table 3 also indicated that the percentage order of resistance strains in broiler chicken meat isolates from highest to lowest is *Serratia* spp. (100%) > *Yersinia* spp. (91.30%) > *Proteus* spp. (89.47%) > *Shigella* spp. (85.71%) > *E. coli* (85.53%) > *Salmonella* spp. (81.43%) > *Enterobacter* spp. (80.00%) > *Klebsiella* spp. (78.95%) > *Citrobacter* spp. (74.19%) > *Edwardsiella* spp. (50.00%); the order in backyard chicken meat isolates is *Serratia* spp. (91.67%) > *Salmonella* spp. (80.52%) > *Proteus* spp. (79.17%) > *Shigella* spp. (75.00%) > *E. coli* (74.07%) > *Edwardsiella* spp. (71.43%)> *Citrobacter* spp. (63.64%) > *Yersinia* spp. (58.33%) > *Enterobacter* spp. (53.33%) >*Klebsiella* spp. (44.00%).

The percentages of antibiotic-resistant strains among genera of *Enterobacteriaceae* from broiler chicken meat isolates were significantly higher ($P < 0.05$) than that from backyard chicken meat isolates (Table 3). In Indonesia, backyard chickens are farmed extensively with no additional feeding and antibiotics, whereas broiler chickens are farmed with antibiotics as therapeutic, prophylactic, and growth promoting agents; therefore, antibiotics influence the contents of resistant bacterial strains in the guts of broiler chickens. This study is supported by several previous studies, where the resistance rates of organic chicken meat isolates to the antimicrobial agents found in commonly used as poultry drugs, such as quinolones (ciprofloxacin), tetracycline (doxycycline), chloramphenicol, gentamicin, penicillin, streptomycin, spectinomycin and sulfonamide (sulfisoxazole), were significantly lower than those obtained from conventional chicken and turkey meat that had been treated with antibiotics (Miranda *et al*., 2008). Cormican *et al*. (2001) dan Koga *et al.* (2015), showed that higher frequency of *Enterobacteriaceae* resistance strains isolated from conventionally raised poultry than free-range or organic poultry; as a result, the use of antimicrobial agents is more common in conventionally poultry farm than free-range or organic poultry.

An interesting finding in our study is the high levels of resistant strains of backyard chicken meat isolates (Table 3). The backyard chickens are farmed extensively, thus increasing their exposure to resistant bacteria from the surrounding environment. Resistant bacteria can

Table 3. Percentage of Resistant Strains for each Genera of *Enterobacteriaceae* from Broiler and Backyard Chicken Meat Isolates

Genera of *Enterobacteriaceae*	Broiler Chicken Meat isolates (n = 304)		Backyard Chicken Meat Isolates (n = 310)	
	No. of Isolates (n)	No. of Resistant Strains (%)	No. of isolates (n)	No. of Resistant Strains (%)
Salmonella spp.	70	57 (81.43)	77	62 (80.52)
E. coli	76	65 (85.53)	81	60 (74.07)
Shigella spp.	28	24 (85.71)	12	9 (75.00)
Citrobacter spp.	31	23 (74.19)	33	21 (63.64)
Klebsiella spp.	19	15 (78.95)	25	11 (44.00)
Yersinia spp.	23	21 (91.30)	24	14 (58.33)
Proteus spp.	38	34 (89.47)	24	19 (79.17)
Enterobacter spp.	5	4 (80.00)	15	8 (53.33)
Serratia spp.	8	8 (100.00)	12	11 (91.67)
Edwardsiella spp.	6	3 (50.00)	7	5 (71.43)
P-value: $P < 0.05$				

n = number of isolates tested

also be carried by other hosts living in the same environment. Therefore, the digestive tract of backyard chickens contains antibiotic-resistant bacteria as a source of contamination. According to Belanger *et al*. (2011) and Rossa *et al*. (2013), extensively farmed chickens and those raised as scavengers can be exposed to large numbers of microorganisms from the environment and may come into contact with soil contaminated with feces of wild animals that carry antibiotic-resistant microorganisms. Backyard chickens can also be exposed to resistant bacteria via contact with carriers, by ingesting food or water contaminated with feces of other animals that have previously received antimicrobial action, or via direct exposure to improperly disposed containers of antimicrobial agents used by humans (Ojo *et al*., 2012).

Poor sanitary and waste management conditions at the slaughtering location, and sites of chicken meat sales in the traditional markets also contribute to environmental pollution , thus increasing the possibility that chicken meats will be exposed to resistant bacteria (Ojo *et al*., 2012). Enteric bacteria derived from humans and animals can pollute the domestic environment via cross-contamination (Azevedo *et al*., 2015). The results of this study emphasize the possibility of high levels of resistance from bacteria present in the traditional markets of Surabaya. The horizontal gene transfer mechanism is responsible for increasing the spread of antibiotic resistance to food-borne bacterial pathogens. Conjugation, transformation, and transduction are the main mechanisms for the spread of antibiotic-resistant genes (von Wintersdorff *et al*., 2016). Furthermore, phage-mediated transduction is considered a major driver of antibiotic resistance gene transfer from food-borne pathogens and the environment to animals and humans. Phages are the most abundant organisms in the biosphere and are found in various environments (Clokie *et al*., 2011). Antibiotic resistance genes are often found in various mobile genetic elements, such as plasmids, genomic islands, and transposons; therefore, these genes can be transferred horizontally by phage transduction (Colavecchio *et al*., 2017).

Multi-drug Resistance *Enterobacteriaceae*

Table 4 indicates that the *Enterobacteriaceae* isolates from broiler and backyard chicken meats contained a high percentage of MDR strains against the antibiotics tested. Overall, 184 (60.53%) of the 304 broiler chicken meat isolates and 125 (40.32%) of the 310 backyard chicken meat isolates displayed MDR strains to two or more antimicrobial agents. The percentage of MDR strains of the broiler chicken meat isolates was significantly higher

Table 4. MDR Strains of *Enterobacteriaceae* from Broiler and Backyard Chicken Meat Isolates

Number of Resistant Antibiotics	Resistant Strains (%)	
	Broiler Chicken Meat Isolates (n =304)	Backyard Chicken Meat Isolates (n = 310)
0*	50 (16.45)	90 (29.03)
1	70 (23.03)	95 (30.64)
2	50 (16.45)	68 (21.94)
3	57 (18.75)	33 (10.65)
4	45 (14.80)	16 (5.16)
5	28 (9.21)	6 (1.94)
6	4 (1.31)	2 (0.64)
Resistance to ≥1 antibiotic	254 (83.55)	220 (70.97)
Multidrug resistance **	184 (60.53)	125 (40.32)
P-value: P > 0.05		

n= number of isolates tested; 0* = sensitive (not resistant) to antibiotics tested; Multidrug resistance ** = resistant to two or more types of antibiotics

Table 5. Antimicrobial Resistance Profiles of *Enterobacteriaceae* from Broiler and Backyard Chicken Isolates

Number of Resistant Antibiotics	Broiler Chicken Meat Isolates (n = 304)		Backyard Chicken Meat Isolates (n = 310)	
	Antimicrobial Resistance Profiles (ARP)	ARP Frequency	Antimicrobial Resistance Profiles (ARP)	ARP Frequency
0*	-	50	-	90
1	TE	37	TE	71
	NA	25	NA	9
	SXT	2	CN	2
	FOX	5	SXT	2
	C	1	FOX	7
			C	4
2	TE-NA	23	TE-NA	34
	TE-SXT	14	TE-SXT	22
	TE-C	1	TE-C	2
	TE-CN	5	NA-C	2
	NA-C	1	TE-FOX	2
	TE-FOX	1	FOX-NA	1
	FOX-NA	1	SXT-NA	4
	STX-NA	4	SXT-C	1
3	TE-NA-C	3	TE-NA-C	1
	TE-SXT-NA	38	TE-SXT-NA	17
	TE-SXT-C	1	TE-SXT-C	5
	TE-FOX-NA	4	TE-FOX-NA	2
	TE-CN-NA	5	TE-CN-SXT	1
	FOX-NA-C	2	TE-FOX-SXT	1
	FOX-SXT-NA	3	TE-CN-NA	1
	SXT-NA-C	1	CN-SXT-NA	2
			FOX-SXT-NA	2
			SXT-NA-C	1
4	TE-SXT-NA-C	29	TE-SXT-NA-C	6
	TE-FOX-SXT-NA	4	TE-FOX-NA-C	2
	TE-CN-SXT-NA	9	TE-FOX-SXT-NA	4
	TE-CN-NA-C	1	TE-CN-SXT-NA	4
	TE-CN-FOX-NA	1		
	CN-SXT-NA-C	1		
5	TE-CN-SXT-NA-C	20	TE-CN-SXT-NA-C	4
	TE-FOX-SXT-NA-C	8	TE-FOX-SXT-NA-C	2
6.	TE-CN-FOX-SXT-NA-C	4	TE-CN-FOX-SXT-NA-C	2
	Total	304		310

n = number of isolates tested, 0* = sensitive (not resistant) to antibiotics tested. TE: tetracycline; CN: gentamicin; FOX: cefoxitin; SXT: sulfamethoxazole–trimethoprim: NA: nalidixic acid; C: chloramphenicol

than that of the backyard chicken meat isolates (P<0.05).

The National Antimicrobial Resistance Monitoring System defines multidrug resistance as resistance to two or more antimicrobial classes (NARMS, 2009). In this research, *Enterobacteriaceae* isolates from broiler and backyard chicken meats showed a high percentage of MDR strains against the antibiotics tested. The percentage of MDR *Enterobacteriaceae* strains from broiler chicken meat isolates was significantly higher than those for backyard chicken meat isolates. Some MDR strains of broiler and backyard chicken meat isolates were resistant to two to six antibiotics (Table 5). From the *Enterobacteriaceae* isolates, 25 different multiresistance profiles were obtained from the broiler chicken meat isolates, and 25 different multiresistance profiles were obtained from the backyard chicken meat isolates (Table 5). It also shows a strong association between TE, SXT, and NA resistance (TE-SXT-NA) in the majorly multiresistant profiles of broiler chicken meat isolates. On the contrary, the strong association in multiresistance profiles was only observed for TE and NA resistance (TE-NA) in backyard chicken meat isolates.

This study is supported by Kamboh *et al.* (2018), which reported MDR *E. coli, Salmonella*, and *Klebsiella* were higher in liver of commercial broilers compared to backyard chickens. The prevalence of extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* in liver of broiler chickens was higher than in backyard chickens (Shoaib *et al*., 2016). Akhtar *et al.* (2016), also reported MDR *E. coli* isolates were higher commercial broiler chicken (64.2%) than backyard chicken (53.5%). The presence of MDR *Enterobacteriaceae* strains is a cause of great concern because of their potential to become widespread and the difficulty of managing infected patients (Karlowsky *et al*., 2003). Therefore, consumers should avoid consuming meat that contains multiresistant bacteria and avoiding cross-contamination during food handling and preparation.

Animals and food products of animal origin are potential sources of MDR bacteria. Unhealthy meat consumption, unhygienic livestock practices, and polluted environments around slaughterhouses contribute to the transmission of antibiotic-resistant bacterial strains and several diseases (Saikia and Joshi, 2010; Marshall and Levy, 2011). In Indonesia, the availability of information regarding the prevalence of MDR *Enterobacteriaceae* in chicken meat is poor. Multidrug resistance in bacteria can be produced by two mechanisms. First, bacteria can accumulate many genes, with each encoding resistance to a single drug in a single cell. This accumulation usually occurs in the resistance plasmid (R). Second, multidrug resistance can also occur via the increased expression of genes that encode multidrug efflux pumps, which extrude various drugs (Nikaido, 2009).

CONCLUSION

This study revealed a higher antibiotic resistance strains in broiler chicken meat isolates than in backyard chicken meat isolates. MDR *Enterobacteriaceae* strains have spread to broiler and backyard chicken meats sold in Surabaya, Indonesia, which easily spread to the human population. Basic hygienic measures and the rational use of antibiotics in animal feed should be promoted.

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