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Prevalence of Antibiotic-resistance *Enterobacteriaceae* strains Isolated from Chicken Meat at Traditional Markets in Surabaya, Indonesia

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Abstract. Antibiotic resistance in bacteria from the family *Enterobacteriaceae* is an important indicator of the emergence of resistant bacterial strains in the community. This study investigated the prevalence of antibiotic-resistant *Enterobacteriaceae* isolated from chicken meat sold at traditional markets in Surabaya Indonesia. In all, 203 isolates (43 *Salmonella* spp., 53 *Escherichia coli*, 16 *Shigella spp.* , 22 *Citrobacter* spp., 13 *Klebsiella* spp*, 24 Proteus* spp., 15 *Yersinia spp.,* 7 *Enterobacter* spp., 6 *Serratia* spp., 3 *Edwardsiella* spp. were resistant to tetracycline (69.95 %), nalidixid acid (54.19 %), sulfamethoxazole/sulfamethizole (42.36 %), chloramphenicol (12.81%), cefoxitin (6.40 %), gentamicin (5.91 %). Tetracycline was the antimicrobial that showed the highest frequency of resistance among *Salmonella, E. coli, Citrobacter, Proteus* and *Erdwardsiella* isolates, and nalidixid acid was second frequency of resistance. Overall, 124 (61.08 %) out of 203 isolates demonstrated *multidrug resistance* to at least two unrelated antimicrobial agents. The high rate of antimicrobial resistance in bacterial isolates from chicken meat may have major implications for human and animal health with adverse economic implications.

1. Introduction

Antibiotic resistanceare significant health, social, and economic problem at this time. Antibiotic resistance of bacteria is biological risk, which increases morbidity and mortality of animal and human [5]. In recent years, accumulating problems with bacteria, which are resistant to antibiotics, leading to predictions that we return to the time before the discovery of antibiotics [8]. Resistant bacteria from the intestines of food animals may be transferred to retail meat products resulting from fecal contamination during various stages of the slaughter process (e.g. evisceration) and subsequent handling of animal tissue [7]. Endogenous bacterial flora may play an important role as acceptor and donor of transmissible drug resistance genes [3,13]. The *Enterobacteriaceae* family is commonly used as an indicator of fecal contamination during food microbiological analyses, and includes important zoonotic bacteria such as *Salmonella* spp., *Yersinia* spp. and *Escherichia coli*. *Enterobacteriaceae* are the significant causes of serious infection, and many of the most important members of this family are becoming increasingly resistant to currently available antimicrobials [14].

In the past decade, *multidrug-resistant Enterobacteriaceae* (MDE) has become an important challenge to disease control [15]. Gram-negative *Enterobacteriaceae* may cause severe infections and unfortunately several of the most important members of this family are becoming progressively more resistant to currently available antimicrobials [4, 6, 14]. Considering their wide spread and few options for treatment, MDE have public health importance [8].

A main goal of the present study was to investigate the prevalence of antibiotic-resistant *Enterobacteriaceae* isolates collected from chicken meat sold at traditional market in Surabaya, Indonesia. The potential repercussions of these results were in terms of microbiological safety, especially concerning the development and spread of antimicrobial resistance in the food chain.

2. Experimental

Two hundred and three (203) isolates of *Enterobacteriaceae* obtained from our preliminary study. The isolates were isolated from chicken meat sold at traditional markets (Kapas Krampung, Pacar Keling, Pucang Anom Timur, Wonokromo Baru, Soponyono, and Pabean) in Surabaya, Indonesia.

The Kirby–Bauer disk diffusion method was used to determine sensitivity or resistance to antimicrobial agents, according to guidelines developed by the Clinical Laboratory Standard Institute [2]. Antimicrobial susceptibility testing was performed for 203 isolates of *Enterobacteriaceae* from chicken meat (43 *Salmonella* spp., 53 *Escherichia coli*, 16 *Shigella spp.*, 22 *Citrobacter* spp., 13 *Klebsiella* spp, 24 *Proteus* spp., 15 *Yersinia spp.*, 7 *Enterobacter* spp., 6 *Serratia* spp., 3 *Edwardsiella* spp. and 1 *Morganella* spp.). Antibiotic discs used in this study were Tetracycline (TE 30 µg/disc, Gentamicin (CN) 10 µg/disc, Cefoxitin (FOX) 30 µg/disc, Sulfamethoxazole/ Sulfamethizole (SXT) 25 µg/disc, Nalidixid acid (NA) 30 µg/disc, Chloramphenicol (C) 30 µg/disc (Oxoid, UK).

The protocol was performed as follows: fresh cultures were inoculated into LB broth and incubated until they reached a turbidity of 0.5 using the McFarland standard. Mueller–Hinton agar plates were swabbed with these cultures, and antibiotic disks (Oxoid, UK) were placed on to inoculated plates in a sterile environment. The plates were incubated at 37 °C for 18 to 20 h. The diameters (in millimeters) of clear zones of growth inhibition around the antimicrobial agent disks were measured using a precision digital caliper (Absolute, Mitutoyo, Japan). Recommendations of the National Antimicrobial Resistance Monitoring System for *Enterobacteriaceae* isolates were utilized to define breakpoints of antibiotics and thus categorize the isolates as resistant, intermediate or sensitive classified base on the Clinical Laboratory Standard Institute [2].

3. Results and Discussion

Research findings on 203 isolates showed resistance to all antibiotics tested. The resistance levels of each antibiotic against isolates that were tested are shown in table 1. The highest resistance was to tetracycline (69.95%), followed by nalidixid acid (54.19%), sulfa-methoxazole/sulfamethizole (42.36%), chloramphenicol (12.81%), cefoxitin (6.40%) and the lowest resistance was to gentamicin (5.91 %). High resistance to tetracycline in this research can be explained by the widespread use and

quite long of tetracycline on poultry farms associated with the prophylaxis, growth promotor agent, and therapeutics or their potential uses of antibiotics were not improper. Habit of improper use of antibiotics in poultry may be one trigger of antibiotic resistance particularly antibiotics are often used on the chicken farm.

Table 1. In vitro susceptibility of *Enterobacteriaceae isolates* to several antibiotics

Class and Antibiotic	Breakpoints (CLSI, 2011) S/I/R (mm)	*Enterobacteriaceae* isolates (n = 203)		
		Number of sensitive isolates (%)	Number of intermediary isolates (%)	Number of resistant isolates (%)
Tetracycline (TE)	$\geq 15/12\text{-}14/ \leq 11$	47 (23.15)	14 (6.90)	142(69.95)
Aminoglycoside - *Gentamicin* (CN)	$\geq 15/13\text{-}14/\leq 12$	187 (92.12)	4 (1.97)	12 (5.91)
Cephalosporin - *Cefoxitin* (FOX)	$\geq 18/15\text{-}17/\leq 14$	185 (91.13)	5 (2.46)	13 (6.40)
Folate Pathway Inhibitors *Sulfamethoxazole/Sulfamethizole*(SXT)	$\geq 16/11\text{-}15/\leq 10$	112 (55.17)	5 (2.46)	86 (42.36)
Quinolones - *Nalidixid acid* (NA)	$\geq 19/14\text{-}18/\leq 13$	76 (37.44)	17 (8.37)	110(54.19)
Phenicols - *Chloramphenicol* (C)	$\geq 18/13\text{-}17/\leq 12$	159 (78.32)	18 (8.87)	26 (12.81)

Notes: R = resistant; I = Intermediate resistant, S = susceptible

The results in table 2 showed differences in the resistance level of *Enterobacteriaceae* isolates against antibiotics that were tested. *Salmonella* spp. and *E. coli* had the highest resistance to tetracycline. This is possible due to *Salmonella* spp. and *E. coli* are the two dominants species of bacteria found in chicken meat, thus the bacteria are most exposed to antibiotics and can survive   that have the highest resistance.

Table 2. Prevalence of antibiotic-resistance *Enterobacteriaceae* strains isolates from chicken meat

Bacterial strain	Prevalence of antibiotic-resistance (%)					
	Tetra-cyline	Genta-micin	Cefoxitin	Sulfamethoxazole/ Sulfamethizole	Nalidixid acid	Chloram-phenicol
Salmonella spp. (n =43)	36 (83.72)	0 (0.00)	2 (4.65)	14 (32.56)	24 (55.81)	6 (13.95)
Escherichia coli (n =53)	42 (79.24)	7 (13.21)	1 (1.89)	28 (52.83)	29 (54.72)	5 (9.43)
Shigella spp. (n=16)	8 (50.00)	0 (0.00)	1 (6.25)	8 (50.00)	14 (87.50)	2 (12.50)
Citrobacter spp. (n=22)	17 (77.27)	1 (4.54)	2 (9.09)	10 (45.45)	12 (54.54)	5 (22.73)
Klebsiella spp. (n=13)	8 (61.54)	2 (15.38)	1 (7.69)	5 (38.46)	7 (53.85)	2 (15.38)
Yersinia spp. (n=15)	7 (46.67)	1 (6.67)	2 (13.33)	2 (13.33)	7 (46.67)	1 (6.67)
Proteus spp. (n=24)	17 (70.83)	1 (4.17)	1 (4.17)	12 (50.00)	12 (50.00)	3 (12.50)
Enterobacter spp. (n=7)	3 (42.86)	0 (0.00))	1 (14.28)	3 ((42.86)	2 (28.57)	1 ((14.28)
Serratia spp. (n=6)	2 (33.33)	0 (0.00)	2 (33.33)	2 (33,.33)	3 (50.00)	1 (16.67)
Erdwardsiella spp.(n=3)	2 (66.67)	0 (0.00)	0 (0.00)	2 (66.67)	1 (33.33)	0 (0.00)
Morganella spp. (n=1)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Bacteria cells become resistant to tetracycline by at least three mechanisms: enzymatic inactivation of tetracycline where an acetyl group is added to the molecule causing inactivation of the drug, efflux

resistance gene encodes a membrane protein that actively pumps tetracycline out of the cell, and ribosomal protection which blocking tetracycline from binding to the ribosom [1, 10].

Table 3. Identified *Enterobacteriaceae* strains, number of isolate, percentage of antibiotic resistance and resistance profile

Bacterial strain	Number of isolate	Percentage of antibiotic resistance (%)	Resistance Profile
Salmonella spp.	43	39 (90.70)	TE, FOX, SXT, NA, C
Escherichia coli	53	46 (86.79)	TE, CN, FOX, SXT, NA, C
Shigella spp.	16	15 (93.75)	TE, FOX, SXT, NA, C
Citrobacter spp.	22	19 (86.36)	TE, CN, FOX, SXT, NA, C
Klebsiella spp.	13	10 (76.92)	TE, CN, FOX, SXT, NA, C
Yersinia spp.	15	9 (0.60)	TE, CN, FOX, SXT, NA, C
Proteus spp.	24	20 (83.33)	TE, CN, FOX, SXT, NA, C
Enterobacter spp.	7	4 (57.14)	TE, FOX, SXT, NA, C
Serratia spp.	6	5 (83.33)	TE, FOX, SXT, NA, C
Erdwardsiella spp.	3	2 (66.67)	TE, SXT, NA
Morganella spp.	1	0 (0.00)	----

Resistance profile of *Salmonella spp., Escherichia coli, Shigella spp., Citrobacter spp., Klebsiella spp, Proteus spp., Yersinia spp., Enterobacter spp., Serratia spp., Edwardsiella* isolates against six types of antibiotic exhibited multidrug-resistance to tetracycline, gentamicin, cefoxitin, sulfamethoxazole / sulfamethizol, nalidixid acid, and chloramphenicol (table 3.).

Table 4. Resistance patterns in *Enterobacteriaceae* strain isolates from chicken meat

Enterobacteria-ceae strain	Number of antibiotic resistance							Multi-resistant Strain (%)
	0	1	2	3	4	5	6	
Salmonella (n=43)	4 (9.30)	13 (30.23)	13 (30.23)	9 (20.94)	4 (9.30)	0 (0.00)	0 (0.00)	26 (60.46)
E. coli (n=53)	7 (13.21)	9 (16.98)	18 (33.96)	8 (15.09)	11 (20.75)	0 (0.00)	0 (0.00)	37 (69.81)
Shigella (n=16)	1 (6.25)	5 (31.25)	4 (25.00)	4 (25.00)	2 (12.50)	0 (0.00)	0 (0.00)	10 (62.50)
Citrobacter (n=22)	3 (13.64)	5 (22.73)	4 (18.18)	7 (31.82)	2 (9.09)	1 (4.54)	0 (0.00)	14 (63.64)
Klebsiella (n=13)	3 (23.08)	2 (15.38)	3 (23.07)	3 (23.07)	2 (15.38)	0 (0.00)	0 (0.00)	8 (61.54)
Yersinia (n=15)	4 (26.67)	4 (26.67)	6 (40.00)	0 (0.00)	1 (6.67)	0 (0.00)	0 (0.00)	7 (46.67)
Proteus (n=24)	4 (16.67)	6 (25.00)	6 (25.00)	5 (20.83)	3 (12.50)	0 (0.00)	0 (0.00)	14 (58.33)
Enterobacter (n=7)	3 (42.86)	1 (14.28)	1 (14.28)	1 (14.28)	1 (14.28)	0 (0.00)	0 (0.00)	3 (42.86)
Serratia (n=6)	1 (16.67)	2 (33.33)	1 (16.67)	2 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)	3 (50.00)
Erdwarsiella (n= 3)	1 (33.33)	0 (0.00)	1 (33.33)	1 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)	2 (66.67)
Morganella (n=1)	1 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Total (n = 203)	32 (15.76)	47 (23.15)	57 (28.08)	40 (19.70)	26 (12.81)	0 (0.00)	0 (0.00)	124 (61.08)

Multidrug-resistance is a state of resistance to various antibiotics at once mediated by plasmid R

factor (extrachromosomal DNA) [12]. Factors R as the resistance factor can be transferred and spread by way of conjugation between strains of *Enterobacteriaceae*, such as *Salmonella* spp., *Escherichia coli, Shigella* and so on. Resistance to two or more antibiotics was found in 26 isolates of *Salmonella*, followed by 37, 10, 14, 8, 7, 14, 3, 3 and 2 isolates of *Escherichia coli, Shigella spp., Citrobacter* spp., *Klebsiella* spp, *Yersinia spp., Proteus* spp., *Enterobacter* spp., *Serratia* spp., *Edwardsiella* spp., respectively, as shown in table 4. Antibiotics resistance patterns for *Enterobacteriaceae* strains isolated showed a higher level of multi-resistant bacteria. As many as 61.08 % of *Enterobacteriaceae* isolates demonstrated *multidrug resistance* to at least three unrelated antimicrobial agents. *Salmonella* spp., *Escherichia coli, Shigella spp., Citrobacter* spp., *Klebsiella* spp, *Proteus* spp., *Yersinia spp., Enterobacter* spp., *Serratia* spp. and *Edwardsiella* spp. are *Enterobacteriaceae* strains which *multidrug resistance.* The high prevalence of antimicrobial resistance identified in the present study can be explained by the widespread use of common antimicrobials as prophylactics, growth promoter agents, or in veterinary medicine.

Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs [11]. Food contamination with MDR bacteria is a major problem for public health and could be transferred to bacteria of clinical significance.

4. Conclusion

These results showed that the most spread resistance in *Enterobacteriaceae* were isolated from chicken meat sold at traditional markets in Surabaya Indonesia. The antibiotic resistant against tetracycline, gentamicin, cefoxitin, sulfamethoxazole/sulfamethizole, nalidixid acid, and chloramphenicol. Tetracycline was the highest frequency of resistance among *Salmonella, E. coli, Citrobacter, Proteus* and *Erdwardsiella* isolates. Nalidixid acid showed high frequency of resistance among *Shigella, Salmonella, E. coli, Citrobacter* and *Klebsiella* isolates. High percentage (60.08%) isolates demonstrated *multidrug resistance* to at least three unrelated antimicrobial agents. These findings confirm that chicken meat is a reservoir of multidrug-resistant *Enterobacteriaceae* strains, and suggest that the farming and postharvest technology are needed to improve so that the resulting meat that is free of contaminants or bacterial contamination with a low level of resistance.

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