

Contamination of Antibiotic Resistant Enterobacteriaceae in Duck Meat in Bogor District

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Abstract

This study was aimed to determine Enterobacteriaceae contamination that were resistant against antibiotics in duck meat which was related with food safety for the consumers. Total of 52 samples of ducks were taken from 5 subdistricts in Bogor District, i.e., Ciomas, Gunung Sindur, Klapanunggal, Jasinga, and Parung Panjang. This sample size was calculated based on the assumption of 95% confidence level, 50% predicted prevalence, and 10% standard error. The ducks were collected from small-scale farmers which supplied duck meat to markets, so the ducks were slaughtered by them. The samples of duck meat were handled aseptically and transported in cool box to the laboratory. The total of Enterobacteriaceae was examined using plate count method with violet red bile glucose agar according to Kornacki and Johnson (2001). The examination of antibiotic resistance was done using disc diffusion method and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines (CLSI 2012). The data was analysed descriptively. The results showed that the total of Enterobacteriaceae were 79679 ± 260463 cfu/g. The majority of Enterobacteriaceae isolated from duck meat (84.6%) showed resistant against 5 of antibiotic (gentamycin, kanamycin, tetracycline, trimethoprim, and ampicillin). The Enterobacteriaceae had resistance against tetracycline (93.2%), trimethoprim (88.6%), and ampicillin (81.8%). Nevertheless, this family was still sensitive against kanamycin (34.1%) and gentamycin (15.9%). The isolated bacteria (69.2%) showed multi-antibiotic

resistant (≥ 3 antibiotics). The antibiotic resistant bacteria could be a potential impact on public health and animal health.

Keywords: *duck meat, Enterobacteriaceae, total counts, antibiotic resistance*

1. INTRODUCTION

The increase of income of community has a positive impacts on the demand of animal protein. In the last years the government develops the poultry sectors as a source of animal protein for the people. One of the popular poultry is ducks since culinary of duck meat is now very popular in some cities.

Duck farms is mostly kept in small scale. They supply live ducks and duck meat for the consumers whereas they slaughter the ducks in farm. Farmers provide feed for ducks by themselves. Antibiotics are added to feed to promote the growth of ducks without supervision of veterinarian. This condition can cause antibiotic residues in meat and antibiotic resistance in bacteria in the intestines.

Antibiotic resistance is the most critical problem in animal and human health now and in the next years. The antibiotic resistant bacteria can cause the failure of treatment on gastrointestinal infection in human¹. The use of antibiotic in farms without prescription and supervision of veterinarian can led to the occurrence of antibiotic residues in animal products and antibiotic resistance of bacteria. The family of Enterobacteriaceae is used a an indicator of hygiene in food industry. Many studies showed that Enterobacteriaceae was resistant to antibiotics. The antibiotic resistant Enterobacteriaceae becomes an important challenge to disease control². The study on antibiotic resistance of Enterobacteriaceae isolated in domestic environments in house in Oporto, Portugal³, showed that 49.6% of isolated Enterobacteriaceae from the domestic food related environment were resistant to at least one antibiotic and 6.4% of those isolates had resistant to multiple antibiotics. This study was aimed to evaluate the total and occurrence of Enterobacteriaceae in duck meat in the Bogor District.

2. MATERIALS AND METHODS

Sampling of Meat

Total of 52 samples of ducks were taken from 5 subdistricts in Bogor District, i.e., Ciomas, Gunung Sindur, Klapanunggal, Jasinga, and Parung Panjang. This sample size was calculated based on the assumption of 95% confidence level, 50% predicted prevalence, and 10% standard error. The ducks were collected from small-scale farmers which supplied duck meat to markets, so the ducks were slaughtered by them. The sample size was identified according to proportional allocation from the total of duck farmer population. The samples of duck meat were handled aseptically and transported in cool box to the laboratory.

Enumeration of Enterobacteriaceae

The enumeration of Enterobacteriaceae was carried out according to plate count method⁴. 25 g of sample were removed aseptically from duck carcass and added to 225 mL of 0.1% sterile buffered peptone water (OXOID CM1049, England) and blended in a

stomacher machine for 1 minute to give dilution 1:10. Ten-fold serial dilutions up to 10^{-4} were prepared and subsequently 1 mL of each dilution was placed in a sterile Petri dish. Approximately 15 mL of molten cooled violet red bile glucose agar (OXOID CM0361, England; temperature of molten agar 44°C - 46°C) were poured to each Petri dish and mixed well. After the solidification of the agar, the plate was inverted and incubated at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24-48 hours. The colonies were enumerated manually and recorded.

Determination of Antimicrobial Susceptibility

The antimicrobial susceptibility test was determined using the Bauer and Kirby diffusion technique on Mueller-Hinton agar (OXOID CM0337, England) and susceptibility classification (as sensitive, intermediate or resistant) was carried out according to Clinical and Laboratory Standards Institute⁵. The isolated colonies was evaluated for antibiotic resistance. The antibiotics were used in this test including gentamicin (20 $\mu\text{g}/\text{mL}$), kanamycin (25 $\mu\text{g}/\text{mL}$), tetracyclin (10 $\mu\text{g}/\text{mL}$), trimethoprim (25 $\mu\text{g}/\text{mL}$), and ampicillin (100 $\mu\text{g}/\text{mL}$).

Data Analysis

Data were analysed descriptively to describe Enterobacteriaceae counts and occurrence of antibiotic resistant Enterobacteriaceae isolated from duck carcass.

3.

RESULTS

Enterobacteriaceae in Duck Meat

The average of Enterobacteriaceae counts in duck meat were 81212 ± 262763 cfu/g (TABLE 1). The highest contamination of Enterobacteriaceae was found in the Jasinga Subdistrict (226553 ± 445306 cfu/g) and the lowest one was found in Klapanunggal Subdistrict (887 ± 1039 cfu/g).

TABLE 1. Enterobacteriaceae counts in duck meat in Bogor District

Subdistrict	Enterobacteriaceae counts (cfu/g)
Ciomas (n=8)	4794 ± 11422
Gunung Sindur (n=5)	6334 ± 13788
Klapanunggal (n=6)	887 ± 1039
Jasinga (n=16)	226553 ± 445306
Jonggol (n=17)	30754 ± 56119
Total (n=52)	81212 ± 262763

The isolated Enterobacteriaceae from duck meat had mostly (84.6%) resistant to 5 tested antibiotics (gentamicin, kanamycin, tetracyclin, trimethoprim, and ampicillin). In general the isolated Enterobacteriaceae were resistant to tetracyclin (93.2%), trimethoprim (88.6%), and ampicillin (81.8%). Nevertheless, the Enterobacteriaceae was relatively still sensitive to trimethoprim (34.1%) and gentamicin (15.9%). The antibiotic resistance of Enterobacteriaceae that were isolated from duck meat in Bogor District showed in TABLE 2.

TABLE 2. Occurance (%) of antibiotic resistant Enterobacteriaceae isolated from duck meat in Bogor District

Subdistrict	Sample size	Antibiotic resistant	Gentamicin	Kanamycin	Tetracyclin	Trimethoprim	Ampicillin
Ciomas	8	6 (75.0%)	2 (33.3%)	5 (83.3%)	6 (100.0%)	5 (83.3%)	5 (83.3%)
Gunung Sindur	5	5 (100%)	0 (0.0%)	0 (0.0%)	4 (80.0%)	4 (80.0%)	4 (80.0%)
Klapanunggal	6	3 (50.0%)	0 (0.0%)	0 (0.0%)	3 (100.0%)	3 (100.0%)	2 (66.7%)
Jasinga	16	14 (87.5%)	0 (0.0%)	0 (0.0%)	12 (85.7%)	12 (85.7%)	11 (78.6%)
Jonggol-Cariu	17	16 (94.1%)	5 (31.3%)	10 (62.5%)	16 (100.0%)	15 (93.8%)	14 (87.5%)
Total	52	44 (84.6%)	7 (15.9%)	15 (34.1%)	41 (93.2%)	39 (88.6%)	36 (81.8%)

DISCUSSION

Enterobacteriaceae is distributed widely in nature and in the gastrointestinal tract of humans, mammals, and birds. Contamination of *Enterobacteriaceae* in chicken carcass came from contents of intestinal tract during evisceration⁶. *Enterobacteriaceae* is used as hygiene indicator in food industry. The study on contamination of *Enterobacteriaceae* in meat from retail stores in Tennessee, USA⁶ found the contamination level of *Enterobacteriaceae* in raw meats ranged from 3.26 log₁₀ cfu/g to 4.94 log₁₀ cfu/g. The contamination level of *Enterobacteriaceae* in duck meat in this study was relatively same. The contamination level in this study was high. The development of good practices implementation in farms and slaughtering process should be taken into account since some genera of this family are foodborne pathogens that could be a potential risk for consumers.

Antimicrobial resistance is increasing in several species of *Enterobacteriaceae* and this become a major concern in human and animal health. The widespread use of antibiotics in livestock has implicated to emergence of antibiotic resistant bacteria⁶. *Enterobacteriaceae* showed antibiotic resistant mainly to beta lactam antibiotics including carbapenem, especially genera such as *Escherichia*, *Klebsiella*, *Citrobacter*, *Serratia*, *Salmonella*, *Enterobacter*, and *Proteus*⁷. The resistance of the *Enterobacteriaceae* isolated from meat in retail stores in Tennessee, USA to antibiotics was most frequent with erythromycin, penicillin, and ampicillin at 100, 89, and 65.8%, respectively⁶. The antibiotic resistant bacteria could be a potential impact on public health and animal health. The preventive and control measures to overcome the emergence of antibiotic resistance should be developed and implemented using One Health approach.

4. CONCLUSION

The average counts of *Enterobacteriaceae* in duck meat in Bogor District were 81212±262763 cfu/g. This contamination level was relatively high and could become a risk for consumer health since some genera are foodborne pathogens.

The majority of isolated *Enterobacteriaceae* in duck meat showed resistance to 5 tested antibiotics (gentamicin, kanamycin, tetracyclin, trimethoprim, and ampicillin). In general the isolated *Enterobacteriaceae* were resistant to tetracyclin (93.2%), trimethoprim (88.6%), and ampicillin (81.8%), but still sensitive to trimethoprim (34.1%) and gentamicin (15.9%).

ACKNOWLEDGEMENTS

This research project was financially supported by Ministry of Higher Education through the Penelitian Unggulan Perguruan Tinggi (Decentralisation) in the budget year of 2013/2014. The authors are grateful to research assistants and students (Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agricultural University).

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