Escherichia coli CONTAMINATION AND ITS RESISTANCE TO ANTIBIOTICS IN SE’I MEAT

Gabryella Fransina Amalo¹*, Trioso Purnawarman², and Herwin Pisestyani²
¹Veterinary Public Health Study Program, Postgraduate School, Bogor Agricultural University, Bogor, Indonesia
²Department of Veterinary Public Health, Department of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia
*Corresponding author: gabryella.amalo@gmail.com

ABSTRACT

The purpose of this study was to obtain Escherichia coli isolates from 11 se’i meat sellers in Kupang City and to observe their resistance to 13 types of antibiotics. Escherichia coli were isolated and identified based on SNI 2897: 2008. Antibiotic resistance of the Escherichia coli isolates was determined using the Kirby-Bauer method. The results showed that 13 isolates (39.39%) of Escherichia coli in se’i meat samples had a high level of resistance to erythromycin (100%), tetracycline (76.92%), and doxycycline (61.54%). Isolates demonstrated increased resistance to streptomycin (46.15%), cephalothin (38.46%), trimethoprim-sulfamethoxazole (38.46%), amoxicillin (30.77%), chloramphenicol (30.77%), and choline sulfate (30.77%). The antibiotics nalidixic acid and ciprofloxacin demonstrated low Escherichia coli resistance (7.69%). Escherichia coli are sensitive to cefotaxime and gentamicin. A total of 12 isolates (92.31%) experienced MDR. The presence of non-MDR and MDR resistant Escherichia coli in se’i meat can seriously threaten community health.

Key words: antibiotic resistance, Escherichia coli, multidrug resistance, se’i meat

INTRODUCTION

Se’i meat is a processed smoked meat product originating from Rote Ndao Regency, East Nusa Tenggara Province (NTT). Se’i meat is highly consumed in Kupang City because it is popular with the community and a typical souvenir of NTT (Bontong et al., 2012). Se’i meat is made by cutting the meat lengthwise and seasoning it, after which it is brooded for 24 hours. Next, the meat is smoked with kosambi wood for ±15-45 minutes and every 15 minutes the meat is turned over so it does not burn (Bontong et al., 2012; Raza et al., 2012; Hutasoit et al., 2013; Cruz et al., 2018). Se’i meat has water content of 40-60%, which leaves the product vulnerable to contamination by bacteria (Ma'alek et al., 2016). Bacterial contamination of se’i meat can be caused by several factors, such as through contact with raw materials, hygiene and sanitation of workers and equipment, manufacturing processes, and sources of water (Bontong et al., 2012; Raza et al., 2012).

Foodborne disease can occur due to the consumption of food or drink that has been contaminated by pathogenic organisms such as bacteria. One type of pathogenic bacteria that causes foodborne disease is Enterohemorrhagic Escherichia coli (EHEC); its main clinical symptom is bloody diarrhoea (Odumeru, 2012). Escherichia coli O157: H7 (E. coli O157: H7) is a serotype of EHEC which can cause foodborne zoonosis. Guardana et al. (2007) found this serotype in beef in Badung Regency, Bali Province.

E. coli is a Gram-negative bacterium from the Enterobacteriaceae family. The rod-shaped bacterium is motile and can ferment lactose as well as produce indole. Some strains of E. coli can dissolve blood (Quinn et al., 2011). E. coli is usually transmitted by ingestion of contaminated liquid or food, such as undercooked meat or raw milk (WHO, 2019). Research conducted by Bontong et al. (2012) states that the number of E. coli in se’i beef marketed in Kupang City has exceeded 3 MPN/g, the maximum limit required by the maximum limit of microbial contamination in food for smoked meat (SNI 7388: 2009). Research conducted by Raza et al. (2012) also stated that E. coli in se’i pork in Kupang City exceeds the maximum limit of E. coli contamination in smoked meat.

Several studies have reported that E. coli has been resistant to antibiotics. Research conducted by Melo et al. (2015) found that E. coli isolates have been resistant to tetracycline, ampicillin, trimethoprim-sulfamethoxazole, and cephalothin antibiotics in human and food samples in Salvador, Brazil. E. coli is resistant to 8 antibiotics found in animals and animal food products originating from Tunisia (Badi et al., 2018). Research conducted by Kallau (2019) stated that E. coli isolates from pig farms in Kupang City were resistant to 12 antibiotics.

Revised 06-08-2020, Accepted 25-02-2021
Research conducted on pig farms in Kupang City found the resistance of *E. coli* bacteria to antibiotic that are resistant to antibiotics and may present in se’i meat. Research on the amount of *E. coli* contamination in se’i beef and pork sold in Kupang City has been conducted, but the nature of its resistance to antibiotics has not yet been determined. Considering the high risk of disease caused by *E. coli* contamination and its resistance to antibiotics, it is necessary to research antibiotic-resistant *E. coli* in beef and pork se’i meat sold in Kupang City.

**MATERIALS AND METHODS**

The study was conducted from July 2019 to March 2020. Beef and pork se’i meat samples were obtained from meat producers in traditional markets and meat stalls in Kupang City. Se’i meat samples were taken from all 11 beef and pork se’i meat producers in Kupang City. Samples were taken from 5 beef se’i meat producers and 6 pork se’i meat producers. At least one sample with 3 replications was taken at each manufacturer. The total sample taken was 33 samples. Samples were taken within less than 1 week of each other and replications were taken after 3 days. Sampling was carried out from 09.00 to 12.00. Each sample was stored in a sterile plastic bag, labeled and transported using a cooler containing an ice pack. Samples were examined and tested at the Veterinary Public Health Laboratory (Kesmavet) of Veterinary Technical Implementation Unit, part of the East Nusa Tenggara Province Animal Husbandry Service (UPT Veteriner Disnak, NTT Province). Antibiotic resistance testing was conducted at the Veterinary Public Health Laboratory (Kesmavet), Veterinary Public Health and Epidemiology Division, Faculty of Veterinary Medicine, Bogor Agricultural University (FKH-IPB).

**Escherichia coli Isolation and Identification**

Isolation and identification of *E. coli* were carried out based on SNI 2897: 2008 concerning Testing Methods for Microbial Contamination in Meat, Eggs and Milk and Their Processed Products. The positive control used was *E. coli* American Type Culture Collection (ATCC) 25922. Beef and pork se’i meat samples were each aseptically weighed up to 25 g and stored in sterile plastic. The sample received 225 mL 0.1% buffered peptone water (BPW), which was then homogenized using a stomacher for 1 minute. It was then transferred to an Erlenmeyer for 10⁻¹ dilution to 10⁻³ dilution. A total of 1 mL from each dilution was taken and transferred to a lauryl sulfate tryptose broth (LSTB) tube containing a Durham tube and incubated at 35° C for 48±2 hours. If gas formed in the Durham tube indicated positive. Positive cultures were transferred into EC broth tubes containing Durham tubes and incubated at 45.5° C for 48±2 hours. A positive result was indicated by the presence of gas in the Durham tube. The EC broth culture, which was inoculated positively on Levine eosin methylene blue agar (L-EMBA) media, was then incubated for 18-24 hours at 35° C. Colonies suspected of being *E. coli* on L-EMBA media were 2-3 mm in diameter, black or dark at the centre of the colony, with or without shiny greenish metallic appearance. Positive *E. coli* colonies were cultured on nutrient agar (NA) media and incubated at 35° C for 18-24 hours for further biochemical tests. Biochemical tests were carried out on Indole, Methyl Red, Voges Proskauer and Citrate (IMViC) tests. The IMViC biochemical test was carried out using isolates from NA. Colonies suspected of being *E. coli* in the IMViC biochemical test showed positive result in the indole and MR tests, and negative results on the VP and citrate tests.

**Test of Escherichia coli Isolate Resistance to Antibiotics**

The antibiotic resistance of *E. coli* isolates was tested by disc diffusion method, or Kirby-Bauer, on MHA media. Colony suspensions were prepared using the broth culture method or colony suspension equivalent to 0.5 McFarland standards (1-2x10⁹ CFU/mL). The culture was spread on the surface of the MHA via a sterile cotton and left to stand for 5 minutes. Then a filter paper disc containing a standard was placed on the MHA, which had been spread with the pure culture at a distance of 25-30 mm. The cultures were then incubated at 35° C for 18-24 hours. The size of the inhibition zone formed was determined based on the standards of the Clinical and Laboratory Standards Institute. The standards describe 3 categories: susceptible (S), intermediate (I), and resistant (R) (CLSI, 2018).

**Data Analysis**

Laboratory data was analyzed descriptively and the resulting data was presented here in the form of tables and figures.

**RESULTS AND DISCUSSION**

**Escherichia coli in Se’i Meat**

The results of the isolation and identification tests for *E. coli* in meat samples found 13 isolates (39.39%) of *E. coli* from the 33 samples studied (Figure 1). The numbers of *E. coli* isolates found in se’i meat samples were 9 *E. coli* isolates from 15 beef se’i meat samples and 4 *E. coli* isolates from 18 pork se’i meat samples, respectively. The results find lower levels of *E. coli* than the research conducted by Bontong et al. (2012) and Raza et al. (2012). Bontong et al. (2012) stated that of the five samples of beef se’i meat analyzed, all sample was positive for *E. coli* (100%). Research conducted by Raza et al. (2012) also reported positive results for *E. coli* in all samples of se’i meat studied with a total of 6 samples (100%).

The results of this study indicated that se’i meat sold in Kupang City contains pathogenic *E. coli* which can cause foodborne illness. *E. coli* contamination of se’i meat can be caused by several factors including the cleanliness of the building, workers, and equipment. Inadequate workplace awareness about hygiene and sanitation in se’i meat processing also contributes to incidence of *E. coli* contamination (Cruz et al., 2018). Adu (2005) stated that microbial contamination of se’i meat can occur at every step of the handling process, starting from handling raw materials to handling se’i...
meat after smoking. Raw materials can also be a source of microbial contamination.

**Antibiotic Resistance in *Escherichia coli***

A total of 13 *E. coli* isolates were charged using the disc diffusion method to determine the level of resistance of *E. coli* to the 13 antibiotics used in this study. The results of the resistance test are presented in Figure 2.

The results of the resistance test showed that 13 *E. coli* isolates had resistance to erythromycin (100%), tetracycline (76.92%), and doxycycline (61.54%). Several *E. coli* isolates also demonstrated an increase in resistance to several antibiotics such as streptomycin (46.15%), cephalothin (38.46%), trimethoprim-sulfamethoxazole (38.46%), amoxicillin (30.77%), chloramphenicol (30.77%), and choline sulfate (30.77%). The nalidixic acid and ciprofloxacin demonstrated low resistance (7.69%). *E. coli* was still sensitive to ceftoxime and gentamicin. This is indicated by a resistance level of 0%.

The results showed that 13 *E. coli* isolates were resistant to erythromycin, tetracycline, and doxycycline. This differs from the research of Jakovele et al. (2018) whose study on frozen smoked meat in Latvia found 17 positive isolates of *E. coli* resistant to vancomycin and erythromycin as well. Rizaldi et al. (2019) found 17 samples of pork sold in the Tamiang Layang market were contaminated with *E. coli* and were resistant to erythromycin, streptomycin, penicillin G, chloramphenicol (100%), tetracycline (94.1%), ampicillin (76.5%), and acid nalidixate (23.5%). In addition, in an analysis of products sold at meat stalls in Tamaulipas, Mexico, Martinez-Vazquez et al. (2018) reported that 73 *E. coli* strains obtained from beef samples and 85 *E. coli* strains obtained from pork samples were resistant to cephalothin, ampicillin, cefotaxime, nitrofurantoin, and tetracycline antibiotics.

Erythromycin is a macrolide class of antibiotics. This antibiotic binds to the 50S ribosomal subunit to inhibit protein synthesis (Papich, 2011). Erythromycin therapy is administered to bees, poultry, cattle, goats, horses, rabbits, sheep, fish, and pigs (OIE, 2018). In general, it is bacteriostatic, but can be bactericidal if given in high doses. Erythromycin can be used to treat diseases caused by Gram-positive bacteria and some Gram-negative bacteria. Most of the strains from the Enterobacteriaceae family such as Pseudomonas, *E. coli*, Klebsiella, and others have resistance to erythromycin (Plumb, 2011). Research conducted by Kallau et al. (2018) found that *E. coli* in pigs were resistant to erythromycin, even though it was not used in pig farms but rather in poultry and humans. This shows that the incidence of erythromycin resistance can be found in the environment, humans, poultry and other livestock, including pigs. This resistance is caused by the *E. coli* ribosomal erythromycin methyltransferase gene. This gene encodes the expression of a methyl group which can inhibit erythromycin in binding to the 50S ribosomal subunit.

**Percentage of Multidrug Resistant (MDR) *E. coli* Isolated from Se’i Meat**

A total of 12 *E. coli* isolates (92.31%) isolated from *se’i* meat samples showed resistance to two or more antibiotics, as presented in Table 1. The results of the resistance test are shown in Figure 2.

![Figure 1](image1.png)  
*Figure 1. Percentage of *E. coli* contamination in se’i meat from 11 producers in Kupang City*  

![Figure 2](image2.png)  
*Figure 2. The percentage of antibiotics susceptibility of *E. coli* in se’i meat sold in Kupang City*

**Table 1. Prevalence of MDR *E. coli* and its resistance pattern in se’i meat sold in Kupang City**

| Resistance type | Number of isolates | % Isolates | Resistance pattern |
|-----------------|--------------------|------------|-------------------|
| Non-MDR resistant | 1 | 7.69 |  |
| 2 Types of antibiotics | 1 | 7.69 | KF-E |
| 3 Types of antibiotics | 1 | 7.69 | E-C-DO |
| 4 Types of antibiotics | 3 | 23.08 | E-SXT-TE-DO, E-TE-DO-CT, E-TE-DO-CT |
| 5 Types of antibiotics | 4 | 30.77 | E-C-SXT-TE-CT, KF-S-E-C-TE, AML-S-E-SXT-TE, S-E-C-TE-DO |
| 7 Types of antibiotics | 2 | 15.38 | AML-KF-S-E-SXT-TE-DO, AML-KF-S-E-SXT-TE-DO |
| 9 Types of antibiotics | 7 | 60.61 | AML-KF-S-NA-CIP-E-TE-DO-CT |

KF= Cephalothin; E= Erythromycin; C= Chloramphenicol; DO= Doxycycline; SXT= Trimethoprim-sulfamethoxazole; TE= Tetracycline; CT= Colistin sulfate; S= Streptomycin; AML= Amoxicillin; CIP= Ciprofloxacin
classes of antibiotics, which are known as multidrug resistant (MDR). The results show MDR resistance with 11 different resistance patterns (Table 1). Four isolates (30.77%) had an MDR pattern with five antibiotic classes. The highest MDR pattern was 9 antibiotic classes. Erythromycin, tetracycline, and doxycycline resistance dominated most of the MDR patterns that emerged from this study. Research conducted by Kallau et al. (2018) showed 57.3% prevalence of MDR E. coli isolates with 39 resistance patterns. Jaja et al. (2020) reported a total of 20 MDR patterns with 3-10 antibiotic classes of E. coli isolates.

Antibiotic-resistant E. coli in animal products can seriously threat public health (Rahman et al., 2017). Research conducted by Ho et al. (2011) stated that food of animal origin is the main reservoir for E. coli with MDR, to which antibiotics are important treatments.

CONCLUSION

The results show that 13 isolates (39.39%) of E. coli in se'i meat samples sold in Kupang City had high levels of resistance to three types of antibiotics: erythromycin (100%), tetracycline (76.92%), and doxycycline (61.54%). As many as 12 isolates (92.31%) had MDR. Se'i meat contaminated by E. coli with MDR or non-MDR resistance can pose a serious health threat to consumers

ACKNOWLEDGEMENT

We would like to thank the staff of the Veterinary Public Health Laboratory (Kesmavet) of the Veterinary Technical Implementation Unit of the East Nusa Tenggara Province Animal Husbandry Service (UPT Veterinter Disnak NTT Province). Thank also goes to staff of the Veterinary Public Health Laboratory (Kesmavet), Veterinary Public Health Division and Epidemiology, Bogor Agricultural University (FKH)-IPB), as well as all parties who directly or indirectly assisted with the implementation of this research.

REFERENCES

Adu, A.A. 2005. Perbedaan Tingkat Pencernaman Mikroba pada Proses Pengolahan Daging Se’i antara Perusahaan Pengolahan Daging Se’i Sapi Modern dan Tradisional di Kota Kupang. Thesis. Universitas Airlangga. Surabaya.

Adzrie, F. 2015. Antibiotic resistance of Escherichia coli isolated from beef and its related samples in Techman Municipality of Ghana. Asian. J. Anim. Sci. 9(3):233-240.

Badi, S., P. Cremonesi, M.S. Abbassi, C. Ibrahim, M. Snoussi, G. Bignoli, M. Luini, B. Castiglioni, and A. Hassen. 2018. Antibiotic resistance phenotypes and virulence-associated genes in Escherichia coli isolated from animals and animal food products in Tunisia. FEMS. Microbiol. Letters. 365(10):1-7.

Bontong, R.A., H. Mahatmi, and I.K. Suada. 2012. Kontaminasi bakteri Escherichia coli pada daging se’i sapi yang dipasarkan di Kota Kupang. Indones. Medicus. Vet. 1(5):699-711.

CLSI [Clinical and Laboratory Standards Institute]. 2018. M100S - Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI, West Valley.

Cruz, E.S.D.C., H.J.D. Lalé, and P.R. Kale. 2018. Evaluasi penerapan Hazard Analysis Critical Control Point (HACCP) pada mutu daging se’i babi di Kota Kupang. J. Petern. Indones. 20(3):201-210.

Ho, P.L., K.H. Chow, E.L. Lai, W.U. Lo, M.K. Yeung, J. Chan, P.Y. Chan, and K.Y. Yuen. 2011. Extensive dissemination of CTX-M-producing Escherichia coli with multidrug resistance to ‘critically important’ antibiotics among food animal in Hong Kong. 2008-10. J. Antimicrob. Chemother. 66:765-768.

Hutasoit, K., J.G.K. Surajana, and I.K. Suada. 2013. Kualitas daging se’i sapi di kota Kupang ditinjau dari jumlah bakteri coliform dan kadar air. Indones. Medicus. Vet. 2(3):248-260.

Jaja, I.F., J. Oguttu, C-J.J. Jaja, and E. Green. 2020. Prevalence and distribution of antimicrobial resistance determinants of Escherichia coli isolates obtained from meat in South Africa. PLoS ONE. 15(5):1-13.

Jovekole, A., V. Nikolajeva, J. Trofinova, and N. Ivanova. 2018. Serogroups, virulence genes and antibiotic resistance of Escherichia coli isolated from cold smoked meat products and sprouted grains in Latvia. Proceedings of the Latvian Academy of Sciences Section B Natural Exact and Applied Sciences. Berlin:290-297.

Kallau, N.H.G. 2019. Escherichia coli Resisten Antibiotik pada Peternakan Babi di Kota Kupang. Dissertation. Institut Pertanian Bogor. Bogor.

Kallau, N.H.G., I.W.T. Wibawan, D.W. Lukman, and M.B. Sudarwanto. 2018. Detection of multidrug-resistant (MDR) Escherichia coli and tet gene prevalence at a pig farm in Kupang, Indonesia. J. Adv. Vet. Anim. Res. 5(4):388-396.

Malelak, G.E.M., G.M. Sipahelt, and I.G.N. Jelantik. 2016. Evaluation of using Hibiscus sabdariffa calyces in the production of rottenesse smoked beef (se’i). Proceeding of International Seminar on Livestock Production and Veterinary Technology 2016. Denpasar:514-519.

Martinez-Vazquez, A.V., G. Rivera-Sanchez, K.L. Mendez, M.A. Reyes-Lopez, and V. Bocanegra-Garcia. 2018. Prevalence, antimicrobial resistance and virulence genes in Escherichia coli isolated from retail meats in Tamaulipas, Mexico. J. Glob. Antimicrob. Resist. 14:266-272.

Melo, D.B., A.P.D.O. Menezes, J.N. Reis, and A.G. Guimarães. 2015. Antimicrobial resistance and genetic diversity of Escherichia coli isolated from humans and foods. Braz. J. Microbiol. 46(4):1165-1170.

Odumuru, J.A. 2012. Microbial Safety of Food and Food Products. In Food Biochemistry and Food Processing. Simpson, B.K. (Ed.). 2nd ed. John Wiley & Sons Inc, New Jersey.

OIE [World Organisation of Animal Health]. 2018. OIE list of antimicrobial veterinary importance. https://www.oie.int/fileadmin/Home/eng/Our_scientific_experti se/docs/pdf/AMR/A_OIE_List_antimicrobials_May2018.pdf.

Papich, M.G. 2011. Saunders Handbook of Veterinary Drugs: Small and Large Animal. 3rd ed. Saunders Elsevier Inc, St. Louis.

Plumb, D.C. 2011. Plumb’s Veterinary Drug Handbook. 7th ed. Pharma Vet Inc, Stockholm.

Quinn, P.J., B.K. Markey, F.C. Leonard, E.S. FitzPatrick, S. Fanning, and P.J. Hartigan. 2011. Veterinary Microbiology and Microbial Disease. John Wiley & Sons Ltd, West Sussex.

Rahman, M.A., A.K.M.A. Rahman, M.A. Islam, and M.M. Alam. 2017. Antimicrobial resistance of Escherichia coli from milk, beef and chicken meat in Bangladesh. Bangl. J. Vet. Med. 15(2):141-146.

Raza, E.M.B., I.K. Suada, and G. Mahatmi. 2012. Beban cemaran bakteri Escherichia coli pada daging asap se’i babi yang dipasarkan di Kota Kupang. Indones. Medicus. Vet. 1(5):453-470.

Rizaldi, A., D.W. Lukman, and H. Pisestyani. 2019. Antibiotic resistance of Escherichia coli in pork sold at Tamiamg Layang Market, East Barito District. Adv. Anim. Vet. Sci. Tech. 79(9):791-797.

Suardana, I.W., B. Bumiarto, and D.W. Lukman. 2007. Isolasi dan identifikasi Escherichia coli O157:H7 pada daging sapi di Kabupaten Badung Provinsi Bali. J. Vet. 5(1):16-23.

WHO [World Health Organization]. 2019. Food Safety: Escherichia coli Infection. https://www.who.int/foodsafety/areas_work/foodborne-diseases/ecoli/en/.