Jurnal Kedokteran Hewan March 2021, 15(1):27-30
P-ISSN : 1978-225X; E-ISSN : 2502-5600 DOI: https://doi.org/10.21157/j.ked.hewan.v15i1.18204

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

Gabryella Fransina Amalo[1]*, Trioso Purnawarman[2], and Herwin Pisestyani[2]
[1]Veterinary Public Health Study Program, Postgraduate School, Bogor Agricultural University, Bogor, Indonesia
[2]Department 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

ABSTRAK

Tujuan penelitian ini adalah memperoleh isolat Escherichia coli dari 11 penjual daging se'i di Kota Kupang dan mengevaluasi resistensinya terhadap 13 jenis antibiotik. Isolasi dan identifikasi Escherichia coli dilakukan berdasarkan SNI 2897:2008. Pengujian resistensi isolat Escherichia coli terhadap antibiotik dilakukan dengan menggunakan metode Kirby-Bauer. Hasil penelitian menunjukkan bahwa sebanyak 13 isolat (39,39%) Escherichia coli pada sampel daging se'i memiliki tingkat resistansi yang tinggi terhadap eritromisin (100%), tetrasiklin (76,92%), dan doksisiklin (61,54%) serta mengalami peningkatan resisten terhadap streptomisin (46,15%), sefalotin (38,46%), trimetoprim-sulfametoksasol (38,46%), amoksisilin (30,77%), kloramfenikol (30,77%), dan kolistin sulfat (30,77%). Antibiotik asam nalidiksat dan siprofloksasin menunjukkan hasil resistan Escherichia coli yang rendah sebesar 7,69%. Escherichia coli sensitif terhadap sefotaksim dan gentamisin. Sebanyak 12 isolat (92,31%) mengalami MDR. Keberadan Escherichia coli resistan non MDR maupun MDR dalam daging se'i dapat menimbulkan ancaman kesehatan bagi masyarakat.

Kata kunci: resistensi antibiotik, Escherichia coli, multidrug resistance, daging se'i

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 (Malelak *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 *Enterohaemorrhagic 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. Suardana *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 ($1\text{-}2x10^8$ 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 *E. coli* resistance (7.69%). However, *E. coli* was still sensitive to cefotaxime 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 *E. coli* samples resistant against tetracyclines, ampicillin, and sulfamethoxazole. *E. coli* can contaminate every step in the food chain, from the production process to sales or serving. Research conducted by Adzitey (2015) on contaminated beef in Ghana showed that *E. coli* isolates have resistance 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, Martınez-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 methylation 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

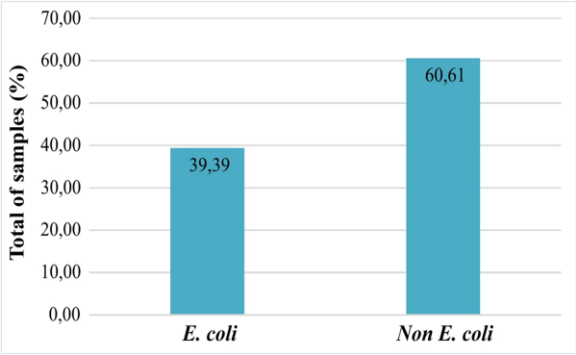

Figure 1. Percentage of *E.* coli contamination in *se'i* meat from 11 producers in Kupang City

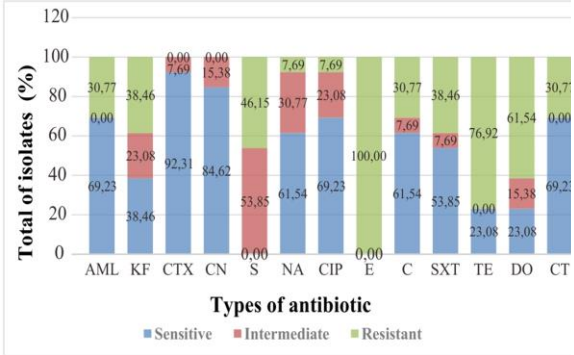

Figure 2. The percentage of antibiotics susceptibility of *E.* coli in *se'i* meat sold in Kupang City

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	1	7.69	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

ACKNOWLEGEMENT

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.

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