



Occurrence and Antibiotic Resistance of *Escherichia coli* in Street-Vended Kebab in Dramaga, Bogor, Indonesia

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HIGHLIGHTS

- The mean Total Plate Count (TPC) in kebab samples was 5.3 log₁₀ Colony Forming Unit (CFU)/g.
- Based on TPC, 13 out of 43 (30.2%) of kebab samples did not comply with the Indonesia National Standard.
- *Escherichia coli* was identified in 5 out of 43 samples (11.6%) with mean of 39.2 Most Probable Number (MPN)/g.
- Four out of 5 *E. coli* isolates were resistant to gentamycin; and all of them were susceptible to amoxicillin/clavulanate and chloramphenicol.

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Acronyms and abbreviations

CFU=Colony Forming Unit
MDR=Multi Drug Resistant
MPN=Most Probable Number
TPC=Total Plate Count

ABSTRACT

Background: Kebab is one of the popular ready-to-eat foods in Indonesia and sold as a street food. The purpose of this study was to determine occurrence and antibiotic resistance of *Escherichia coli* in street-vended kebab in Dramaga, Bogor, Indonesia.

Methods: Totally, 43 samples of kebab meat were collected from street food kebab vendors. Examinations on Total Plate Count (TPC), and total *E. coli* using Most Probable Number (MPN) method were referred to the Indonesia National Standard. The antibiotic resistance test was carried out using the Kirby-Bauer disk diffusion method.

Results: The mean of TPC in kebab samples was 5.3 log₁₀ Colony Forming Unit (CFU)/g. Based on TPC, 13 out of 43 (30.2%) of kebab samples did not comply with the Indonesia National Standard with maximum acceptable level of 5 log₁₀ CFU/g. *E. coli* was identified in 5 out of 43 samples (11.6%) with mean of 39.2 MPN/g ranged from 7.4 to 150 MPN/g which were higher than standard level (0.5 log₁₀ CFU/g). Four out of 5 *E. coli* isolated from kebab samples were resistant to gentamycin. All *E. coli* isolates were susceptible to amoxicillin/clavulanate and chloramphenicol.

Conclusion: The occurrence of antibiotic resistant *E. coli* in ready-to-eat kebab in this area of Indonesia could cause the health problem in consumers. The local government should conduct the monitoring and surveillance on the occurrence of pathogens and the antibiotic resistance in food chain.

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Introduction

Kebab or Döner kebab is a traditional Middle Eastern meat product that has different names, e.g., Gyro, Donair, Dona kebab, and Chawarma. This ready-to-eat food is

very popular in Europe, Asia, and many parts of the world today. Kebab can be produced using marinating lamb, veal, beef, chicken, ostrich or turkey meat, roasted

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vertically on heating element. During cooking, the cooked part is cut away with a long-bladed or circular knife, put into bread, and added with sliced tomatoes, onions, lettuce, and yogurt (Bonilauri et al., 2018; Ergönül et al., 2012; Kaya et al., 2018; Liuzzo et al., 2016).

Detection of food-borne pathogens in doner kebabs has been reported such as *Salmonella* (Elmali et al., 2005; Kaya et al., 2018; Nemati et al., 2008; Wimalasekara and Gunasena, 2016), *Escherichia coli* (Agbodaze et al., 2005; Amani et al., 2017; Bonilauri et al., 2018; Elmali et al., 2005; Kaya et al., 2018; Ulukanli et al., 2006), *Listeria monocytogenes* (Bonilauri et al., 2018; Haskaraca and Kolsarici, 2019; Kaya et al., 2018), *Clostridium perfringens* (Elmali et al., 2005; Ergönül et al., 2012; Haskaraca and Kolsarici, 2019; Kayisoglu et al., 2003; Lopašovský et al., 2016), and *Staphylococcus aureus* (Elmali et al., 2005; Ergönül et al., 2012; Haskaraca and Kolsarici, 2019). Elmali et al. (2005) found 54% of cooked beef doner kebabs sold in restaurants in the City of Kars, Turkey contaminated with *E. coli*. Furthermore, Amani et al. (2017) reported that 33.3% of beef Shawarma sold in Ashmoun City, Menofia Governorate, Egypt were positive for *E. coli* serotypes O111:H2, O91:H21, O113:H7, and O86.

Some studies reported the Total Plate Count (TPC) in doner kebabs. Elmali et al. (2005) found 56% of cooked doner kebabs containing TPC between 10^4 and 10^6 Colony Forming Unit (CFU)/g. Total bacteria were reported by Nemati et al. (2008) in cooked kebab sold in Tabriz, Iran in range from 3.22 to 6.75 \log_{10} CFU/g. Kebab and Shawarma sold in Colombo City, Sri Lanka, contained total bacteria in range from 4.34 to 8.53 \log_{10} CFU/g and from 4.15 to 4.48 \log_{10} CFU/g, respectively, and total *E. coli* in kebab in range from 2.66 to >3.04 Most Probable Number (MPN)/g (Wimalasekara and Gunasena, 2016).

Antibiotic resistance is increasing to dangerous level in the world since it is emerging and spreading globally which cause the threat to the treatment of infectious diseases. The emergence and spread of antibiotic resistance can be increased with the improper use or misuse of antibiotics in human and animals (WHO, 2020). *E. coli* is one of the bacteria that is mostly used by researchers to determine the antibiotic resistance since *E. coli* exists in human and animal intestinal tract and in environment. Some studies reported the antibiotic resistance in *E. coli* isolated from meat (Adzitey, 2020; Dsani et al., 2020; González-Gutiérrez et al., 2020), and retail foods (Li et al., 2020), nevertheless it is still a lack of studies on antibiotic resistance in *E. coli* isolated from street-vended kebab. Amani et al. (2017) found the *E. coli* isolated from beef shawarma resistant to amoxicillin/clavulanic acid, streptomycin, norfloxacin, and doxycycline. The purpose of this study was to determine the occurrence

and antibiotic resistance of *E. coli* in street-vended kebab in Dramaga, Bogor, Indonesia.

Materials and methods

Material

The materials used in this research included 0.1% Buffered Peptone Water (BPW; Oxoid M1049, England), Plate Count Agar (PCA; Oxoid CM0325, England), Lauryl Tryptose Broth/Lauryl Sulphate Broth (LTB/LSB; Oxoid CM0451, England), *Escherichia coli* broth (EC; Oxoid CM0853, England), Eosin Methylene Blue agar (Levine) (L-EMB; Oxoid CM0069, England), Methyl Red-Voges Proskauer broth (MR-VP; Oxoid CM0043, England), SIM Medium (Oxoid CM0435, England), Koser Citrate broth (Conda Cat. 1200.00, Spain), Nutrient Agar (NA; Oxoid CM0003, England), Kovacs Indole Reagent (Merck 1.09293.0100, Germany), KOH 40%, Mueller Hinton broth (Oxoid CM0129, England), *E. coli* American Type Culture Collection (ATCC) 25922 (Thermo Scientific™, USA), antibiotic disks (8 antibiotics), i.e., amoxicillin/clavulanate (AMC) 30 μ g (Oxoid AMC 30 CT0223B, England), cefotaxime (CTX) 30 μ g (Oxoid CTX 30 CT0166B, England), gentamycin (CN) 10 μ g (Oxoid CN 10 CT0024B, England), trimethoprim/sulfamethoxazole (SXT) 25 μ g (Oxoid SXT 25 CT0052B, England), ciprofloxacin (CIP) 5 μ g (Oxoid CIP 5 CT0425B, England), enrofloxacin (ENR) 5 μ g (Oxoid ENR 5 CT0639B, England), oxytetracycline (OT) 30 μ g (Oxoid OT 30 CT0041B, England), as well as chloramphenicol (CL) 30 μ g (Oxoid CL 30 CT0013B, England).

Samples

During August to September 2019, 43 samples of kebab meat were randomly collected from all street food kebab vendors in Dramaga, Bogor, Indonesia. There were totally 9 vendors and from every vendor was taken 4-5 samples. Since the kebab vendors opened in the evening, the samples were taken at 7.00-9.00 p.m. (peak hours for dinner). The samples of kebab consisted of bread and meat without sauces and vegetables. Then, the samples were put inside the sterile plastic bags, and stored in the cool box with temperature of below 7 °C and immediately transported to the Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, IPB University for examination on TPC (CFU/g), enumeration of *E. coli* (MPN/g), and also identification of antibiotic resistance against 8 different antibiotics, i.e., amoxicillin/clavulanate, cefotaxime, gentamycin, trimethoprim/sulfamethoxazole, ciprofloxacin, enrofloxacin, oxytetracycline, and chloramphenicol.

TPC, Enumeration, isolation and identification of *E. coli*

In this study, the examination of TPC, enumeration, isolation, and identification of *E. coli* was referred to the Indonesia National Standard concerning the guideline for laboratory analysis on examination of microbial contamination in meat, egg, as well as milk (BSN, 2008). The TPC was carried out using plate count method and the enumeration of *E. coli* was conducted using MPN method with 3 tubes. From the cultured bacteria in tubes, *E. coli* was isolated and identified using indole, methyl red, Voges-Proskauer, and Citrate (IMViC). Quality control of each test was conducted using *E. coli* ATCC 25922.

The sample of kebab meat was weighed to 25 g and put into 225 ml of 0.1% BPW and homogenized using stomacher for 1 min. The homogenized samples were made decimal dilution of 10^{-2} and 10^{-3} by transferring 1 ml previous dilution into 9 ml of BPW. From every dilution, 1 ml was transferred to petri dish. Then, 12-15 ml PCA (45 ± 1 °C) was poured into petri dish, mixed thoroughly, and let the agar solidify. The petri dishes were incubated at 35 °C for 48 h.

For enumeration of *E. coli*, 1 ml of dilution was transferred into Lauryl Tryptose Broth/Lauryl Sulphate Broth and incubated at 35 °C for 24-48 h for MPN presumptive test. The positive results, which were shown as gas formed in the Durham tube, were transferred into *E. coli* (EC) broth and incubated at 45.5 °C for 48 h for MPN-confirmed test. Positive results in EC broth then inoculated to selective medium Levine Eosin Methylene Blue agar (LEMB) and incubated at 35 °C for 18-24 h. The expected *E. coli* colony was shown as black/dark color, while the center of the colony was metallic green. Suspicious colonies from each LEMB plate inoculated to nutrient agar slant and incubated for 18-24 h at 35 °C for further test. The biochemical tests of indole, methyl red, Voges-Proskauer, and citrate (IMViC) were carried out for confirmation.

Antibiotic susceptibility testing

All *E. coli* colonies isolated from MPN were subjected to antibiotic susceptibility testing that was referred to standard on Clinical Laboratory Standard Institute (CLSI, 2018) using the Kirby-Bauer disk diffusion method. Antibiotic susceptibility of *E. coli* isolates was tested against 8 antibiotic disks, i.e., amoxicillin/clavulanate (AMC) 30 µg, cefotaxime (CTX) 30 µg, gentamycin (CN) 10 µg, trimethoprim/sulfamethoxazole (SXT) 25 µg, ciprofloxacin (CIP) 5 µg, enrofloxacin (ENR) 5 µg, oxytetracycline (OT) 30 µg, and chloramphenicol (CL) 30 µg. The bacterial suspension equivalent to 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml) was prepared. The culture of *E. coli* was spread on the surface of Mueller Hinton Agar

(MHA) using a sterile cotton swab and left for ± 5 min. The paper disk contained specific antibiotic was put on MHA, which had been previously spread by the pure culture at a distance more than 24 mm. The cultured bacteria were incubated at 35 °C for 18-24 h. The categories of susceptible, intermediate, and resistant were based on the size of the inhibition zone formed according to the standard of CLSI (2018). A blank disk without antibiotic was used as a negative control for each test. *E. coli* ATCC 25922 was used for quality control of antibiotic disks.

Results

The mean of TPC in kebab samples was $5.3 \log_{10}$ CFU/g. Based on TPC, 13 out of 43 (30.2%) of kebab samples did not comply with the Indonesia National Standard with maximum acceptable level of $5 \log_{10}$ CFU/g (BSN, 2009). *E. coli* was identified in 5 out of 43 samples (11.6%) with the mean of 39.2 MPN/g ranged from 7.4 to 150 MPN/g which were higher than standard level ($0.5 \log_{10}$ CFU/g).

Four out of 5 *E. coli* isolated from kebab samples were resistant to gentamycin. All of *E. coli* isolates were susceptible to amoxicillin/clavulanate and chloramphenicol. The study found the multi-drugs resistance occurring in only 1 isolate, i.e., resistant to gentamycin-trimethoprim/sulfamethoxazole-ciprofloxacin-oxytetracycline (Table 1).

Discussion

The mean of TPC in street-vended kebab in this study was $5.3 \log_{10}$ CFU/g which was higher than TPC in Bonab kebab sold in Tabriz market, Iran (Nemati et al., 2008), in beef döner manufactured in Izmir, Turkey (Ergönül et al., 2012), beef kebab in Italy (Bonilauri et al., 2018), beef kebab sold in Malang, Indonesia (Adiyastiti et al., 2019) which had TPC below $5.0 \log_{10}$ CFU/g, nevertheless this TPC compared favorably with the study of Kayisoglu et al. (2003) which found TPC in beef doner kebabs sold in fast food markets in Terkidag, Turkey, i.e., $5.59 \log_{10}$ CFU/g for raw beef kebabs and $4.99 \log_{10}$ CFU/g for cooked beef kebabs. Adiyastiti et al. (2019) found mean total bacteria count in kebab sold in Malang, Indonesia 2.2×10^5 CFU/g in day time and 44.3×10^5 CFU/g in the evening. The total *E. coli* in kebab sold in the market in Italy were $1.0 \log_{10}$ CFU/g (Bonilauri et al., 2018).

The contamination in kebab and meat products is generally related to lack of hygiene practices (Haskaraca and Kolsarici, 2019; Kaya et al., 2018; Kwiri et al., 2014), poor sanitary environment (Albarri et al., 2017), and

Table 1: Number of *E. coli* isolated from kebab meat sold in Dramaga, Bogor based on their resistance to antibiotics*

Resistance level	AMC	CTX	CN	SXT	CIP	ENR	OT	CL
Resistant	0/5	1/5	4/5	2/5	2/5	1/5	1/5	0/5
Intermediate	0/5	3/5	0/5	0/5	0/5	4/5	0/5	0/5
Susceptible	5/5	1/5	1/5	3/5	3/5	0/5	4/5	0/5

* Total number of *E. coli* isolates is 5.

AMC=amoxicillin/clavulanat; CTX=cefotaxime; CN=gentamycin; SXT=trimethoprim/sulfamethoxazole; CIP=ciprofloxacin;

ENR=enrofloxacin; OT=oxytetracyclin; CL=chloramphenicol

inappropriate temperature of cooking and storage (Haskaraca and Kolsarici, 2019). Our observations on the kebab preparation in vendors in Dramaga, Bogor, Indonesia showed that particular vendors had cut away the meat and put in a container stored in room temperature until it was stuffed into the bread. WHO (1996) has set the essential food safety requirements for street-vended food. It is recommended to keep the prepared foods served hot at a temperature of at least 50 °C to prevent microbial growth.

Similar to our findings, Lopašovský et al. (2016) found 60% satisfactory samples of beef kebab taken from fast food establishment in Nitra Region, Slovakia based on microbiological examination; nevertheless the result of this survey was much better than study of Choiriyah et al. (2016) that 25% of cooked kebab samples sold in Semarang, Indonesia were satisfactory based on TPC. The high bacterial contamination in kebab in Semarang City was related to inappropriate cooking temperature (82.5%), lack storage conditions (50.0%), unhygienic equipment (22.5%), and poor personal hygiene (17.5%) (Choiriyah et al., 2016).

E. coli in kebab sold in Dramaga, Bogor was identified in 5 samples (11.6%) and the occurrence was lower than the findings of Elmali et al. (2005), Ziino et al. (2013), and Amani et al. (2017), i.e., 54, 13.6, and 33.3%, respectively. Ziino et al. (2013) recorded the number of *E. coli* in cooked döner kebab retailed in Palermo and Messina, Italy from <1.0 to 6.18 log₁₀ CFU/g. According to Haskaraca and Kolsarici (2019), döner kebab is considered microbiologically safe for consumption after cooking; nevertheless the contamination with pathogens such as *E. coli* may occur after cooking due to low microbiological quality, poor hygiene, and sanitation at production or preparation of kebab. The bacteria in döner kebab can survive during cooking process or bacteria contaminating döner kebab can grow and reach the level that may cause food-borne disease.

The isolates of *E. coli* from street-vended kebab in Dramaga, Bogor were resistant to five group of antibiotics, i.e., gentamycin (4/5), trimethoprim/

sulfamethoxazole and ciprofloxacin (2/5), and cefotaxime, and oxytetracycline (1/5), nevertheless the isolates were still susceptible to three kinds of antibiotics (amoxicillin/clavulanate, enrofloxacin, and chloramphenicol). The study on antibiotic resistant *E. coli* isolated from the environment of ruminant slaughterhouse in Bogor, Indonesia reported that *E. coli* was resistant to gentamycin (60.0%), trimethoprim/sulfamethoxazole (60.0%), tetracycline (40.0%), ciprofloxacin (40.0%), and enrofloxacin (20.0%) (Sudarwanto et al., 2017). Dsani et al. (2020) found *E. coli* isolated from raw meat in Greater Accra region, Ghana have resistance to cefotaxime (98%), chloramphenicol (97%), gentamycin (97%), ciprofloxacin (92%).

E. coli isolated from kebab meat samples in the current research was resistant to gentamycin (4/5) whereas this occurrence was the highest in this study. This finding was comparable to the investigation of Badi et al. (2018) which reported *E. coli* isolated from healthy animals and animal products from raw meat samples from selected slaughterhouses showing resistant to gentamycin. Gentamycin resistance in *E. coli* isolated from the feces samples of healthy animals and food products of animal origin taken from different Tunisian regions has been increasing.

Davis et al. (2018) also found the highest prevalence of resistance against gentamycin in *E. coli* isolated from chicken sold in stores in Flagstaff, Arizona, America. Based on the study of Bakhshi et al. (2017), among 100 *E. coli* isolated from chicken samples farmed in Yazd, Iran, the highest antibiotic resistance was found against nalidixic acid (100%), enrofloxacin (87%), ciprofloxacin (86%), and erythromycin (82%), respectively.

The resistance of *E. coli* against trimethoprim/sulfamethoxazole and ciprofloxacin in this study were 2 out of 5 samples. Also, Badi et al. (2018) reported that 36.9% of *E. coli* isolated from the feces and food products of animal origin were resistant to trimethoprim/sulfamethoxazole. In addition, Messele et al. (2017) showed 21.1% of *E. coli* isolated from raw meat from abattoir in Addis Ababa City and

Bishoftu Town, Ethiopia was resistant to ciprofloxacin. In the contrary, other researchers found that *E. coli* was still susceptible to trimethoprim/sulfamethoxazole and ciprofloxacin (Adzitey, 2020; Dsani et al., 2020).

The Multi-Drugs Resistance (MDR) was found in only 1 isolate (20.0%), i.e., resistant to gentamycin-trimethoprim/sulfamethoxazole-ciprofloxacin-oxytetracycline (CN-SXT-CIP-OT). Some studies reported the occurrence of MDR in *E. coli* higher, such as 46.0% in meat sample of different livestock species in Addis Ababa City and Bishoftu Town, Ethiopia (Messele et al., 2017) and 22.0% in meat samples from slaughterhouse in the Greater Accra Region of Ghana (Dsani et al., 2020). Genes of drug resistance carried by *E. coli* can be transmitted to other bacteria, and, due to the excessive use of antibiotics, selection pressure is very high, resulting in bacterial strains resistant to a variety of antibiotics. MDR strains are characterized by the presence of multiple genes conferring drug resistance, which results in resistance to many different antibiotics (Li et al., 2020). The increasing occurrence of MDR in *E. coli* in human and veterinary medicine has become an alarming issue (Poirel et al., 2018). MDR can increase failure on treatment of infectious diseases (Ramírez-Castillo et al., 2018). The occurrence of antibiotic resistant *E. coli* in ready-to-eat foods could cause health problem in the consumers.

Conclusion

The occurrence of antibiotic resistant *E. coli* in ready-to-eat kebab in this area of Indonesia could cause the health problem in consumers. The local government should conduct the monitoring and surveillance on the occurrence of pathogens and the antibiotic resistance in food chain.

Author contributions

D.W.L., D.Y.S., and H.P. designed the study, analyzed the data and wrote the manuscript. D.Y.S. conducted the experimental work. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest.

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