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FULL PAPER

Prevalence of multidrug-resistant *Escherichia coli* isolated from *Ocimum basilicum* sold at the traditional market in Indonesia

*Suswati, E., Nurdian, Y., Mufida, D.C., Raharjo, A.M., Putri, E.R.M. and Haq, H.S.

Medical Faculty, University of Jember, East Java, Indonesia

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Abstract

Ocimum basilicum, also known as basil, is commonly consumed uncooked and can spread foodborne infections. *Escherichia coli* is one of the germs that can cause food poisoning. Antibiotic resistance has previously been linked to these microorganisms. This study aimed to analyze *E. coli* resistance in basil sold in traditional markets in Jember. The basil used in the study was acquired at eight local markets in Jember. Isolation, identification of bacteria, and antibiotic susceptibility tests from basil were carried out in the microbiology laboratory of the medical faculty of the University of Jember. This investigation included 40 basil vendor samples. Isolation and identification test used Eosin Methylene Blue agar. The resistance of *E. coli* to Amoxicillin, Ciprofloxacin, Ceftriaxone, Cefixime, Ampicillin, Azithromycin, Cefotaxime, and Cotrimoxazole was determined using the Kirby Bauer diffusion method. According to this analysis, all basil samples were dangerous for direct consumption, with the highest incidence of *E. coli* resistance to Amoxicillin and Cefixime antibiotics and the lowest prevalence of Ciprofloxacin resistance. Multi-drug resistant (MDR) *E. coli* strains were found in most basil *E. coli* isolates. Good agricultural practices on farms and sterilizing fresh vegetables before eating are highly suggested to avoid potential public health hazards.

1. Introduction

Foodborne disease is an infectious or toxic disease that enters the body due to food contamination by bacteria, parasites, and other toxic compounds (Kim *et al.*, 2018). Bacteria cause most cases of foodborne diseases. Symptoms of the disease are dominated by gastrointestinal disorders such as vomiting, nausea, abdominal pain, and diarrhoea. According to Kirk *et al.* (2015), more than 500 million people have been exposed to foodborne illnesses. More than 1 million individuals died because of these cases around the world, with children accounting for more than a third of those who perished. Foodborne sickness affects 1.9 million people each year in underdeveloped nations, according to the World Health Organization (WHO 2015). Food ingredients like vegetables, especially ready-to-eat meals like fresh vegetables, can be a source of foodborne disease transmission. Basil is one of the fresh vegetables that is commonly consumed and served raw in Indonesia. Bunsal (2015) study on the identification of *E. coli* in basil discovered that all basil samples tested positive for *E. coli*, with levels exceeding the BPOM maximum limit.

It should be noted that fresh vegetables provide nutritious materials such as vitamins and micronutrients (Wijaya *et al.*, 2021a; Wijaya *et al.*, 2021b) and a potential source of income (Mariyono, 2019; Mariyono, 2020). However, consuming fresh vegetables might have unexpected impacts resulting from agrochemical residue (Mariyono, 2008) and infectious microorganisms. Thus, vegetables' health and economic benefits will be more pronounced when the materials are free of harmful contaminants of microorganisms and agrochemicals.

Cases of foodborne disease due to bacterial contamination increment have also increased the administration of antibiotic drugs. One problematic issue of healthcare management regarding antibiotics usage is the occurrence of cases of antibiotic resistance. A previous study by Martha in 2014 concluded that *E. coli* bacteria culture from patients' faecal samples had various levels of antibiotic resistance, specifically Ampicillin by 100%, Amoxicillin by 77.42%, Cefixime 67.74%, Cefotaxime 54, 84%, Ceftriaxone 51.65%, Ciprofloxacin 74.19% Cotrimoxazole 70.97%. Another research by Endriani *et al.* (2012) found that 80% of *E. coli* from the samples tested were resistant to Azithromycin.

*Corresponding author.
Email: *ennysuswati.fk@unej.ac.id*

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Research on the identification of *E. coli* in basil has been carried out by Nissa (2017) who pointed out that all samples of basil in the city of Semarang were contaminated with *E. coli* and were resistant to Ampicillin. Vegetables, such as basil, are essential sources of resistant foodborne pathogens in Indonesia and should not be disregarded. In *E. coli*, there is no information on antibiotic resistance. As a result, for the first time, this study examined the prevalence and distribution of antibiotic-resistant *E. coli* in basil samples from Jember, Indonesia.

2. Materials and methods

2.1 Sample collection

The Ethics Committee approved this study of Medical Faculty at the University of Jember with letter number: 1447/H25.1.11/KE/2020. This laboratory research was conducted in the Microbiology Laboratory of the Faculty of Medicine at the University of Jember between January and March 2021. This study was started by getting basil from several vendors at eight different markets in Jember: Kepatihan, Tanjung, Gebang, Arjasa, Pelita, Kreongan, Mangli, and Sabtuan. These markets were chosen because they are the most important traditional markets in the Jember district and where customers can quickly obtain basil. A total of forty basil samples were collected at random from seven markets in Jember. A total of five basils were collected from each market. Five basil merchants are chosen and purchased from each market. The basil samples are then placed in sterile plastic, labelled, and transported to the laboratory to be processed. The time it took to collect and analyze a sample was less than two hours. After being collected, the samples had no more washing processes. The analysis began as soon as the sample collection for each market was completed.

2.2 Identification of Escherichia coli

Basil samples were kept in a more excellent box at 4 -6°C until they were sent to the Microbiology Laboratory of the Faculty of Medicine, University of Jember. After safely arriving, the basil samples were removed from the more excellent box, and the polythene sacs were sterilized. The samples were then weighed at 25 g. The samples were placed in a beaker glass filled with 225 mL of sterile equates and left to soak for 15 mins. Next, the basil sample was separated from equates by shaking the beaker glass vigorously. The left-over water from the basil whisk is referred to as basil rinse water. The Most Probable Number (MPN) test, which includes a presumptive test with lactose broth, a complete test with eosin methylene blue (EMB) media, and Gram staining, was then used to identify *Escherichia coli* bacteria using basil rinse water.

Lactose broth was used as a presumptive test media to confirm the presence of coliform bacteria. A change in colour haziness of the media and the appearance of gas in the Durham tube showed the presence of coliform bacteria. EMB is a gram-negative selective medium. This media was used as a comprehensive test to identify E. coli bacteria. The appearance of a metallic sheen/ green tint in this media is a hallmark of *E. coli*.

2.3 Antimicrobial susceptibility testing

This study used a test on medium Mueller-Hilton agar with the diffusion method Kirby-Bauer and using the standard 0.5 Mac Farland. The disc diffusion test was used to determine antimicrobial resistance in all *E. coli*. Isolates, using the following antibiotic discs (B.D.): Amoxicillin (Aml 20/10 µg), Ampicillin (Amp 10 µg), ceftriaxone (Cro. 30 µg), Cefixime (Cfm 30 µg), Cefotaxime (Ctx 30 µg), Ciprofloxacin (Cip 5 µg), Azithromycin (Azm 15 µg), dan sulfamethoxazole/ trimethoprim (23.75/1.25 µg). Following the Clinical and Laboratory Standards Institute guidelines, antibiotic resistance was interpreted through inhibitory zone diameter measurement formed in the study (Clinical and Laboratory Standard Institute (CLSI), 2017). MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial groups.

3. Results

From forty basil samples gathered from the eight marketplaces (Kepatihan, Tanjung, Gebang, Kreongan, Pelita, Arjasa Mangli, and Sabtuan), thirty-three *E. coli* colony samples were obtained (Table 1). Kepatihan (12.5%), Tanjung (12.5%), Pelita (12.5%), Sabtuan (12.5%), Kreongan (10%), Gebang (7.5%), Mangli (7.5%), and Arjasa (7.5%) were among the marketplaces that were impacted (7.5%). The levels of resistance to eight medicines detected in all bacterial samples are shown in Table 2.

Table 1. Prevalence of *Escherichia coli* isolated from basil in Jember Traditional Market

Market	*Escherichia coli*			
	Negative		Positive	
	n	%	n	%
Kepatihan	0	0.00%	5	12.50%
Tanjung	0	0.00%	5	12.50%
Kreongan	1	2.50%	4	10.00%
Gebang	2	5.00%	3	7.50%
Pelita	0	0.00%	5	12.50%
Arjasa	2	5.00%	3	7.50%
Mangli	2	5.00%	3	7.50%
Sabtuan	0	0.00%	5	12.50%
Total	7	17.50%	33	82.50%

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Table 2. Percentage of antibiotic resistance of *Escherichia coli* isolated from basil sold in Jember Traditional market Indonesia

Antibiotics	*Escherichia coli*					
	Sensitive		Intermediate		Resistant	
N:33	N	%	N	%	N	%
Amoxicillin (Aml)	2	6.06%	1	2.50%	30	90.91%
Ciprofloxacin (Cip)	2	6.06%	21	52.50%	10	30.30%
Ceftriaxone (Cro)	2	6.06%	2	5.00%	29	87.88%
Cefixime (Cfm)	1	3.03%	1	2.50%	31	93.94%
Ampicillin (Amp)	0	0.00%	5	12.50%	28	84.85%
Azithromycin (Azm)	19	57.58%	0	0.00%	14	42.42%
Cefotaxime (Ctx)	1	3.03%	3	7.50%	29	87.88%
Sulphamethoxazole (Stx)	12	36.36%	2	5.00%	19	57.58%

Table 3. Prevalence of MDR *Escherichia coli* from basil sold in Jember Traditional market, Indonesia

Market	Pattern of MDR	MDR	Percentage
Kepatihan, n = 5	Aml, Amp, Cfm, Ctx, Cro, Azm Aml, Amp, Cfm, Ctx, Cro, Stx Aml, Amp, Cfm, Ctx, Cro, Stx Aml, Amp, Cfm, Ctx, Cro, Cip, Azm, Stx Aml, Amp, Cfm, Ctx, Cro, Cip, Azm, Stx	5	15.20%
Tanjung, n = 5	Aml, Amp, Cfm, Ctx, Azm Aml, Amp, Cfm, Ctx, Cro, Stx Aml, Amp, Cfm, Ctx, Cro, Cip, Stx Aml, Amp, Ctx, Cro, Cip, Azm, Stx Aml, Amp, Cfm, Ctx, Cro, Cip, Azm, Stx	5	15.20%
Kreongan, n = 5	Aml, Amp, Cfm, Ctx, Cro Azm, Stx Aml, Amp, Cfm, Ctx, Cro, Stx Aml, Amp, Cfm, Ctx, Cro, Cip, Azm, Stx	3	9.10%
Gebang n = 5	Aml, Amp, Cfm, Ctx, Cro, Cip, Azm, Stx	1	3.00%
Pelita, n = 5	Aml, Amp, Cfm, Ctx, Cro, Azm, Stx Aml, Amp, Cfm, Ctx, Cro, Stx Amp, Cfm, Ctx, Cro, Azm Aml, Amp, Cfm, Ctx, Cro, Azm	4	12.10%
Arjasa, n = 5	Amp, Cfm, Ctx, Cro, Cip	1	3.00%
Mangli, n = 5	Aml, Amp, Cfm, Azm Aml, Amp, Cfm, Ctx, Cro, Cip	2	6.10%
Sabtuan, n = 5	Aml, Cfm, Cro, Stx Aml, Cfm, Cro, Stx Aml, Cfm, Ctx, Cro, Azm, Stx	3	9.10%
Total		24	72.70%

Cefixime resistance was found in the greatest number of *E. coli* (31/33, 93.94%), followed by Amoxicillin resistance (30/33, 90.91%), Cefotaxime and Ceftriaxone resistance (29/33, 87.88%), Ampicillin resistance (28/33, 84.85%), sulfamethoxazole/trimethoprim resistance (19/33, 57.68%), Azithromycin resistance (10/33, 42%), Ciprofloxacin (30.30%).

Multidrug resistance was found in twenty-four *E. coli* isolates (72.70%), which means they were resistant to at least three antimicrobial drugs (Table 3). The presence of MDR *E. coli* indicates a high-risk source of contamination. The MDR of *E. coli* isolates from Tanjung and Kepatihan marketplaces were the highest. The strain was resistant to eight antimicrobials, making it resistant to the antimicrobial classes of penicillin, macrolide quinolone, cephalosporin, and sulfonamide.

4. Discussion

Escherichia coli is a common inhabitant of humans and other warm-blooded animals' gastrointestinal tracts; nevertheless, it can also be found outside the host, in a transitory form, and can contaminate water and soil (Hesperia *et al*., 2018). *Escherichia coli* was detected in 33 of the 40 basil samples, as shown in Table 1. This

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study's findings are consistent with those of Bansal Tindry's (2015) study, which reported that *E. coli* in basil found in 8 food booths in Manado was 100% positive for *E. coli*. In 96.7% of samples, Sari *et al*. (2019) revealed that all basil samples containing the coliform bacteria had values ranging from 210 MPN/g to > 1100 MPN/g. Other vegetables, such as lettuce cultivated on California farms, were contaminated with *E. coli* bacteria (Jeamsripong *et al*., 2019). Pre-harvest and post-harvest *E. coli* contamination in vegetables can be generated by a variety of events and phases.

Soil, irrigation water, improperly composted fertilizer, and human handling are preharvest variables that can lead to bacterial infection. Contaminated water used for washing, transporting trucks, processing equipment, dirty utensils, inadequate hand hygiene, and cross-contamination are post-harvest issues that can lead to bacterial infection (Luna-Guevara *et al.*, 2019). There were five fundamental procedures to prevent *E. coli* and other enteropathogens from contaminating food: (1) separating raw and cooked meals, (2) keeping the work environment clean, (3) thoroughly cooking the food, (4) maintaining safe temperatures for the food, and (5) using safe water and raw materials (Thapa, Shrestha, and Anal 2020).

In this research sample, the resistance test yielded different findings in each group. The β-lactam group was separated into two groups in this investigation, specifically penicillin and cephalosporin. In this investigation, antibiotic resistance to Amoxicillin was found to be 90.91% in the penicillin group and 84.85% in the β-lactam group. According to Shakerian *et al*. (2016)'s research in Iran, all basil samples carrying *E. coli* were resistant to Amoxicillin. Adzitey (2018) found that the antibiotic Ampicillin had a resistance rate of 90.91% in a comparative investigation of another food, namely cabbage in Ghana. However, this study differs from one conducted by (Al-Kharousi *et al*., 2019) on vegetables sold in traditional markets, specifically carrots, cucumbers, tomatoes, and lettuce, in which *E. coli* from the sample had 13% resistance to Ampicillin and 0% resistance to Amoxicillin. The resistance to antibiotics Cefixime, Cefotaxime, and Ceftriaxone was 90.91%, Cefotaxime was 87.88%, and Ceftriaxone was 87.88% in this study's β-lactam antibiotic test. This study's findings are identical to those of Kim *et al*. (2015), who used a sample of sprouts with a Cefotaxime resistance rate of 100%. On the other hand, the earlier study was not the same as the one by Aabed *et al*. (2021); resistance to Cefixime was found in 29.2% of carrot and cucumber samples in Saudi Arabia, whereas resistance to Cefotaxime and Ceftriaxone was found in 20%. Although the prevalence of β-lactams antibiotic resistance varies by region, it is frequently reported as a high percentage in research. The existence of an efflux pump, alteration of antibiotic targets in Penicillin Binding Protein (PBP), and decreased drug penetration due to diminished porin and β-lactamase enzyme synthesis are all factors contributing to this group's high resistance (Katzung and Vanderah, 2012)

The study results of *E. coli* bacteria on Macrolide antibiotics, namely Azithromycin, had a resistance of 42.42%. Another study by Attien *et al*. (2020) in Daloa investigated that macrolide antibiotics have resistance to *E. coli* by 60%. The mechanism of resistance to antibiotics can occur due to the efflux pump (Hooda *et al*., 2019)

This study discovered that *E. coli* from basil samples exhibited 27.27% resistance to the quinolone group, specifically Ciprofloxacin. Furthermore, Aabed *et al*. (2021) in Arabia discovered that 50% of lettuce samples were resistant to Ciprofloxacin. Meanwhile, their findings differ significantly from those of Lima *et al*. (2017) from Brazil, who found that all samples of *E. coli* in salad greens were 100% responsive to the antibiotic Ciprofloxacin. Even though the level of resistance to Ciprofloxacin varies between studies, Sumampouw's (2018) study found that Ciprofloxacin is the best antibiotic for treating diarrhoea caused by *E. coli* infection. Resistance to Ciprofloxacin is caused by a plasmid mutation that results in the creation of the Qnr protein, which protects D.N.A. gyrase from destruction (Ruiz, 2019).

The findings revealed that *E. coli* was resistant to the Sulfamethoxazole-trimethoprim antibiotic class, namely Cotrimoxazole, in 57.58% of cases. This research backs up the findings of a Ghanaian study that looked at a 70% lettuce sample (Adzitey, 2018). Similarly, Faour-Klingbeil *et al*. (2016) found that the level of antibiotic resistance to Cotrimoxazole was 72.7% in salad vegetables grown in Lebanon. Resistance to the antibiotic trimethoprim in the plasmid can result in the formation of the antibiotic-resistant dihydrofolate reductase enzyme, whereas resistance to sulfonamides is thought to be due to excessive PABA (p-aminobenzoic acid) production and decreased affinity for antibiotics due to changes in the enzyme molecule in dihydropteroate synthetase (Griffith *et al*., 2018).

The results showed that 72.7% of the basil samples were MDR The results of this study are identical to the study in Lebanon by Faour-Klingbeil *et al.* (2016), which claimed that 60% of ready-to-eat salad vegetables are MDR Another study by Al-Kharousi *et al*. (2019) used samples of tomatoes, local cucumbers, and zucchini cucumbers purchased from supermarkets had MDR

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bacteria with the rate of 26%.

The hike in antibiotic resistance cases happens because of the irrational use of antibiotics. The criteria for proper and rational use of drugs are divided into three. The first criteria match the patient's diagnosis and disease with the used dosages and selected antimicrobial drugs. Secondly, prescribing antibiotics must apply the proper method of administration, instructions for use, and the recommended duration of therapy. Finally, the physician must educate the patient about drug information to ensure that the antibiotic medication should be finished since it can affect patient compliance and success in treatment (Andrajati *et al.*, 2017). The disadvantage of increasing cases of resistance will harm patients, such as narrowing the choice of drugs, prolonging the treatment period, and causing the cost of the treatment to be more expensive.

4. Conclusion

All samples of basil isolates did not meet the food quality requirements that were safe for direct consumption; most of the basil isolates sold in 8 traditional markets in the Jember district were contaminated with *E. coli* bacteria, the highest resistance of *E. coli* basil isolates to Amoxicillin and Cefixime. In contrast, the lowest to Ciprofloxacin, and most samples were MDR

Conflict of interest

The authors declare no conflict of interest.

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