Public Awareness for Antimicrobial Resistance from Escherichia coli Isolated from Beef Sold on Several Wet Market in Surabaya, Indonesia

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Public Awareness for Antimicrobial Resistance from *Escherichia coli* Isolated from Beef Sold on Several Wet Market in Surabaya, Indonesia

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Abstract

Objective: This study aims to phenotypically identify and confirm the presence of multi-drug resistant (MDR) and Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* in the swab surface samples of beef using VITEK-2 method.

Materials and Methods: Swab samples were taken from five wet markets: Pucang market, Wonokromo market, Keputran market, Pabean market, and Manukan market; 10 swab samples of beef were collected from each market. Then, isolation and identification in terms of bacteria using selective media and biochemical test were conducted. Resistance testing using disc diffusion method was performed on 6 types of antibiotics: Ampicillin, Cefazolin, Ceftriaxone, Cefotaxime, Ceftazidime, Tetracyclin. Positive isolates resistant to ≥ 2 types of Beta-Lactam antibiotics using disc diffusion method then were tested using VITEK-2 method.

Results: Positive samples containing *Escherichia coli* are found in 29 samples out of 50 swab samples. Of the 29 *Escherichia coli* isolates, 17 isolates are found resistant to the disc diffusion method. After testing those 17 isolates using VITEK-2 method, 5% (1/17) of ESBL-producing *Escherichia coli* are obtained which is resistant to all Beta-Lactam antibiotics. Besides, this study also reveals that 35% (6/17) of the *Escherichia coli* are positive multidrug resistance. Those *E.coli* MDR are found to be resistant towards antibiotic class Beta-Lactams, Aminoglycoside, Quinolon, and Sulfonamida.

Conclusion: This study has encouraged the need for public awareness for the understanding that beef from wet market can be potential as reservoir to spread multi-drug resistant bacteria that can cause health problems in humans.

Key words: *MDR, ESBL, Escherichia coli, Beef, Vitek-2 System, Public Health*

Introduction

Inappropriate use of antibiotics is one of the main factors in the occurrence of antibiotic resistance [(1)]. Antibiotic resistance is a change in the ability of bacteria to become resistant to antibiotics. Antibiotic resistance has now become a global public health problem and has been reported by the global agenda as one of the biggest threats to health [(2)]. Antibiotic resistance in bacteria can cause complications, longer treatment periods, treatment failure and death due to infection with resistant bacteria [(3)]. Humans can be infected by bacteria that are resistant to antibiotics through direct contact, consumption of contaminated meat, and the environment [(4)]. Extended spectrum Beta-Lactamase (ESBL) is an enzyme produced by gram negative bacteria and is a cause of resistance to almost all Beta-Lactam group antibiotics

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such as Penicillin, Cephalosporin and Monobactam Aztreonam [5]. Escherichia coli is a normal flora of the mammalian digestive tract which can also be a cause of diseases such as gastroenteritis, cystitis, pneumonia, septicemia in humans and animals [6].

Escherichia coli can also act as a reservoir for the spread of antibiotic resistance because it can easily move resistance genes to other bacteria [7]. ESBL producing Escherichia coli has been isolated from food from animals, hospital environments, plants, and feces [8]. Some studies also report the high prevalence of ESBL-producing Escherichia coli in food-consuming animals [9], food products [10], and the environment [11]. Escherichia coli is a contaminant bacterium commonly found in meat [12]. The chemical composition and moisture of the meat is ideal for the life process of bacteria, this causes the meat can not last long when stored at room temperature [13]. This study focuses on identifying and confirming the presence of ESBL-producing Escherichia coli in beef through its resistance to Beta-lactam type antibiotics using the Vitek-2 method.

Vitek-2 advance expert system (Biomerieux) is an automated system used to show the phenotype of the isolates tested and this method is able to determine the sensitivity or resistance of an isolate to antibiotics. The Vitek-2 test method has proven to be more reliable in detecting bacterial resistance to antibiotics because there is no subjective interpretation of the results [14].

Materials and Methods

Ethical approval

Fresh beef samples were used in this study; hence, ethical approval was not necessary. Fresh beef samples were collected from Surabaya wet market.

Sampling

Sampling uses purposive sampling method. Samples were taken from five wet markets with criteria such as the market environment and the condition of dirty beef stalls. The number of samples is 10 samples of beef swabs from each market. The total samples examined were 50 samples of beef swabs. Swab results are labeled and sample swabs must be carried out aseptically. Using sterile swab sticks (Oxoid, Bangistoke, UK) which are placed in tubes containing media transport. After the sample is taken, the sample is stored in the cooler box and taken to the laboratory.

Isolation and Identification of Escherichia coli Bacteria

Each sample produced by beef swabs was planted on Brillilliant Green Bile Broth (BGBB) media (E. Merck, Darmstadt, Germany) then incubated at 37ºC for 24 hours. Positive results are indicated by the presence of gas bubbles in the durham tube and the change in color of the media to cloudy green. After being positive, it was then planted in Eosin Methylene Blue Agar (EMBA) media (E. Merck, Darmstadt, Germany) by streaking and incubated at 37ºC for 18-24 hours. Typical colonies of Escherichia coli on metallic EMBA media.

Typical colonies of Escherichia coli grown in EMBA are planted again in the Triple Sugar Iron Agar (TSIA) media (E. Merck, Darmstadt, Germany) and Pepton Water Buffers and then incubated at 37ºC for 24 hours. The Pepton Water 1 buffer, which has been incubated, is then dropped with a kovach reagent of two or three drops. A positive test for Escherichia coli is characterized by the formation of a red ring on the surface of 1% Pepton Water [15; 16].

Antibiotic Sensitivity Test

The Escherichia coli colonies found on the EMBA media were planted in a test tube containing 8 ml of physiological Nacl, homogenized using vortex until the same turbidity was obtained with standard Mc Farland 0.5. Then 0.2 ml was taken and gently rubbed on the entire surface of Mueller Hinton Agar (MHA) media (E. Merck, Darmstadt, Germany) using sterile cutton swabs.

Sensitivity tests using the disc diffuscion method were performed on 6 types of antibiotic disks namely Ampicillin 10 µg (Oxoid CT0003, UK) cefotaxime 30 µg (Oxoid CT0166, UK), ceftazidime 30 µg (Oxoid CT0412, UK), ceftriaxone 30 µg (OxoidCT0417, UK) , tetracycline 30 µg (Oxoid CT0054, UK), cefazolin 30 µg (Oxoid CT0011, UK) (CLSI, 2016). Culture of bacteria was incubated at 37ºC for 24 hours. The results of the tests are interpreted based on the provisions of the Standard Laboratory Clinical Institute (CLSI, 2016). Positive isolates resistant to ≥ 2 types of Beta-Lactam antibiotics using disc diffusion method then were tested

using VITEK-2 method [14].

ESBL confirmation with Vitek-2 system

All isolates tested with the disk diffusion test were then identified and confirmed phenotypically using the Vitek-2 system (BioMerieux, Marcy L'Etoile, France) at the Microbiology Laboratory at Hospital of Airlangga University, Surabaya. Tests with Vitek-2 were carried out based on the factory protocol (BioMerieux, Marcy L'Etoile, France) that had been printed on the device.

Findings

The results of isolation and identification of bacteria from a total of 50 beef swab samples taken from 5 wet markets found 29 (58%) samples that were positive for *Escherichia coli* (Fig. 1). The high level of *Escherichia coli* contamination in beef found in this study is in accordance with the study conducted by Chuku et al (17) which reported that Escherichia coli contamination levels in beef sold in traditional markets in Nigeria reached 90%.

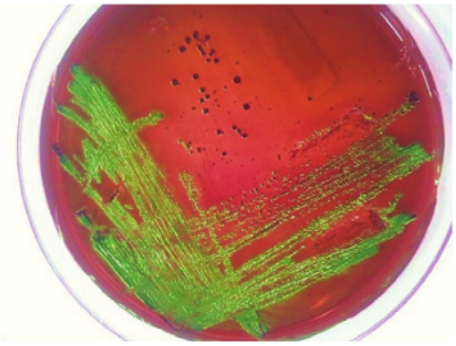

Figure 1. E coli seen metallic green on EMBA media

Factors that cause high levels of *Escherichia coli* contamination in beef sold on the wet market are table surfaces that are in contact with meat, cleanliness of stalls is not maintained [18]. The sensitivity test (Fig. 2.) results using the disk diffusion test method of a total of 29 positive Escherichia coli samples showed 17 (58%) Escherichia coli isolates suspected of producing ESBL due to en 2 types of Beta-Lactam antibiotics with a resistance pattern shown in table 1.

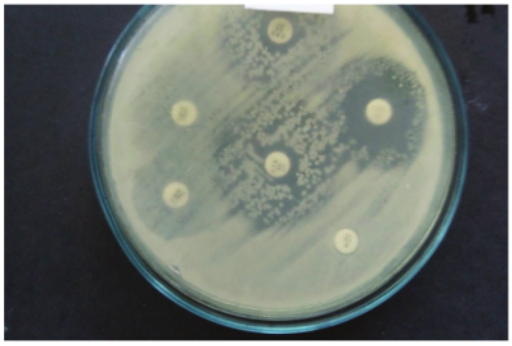

Figure 2. Antibiotic sensitivity test results using the method for the disk diffusion test.

Table 1. Pattern of *Escherichia coli* Resistance to Antibiotics using Disk Diffusion Test

Antibiotics	Isolate number	Pattern of Antibiotic Resistance		
		S % (n)	I % (n)	R % (n)
Ampicillin	29	0	0	100 (29)
Cefazolin	29	10 (3)	31 (9)	58 (17)
Ceftriaxone	29	89 (26)	6 (2)	3 (1)
Cefotaxime	29	86 (25)	10 (3)	3 (1)
Ceftazidime	29	93 (27)	0 (0)	6 (2)
Tetracyclin	29	6 (2)	3 (1)	89 (26)

Description: R=*Resistant*, I= *Intermediate*, S= *Susceptible.*

A total of 17 isolates were then confirmed by phenotype using the Vitek-2 system. Confirmation test results using the Vitek-2 system showed that from 17 Escherichia coli isolates suspected of producing ESBL in the disc diffusion test, only 1 (5%) ESBL-producing positive Escherichia coli isolates were found in the pattern of resistance shown in Table 3. Although in the last three years there has been a dynamic increase in research on antibiotic resistance caused by ESBL [19; 20], there have been relatively few publications that report the presence of ESBL-producing Gram Negative Bacteria [21].

Table 2. Identification of ESBL producing E. coli using Vitek-2 System

No	Isolate	Location	Antibiotics Resistance
1	A4	Pabean Market	AMX, AMP, SAM, CZ, CAZ, CRO, CTX, FEP, ATM, GM, CIP, SXT

The available data mostly discusses the existence of these microorganisms in livestock animals [22; 23], only a few data about ESBL-producing Escherichia coli contamination in meat and processed meat products [9]. Research on meat is mostly done on chicken meat compared to meat from other animal species such as cattle [24]. From the results found in this study, it can be confirmed phenotypically the presence of ESBL producing *Escherichia coli* in beef sold in wet markets using the Vitek-2 method shown in table 2.

Table 3. Identification of MDR *E. coli* using Vitek-2 System

No	Isolate	Location	Antibiotics Resistance
1	C4	Pucang Market	AMX, AMP, GM, CIP, SXT
2	C5	Pucang Market	AMX, AMP, CIP, SXT
3	C7	Pucang Market	AMX, AMP, CIP, SXT
4	C9	Pucang Market	AMX, AMP, CIP, SXT, SAM
5	C10	Pucang Market	AMX, AMP, CIP, SXT
6	E3	Keputran Market	AMX, AMP, CIP, SXT

Description: AMX = Amoxicillin, AMP = Ampicillin, SAM = Ampicillin-sulbactam, CZ = Cefazolin, CAZ = Ceftazidime, CRO = Ceftriaxone, CTX= Cefotaxime, FEP = Cefepime, ATM =Aztreonam, GM = Gentamycin, CIP = Ciprofloxacin, SXT = Trimethoprim-Sulfamethoxazole.

This study also succeeded in obtaining 6 (35%) positive multidrug resistance *E. coli* isolates, MDR *E. coli* isolates were found to be resistant to Amoxycillin antibiotics by 35% (6/17), Gentamycin 5% (1/17), Ciprofloxacin 35% (6/17), Trimetophrim-Sulfamethoxazole 35% (6 /17), and Ampicillin-sulbactam 5% (1/17) shown in Table 3. These results are consistent with the study by Adinepekun et al [25]

which states that resistance to a number of antibiotics such as aminoglycoside, Beta-Lactam, cephalosporin, fluoroquinolones, sulfonamide, tetracyclin, and trimethoprim have been found in Escherichia coli isolated from food from animal origin. Multidrug Resistance (MDR) is a condition where bacteria are resistant to ≥ 3 types of antibiotics. MDR has recently spread widely, especially in gram negative bacteria such as *Escherichia coli* [(7)].

Food contamination by antibiotic-resistant bacteria can be a serious threat to public health, the presence of resistant genes in plasmids, transposons and integrons facilitates the rapid spread of resistance genes between bacteria. Many resistant genes in *Escherichia coli* MDR are located on plasmids, which increase the likelihood of spreading these genes in the community [(26)].

Conclusion

In this study testing using the Vitek-2 method has succeeded in confirming the presence of ESBL-producing Escherichia coli with the discovery of one isolate (5%) positive ESBL-producing *Escherichia coli* that is resistant to all Beta-Lactam group antibiotics, Penicillin, Cephalosporin and Aztreonam. This study also succeeded in confirming the presence of *Escherichia coli* as MDR bacterial isolated from fresh beef swab samples which amounted to 35% of 17 samples examined using the Vitek-2 method. This results can be concluded that the need for public awareness for the understanding that beef from wet market can be potential as reservoir to spread multi-drug resistant bacteria that can cause health problems in humans.

Conflicts of Interest : The authors declare no conflict of interest.

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