Detection of Multidrug-Resistant (MDR) *Escherichia coli* Isolated from Raw Milk in East Java Province, Indonesia

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Abstract

The aim of this study is to figure out the profile of antibiotic resistance in *E. coli*, sourced from raw milk, obtained from five districts in East Java Province, Indonesia. A total of 250 samples were tested using the Kirby-Bauer Test method. The result revealed that the highest percentage of antibiotic resistance was Tetracycline (17.05%), followed by Streptomycin (14.2%), Trimethoprim (9.66%), Chloramphenicol (7.95%) and Aztreonam (1.7%). A total of 16 *E. coli* isolates (9.1%) were detected as MDR, and 3 *E. coli* isolates (1.7%) were suspected as presumptive ESBL. Thus, threat of multidrug resistance *E. coli* possibly sourced from the milk.

Keywords: *Escherichia coli*, multidrug resistant, raw milk, antibiotic susceptibility test.

Introduction

Contamination of milk with microorganisms is something that cannot be completely avoided, therefore microbial contamination in milk is an important point in measuring its quality[1]. Some microorganisms can be found in milk and other dairy products, one of which is *E. coli*. Pathogenic *E. coli* infections in humans, which originate from milk, are widely reported in several cases in developing countries[2].

The case of *E. coli* contamination that is resistant to various antibiotics has been widely studied. At present, a focus on public health is being developed regarding antibiotic resistance due to its use in the treatment of livestock, following the conditions of human diseases in developing countri[3]. Antibiotics are used as prevention and treatment of various cases of *E. coli* bacterial infections that attack livestock[4]. However, inappropriate and irrational administration of antibiotics results in the emergence of antibiotic resistance in *E. coli* bacteria[5].

E. coli can be a reservoir of various antibiotic resistance genes, which can be transmitted to other bacteria. Raw milk, including one of the foodstuffs of animal origin which is a potential source in the spread of multidrug-resistant bacteria, which impacts on human health[6]. Therefore, the objective of this study was to detect the presence of multidrug resistant *E. coli* from raw milk in East Java Province, Indonesia.

Materials and Methods

Raw milks were taken from dairy farm with a total of 250 samples, located in Kediri, Probolinggo, Pasuruan, Batu and Blitar Region, East Java Province, Indonesia, in the period between August-November 2019. Raw milk samples contained in wrapped sterile plastic and were taken to the laboratory using cool box container at 4˚C.

Each sample were cultured into enrichment media Brilliant Green Bile Lactose Broth (Merck, 105454) and incubated at 37ºC for 18-24 hours. The positive results characterized by change of media from green colour to cloudy green and gas presence in Durham tube[7]. Then were streaked onto Eosin Methylene Blue (EMB)

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agar (Merck, 101347) and incubated at 37 °C for 18-24 hours[8]. Colonies that showed metallic green colour (Figure. 1) was purified and continued to identification with IMViC test.

Antibiotic sensitivity testing was done using Kirby-Bauer disc diffusion assay on medium Mueller-Hinton agar (Oxoid, CM0337). Antibiotics disks used was Tetracycline 30 µg (Oxoid, CT0054), Streptomycin 10 µg (Oxoid, CT0047), Chloramphenicol 30 µg (Oxoid, CT0013), Trimethoprim 5 µg (Oxoid, CT0057), Aztreonam 30 µg (Oxoid, CT0264) (Fig. 2 and 3). Interpretation of results by measuring the diameter of the inhibitory zone formed, based on Clinical and Laboratory Standards Institutions[9].

Table 1. Antimicrobial Resistance Profile

Location (Code)	Sample Size	Confirmed E. coli	Resistant to					Multidrug Resistant	Presumptive ESBL
			TE	S	W	C	ATM		
Kediri (K)	50	35	7	5	1	4	0	3	0
Probolinggo (A)	50	36	3	2	3	2	0	2	0
Pasuruan (G)	50	30	7	5	8	1	1	4	1
Batu (H)	50	38	4	5	1	1	2	1	2
Blitar (S)	50	37	9	8	4	6	0	6	0
Total	250	176	30	25	17	14	3	16	3
Percentage (%)	100	70.4	17.05	14.2	9.66	7.95	1.7	9.1	1.7

Info: TE: Tetracycline, S: Streptomycin, W: Trimethoprim, C: Chloramphenicol, ATM: Aztreonam

Table 2. Measurement of inhibition zone on antibiotic sensitivity test of Multidrug Resistant and Presumptive ESBL *E. coli*

No.	Sample Code	Diameter of inhibition zone of antibiotics in mm					MDR	Presumptive ESBL
		TE	S	W	C	ATM		
		30 µg	10 µg	5 µg	30 µg	30 µg		
1.	K-03	6 (R)	6 (R)	6 (R)	6 (R)	36 (S)	+	-
2.	K-04	6 (R)	10 (R)	30 (S)	6 (R)	33 (S)	+	-
3.	K-36	6 (R)	6 (R)	30 (S)	6 (R)	34 (S)	+	-
4.	A-26	6 (R)	6 (R)	6 (R)	6 (R)	31 (S)	+	-
5.	A-44	16 (S)	9 (R)	6 (R)	6 (R)	40 (S)	+	-
6.	G-12	6 (R)	6 (R)	6 (R)	22 (S)	30 (S)	+	-
7.	G-31	17 (S)	11 (R)	7 (R)	16 (I)	6 (R)	+	+
8.	G-35	6 (R)	6 (R)	6 (R)	23 (S)	31 (S)	+	-
9.	G-43	8 (R)	6 (R)	26 (S)	6 (R)	30 (S)	+	-
10.	H-11	6 (R)	6 (R)	6 (R)	6 (R)	30 (S)	+	-
11.	H-15	9 (R)	6 (R)	6 (R)	28 (S)	34 (S)	+	-
12.	H-28	10 (R)	6 (R)	25 (S)	7 (R)	28 (S)	+	-
13.	H-37	9 (R)	9 (R)	22 (S)	6 (R)	27 (S)	+	-
14.	H-44	7 (R)	10 (R)	30 (S)	6 (R)	29 (S)	+	-
15.	H-45	7 (R)	10 (R)	31 (S)	6 (R)	30 (S)	+	-
16.	S-25	7 (R)	16 (S)	24 (S)	7 (R)	15 (R)	+	+
17.	S-38	18 (S)	11 (R)	27 (S)	30 (S)	10 (R)	-	+

Info: R: Resistant, I: Intermediate, S: Sensitive.

Figure 1. E coli in EMB Agar

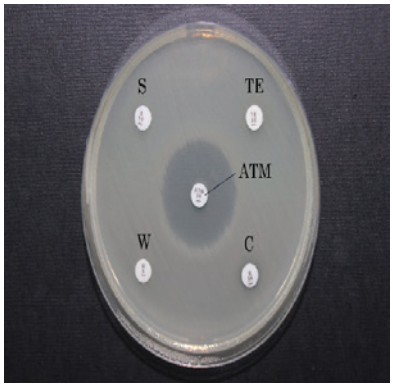

Figure 2. Isolate resistant to 4 antibiotic discs (K-03)

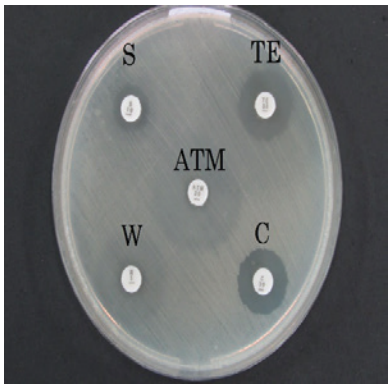

Figure 3. Isolate of Presumptive ESBL (G-31)

Results and Discussion

Among the total 250 raw cow milk samples collected from different dairy farms, 176 samples (70.4%) were found to be positive for *E. coli* (Figure 1). The antimicrobial resistance profiles of the bacterial isolates were summarised in Table 1. *E. coli* showed resistance to antibiotics like Tetracycline (17.05%), Streptomycin (14.2%), Trimethoprim (9.66%), Chloramphenicol (7.95%) and Aztreonam (1.7%). A total of 16 multiple drug resistance (MDR) isolates were found (8.52%) with six different patterns (Figure 2.). The highest MDR patterns noted was Tetracycline, Streptomycin, and Chloramphenicol in 7 out of 16 MDR isolates (Table 2). Three presumptive ESBL isolate detected from Pasuruan and Blitar, which resistant to Aztreonam disc (Figure 3).

The result of this study showing high level for *E. coli* found in milk indicating a poor sanitation practices of farmers during milking process[10]. This number has the same level reported by Chey et al.[11] in Malaysia, stated that the highest prevalence (72.2%) of *E. coli* originated from the Eastern zone of the research location. Compared to other developing countries, namely Bangladesh, as much as 75% of milk samples studied contain E coli bacteria[12].

Tetracycline has the highest antibiotic resistance because it is commonly used in the veterinary field, as well as other antibiotics used in this study such as aminoglycoside (Streptomycin), macrolide (Chloramphenicol) and sulphonamide (Trimethoprim)[13]. The use of broad-spectrum antibiotics such as the tetracycline and beta-lactam classes is more common in clinical mastitis cases of dairy cows in Europe, due to their effective treatment results. For respiratory and digestive tract problems, the tetracycline and aminoglycoside groups are the first choice antibiotics, while the second choice is the macrolide group and the combination of sulfonamid-trimethoprim drugs which have a significant impact on microbial activity in the rumen, and the last choice is the cephalosporin class three and four antibiotics (the sulfonamide-trimethoprim drug combination which has a significant impact on microbial activity in the rumen, and the final choice is the cephalosporin class three[14].

Seventeen MDR and Presumptive ESBL *E. coli* isolates that have been studied in this study, have almost the same pattern of resistance to one antibiotic, that is resistant to aminoglycoside class drugs, (Streptomycin), except for S-38 isolates which are still intermediate status. This resistance can be attributed to the effectiveness of the drug streptomycin in the treatment of dairy cows affected by mastitis in Indonesia. According to Riyanto et al.[15], a good treatment for dairy cows suffering from mastitis is a combination of the Penicillin-Streptomycin antibiotic. Although in this study the level of resistance of the drug Streptomycin is quite low (14.2%), but there is a need to monitor the use of antibiotics Streptomycin in dairy cows in Indonesia.

Based on this study, Trimethoprim and Aztreonam antibiotics may still be used to treat MDR bacteria that are found because there are still several isolates of *E. coli*

MDR that are sensitive to them. Trimethoprim is usually combined with Sulfonamide which works synergistically with broad spectrum activity. In the case of urinary tract infections, Trimethoprim has been shown to be effective in killing infections caused by *E. coli* bacteria[16].

While monobactam (Aztreonam) has not been defined for veterinary applications, the present of resistant isolates has the possibility of ESBL producing *E. coli* found in raw milk. The finding of ESBL Enterobacteriaceae sourced from milk, indicates the presence of environmental contamination, and the lack of environmental sanitation when milking is carried out[17]. *E. coli* is a bacterium that can be a reservoir of various antibiotic resistance genes, including the beta lactam antibiotic resistance gene, which makes *E. coli* capable of producing the β-lactamase enzyme[7,18]. Cage sanitation, enclosure base, and drainage of the cage need to be considered by breeders to prevent contamination of milk by the ESBL *E. coli* presumptive bacteria.

The occurrence of antibiotic resistance is known to originate from the bacterial plasmid which is able to decrease the resistance gene and spread it to other bacteria around it. *E. coli* is a bacterium that is well known as the owner of resistance genes for various antibiotics[19]. Various resistance genes can accumulate in bacterial plasmids, usually in plasmid R (resistance), which is the cause of the discovery of bacterial isolates that are resistant to various antibiotics. In the R plasmid, there is an integron sequence, which is able to add various kinds of resistance coding genes to the base arrangement in the plasmid, so that a series of resistance genes is created in a bacterium[20, 21].

E. coli MDR isolates are very common in various countries and are responsible for a series of infections with high severity and difficult to treat. In Canada, there are studies on cases of urinary tract infections due to *E. coli* bacteria, of which 60% are cases of *E. coli* infections with resistance to more than 3 classes of antibiotics. Consumption of food from raw uncooked animals, inter-regional travel habits, and contact with reservoir animals is associated with an increased risk of urinary tract infections caused by *E. coli* MDR[22].

The multidrug resistance reported in the current study might be due to high and irrational antimicrobial use in individual cows to treat various diseases affecting the dairy sector. As a presence of resistance to many antimicrobial drugs has become a public health problem according to the fact that food borne outbreaks would be difficult to treat and this findings of MDR *E. coli* in food supply establish a source for communicable resistant genes[23].

Conclusion

Multidrug resistant E coli is a public health threat that can be sourced from the consumption of foods derived from animals, one of which is raw milk. From this study it was concluded that E. coli is prevalent in raw milk and has multidrug resistance (MDR) properties from East Java Province, Indonesia. E. coli found in milk is expected to increase public awareness of the dangers it poses. In this way, the application of sanitation and monitoring of antibiotic use must continue to be promoted.

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Ethical Clearance: Raw milk were used in this study, hence ethical clearance was not necessary. Raw milk samples were collected from dairy farms East Java province, Indonesia.

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Conflict of Interest: Nil.

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