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Prevalence of Pathogenic *Escherichia Coli* Isolated from Subclinical Mastitis in East Java Province, Indonesia

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Abstract

A study was conducted to isolate *E. coli* from raw milk samples and to determine the antibiogram pattern of *E. coli* isolates. A total of 150 milk samples were collected from East Java Province, Indonesia, and tested with CMT for detection of subclinical mastitis. MacConkey agar and Eosin Methylene agar were used as culture media. Several biochemical tests and Antibiogram pattern of *E. coli* by the disk diffusion method were used. Results of the present study have revealed that out of the 150 samples tested, 128 (85.33%) were found to have subclinical mastitis due to *E. coli* 94 (74.33%). Antibiogram pattern assessed, revealed a higher resistance against erythromycin (100%) and gentamicin (26%), followed by oxytetracycline (17.78%), and chloramphenicol (0%). The presence of antimicrobial resistance indicates alarming situation for timely designing of control and prevention methods to reduce its prevalence and antimicrobial resistance in foods of animal origin.

Key words: Antimicrobial resistance, raw milk, subclinical mastitis, *E. coli.*

The nutritional composition of milk is very high and also serves as a good medium for the growth of microorganisms (Harpini, 2008).

The presence of microbial contamination in raw milk, which endangers the health of consumers (Adam and Moss, 2008) can also be used as an indicator that the cow is infected with the disease (Kusumaningsih, and Aryianti, 2013). Mastitis is a major problem which leads to decline in milk production (Oliver *et al*., 2005). Based on a study conducted by Kusumaningsih and Aryianti (*loc. cit*), identified pathogenic or commensal bacteria which are antibiotic-resistant in milk has important public health significance, leading to treatment failure, increased hospitalization time, medical costs, and presence of antibiotic residue in the product of livestock origin.

The studies were undertaken to isolate Escharichia coli from raw milk samples and to study the antibiotic resistance of pattern of the isolates to certain antibiotics.

Materials and Methods

Raw milk sampling is done as per (Alfiasari, 2012). A total of 150 samples of milk were taken from 4 dairy farms in East Java (Nongkojajar, Grati, Senduro and Batu). During milking, 10 ml of milk was taken aseptically from milk can after milking (Effendi *et al*., 2017). Each milk sample was stored in sterile plastic bag in a thermos flask.

California Mastitis Test (CMT) was conducted. Positive reactions occur in the CMT paddle and are subjectively assessed (0, +1, +2, +3). The subjective value for positive 1 indicates the presence of clear, positive sediment; 2 mixture immediately thickens and the sediment moves to the center of paddle and positive 3 showed more sediments and the surface become convex (Setiawan, *et al*., 2012). CMT test results from cow milk samples taken from several farms in East Java is presented and the results of CMT in the 4 farms evaluated in Table I.

Based on CMT test results from cow milk samples taken in several farms in East Java there are 128 milk samples that showed positive results, which has shown that as many

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Table I. Number of Subclinical mastitis and *E. coli* from several dairy farms

Name of farm	Number of samples	Subclinical mastitis	Positive *E. coli*
Nongkojajar	30	23	13
Grati	37	33	26
Batu	48	39	30
Senduro	35	32	25
Total	150	128	94

Table II. The results of antibiotics sensitivity test on *E. coli*.

Name of farm	*E. coli*	Erythromycin	Oxytetracycline	Gentamicin	Chloramphenicol
Nongkojajar	13	R : 100%	R : 23,1%	R : 23,1%	R : 0%
		I : 0%	I : 23,1%	I : 46,1%	I : 0%
		S : 0%	S : 53,8%	S: 30,8%	S : 100%
Grati	26	R : 100%	R : 15,4%	R : 30,8%	R : 0%
		I : 0%	I : 19,2%	I : 46,1%	I : 0%
		S : 0%	S : 65,4%	S : 23,1%	S : 100%
Batu	30	R : 100%	R : 16,7%	R : 30%	R : 0%
		I : 0%	I : 10%	I : 36,8%	I : 0%
		S : 0%	S : 73,3%	S : 33,2%	S : 100%
Senduro	25	R : 100%	R : 16%	R : 20%	R : 0%
		I : 0%	I : 16%	I : 52%	I : 0%
		S : 0%	S : 68%	S : 28%	S : 100%

as 85.33% (94) of the 150 samples of cow milk in East Java was found to have a positive reaction on the CMT test.

Isolation and identification of Escherichia coli bacteria was done from milk samples which had positive results on CMT test. The positive results of planting milk samples on Brilliant Green Bile Broth (BGBB) media are characterized by the discolouration from clear green colour changing to turbid green followed by gas formation. There are 109 (85.16%) isolates which gave positive results on planting in BGBB media. Positive isolates were planted on Eosin Methylene Blue (EMBA) media and the presence of metallic green colonies. 94 (100%) isolates were positive of *Escherichia coli* (Table I).

Escherichia coli isolates obtained were used to test the sensitivity of 4 antibiotics namely erythromycin, oxytetracycline, gentamicin, and chloramphenicol. The reading of the results of the sensitivity test is done by measuring and comparing the clear zone formed with the standard zone according to Clinical and Laboratory Standard Institute (CLSI 2007).

Results and Discussion

The results of antibiotics sensitivity test on *E. Coli* are presented in Table II. Out of a total number of 150 samples of cow's milk taken from 4 dairy farms in East Java, 128 samples of were positive for CMT test, which indicates the presence of mastitis. The advantages of using CMT is that an inflammatory reaction of the udder can be known as early as possible, less treatment cost, easy to perform and can be done for multiple milk samples simultaneously (Karimuribo *et al.*, 2006).

Mastitis can be divided into two types based on the causes as contagious mastitis and environmental mastitis (Gianneechini *et al.*, 2002).. The most common contagious mastitis causing bacteria are *Staphylococcus aureus*, *Streptococcus agalactiae* and Mycoplasma (Subronto and Tjahjati, 2001). Environmental

mastitis is mostly caused by coliform bacteria such as *Escherichia coli, Enterobacter aerogenes* and *Klebsiella pneumonia* (Haftu *et al*., 2012). In the present study, involvement of Eschericia coli in mastitis was taken up.

Based on isolation and identification test of *Escherichia coli* bacteria from 128 positive milk samples by CMT test, 94 samples were positive for *Escherichia coli. Escherichia coli* can ferment lactose to acid and gas within 24-48 hours at a temperature of 30°C - 37°C. In addition, *Escherichia coli* can also produce acids and gases from glucose, fructose, galactose, arabinose, xylose and mannitol. In EMBA media, colonies are metallic green with a black colony center. Escherichia coli is also capable of forming indole from tryptophan which is indicated by the appearance of red colour in indole test.

Escherichia coli bacteria can live on the floor of a cage, grass or water contaminated by livestock manure (Haftu *et al*., *loc. cit*). *E.coli* is aerobic and facultative anaerobic. The growth temperature of *E.coli* is 15-45°C with the optimum temperature of *E.coli* growth at 37.5°C (Chusniati, *et al.,* 2011).

Mastitis caused by *Escherichia coli* bacteria occurs during the rainy season (Gianneechini, *et al., loc. cit*), where the cotton bed is dirty and damp (Radostits *et al*., 2007). *Escherichia coli* bacteria entering the nipple will infect the udder by releasing endotoxin (Radostits *et al., loc. cit*). This endotoxin then spreads to the microvascular wall of the alveoli and the intertisial tissue of the udder causing hyperemia, hemorhagidanoedem (Quinn *et al.,* 2011).

The results of antibiotic sensitivity test showed that 100% *Eschericia coli* isolates experienced resistance to erythromycin, while against chloramphenicol as much as 100% isolates were still sensitive. The data also showed that *Escherichia coli* isolates are also still sensitive to oxytetracycline. This is evident from 53.8% isolate N, 65.4% isolate G, 73.3% isolate B, and 68% isolate S which is still sensitive to oxytetracycline. The sensitivity test for gentamicin antibiotics showed intermediate results where N and G isolates had the same percentage of 46.1%, 36.8% for isolate B, and 52% for S. isolates. This indicates that *Escherichia coli* is still sensitive to chloramphenicol antibiotics and oxytetracycline, are less sensitive to gentamicin, and are resistant to erythromycin.

Eschericia coli bacterial resistance to erythromycin antibiotics shows that erythromycin loses its ability to effectively control or eradicate the growth of *Escherichia coli* bacteria, in other words, bacteria are resistant and continue to proliferate despite adequate antibiotics treatment. Non-genetic mechanisms of resistance can occur through excessive use of antibiotics, low doses continuously or irregularly (Effendi *et al., loc. cit*). Pasteurization failures and improper storage may create toxicity among young ones when raw milk is used. While genetically, antibiotic resistance spreads through the bacterial population both vertically as new generations inherit genes that are resistant to antibiotics and horizontally, when bacteria share or exchange materials with other bacteria. WHO (2012), the ereA gene encodes a microlide enzyme -inactivating or enzymatic hydrolysis activity of the macrolactone ring catalyzed by erythromycin esterase (ereA) resulting in drug inactivation leading to erythromycin resistance.

Summary

Clean milk production practices safeguards quality of milk, the health of dairy animals and in turn the consumers. Regular testing of milk samples from the herds for Mastitis, the sensitivity of microbs to the antibiotics used in the treatment of mastitis is useful. The pasteurization of milk and proper storage will play a major role in the preservation and safeguarding the consumers.

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Indian Vet. J., March 2019, 96 (03) : 25 - 28

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