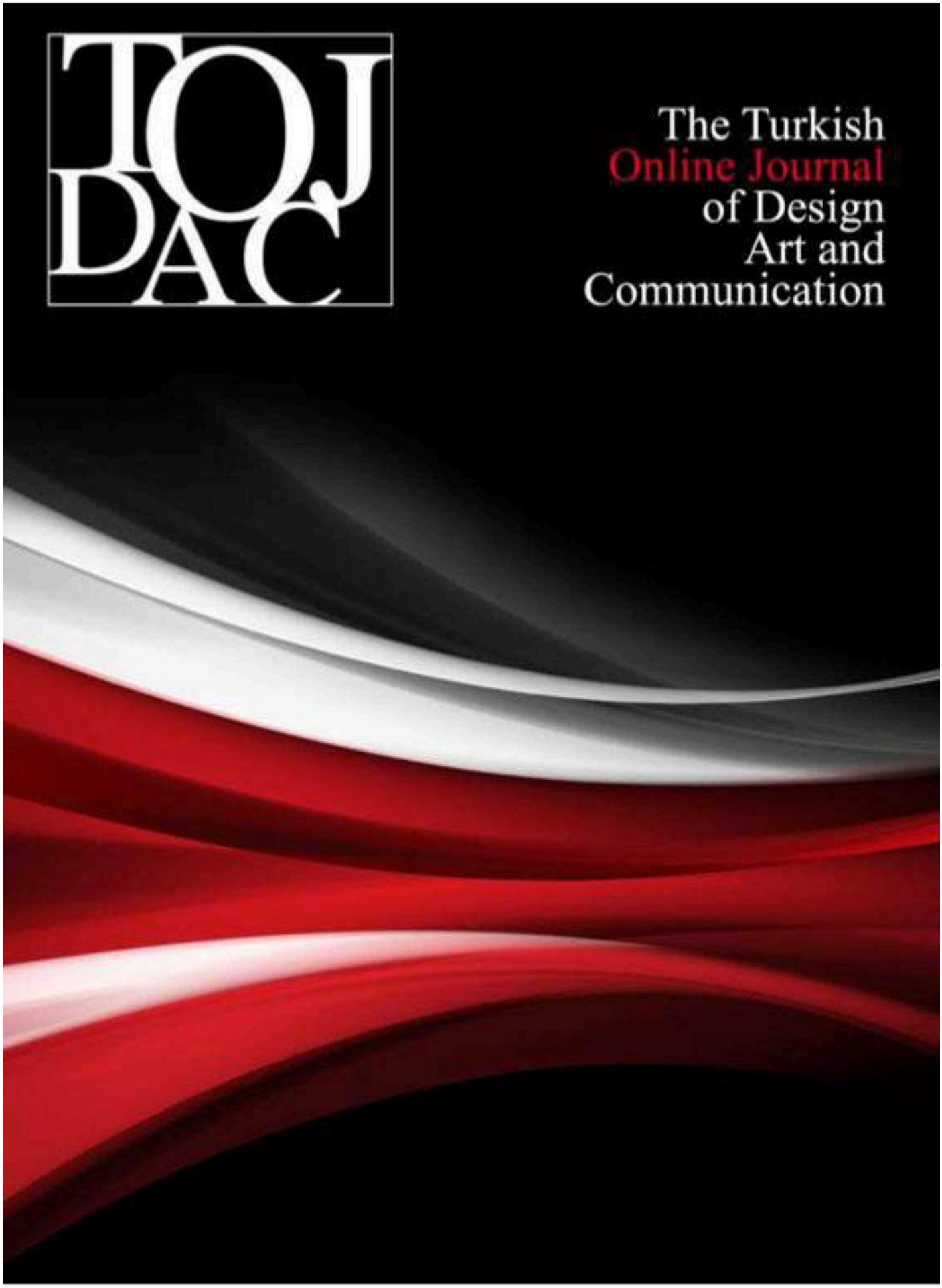
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PROFILE ANTIBIOTICS RESISTANCE ON ESCHERICHIA COLI ISOLATED FROM RAW MILK IN SURABAYA DAIRY FARMS, INDONESIA

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ABSTRACT
Aim : The study was undertaken to isolate pathogenic E. coli from raw milk in Surabaya area and investigate their antibiotic sensitivity pattern. **Materials and Methods:** During 4 months duration of study a total of 128 raw milk samples were collected from different places of dairy farms located in Surabaya, under aseptic precautions. For the enrichment of the organism from the collected samples, MacConkey broth was used and inoculation was carried out on MacConkey agar and EMB agar, to confirm the isolates, various biochemical tests such as IMViC test were performed. Antibiotic sensitivity pattern of E.coli to antibiotic agents was evaluated by disk diffusion method. **Results:** The result of present study revealed that out of 128 samples, 86 samples were found contaminated with E.coli. Antibiotic sensitivity pattern revealed high resistance against ampicillin (95.3 %), whereas moderate resistance was observed for chloramphenicol (39.5 %). Also sensitive antibiotic was observed for ciprofloxacin, gentamicin and trimethoprim. **Conclusion:** Current study supports the finding that raw milk can be regarded as critical source of pathogenic E. coli and pose potential public health hazards

Keywords: Raw milk, Escherichia coli, antibiotic sensitivity test.

INTRODUCTION

The emergence of antibiotic resistant pathogenic bacteria led to improvements in patients infected with the bacteria more difficult and thus require a longer healing time and new types of antibiotics. Furthermore, deaths from bacterial pathogens resistant pathogens has increased, even cases of infection by antibiotic-resistant pathogens is estimated to reach 54% in the United States (CDC, 2016). The main reservoir of multi-drug resistant Gram-negative bacilli group Enterobacteria (Escherichia coli, Salmonella sp.) Is the gastrointestinal tract of animals and humans, especially those who receive antibiotic treatment. The spread of bacterial resistance from animals or humans into the environment through water, food, and the environment is one of the routes that are important to be controlled (Wellington et al., 2013). Misuse of antibiotics in livestock farming, especially as growth promoter causing bacteria resistant to antibiotics (Bouki, VENIERI, & Diamadopoulos, 2013). Therefore, bacterial resistance profiles in animal husbandry importantly investigated as initial data and prediction of these microbial resistance in an integrated transmission between livestock, food and human health.

Prevention of microbial resistance in livestock sector is important because the products of animal origin to be consumed by humans. Residues of antibiotics and antibiotic-resistant bacteria in livestock products produced can lead to resistance in human intestinal microflora. Therefore, a survey of the type and amount of antibiotics that are commonly used in the community farmers and fish farmers, it is important to do as initial data estimate the use of antibiotics in animal husbandry in Indonesia.

Besides this, testing the resistance profile of bacteria commonly found in dairy cows is also a challenge in controlling microbial resistance. One type of bacteria commonly found in the environment so that it can

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enter the digestive tract of animals is Escherichia coli. Escherichia coli is a Gram-negative bacterium that is commonly found as normal microflora in the human digestive tract. The existence of E. coli in a farm environment and exposure to antibiotic use in livestock enlargement process could be expected to cause resistance to antibiotics. Therefore, resistance profiling of Escherichia coli in raw milk is one important approach to determine the spread of resistance in the region.

Resistance can be caused by a factor already present on the bacteria. E. coli bacteria have genes that function to protect himself from the influence of antibiotics derived from the plasmid. Plasmids carrying some drug resistance genes often detected in E.coli (Costa et al., 2010). Plasmids can transfer the resistance genes in bacteria sensitive to antibiotics. Therefore, assessment and monitoring of resistance at the genetic level is very important (Pyatov et al., 2014).

This study uses five antibiotics are often associated with data about the problem of resistance in E. coli and then the antibiotic sensitivity test to determine the sensitivity of E. coli to antibiotics, the antibiotics ampicillin, ciprofloxacin, gentamicin, chloramphenicol and trimethoprim in E. coli isolated from raw milk in Surabaya.

MATERIALS AND METHODS

Sampling

Sampling using purposive sampling was done by selecting a subject based on specific criteria set researcher. Milk samples were taken from four dairy farms in Surabaya

Isolation and Identification of Bacteria

a. Isolation of Bacteria Escherichia coli (Um et al., 2016)

Specimens obtained from raw milk brought to the laboratory in plastic wrapped sterile conditions and were taken using a cool-box. Samples will be included in media enrichment using tryptic soy broth (TSB) supplemented with novobiocin (16mg / L) for 18 hours at 37 ° C and then streaked onto MacConkey media agar and incubated at 37 ° C for 18 ± 2 hours. Colonies that showed lactose-fermenting was purified and continued positive presumptive test of E. coli.

Identification of Escherichia coli

Pure culture isolates plating results that have been stored in glycerol 3% will be cultured in medium TSA. A total of 20 mL of stock culture will be grown in 1 mL of TSA on microtube and incubated at 37 ° C for 18 hours prior to identification. Identification of bacteria were performed using morphological approach, and biochemistry. Biochemical tests include tests Indole, Methyl Red, Vagos-Pasteur, simon Citrate (IMViC) to determine the level of genus and continued until the sugar fermentation test to determine the species of E. coli obtained.

Antibiotic Sensitivity Test (Clinical laboratory standards institute, 2007)

Antibiotic sensitivity testing was done using Kirby-Bauer disc diffusion assay on medium Mueller-Hilton agar. Antibiotics and concentration used was ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), ciprofloxacin (10 μg), trimethoprim (5 μg).

RESULTS AND DISCUSSION

This study uses five antibiotics against Escherichia coli, the results of sensitivity test resistance of Escherichia coli to antibiotics showed that the antibiotic ampicillin occurrence of resistance higher at 100% of the antibiotic chloramphenicol in the amount of 35%, but in this study Escherichia coli sensitive to the antibiotic ciprofloxacin, gentamicin, and trimethoprim. Microbes that are sensitive to an antibiotic can become resistant due to the habit of excessive use of antibiotics and improper (Bhaskara, et al., 2012).

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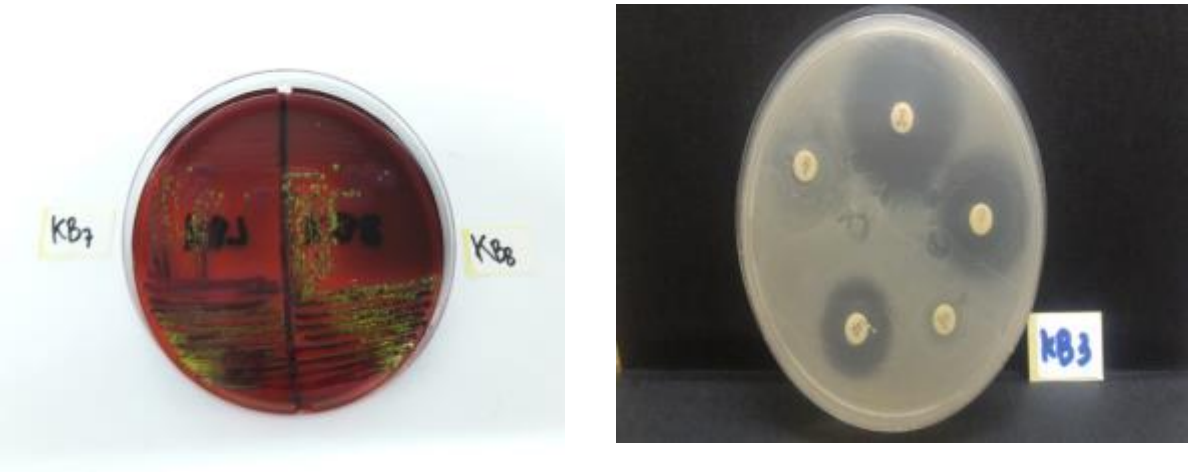
The results of this study are similar to the results of research Indana (2015) in Surabaya showed antibiotic ampicillin resistance against Gram-negative bacteria. While this study was different from the research conducted Chitraningtyas et al (2014) in Surabaya generate Escherichia coli that were resistant to trimethoprim sulfametoxazole amounted to 81.3% and 76.5% resistant to ciprofloxacin, and also different from the research Bhaskara (2012) in Bogor produce that Escherichia coli high resistance to chloramphenicol of 57.1%.



(a) **(b)**

FIGURE 1. (a). E.coli on EMB Agar

(b). Antibiotic sensitivity test

Table 1: Number of milk samples, the number of positive isolates of *E. coli* and Resistant to Antibiotics from Surabaya Farms Area

	P Farm	Kb Farm	Kw Farm	Wn Farm
Number of milk samples	23	32	17	56
E coli (+)	18	21	9	38
Resistant to Ampicilin 10 µg	18	19	9	36
Resistant to chloramphenicol (30 µg)	7	6	2	19
Resistant to gentamicin (10 µg)	0	0	0	0
Resistant to ciprofloxacin (10 µg)	0	0	0	0
Resistant to trimethoprim (5 µg)	0	0	0	0

Resistance to antibiotics in bacteria can be divided into innate resistance (primary), resistance is acquired (secondary) and resistance episomal. Resistance congenital (primary) is the resistance that is the nature of

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microorganisms, so naturally microorganisms can break down antibiotics. For example, Escherichia coli to ampicillin resistance caused by the bacteria's ability to produce β-lactamase enzyme encoded by the gene in the plasmid R factor R. factors including grade plasmid carrying the gene for resistance to one or more antibiotics is often controlling the formation of enzymes that can destroy the drug (Krisnaningsih et al, 2005)

Resistance acquired (secondary) can also take place due to the adaptation or adjustment mechanism metabolic activity of microorganisms to resist the effects of drugs, for example, by changes in the enzyme. Thus, microorganisms can form an enzyme which outlines antibiotics, eg gentamicin and ciprofloxacin, but research is sensitive to gentamicin and ciprofloxacin caused by the absence of mutation. No change in the target enzyme and DNA gyrase or instability of outer membrane permeability to an increase in drug accumulation in bacteria (Costa et al, 2010)

The occurrence of resistance can also be caused by two major ways excessive use of antibiotics and the resistant genes. According to Lopez-Lazaro et al., (2000) that there is a very close relationship between the development of antibiotic resistance by the number of antibiotic use. In addition, inaccuracies and irrational use of antibiotics is the main cause of most bacteria have become resistant (WHO, 2012).

CONCLUSION

Results can be concluded that microbial quality and safety of raw milk produced by local farmers and distributors was unsafe. The presence of *E. coli* as fecal indicator organism not only indicates the poor hygiene but also itself may be pathogenic for human health. Continuous efforts are required to reduce the resistance burden in human by strict monitoring of antibiotic resistance of *E. coli from* raw milk.

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