

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

by Mustofa Helmi

Submission date: 31-Dec-2019 10:58AM (UTC+0800)

Submission ID: 1238821640

File name: 7._Profile_Antibiotics_Resistance_On.....pdf (235.27K)

Word count: 2136

Character count: 12139

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

10 Mustofa Helmi Effendi^{1*}, Nenny Harijani¹ And Budiarto¹

1. Departement of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University, Indonesia

kampus c fkh unair, Jl Mulyorejo Surabaya 60115, Indonesia

*Correspondence author, Email : mheffendi@yahoo.com

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, & Diamadopoulou, 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

4
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 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

17
Sampling 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).

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 Chitrangingtyas 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%.

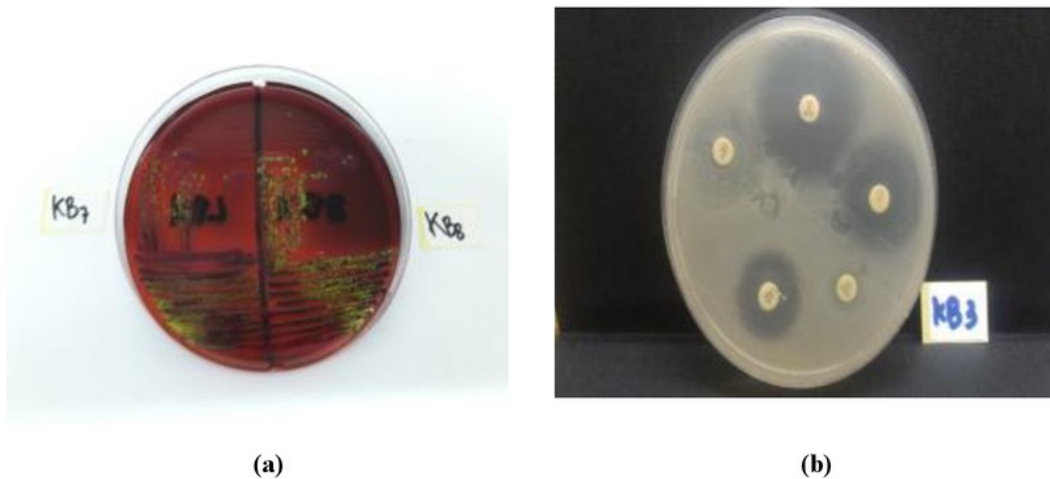


FIGURE 1. (a). *E. coli* on EMB Agar
(b). Antibiotic sensitivity test

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
<i>E. coli</i> (+)	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

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.

REFERENCES

- Bhaskara, I.B.M., K. Budiasa., K. Tono. 2012. Uji Kepekaan *Escherichia coli* sebagai Penyebab Kolibasilosis pada Babi Muda terhadap Antibiotika Oksitetrasiklin, Streptomisin, Kanamisin dan Gentamisin. Indonesia Medicus Veterinus 2012 ISSN : 2301-7848. 1(2) : 186 - 201.
- Bouki, C., Venieri, D., Diamadopoulos, E, 2013. Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. Ecotoxicology and Environmental Safety, 91: 1-9
- CDC, 2016. Methicillin-resistant Staphylococcus aureus (MRSA) infections. Diakses pada 10 April 2016
- Clinical Laboratory Standards Institute, 2007. Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing: 17th informational supplement CLSI document (M100-S17), CLSI, Wayne, PA (2007)
- Costa M.M., G. Drescher., F. Maboni., S.S Weber., A. Schrank., M.H Vainstein., I.S Schrank and A.C Vargas. 2010. Virulence factors, antimicrobial resistance, and plasmid content of *Escherichia coli* isolated in swine commercial farms. Arq. Bras. Med. Vet. Zootec. 62 30-36.
- Indana. 2015. Pembuktian Gen Penyandi Resisten Antibiotik Ampicillin pada *Escherichia coli* yang Diisolasi dari Beberapa Peternakan dan Pengepul Ayam di Surabaya. [Tesis] Fakultas Kedokteran Hewan Universitas Airlangga. Surabaya.
- Krisnaningsih, M.M.F., W.Asmara, dan M.H. Wibowo. 2005. Uji Sensitivitas Isolat *Escherichia coli* Patogen Terhadap Beberapa Jenis Antibiotika. I: 13-18.
- Lopez, L., L. Monnet., D. Yagüe., A. Burgos., A. Gonzalo., N. Campillos., and Saez M. 2000. Modelling and forecasting antimicrobial resistance and its dynamic relationship to antimicrobial use: a time series analysis. International Journal of Antimicrobial Agents 14 (1): 21-31.
- Pyatov, V., I. Vrtkova, and A. Knoll. 2014. Detection of Aminoglycoside, Sulfonamide and Tetracycline Resistance Genes in *Escherichia coli* Isolated from Bovine Milk Sampels. Mendel University. Brno.
- Um, M.M., Barraud, O, Kerouredan, M., Gaschet, M., Stalder, T., Oswald, E., Dagot, C., Ploy, M., Brugere, H., Bibbal, D., 2016. Comparison of the incidence of pathogenic and antibiotic-resistant *Escherichia coli* strains in adult cattle and veal calf slaughterhouse effluents highlighted different risks for public health. Water Research, 88: 30-38

Wellington, E.M., Boxall, A.B., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W. and Thomas, C.M., 2013. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet infectious diseases*, 13(2), pp.155-165.

World Health Organization. 2012. *The Evolving Threat of Antimicrobial Resistance Options for Action*. World Health Organization. France.

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

ORIGINALITY REPORT

17%

SIMILARITY INDEX

7%

INTERNET SOURCES

13%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

- 1 AL-AZZAWI, Sana and INALHAN, Göksenin. "ÇOCUKLUKTA OKUL ÇEVRELERİNİN TECRÜBE EDİLMESİNDEN YANSIMALAR: OKUL BAĞÇELERİ, OKUL YOLU VE ÇOCUĞUN FAVORİ MEKANLARI", İstanbul Kültür Üniversitesi, 2018. 4%

Publication
- 2 ARTUT, Selçuk. "BİLİM KURGU SİNEMASI BAĞLAMINDA VERİ ", İstanbul Kültür Üniversitesi, 2017. 3%

Publication
- 3 Abdullah D. Altalhi, Sabry A. Hassan. "Bacterial quality of raw milk investigated by Escherichia coli and isolates analysis for specific virulence-gene markers", Food Control, 2009 2%

Publication
- 4 www.absoluteastronomy.com 1%

Internet Source

5

www.omicsonline.org

Internet Source

1%

6

Tin Tin Myaing. "Antibiotic Resistance and Plasmid Carriage among Escherichia Coli Isolates From Chicken Meat in Malaysia", Applications of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries, 2005

Publication

1%

7

Trajano Felipe Barrabas Xavier da Silva, Débora Toledo Ramos, Maurício Dziedzic, Cíntia Mara Ribas de Oliveira et al. "Microbiological Quality and Antibiotic Resistance Analysis of a Brazilian Water Supply Source", Water, Air, & Soil Pollution, 2010

Publication

1%

8

academic.oup.com

Internet Source

1%

9

M.M. Costa, G. Drescher, F Maboni, S.S. Weber, A. Schrank, M.H. Vainstein, I.S. Schrank, A.C. Vargas. "Virulence factors, antimicrobial resistance, and plasmid content of Escherichia coli isolated in swine commercial farms", Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 2010

Publication

1%

10	journal.ipb.ac.id Internet Source	1%
11	www.ajas.info Internet Source	1%
12	psr.ui.ac.id Internet Source	1%
13	crwad.org Internet Source	1%
14	www.ijcmr.com Internet Source	1%
15	repository.up.ac.za Internet Source	1%
16	www.mdpi.com Internet Source	<1%
17	vetjournal.ankara.edu.tr Internet Source	<1%
18	P. J. van den Broek. "Determinants of carriage of resistant Escherichia coli in the Indonesian population inside and outside hospitals", Journal of Antimicrobial Chemotherapy, 06/22/2007 Publication	<1%

Exclude bibliography On