Poll Res. 40 (1) : 317-321 (2021)

ISSN 0257–8050

ANTIMICROBIAL RESISTANCE PROFILE OF *ESCHERICHIA COLI* BACTERIA COLLECTED FROM CLOACA SWAB OF BROILER CHICKEN AT SURABAYA TRADITIONAL MARKET, INDONESIA

W. TYASNINGSIH, Y. A. YURIANTI, J. RAHMAHANI, B. SETIAWAN, W. TYASNINGSIH, N. HARIJANI, BUDIARTO, M. H. EFFENDI*, SALAMAH AND A.M. WITANINGRUM

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

(Received 29 May, 2020; Accepted 23 July, 2020)

ABSTRACT

The aim of this research is to identify antimicrobial resistance's profile of *Escherichia coli* from cloaca swab of broiler chicken at the Surabaya traditional market for antibiotics: aztreonam, gentamicin, chloramphenicol, tetracycline, and ciprofloxacin. This research is using 60 broilers chicken sample, with cloaca swab method. The samples are isolated and identified with several procedures, Mac Conkey Agar (MCA), Eosin Methylene Blue Agar (EMBA), Gram staining, indole test, Methyl Red-Voges Proskauer (MR-VP), citrate and Triple Sugar Iron Agar (TSIA). The aim of isolation and identification is finding 60 positive isolate of *Escherichia coli*. Antibiotic sensitivity test using Kirby-Bauer (Disc Diffusion Method) based on Clinical and Laboratory Standard Institute 2018. *Escherichia coli* sensitivity test proved that resistant bacteria for antibiotic ciprofloxacin is 67%, tetracycline is 65%, gentamicin is 37%, chloramphenicol is 23%, and aztreonam is 3%.

KEY WORDS : Antimicrobial, Resistance, *Escherichia Coli*

INTRODUCTION

Antimicrobial Resistance (AMR) is a threat to health in Indonesia. Resistance has an impact on morbidity and mortality, as well as a negative impact on the health, economic, and future generations. Resistance initially occurs at the hospital level and then develops in the community. AMR in humans can be transmitted through food sources from animals consumed by the community cannot be separated from the presence of microorganism contamination, which has the opportunity to transmit the nature of resistance. One of the microorganisms that become contaminated in food sources of animal origin is the bacterium *Escherichia coli* (Erfianto, 2014; Rahmahani *et al*., 2020).

Resistance levels of *E. coli* isolated from broiler chicken meat from the traditional Bogor market, there is a resistance level of 80.6% against tetracycline, 14.2% against gentamicin, and 11.4% against chloramphenicol. From these data it was concluded that the level of resistance *E. coli* against some antibiotics is quite high due to excessive use of antibiotics (Suandy, 2011)

The WHO statement on the selection of appropriate antibiotics based on bacterial resistance patterns is important for the assessment of bacterial resistance factors and controlling the incidence of bacterial resistance in animal to human food. So there needs to be supervision related to the emergence of patterns of resistance to these antibiotics (WHO, 2019). The study was conducted to obtain a study of the resistance profile of *E. coli* bacteria in broiler chickens related to bio safety where *E. coli* bacteria with resistant nature have the opportunity to transmit the nature of resistance to humans through the food chain pathway.

The purpose of this study was to determine the antibiotic resistance profile of *E. coli* bacteria resulting from the isolation of broiler chicken cloaca from the Surabaya traditional market against aztreonam, gentamicin, chloramphenicol,

tetracycline and ciprofloxacin antibiotics. This research is expected to provide information to the public, especially for related institutions regarding the antibiotic resistance profile of *E. coli* bacteria resulting from the isolation of broiler chicken cloaca from the Surabaya traditional market to the antibiotics tested.

MATERIALS AND METHODS

The research materials are broiler cloaca swab samples, Buffered Peptone Water (BPW), Mac Conkey Agar (MCA), Eosin Methylen Blue Agar (EMBA), Sulfide Indol Motility (SIM), Methyl Red Voges Proskauer (MR-VP), Simons Citrate Agar (SCA), Triple Sugar Iron Agar (TSIA), Standard Mc Farland number 0.5, Mueller Hinton Agar (MHA), Nutrient Agar (NA), physiological NaCl, 70% alcohol, antibiotic disks, Gram staining (violet crystals, lugol, acetone alcohol, and safranin). Research tools include vacutainer tubes, S size sterile cotton swabs, test tubes, disposable petri dishes, ose, needles, microscopes, glass objects, cool boxes, and calipers. The research samples were 60 broilers cloaca swabs taken from three Surabaya traditional markets (Pucàng Market, Keputran Market and Wonokromo Market).

Sixty samples taken by the cloaca swab method were then put into a vacutainer tube containing Buffered Peptone Water (BPW) and put in a cool box. Samples were taken to the Bacteriology and Mycology Laboratory of the Veterinary Microbiology Department of the Faculty of Veterinary Medicine, Universitas Airlangga for further research. Samples were cultured on Mac Conkey Agar (MCA) media for 24 hours at 37 ºC. *Escherichia coli* bacterial colonies on MCA media grew with characteristic red, convex, and clear boundaries (Dewanti and Wahyudi, 2011; Effendi *et al*., 2018).

Colonies suspected of being *E. coli* bacteria were Gram stained with the result that all samples had morphology in the form of a short stem (coccobasil) and red in color. *E. coli* bacteria cannot maintain violet crystalline dyes during the Gram staining process due to the structure of Gram negative bacterial cell walls consisting of lipoproteins and thin peptidogly can (Fitrinaldi, 2011)

Colonies that were suspected as *E. coli* bacteria on MCA media and showed Gram negative results with morphology of the short stem were then cultured on Eosin Methylene Blue Agar (EMBA) media. *E. coli* bacteria in EMBA media have the characteristics of the growth of metallic green colonies and convex colony surfaces with flat edges. Metallic green discoloration in EMBA caused by *E. coli* bacteria can ferment glucose, lactose, maltose, and mannitol (Matuwo, 2012; Effendi *et al.,* 2019). Colonies suspected of being *Escherichia coli* bacteria on the EMBA medium were again stained with Gram to confirm the morphology and nature of the bacteria. Separate colonies that have been tested for Gram staining are followed by biochemical IMViC tests (Indol, MR-VP, citrate) and TSIA (Sridhar, 2006; Wibisono *et al.,* 2020).

E. coli bacteria showed positive indole results and there was motility on the SIM media. In the Methyl-Red (MR) test, *E. coli* bacteria showed positive results and Voges-Proskauer (VP) with negative results. In the citrate test, *E. coli* bacteria showed negative results. TSIA test results showed Acid / Acid results, negative H2S, and positive gas.

The *E. coli* bacterial isolate was made in the form of a suspension that was synchronized with McFarland 0.5 standard which was then subjected to antibiotic sensitivity testing using the Kirby-Bauer diffusion method against aztreonam antibiotics, gentamicin, chloramphenicol, tetracycline, and ciprofloxacin on Mueller Hinton Agar (MHA) media. MHA media were incubated for 16-18 hours at 37 ºC. Inhibition zone diameters were measured using a calipers measuring instrument with an accuracy of 0.02 millimeter (mm) and adjusted to the 2018 Clinical and Laboratory Standards Institute (CLSI)(CLSI, 2018; Putra *et al.,* 2020).

RESULTS AND DISCUSSION

The results of 60 positive samples of *Escherichia coli* bacteria were marked on MCA media. The results were obtained from all samples with a growth of red and convex colonies, Gram staining results obtained were Gram negative and colony-shaped short stems (coccobasil), on EMBA media the colonies were metallic green and spherical in shape and biochemical tests on SIM media with positive indole and motility. The MR test showed positive results, negative VP, the citrate test showed negative results, and the TSIA test showed Acid/Acid results, negative H2S, positive gas. The results of isolation and identification can be seen in Figure 1 and 2.

Sixty *Escherichia coli* bacterial isolates were followed by sensitivity testing to the antibiotics aztreonam, gentamicin, chloramphenicol,

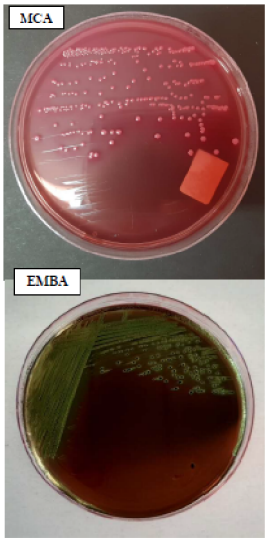

Fig. 1. Isolation results and identification of *Escherichia coli* bacteria on Mac Concey Agar (MCA) and Eosin Methylene Blue Agar (EMBA) media

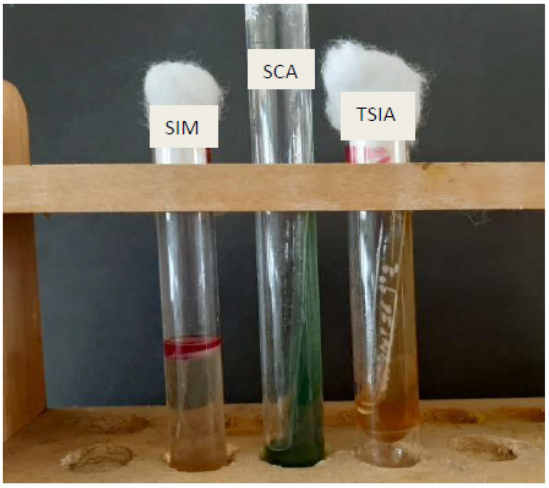

Fig. 2. The results of the identification of *Escherichia coli* bacteria on Sulfide Indol Motility (SIM), Simons Citrate Agar (SCA), and Triple Sugar Iron Agar (TSIA) media

tetracycline and ciprofloxacin. The sensitivity test showed that 40 samples (67%) were resistant to ciprofloxacin antibiotics, 39 samples (65%) were resistant to tetracycline antibiotics, 22 samples (37%) were resistant to gentamicin antibiotics, 14 samples (23%) were resistant to chloramphenicol antibiotics, and 2 samples (3%) were resistant to aztreonam antibiotics.

The results of the sensitivity test of *E. coli* bacteria to ciprofloxacin are classified as the highest resistance with a percentage of 67%. Ciprofloxacin antibiotics include fluoroquinolone antibiotics that work to influence DNA gyrase acid in bacteria, thus

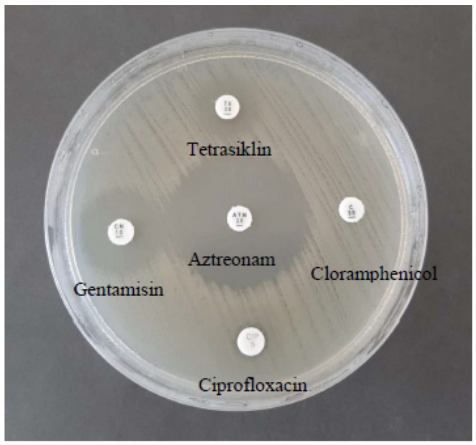

Fig. 3. Test results for the sensitivity of *Escherichia coli* bacteria to the antibiotics aztreonam, gentamicin, chloramphenicol, tetracycline, and ciprofloxacin.

inhibiting DNA synthesis. The resistance of *E. coli* bacteria to ciprofloxacin causes that ciprofloxacin cannot inhibit the existing DNA gyrase enzymes. Ciprofloxacin resistance is a result of the gyrA subunit mutation from gyrase which reduces the ability of antibiotics to bind to bacteria (Reygaert, 2013).

Sensitivity of *E. coli* bacteria to tetracycline showed the greatest 65% resistance after ciprofloxacin. Tetracycline antibiotics inhibit bacteria by inhibiting the process of ribosome 30S from prokaryotics by binding to aminoacyl-tRNA. The mechanism of resistance to tetracycline can occur because of changes in the permeability of bacterial cell envelopes. Bacterial cells that have resistance to tetracycline antibiotics cannot be actively transported into cells, so that the minimum inhibitory concentration cannot be maintained (Sudigdoadi, 2008).

Bacterial sensitivity test showed 37% of *E. coli* samples were resistant to gentamicin antibiotics. Gentamicin is an aminoglycoside class of antibiotics that has two or more amino groups that are bound to the benzene group and are bacteriocid (Juniastuti *et al.*, 2015). The mechanism of bacterial resistance to gentamicin occurs through inactivation of antibiotics by aminoglycosides modifiying enzymes, namely the attachment of aminoglycosides to specific protein receptors of the 30S subunit on bacterial ribosomes, and subsequently aminoglycosides will inhibit the complex activity of the initiation of peptide formation (Dwidjoseputro, 2005).

The sensitivity test of *E. coli* bacteria to the chloramphenicol antibiotic showed a resistance of

23%. Chloramphenicol is an antibiotic that works to inhibit strong protein synthesis in bacteria. Blocking the attachment of amino acids to the newly emerging peptide chain of the 50S unit in the ribosome, by interfering with the action of peptidyl transferase. Chloramphenicol resistance is caused by *E. coli* bacteria producing the enzyme chloramphenicol acetyltransferase which can damage drug activity (Jawetz *et al*., 2001)

Bacterial sensitivity test to aztreonam antibiotics showed a resistance of 3%. Aztreonam works by inhibiting bacterial cell wall synthesis to overcome severe infections by aerobic Gram-negative bacteria. The aztreonam antibiotic spectrum is similar to aminoglycosides, making it a special alternative to Gram-negative bacterial infections (Stringer, 2006). The mechanism of resistance to aztreonam occurs through the inactivation of antibiotics by the beta-lactamase enzyme which causes the bacterial cell wall to become resistant to antibiotics and reduced membrane permeability to antibiotics (Paterson and Bonomo, 2005; Wibisono *et al.,* 2020)

Antibiotic resistance occurs both intrinsically and in an acquired manner. Intrinsic resistance occurs chromosomally and takes place through cell multiplication which will be inherited in subsequent strains. Resistance can occur due to chromosomal mutations or due to DNA transfer through the process of transduction, transformation, and conjugation which is responsible for the emergence of resistance (Sudigdoadi, 2008; Putra *et al.,* 2019).

Genetic factors that underlie resistance can be caused by the mechanism by which enzymes are produced that can decipher antibiotics, changes in membrane permeability, increased endogenous substances that work antagonist to drugs, and changes in the number of drug receptors on bacterial cells or the nature of the component that binds the drug to its target (Sudigdoadi, 2008; Kristianingtyas *et al.,* 2020).

The results of the resistance profile of *E. coli* bacteria to antibiotics found that high isolates were resistant such as ciprofloxacin and tetracycline. *E. coli* bacteria resistant to antibiotics can transfer genetic factors to humans through the food chain or direct contact. The use of antibiotics in animals contributes to the occurrence of foodborne bacterial resistance in humans and animals.

CONCLUSION

The conclusions from the results of the study of antibiotic resistance profile against *E. coli* bacteria isolated from broiler chickens swabs from the Surabaya traditional market found 100% positive samples of *E. coli* are resistant to ciprofloxacin antibiotics (67%), tetracycline (65%), gentamicin (37% %), chloramphenicol (23%), and aztreonam (3%).

ACKNOWLEGEMENT

This research is expected to provide information to the public. Required for institutions related to the antibiotic resistance profile of *E. coli* bacteria and thank you for your guidance and support until the expected completion of this research.

REFERENCES

Clinical and Laboratory Standards Institute (CLSI). 2018. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Wayne, PA: CLSI.

Dewanti, S. and Wahyudi, M.T. 2011. Antibacteri activity of bay leaf infuse (Folia *Syzygium polyanthum* Wight) to *Escherichia coli in-vitro. J. Med. Planta*. 1 (4) : 78-81.

Dwidjoseputro, D. 2005. *Fundamentals of Microbiology*. Djambat, Jakarta. [Text in Indonesia].

Effendi, M. H., Bintari, I. G., Aksoro, E. B. and Hermawan. I. P. 2018. Detection of blaTEM Gene of *Klebsiella pneumoniae* Isolated from swab of food-producing animals in East Java. *Trop. Anim. Sci. J.* 41(3):174-178.

Effendi, M.H., Harijani, N., Budiarto, Triningtya, N.P., Tyasningsih, W. and Plumeriastuti, H. 2019. Prevalence of Pathogenic *Escherichia Coli* Isolated from Subclinical Mastitis in East Java Province, Indonesia. *Indian Vet. J.* 96 (03): 22-25.

Effendi**,** M.H., Harijani, N., Yanestria, S.M., Hastutiek, P. 2018. Identification of shiga toxin-producing *Escherichia coli* in raw milk samples from dairy cows in Surabaya, Indonesia. *Philippine J Vet Med.* 55(SI) : 109-114.

Erfianto, G.I. 2014. *Escherichia coli* Resistance to Antibiotics Isolated from Beef Cattle Imported through Jakarta's Tanjung Priok Port [Thesis]. Faculty of Veterinary Medicine. Bogor Agricultural Institute. [Text in Indonesia].

Fitrinaldi, 2011. Microbial Fuel Cell as Alternative Energy Using *Escherichia coli* Bacteria. Postgraduate Chemistry Study Program. Andalas University. Padang. [Text in Indonesia].

Juniastuti, T., Dewa, K.M., Sri, U.S., Iwan, S.H. and Rochma, K. 2015. *Textbooks on Pharmacotherapy and Toxicology*. Duta Persada Press: Surabaya. 10-22. [Text in Indonesia].

Jawetz, Z.E., M.E.A.A. Joseph and Delberg. 2001. *Medical Microbiology.* Translation by Eddy Mudihardi, *et al.* Microbiology Section, Faculty of Medicine, Airlangga University. Jakarta: Salemba Medika. [Text in Indonesia].

Kristianingtyas, L., Effendi, M.H., Tyasningsih, W. and Kurniawan, F. 2020. Genetic Identification of blactx-M Gene and blatem Gene on Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia Coli* from Dogs. *Indian Vet. J.* 97 (01): 17 – 21.

Leboffe, M.J. and Pierce, B.E. 2011. *A Photographic Atlas for the Microbiology Laboratory* 4th Edition. Morton Publishing. Colorado. 110-113.

Matuwo, A. 2012. Microbiological Quality of Chicken Meat in Modern and Traditional Markets in Makassar [Thesis]. Faculty of Animal Husbandry. Makassar Hasanuddin University. [Text in Indonesia].

Paterson, D.L. and Bonomo, R.A. 2005. Extended-spectrum β-lactamases: a clinical update. *Clinical Microbiology Reviews*. 18(4): 657-686. [Text in Indonesia].

Permatasari, D.A., Witaningrum, A.M., Wibisono, F.J., Effendi, M.H**.** 2020. Detection and prevalence of multidrug-resistant *Klebsiella pneumoniae* strains isolated from poultry farms in Blitar, Indonesia. *Biodiversitas.* 21 (10): 4642-4647.

Pratiwi, P. B., Hidanah, S., Effendi, M. H., Estoepangestie, A. T. S., Rahardjo, D. and Tyasningsih, W. 2020. Antibiotic Resistance Profile of *Escherichia coli* Isolated from Beef Sold at Several Wet Market In Sidoarjo, Indonesia. *Poll Res.* 39 (4) : 1262-1265

Putra, A.R. Effendi, M.H. Koesdarto, S. Suwarno, S. Tyasningsih, W. and Estoepangestie, A.T. 2020. Detection of the extended spectrum β-lactamase produced by *Escherichia coli* from dairy cows by using the Vitek-2 method in Tulungagung regency, Indonesia. *Iraqi Journal of Veterinary Sciences*. 34 (1): 203-207.

Putra, A.R.S., Effendi, M.H., Koesdarto, S. and Tyasningsih, W. 2019. Molecular Identification of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* Isolated from Dairy Cows in East Java Province, Indonesia. *Indian Vet. J.* 96 (10): 26 – 30.

Rahmahani, J., Salamah, Mufasirin, Tyasningsih, W. and Effendi, M.H. 2020. Antimicrobial Resistance Profile of *Escherichia coli* from Cloacal Swab of Domestic Chicken in Surabaya Traditional Market. *Biochem. Cell. Arch.* 20 (1): 2993-2997.

Reygaert, W.C. 2013. Antimicrobial Resistance Mechanisms of *Staphylococcus aureus*. Microbial Pathogens and Strategies for Combating them: Science, Technology, and Education. 297-305.

Riwu, K.H.P., Effendi, M.H. and Rantam, F.A**.** 2020. A review of extended-spectrum β-Lactamase (ESBL) producing *Klebsiella pneumoniae* and Multidrug-Resistant (MDR) on companion animals. *Sys Rev Pharm.* 11 (7): 270-277.

Suandy, I. 2011. Antimicrobial resistance in *Escherichia coli* isolated from commercial broiler farms in Bogor District, West Java [tesis]. Chiang Mai (TH): Chiang Mai University.

Sridhar, R.P.N. 2006. IMViC Reaction. JJMMC. https:// www.microrao. com/micronotes/imvic.pdf [20 Juli 2019].

Sudigdoadi, Sunarjati, 2008. Antibiotic Resistance Mechanisms in Bacterial Infection. Faculty of Medicine, Padjadjaran University. 8-13. [Text in Indonesia].

Stringer, J.L. 2006. *Basic Concepts of Pharmacology Guide for Students*. Huriawati, editor. Jakarta (ID): EGC.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. 2020. The Presence of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* on Layer Chicken Farms in Blitar Area, Indonesia. *Biodiversitas.* 21 (6) : 2667-2671.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. 2020. CTX Gene of Extended Spectrum Beta-Lactamase (ESBL) Producing Escherichia coli on Broilers in Blitar, Indonesia. *Sys Rev Pharm.* 11 (7) : 396-403.

Wibisono, F.J., Sumiarto, B., Untari, T., Effendi, M.H., Permatasari, D.A. and Witaningrum, A.M. 2020. Short Communication: Pattern of antibiotic resistance on extended-spectrum beta-lactamases genes producing *Escherichia coli* on laying hens in Blitar, Indonesia. *Biodiversitas*. 21 (10): 4631- 4635.

Widodo, A., Effendi, M.H., Khairullah, A.R. 2020. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from livestock. *Sys Rev Pharm.* 11 (7): 382-392.

World Health Organization (WHO). 2019. Let's Together Overcome Antimicrobial Resistance (AMR). // http.www.depkes.go.id. [July 20, 2019]. [Text in Indonesia].

Yanestria, S.M., Rahmaniar, R.P., Wibisono, F.J., Effendi, M.H. 2019. Detection of *invA* gene of *Salmonella* from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. *Veterinary World*. 12 (1) : 170-175.