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Antibiotic resistance profile of *Escherichia coli* isolates collected from cloaca swabs on laying hens in Udanawu Sub-District, Blitar District, Indonesia

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ABSTRACT

The use of antibiotics in poultry can encourage antibiotic resistance, one of which is the bacterium *Escherichia coli*. This research was carried out in a cross-sectional descriptive, aimed at finding out the resistance profile of *Escherichia coli* bacteria on laying hens in Udanawu sub-district, Blitar district. Samples of 30 cloaca swabs were taken from the layer chicken. Positive isolates of *Escherichia coli* were tested for antibiotic sensitivity on Mueller-Hinton agar. The results showed that there were 90% positive *Escherichia coli* on laying hens in Udanawu sub-district, Blitar district. The highest percentage of antibiotic resistance in *Escherichia coli* was 96.3% in the erythromycin antibiotic, 77.78% in ampicillin, 66.67% in streptomycin, 59.26% in sulfamethoxazole-trimethoprim, and 44.44% in tetracycline. *Escherichia coli* which has multidrug resistance properties on laying hens in Udanawu sub-district is quite high, amounting to 70.37%. The overall resistance in *Escherichia coli* to some antibiotics shows that the resistance on laying hens is relatively high, so that the supervision of antibiotic use in animal husbandry needs to be increased as an effort to prevent broader antibiotic resistance.

Key words: *Escherichia coli, Laying hens, Multidrug resistance.*

Introduction

Poultry is one of the livestock industries in Indonesia that is able to develop rapidly. The poultry industry plays an important role in the economy, being able to produce self-sufficiency in poultry meat and eggs. Poultry is one of the important reservoirs of zoonotic bacterial agents (Suardana *et al*., 2014). Pathogenic microorganisms can cause various kinds of infectious diseases, which are the main diseases and the highest cause of death in animals and humans. High incidence of infectious diseases causes the use of antibiotics is still the most dominant in health care (Noor and Poeloenga, 2014). The consumption of antibiotics medically in the animal sector reaches around 80%, mostly to promote growth in healthy animals (WHO, 2017).

The use of antibiotics in animals can encourage antibiotic resistance. Antibiotics that are often used in animal agriculture are beta-lactam antibiotics. Beta-lactam antibiotics work to inhibit cell walls, derived from penicillin and cephalosporins (Hammerum *et al*., 2014; Santos *et al*., 2013). Animal husbandry is one source of antimicrobial resistance. Microorganisms that experience antimicrobial resistance make microorganisms more resistant so that

drugs become ineffective and infections persist in the body and increase the risk of spread in subsequent hosts. Antimicrobial resistance is a threat to global public health that requires action in all sectors of government and society.

Escherichia coli is one of the causes of infection in humans and animals which can easily cause antimicrobial resistance (Paterson and Bonomo, 2005). *Escherichia coli* as commensal bacteria that are multidrug resistance can be a health problem because *Escherichia coli* is able to transfer resistant genes to pathogenic bacteria (Masruroh *et al*., 2016). Antimicrobial resistance causes a decrease in the effectiveness of treatment, increased transmission of infection, increased mortality, and significantly increases the cost of health care, while the discovery of new antibiotics is getting less and less (Handayani *et al*., 2017). The level of resistance to antibiotics other than third generation cephalosporins is 93.7% (ampicillin), 75% (streptomycin), 68.75% (erythromycin), 62.5% (sulfamethoxazole), 18.75% (doxycycline), and 6.25% (kanamycin), these results indicate that *Escherichia coli* in Bogor City is multidrug resistance (Masruroh *et al*., 2016). The results of sensitivity testing of *Escherichia coli* isolates isolated from laying hens cloaca swabs revealed that there was resistance to doxycycline and ciprofloxacin with resistance levels of 36.36% and 14.77% (Ariyani *et al*., 2018).

Materials and Methods

Sampling and bacterial isolation

This research is an observational study of laying hens in Udanawu sub-district, Blitar district. Samples were taken randomly from 30 laying hens cloaca swabs, using sterile cotton swabs. Samples are stored at cold temperatures and taken to the laboratory for isolation and identification of *Escherichia coli* bacteria. Bacterial isolation was carried out on selective media MacConkey Agar no. 3 (Oxoid, England) incubated at 35-37 °C for 20-24 hours, then biochemical identification of bacteria was carried out with the IMVIC and TSIA tests (Effendi *et al*., 2018).

Antibiotic sensitivity test

Resistance tests in this study used 5 different types of antibiotics, including the beta lactam (ampicillin) group, the aminoglycoside group (streptomycin), the macrolide group (Erythromycin), the tetracycline (tetracycline) group, and the sulphonamide group (Sulfamethoxazole-Trimethoprim) so that the macrolide group (Erythromycin), the tetracycline group (tetracycline), and the sulphonamide group (Sulfamethoxazole-Trimethoprim), The results of resistance can be seen the existence of multidrug resistance, namely the sensitivity of *Escherichia coli* bacteria to more than 3 classes of antibiotics. Positive isolates of *Escherichia coli* were tested for antibiotic sensitivity tests on Mueller-Hinton agar (Merck, Germany), (CLSI, 2017) using an antibiotic disk, namely the antibiotic ampicillin 10 µg (Oxoid, England), streptomycin 10 µg (Oxoid, England), erythromycin 15 µg (Oxoid, England), tetracycline 30ìg (Oxoid, England), sulfamethoxazole-trimethoprim 25µg (Oxoid, England). Bacterial isolates were determined their sensitivity to antibiotics by measuring the diameter of bright or inhibited zones formed, using calipers. Determination of susceptible (S), intermediate (I), and resistant (R) is determined through the size of the diameter of the light zone or inhibition formed based on CLSI standard recommendations (CLSI, 2017; Effendi *et al*., 2018).

Results and Discussion

The results of isolation and identification based on testing using MacConkey agar selective media and biochemical testing of IMVIC and TSIA, showed 27 samples (90%) were positive for *Escherichia coli* from 30 samples identified in this study (Table 1). Most *Escherichia coli* bacteria are non-harmonic (commensal) strains, but certain strains such as *Avian Pathogenic Escherichia coli* (APEC) can cause poultry disease. *Escherichia coli* becomes a pathogen if the number of these bacteria in the digestive tract increases or is extraintestinal (Brooks *et al*., 2013).

Table 1. Prevalence of *Escherichia coli* in chicken cloaca swabs

Location	Number of Samples	*Escherichia coli*	
		Positive	Negative
Farm 1	5	5	-
Farm 2	5	5	-
Farm 3	5	4	1
Farm 4	5	4	1
Farm 5	5	5	-
Farm 6	5	4	1
Udanawu	30	27 (90%)	3 (10%)

Positive samples of *Escherichia coli* on MacConkey Agar were identified with a small, reddish pink semi-circular colony (Figure 1). The reddish pink turbid colour indicates that *Escherichia coli* bacteria ferment lactose, this is in accordance with previous research which states that *Escherichia coli* bacteria grow well on MacConkey agar with round and convex colonies (Dewanti and Wahyudi, 2011).

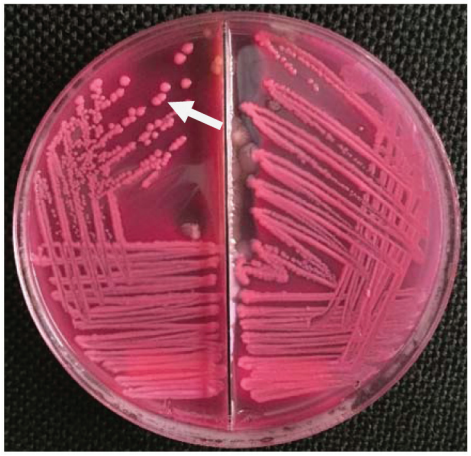

Fig. 1. *Escherichia coli* colony (arrow) on MacConkey

Sensitivity test or sensitivity test of *Escherichia coli* bacteria in chicken cloaca swab samples at layer chicken farms in Udanawu sub-district, Blitar district, was carried out on Mueller-Hinton agar according to recommendations from Clinical Laboratory Standard Institute (CLSI, 2017) on 5 classes of antibiotics, namely ampicillin, streptomycin, erythromycin, tetracycline, and sulfamethoxazole-trimethoprim, seen in Figure 2. The culture was incubated at 37 ° C for 18-24 hours.

The highest percentage of antibiotic resistance in *Escherichia coli* in the Udanawu sub-district layer chicken farm was 96.3% in the erythromycin antibiotic, then 77.78% in ampicillin, 66.67% in streptomycin, 59.26% in sulfamethoxazole-trimethoprim, and 44.44% in tetracycline. The results of the *Escherichia coli* sensitivity test on antibiotics in chicken cloaca swabs in the Udanawu sub-district layer farm are shown in Figure 3.

The results showed a relatively high incidence of MDR in layer chicken farms in Udanawu sub-district, Blitar district, which amounted to 70.37% (Figure 4). The nature of Multidrug resistance on antibiotics, including beta lactam, streptomycin, and tetracycline, often found in *Salmonella* sp. and *Escherichia coli* (Erviani, 2013). Antimicrobial resistance,

Fig. 2. *Escherichia coli* resistance patterns Antibiotics (1) Ampicillin, (2) Streptomycin, (3) Erythromycin, (4) Tetracycline, and (5) Sulfamethoxazole-Trimethoprim

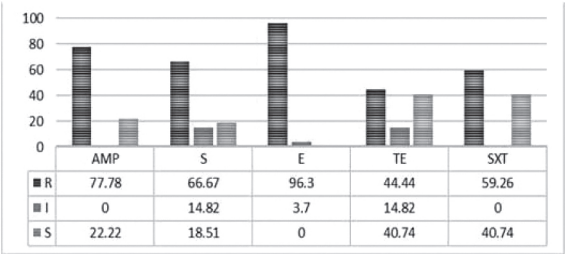

Fig. 3. Percentage of *Escherichia coli* resistance S (Susceptible); I (Intermediate); R (Resistant) Antibiotics (AMP) Ampicillin, (S) Streptomycin, (E) Erythromycin, (TE) Tetracycline, dan (SXT) Sulfamethoxazole-Trimethoprim

especially multidrug resistance is a problem that is difficult to overcome in the treatment of infectious diseases. Multidrug resistance organisms are bacteria that are resistant to three or more different antimicrobial groups (Kurniawati *et al*., 2015). *Escherichia coli* as a commensal bacterium that is multidrug resistance can be a health problem because *Escherichia coli* can transfer resistance to beta lactam antibiotics to other bacteria in the poultry digestive tract (CDC, 2018; WHO, 2018; Wibisono *et al.*, 2020).

A large number of antibiotics used in layer chicken farms are also used for human therapy, resulting in selection of pathogenic bacteria that are resistant to several types of drugs. Infection by *Escherichia coli* can be treated using sulphonamides, ampicillin, cephalosporins, chloramphenicol, tetracycline and aminoglycosides (Kusuma, 2010).

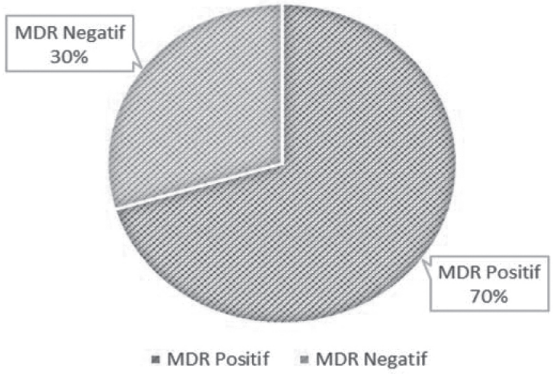

Fig. 4. *Escherichia coli* bacteria Multidrug Resistance (MDR) Event Diagram

Conclusion

Escherichia coli which has MDR resistance properties in Layer Chicken in Udanawu sub-district is quite high, amounting to 70.37%. The highest antibiotic resistance profile in the erythromycin antibiotic. Incidence of overall resistance in *Escherichia coli* against some antibiotics shows resistance in chicken layers is relatively high, so monitoring the use of antibiotics on farms needs to be increased as an effort to prevent antibiotic resistance even broader.

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References

Ariyani, N., Nurhidayah, Istianingsih, Ambarwati and Sari, R.A. 2018. Doxycycline and ciprofloxacin resistance in *Escherichia coli* Isolated from Layer feces. National Veterinary Drug Assay Laboratory.

Brooks, G.F., Carroll, K.C., Butel, J., Morse, S.A. and Mietzner, T. 2013. *Medical Microbiology.* 26th editi. ed. *Medical Microbiology.* Mc Graw Hill. https://doi.org/10.1017/CBO9781107415324.004

CDC, 2018. E.coli (*Escherichia coli*).

CLSI, 2017. M100 Performance Standards for Antimicrobial, 27th ed. Clinical and Laboratory Standards Institute, USA.

Dewanti, S. and Wahyudi, M.T. 2011. Research article antibacteri activity of bay leaf infuse (Folia *Syzygium polyanthum* Wight) to *Escherichia coli In-vitro*. *Jurnal Medika Planta* 1 : 79–80.

Effendi, M.H., Harijani, N., Yanestria, S.M. and 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.

Erviani, A.E. 2013. Analisis Multidrug Resistensi Terhadap Antibiotik Pada *Salmonella typhi* Dengan Teknik Multiplex PCR. *Biogenesis.* 1 : 51–60.

Hammerum, A.M., Larsen, J., Andersen, V.D., Lester, C.H., Skytte, T.S.S., Hansen, F., Olsen, S.S., Mordhorst, H., Skov, R.L., Aarestrup, F.M. and Agersø, Y. 2014. Characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. *Journal of Antimicrobial Chemotherapy*. 69 : 2650–2657. https://doi.org/10.1093/jac/dku180

Handayani, R.S., Siahaan, S. and Herman, M.J. 2017. Resistensi Antimikroba dan Penerapan Kebijakan Pengendalian di Rumah Sakit di Indonesia. jurnal penelitian dan pengembangan pelayanan kesehatan 1 : 131–140.

Kurniawati, A.F., Satyabakti, P. and Arbianti, N. 2015. Perbedaan Risiko Multidrug Resistance Organisms (MDROS) Menurut Faktor Risiko dan Kepatuhan Hand Hygiene. *Jurnal Berkala Epidemiologi*. 3 : 277–289. https://doi.org/http://dx.doi.org/10.20473/jbe.V3I32015.277-289

Kusuma, S.A.F. 2010. *Escherichia coli*.

Masruroh, C.A., Sudarwanto, M.B. and Latif, H. 2016. Tingkat Kejadian *Escherichia coli* Penghasil Extended Spectrum B-Lactamase yang Diisolasi dari Feses Broiler di Kota Bogor. *Jurnal Sain Veteriner*. 34: 42-49.

Noor, S.M. and Poeloenga, M. 2014. Pemakaian Antibiotika Pada Ternak. Lokakarya Nasional Keamanan Pangan Produk Peternakan 56–64.

Paterson, D.L. and Bonomo, R.A. 2005. Extended Spectrum Beta-Lactamases/: a Clinical Update. *Clinical Microbiology Reviews.* 18 : 657–686. https://doi.org/10.1128/CMR.18.4.657

Santos, L.L. dos, Moura, R.A., Aguilar-Ramires, P., Castro, A.P. De, Lincopan, N. 2013. Current status of extended-spectrum β -lactamase (ESBL) -producing Enterobacteriaceae in animals. Microbial pathogens and strategies for combating them: science, technology and education 1600–1607.

Suardana, I.W., Utama, I.H., Putriningsih, P.A.S. and Rudyanto, M.D. 2014. Uji Kepekaan Antibiotika Isolat *Escherichia coli* O157/: H7 asal Feses Ayam. *Buletin Veteriner Udayana.* 6 : 19–27.

WHO, 2018. *E. coli.*

WHO, 2017. Stop using antibiotics in healthy animals to prevent the spread of antibiotic resistance.

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.