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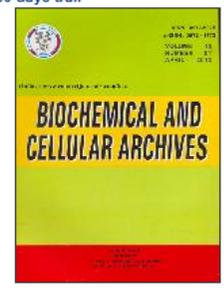
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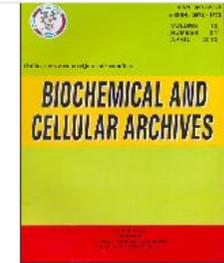
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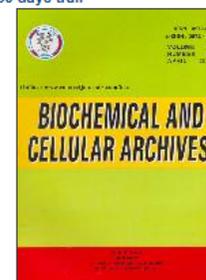
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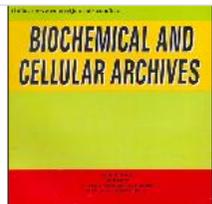
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ANTIMICROBIAL RESISTANCE PROFILE OF *ESCHERICHIA COLI* FROM CLOACAL SWAB OF DOMESTIC CHICKEN IN SURABAYA TRADITIONAL MARKET

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ABSTRACT : The aim of this research is to determine antimicrobial resistance's profile of *Escherichia coli* from cloacal swab of domestic chicken in Surabaya traditional market to several antibiotic such as aztreonam, ciprofloxacin, gentamicin, chloramphenicol, dan tetracycline. Sixty samples were taken from Pucang, Keputran, and Wonokromo traditional market. Those samples were isolated and identified using Mac Conkey Agar (MC Agar), Eosin Methylen Blue Agar (EMB Agar), Gram staining, IMViC and Triple Sugar Iron Agar (TSI Agar). The results shows that 58 out of 60 of the sample was identified as *Escherichia coli*. The isolate of *Escherichia coli* then further tested to sensitivity test using Kirby-Bauer method. Final results indicates that 48% of samples were resistant to tetracycline, 38% resistance to ciprofloxacin, 16% resistance to chloramphenicol, 10% resistance to gentamicin and 7% resistance to aztreonam. The results of this study illustrate the potential of domestic chickens as a reservoir for the spread of antibiotic resistance to the community.

Key words : Domestic chicken, cloacal swabs, antibiotic resistance, *Escherichia coli*.

INTRODUCTION

Contamination of *E. coli* bacteria is often found in food sources of animal origin, *E. coli* bacteria are naturally normal flora of the digestive tract of animals and humans. This bacterium can play a role in preventing pathogenic organisms in the digestive tract. Some strains of *E. coli* usually become pathogenic because of the pathogenic and virulent abilities of different genes in transmissible genetic element (Erfianto, 2014). Pathogenic *E. coli* bacteria can cause diarrhea, urinary tract infections, sepsis, and meningitis (Jawetz *et al.*, 2016).

One source of food of animal origin that is of interest to the public today is free-range chicken, in 2016 the level of consumption of free-range domestic chicken per capita reached 0.626 kg/year, making free-range chicken the second largest poultry meat supplier after broiler chickens (Central Statistics Agency, 2017). *E. coli* bacteria that have antibiotic resistant properties can potentially transfer these resistant genes to other bacteria, especially those belonging to foodborne disease bacteria and if infect in humans can cause harm to human health including failure of treatment with antibiotics against

disease agents that have been resistant (Erfianto, 2014).

The purpose of this study was to determine the resistance profile of *E. coli* bacteria resulting from isolation of native chicken cloaca swabs from traditional markets in Surabaya to aztreonam, ciprofloxacin, gentamicin, chloramphenicol and tetracycline antibiotics. This research is expected to provide benefits in the form of information on the level of resistance of *E. coli* bacteria in domestic chickens that are traded at the traditional market in Surabaya.

MATERIALS AND METHODS

Research sample

The research sample was 60 native chicken cloaca swabs obtained from three traditional markets in Surabaya, namely the Pucang market, the Keputran market, and the Wonokromo market.

Sample isolation and identification

Sixty cloaca swab samples were taken using a sterile cotton swab and then inserted into a vacutainer tube containing Buffered Peptone Water stored in a cool box containing ice gel. Samples obtained were taken to the

Bacteriology and Mycology Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Airlangga University to be isolated on Mac Conkey Agar (MCA) media for 24 hours at 37°C. *Escherichia coli* bacterial colonies on MCA media will appear pink with a perfectly round, convex colonies with clear boundaries (Barcella *et al*, 2016 and Effendi *et al*, 2019).

Colonies suspected of being *E. coli* bacteria were then stained for Gram to see the nature and morphology of the bacteria. Gram stain results of *E. coli* bacteria showed morphology in the form of a short stem and red color, this is caused by the concentration of lipids and the thickness of the peptidoglycan layer on the bacterial cell wall. The alcohol compound increases the porosity of the cell wall by dissolving the lipid layer causing the Violet Crystal to be more easily removed, so that in the provision of a bacterial cell dye comparison with Safranin dyes or the bacteria will show red results (Rahayu and Gumilar, 2017). Colonies suspected as *Escherichia coli* bacteria on MCA media and Gram staining were implanted in isolation media and identification of Eosin Methylene Blue

(MHA) media (Hudzicki, 2016). MHA media were incubated at 37°C for 16-18 hours and calculated the amount of inhibition zone using calipers and adjusted to the standards set by CLSI (2018).

RESULTS AND DISCUSSION

Of the 60 cloaca swab samples obtained, 58 of them were identified as positive. *Escherichia coli* was characterized by the growth of round and red colonies on MCA media, the nature and morphology of rod-shaped and Gram-negative bacteria, on EMBA media, spherical and metallic green colonies followed by biochemical tests. SIM with positive indole test results, negative H₂S and visible motility. Colony growth in the citrate test showed negative results and in TSIA all samples showed Acid / Acid, negative H₂S and positive gas due to the ability of *E. coli* to ferment glucose, lactose and sucrose. The Methyl-Red (MR) test of the samples tested showed positive results, while the Voges-Praskeur (VP) test showed negative results. The results of isolation and identification of *E. coli* bacteria can be seen in Figs. 1 and 2.

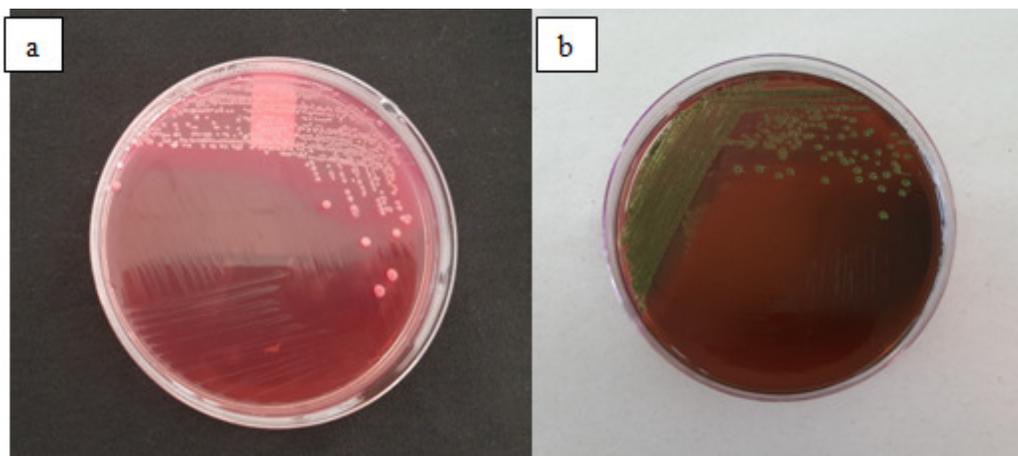


Fig. 1 : Isolation results and identification of *Escherichia coli* bacteria in media. (a). Mac Conkey Agar (MCA) and (b). Eosin Methylene Blue Agar (EMBA).

Agar (EMBA) for 24 hours at 37°C, followed by biochemical tests including Indol, MR-VP, citrate (IMViC) and Triple Sugar Iron Agar (TSIA) tests (Kristianingtyas *et al*, 2020; Putra *et al*, 2019).

Sensitivity test

The *E. coli* bacterial isolate was continued with a sensitivity test using the disk diffusion test method or called the Kirby-Bauer method. *E. coli* bacterial isolates were taken using sterile ose and made a suspension in accordance with Mc Farland's standard 0.5. The suspension was flattened using a sterile cotton swab and placed five antibiotic disks to be tested at a specified distance on the surface of the Mueller Hinton Agar

Fifty-eight isolates of *E. coli* bacteria were then continued with sensitivity tests for five types of antibiotics namely aztreonam, ciprofloxacin, gentamicin, chloramphenicol and tetracycline. Sensitivity test results showed that antibiotic profiles were 48% resistant, 7% intermediate, and 45% sensitive to tetracycline antibiotics, ciprofloxacin showed a percentage of 38% resistant, 3% intermediate and 38% sensitive, to chloramphenicol, showed a resistance amount of 16%, 7% intermediate and 48% sensitive, to Gentamycin showed resistance amount of 10%, 5% intermediate and 84% sensitive, to aztreonam the percentage of resistance was 7% and 93% sensitive. The sensitivity test results can be seen in Fig.

3 and Table 1.

Tetracycline is a broad spectrum bacteriostatic antibiotic with a mechanism of action inhibiting ribosomal protein synthesis by inhibiting the inclusion of aminoacyl t-RNA in the elongation phase, causing blockade of the peptide chain extension. The mechanism of resistance to tetracycline occurs because of the change in permeability of microbial cell envelopes. Bacterial cells that are resistant to tetracycline compounds cannot be actively transported into cells so that the minimum inhibitory concentration cannot be maintained (Suandy, 2011).

The highest resistance level after tetracycline is

ciprofloxacin, that is 22 isolates out of 58 isolates or 38% isolates have inhibition zone diameters less than equal to 15 mm. Ciprofloxacin is a second-generation fluorkuinolone antibiotic that works to deactivate the production of DNA graise enzymes and topoisomerase IV, where both of these enzymes work in helping the synthesis of DNA and bacterial proteins (Rachmad, 2017). The mechanism of resistance to fluorkiunolon antibiotics occurs due to changes in enzyme targets (DNA graise and topoisomerase IV), decreased permeability of the outer membrane of bacterial cells or the development of efflux mechanisms (Kohanski *et al*, 2010).

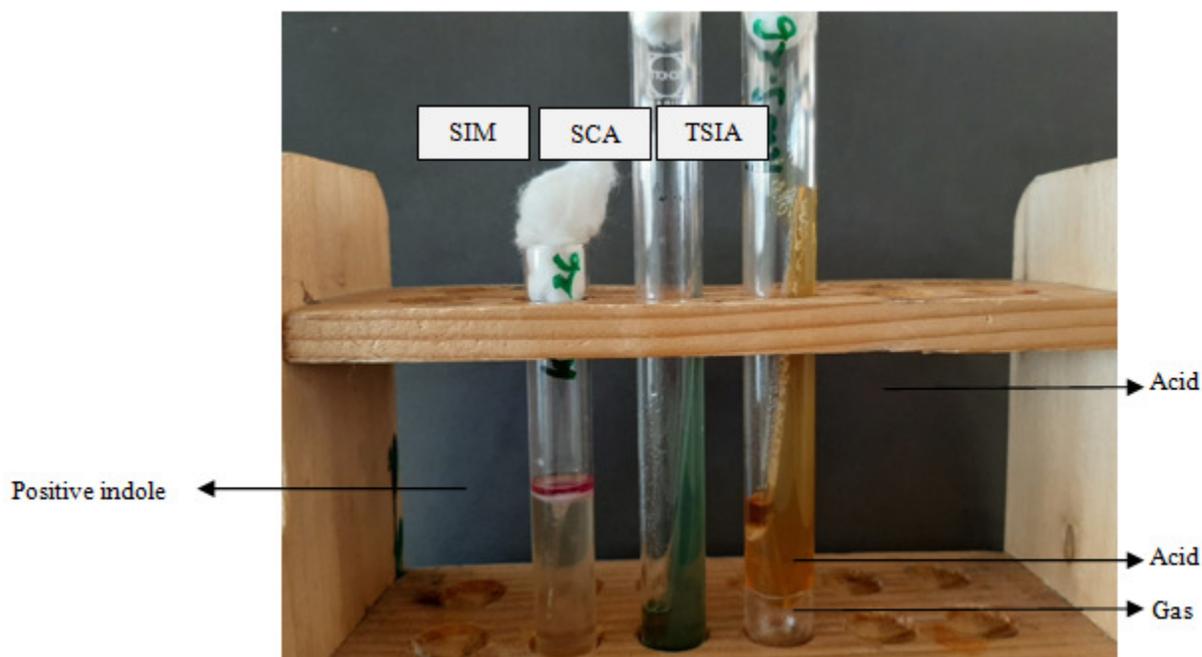


Fig. 2 : Results of *Escherichia coli* bacteria identification in biochemical test of Sulfide Indol Motility (SIM), Simons Citrate Agar (SCA), Triple Sugar Iron Agar (TSIA).

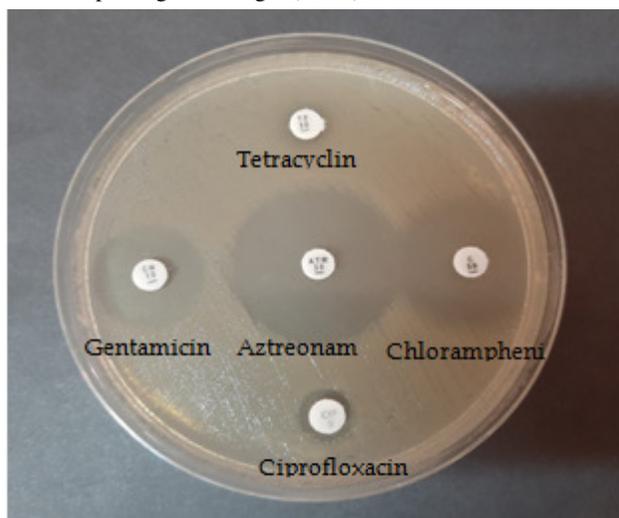


Fig. 3 : Test results of the sensitivity of *Escherichia coli* bacteria to the antibiotics aztreonam, ciprofloxacin, gentamicin, chloramphenicol and tetracycline.

Table 1 : Inhibitory zones of *Escherichia coli* against aztreonam, ciprofloxacin, gentamicin, chloramphenicol and tetracycline antibiotics.

No.	Antibiotics	R	I	S
1.	Aztreonam	4	0	54
2.	Ciprofloxacin	22	2	34
3.	Gentamicin	6	3	49
4.	Chloramphenicol	9	4	45
5.	Tetracycline	28	4	26

Note : R= resistant; I= intermediate; S= sensitive

Chloramphenicol has 9 isolates out of 58 isolates or 16%. Chloramphenicol works by inhibiting protein synthesis by blocking the attachment of peptide chain amino acids that arise in the 50s ribosome unit, by inhibiting the action of peptidyl transferase. (Jawetz *et al*, 2001) mechanism of chloramphenicol resistance caused by bacteria producing the enzyme chloramphenicol

acetyltransferase that can damage drug activity, some cases of resistance to chloramphenicol are also caused by modification of targets, efflux pumps and changes in permeability of the outer membrane (Fernandez *et al*, 2019).

Gentamicin and aztreonam showed the lowest percentage of resistance, namely 10% and 7% of 58 isolates. The mechanism of resistance of gentamicin isolates can occur through several processes, but often resistance to gentamicin occurs due to antibiotic inactivation by aminoglycosides modifying enzymes in addition to that, resistance to gentamicin can also be influenced by membrane modification, efflux pumps, membrane cell modification, mutation and modification of ribosomes (Tsodikova and Laby, 2016).

Aztreonam is a monobactam antibiotic with the mechanism of action of binding to the binding of protein 3 (PBP-3) from Gram negative so that it can cause lysis and death in bacteria. Aztreonam resistance mechanism can be caused by bacteria that produce the enzyme Extended spectrum beta lactamase (ESBL). ESBL can inactivate most beta-lactam antibiotics such as penicillin, cephalosporins and monobactams (Kapoor and Gathwala, 2004).

Antimicrobial Resistance (AMR) mechanism is divided into two aspects, namely genetic and biochemical aspects. The genetic aspect of resistance is mutation and transfer of genetic material horizontally (Susanto, 2014). Biochemical aspects or factors that do not look at the underlying genetic factors are changes in the permeability of bacterial cells to drugs, changes in the number of drug receptors on bacterial cells or the nature of the components that bind the drug to its target, and the production of enzymes that can decompose antibiotics such as the enzyme penicillinase, cephalosporinase, phosphorylase, adenylase and acetylase (Suandy, 2011).

The results of this study indicate that chickens traded in Surabaya traditional markets detected resistance to several antibiotics tested especially against tetracycline and ciprofloxacin antibiotics, which had the highest resistance value of 48% and 38% of 58 *E. coli* bacterial isolates. *E. coli* bacteria that have antibiotic resistant properties can potentially transfer the resistant gene to other bacteria, especially those belonging to foodborne disease bacteria (Erfianto, 2014; Raymond, 2019).

Antibiotic resistance can be caused by several factors, but the most important is the prevalence of resistant genes and the extension of antibiotic use. Misuse of antibiotics such as therapeutic errors, incomplete or prolonged use (Suandy, 2011). Strict supervision is needed related to

the provision of antibiotics in food sources of animal origin, because food sources from animals that have bacteria with antibiotic resistant properties can transmit the nature of resistance through direct contact or through the food chain process (Magiorakos *et al*, 2012; Raymond, 2019).

CONCLUSION

The conclusion that can be drawn from the study of the antibiotic resistance profile of *Escherichia coli* bacteria resulting from isolation of free-range chicken cloacal from the traditional market in Surabaya. The results of this study illustrate the potential of domestic chickens as a reservoir for the spread of antibiotic resistance to the community.

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