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Antibiotics Resistance Patterns of *Escherichia coli* Isolated from Poultry in West Java

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INTRODUCTION

Most of poultry industries use antibiotics for health management program. They use them as bacterial infection treatment and disease prevention, known as *antibiotic growth promotor* (AGP) [1]. Now, worldwide concern is about antibiotics resistance. Monitoring programs are done by countries in the world to protect human and animal health [2]. The monitoring programs usually use indicator bacteria such as *Escherichia coli* [3].

Many researchers studied about antibiotics resistance in Indonesia. *Escherichia coli* isolated from poultry has been resistance to doxycycline (25%) and gentamycin (12.5%) [4]. Seven *E. coli* isolated from fecal samples shown that resistance to methicillin (85.7%), penicillin G (71.4%) and 42,9% were resistance both doxycycline hydrochloride and streptomycin [5]. Start from Januari 1st 2018 Indonesian Misintry of Agricuture banned antibiotics as AGP, based on Permentan No. 14/2017. They tried to prevent the spread of antibiotics resistance. So, it is necessary to determine antibiotics resistance patterns, especially in *E. coli* as indicator bacteria.

MATERIALS AND METHODS

Sample collection

Ninety samples were collected from three difference poultry farm location in West Java. Those were Ubrug Farm and Cigarung Farm in Sukabumi and Cikupa Indah Farm in Bogor. Thrity samples were colected from each farm. Each sample were collected by cloacal swab. The cotton swab were placed on tube containing buffer peptone water (BPW). It was stored on ice condition and transported to Bacteriology Laboratory, Veterinary Medicine, Bogor Agricultural University.

Microbiological analysis

Each sample were cultured on MacConkey agar (MCA) to differences group of fermented lactose or non—fermented lactose bacteria. It was cultured on eosin methylene blue agar (EMBA) to differences *Coliform* and other *Enterobacteriaceae*. Incubation was carried at 37 °C overnight. Then subcultured on triptic soy agar (TSA) and incubated at 37 °C overnight. Gram staining was done to know the cell morphology. Fermentation glucose, lactose, sucrose, and gas production was detected on TSIA. It was incubated at 37 °C overnight. Biochemical test IMViC (indol, methyle red, Voges—Proskauer, citrate) was the key test for *E. coli* isolation. Other biochemical test was urease test and incubated at 37 °C for 48 hours.

Antimicrobial susceptibility testing

Antimicrobial resistance of *E.coli* isolates were tested against six antibiotics using Kirby—Bauer disc diffusion method on Mueller Hinton agar (MHA) following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [6]. The antibiotics which used in this research were commonly used in poultry industry. Those were ampicillin (10 µg), gentamicin (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), erythromycin (15 µg) and oxytetracycline (30 µg). Bacteria was cultured on MHA from colony suspension which equivalent to 0.5 McFarland standard. Disks containing antibiotic were placed on MHA and incubated at 37±2 °C 16—18 hours. Inhibition zones were measured then scored as a sensitive (S), intermediate (I) and resistance (R).

RESULT AND DISCUSSION

Pink colonies which grew on MCA with pink surrounding medium were lactose fermenter. The pink due to acid production from lactose [7]. Green methalic sheen colonies on EMBA due to vigorous lactose and/or sucrose fermentation, a feature unique on *E. coli*. Cell morphology of *E. coli* was bacil and negative Gram. It fermented glucose, lactose and sucrose, also produced gas on TSIA. They were motil and produced tryptophanase enzyme shown by indol ring production. Methyl red test positive and Voges—Proskauer negative. *Escherichia coli* was not use citrate as carbon source. It was not produce urease enzyme so absent on urease acivity [8].

Out of 90 samples collected from poultry farms only 51 (56.04%) were confirmed positive for *E. coli.* Detailed data is shown on Tabel 1. The 51 isolates were tested by antimicrobial sensitivity

following CLSI. Ampicillin, gentamicin, chloramphenicol, nalidixic acid, erythromycin and oxytetracycline were used on antibiotics susceptibility testing.

Table 1. *Escherichia coli* isolated from poultry farms

No	Farm	sample	isolate (%)
1	Ubrug	30	8 (26.7)
2	Cibangbara	30	26 (86.7)
3	Cikupa Indah	30	17 (56.7)
Total		90	51 (56.7)

Table 2 summarized the resistance patterns of *E. coli* to six antibiotics tested in this study. Findings from the current study, erythromycin is the highest percentages of antibiotics resistance. It has zero point on susceptibility of all antibiotics. *Escherichia coli* is also resistance to oxytetracycline, ampicillin, nalidixic acid, gentamycin and chloramphenicol.

Table 2. Antibiotics resistance patterns of *E. coli* isolated from poultry farms

Antibiotics	S (%)	I (%)	R (%)
Ampicillin	5 (9.80)	1 (1.96)	45 (88.24)
Gentamycin	27 (52,94)	1 (1.96)	23 (45.09)
Chloramphenicol	37 (72.54)	5 (9.86)	9 (17.65)
Nalidixic acid	5 (9.80)	6 (11,76)	40 (78.43)
Erythromycin	0 (0.00)	1 (1.96)	50 (98.04)
Oxytetracycline	4 (7.84)	0 (0.00)	47 (92.16)

S: susceptible, I: intermediate, R: resistance

At least, there are many reasons of microorganisms naturally resistance to antibiotics [9]. However, there are two major genetic strategies of bacteria to adapt the antibiotics attack: mutation in gene often associated with the mechanism of the compound and horizontal gene transfer by acquisition of foreign DNA coding for resistance determinants. Antibiotics resistance mechanism can be categorized according to biochemical route involved in resistance: modification of antibiotic molecule, prevention to reach antibiotic target (decreasing antibiotic penetration and efflux), change and/or bypass the target site and global cell adaptive processes [10]. The resistance characteristics can be genetically encoded by the microorganisms on their chromosome or on plasmid called *R* plasmid [9].

Group of β—lactam antibiotic in this study is ampicillin. It shows bad result, 88.24% of isolates are resistance. Ampicillin resistance due to expression of gene encoding β—lactamase enzyme. This enzyme hydrolyze β—lactam ring on ampicillin. Threfore this antibiotic is inactive [9].

Chloramphenicol and erythromycin are the antibiotics that disrupt protein synthesis. They work as 50s ribosomal subunit inhibitor. Chloramphenicol shows good result in this study. Most of *E. coli* are sensitive to chloramphenicol (72.54%). However, high number of *E. coli* isolates are resistance to erythromycin (98.04%). Bacteria inactivates antibiotic by chloramphenicol O—acetil—tranferase enzyme produced by plasmid. Target site of erythromycin in ribosom was modified by enzyme encoded by *erm* gene [10].

Antibiotics which work as 30s ribosomal subunit inhibitor are gentamycin and oxytetracycline [9]. *Escherichia coli* have high percentages at gentamycin (45.09%) and oxytetracycline (92.16%) resistance patterns. The resistance of gentamycin due to modification of antibiotic molecule. The alteration inhibits protein synthesis at the ribosome level. The most frequent mechanism antibiotic resistance of tetracycline group is efflux pumps. The genes encoding efflux pumps is *tet* gene located on MGEs (mobile genetic elements) or in chromosome [10].

Escherichia coli is not enough good treated by nalidixic acid. The result shows that the resistance isolates to nalidixic acid are high, 78.43%. It works to inhibit DNA gyrase and topoisomerase IV on replication and transcription process. Disturbance of enzymes decrease binding complex of enzyme—DNA [10].

CONCLUSION

In conclusion, 51 *E. coli* isolated from 90 samples in this study. Most of them are resistance to six antibiotics used. The findings indicate that the variety of antibiotics administered therapeutically in veterinary practice and poutry farms increase antibiotics resistance patterns to *E. coli*.

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