

# Antimicrobial Resistance on *Escherichia coli* from Poultry Production on Blitar, Indonesia

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## Abstract

The use of antibiotics in poultry can encourage antibiotic resistance, one of which is *Escherichia coli* which produces Extended Spectrum Beta-Lactamase. Extended Spectrum Beta-Lactamase is most produced by *Escherichia coli*. This study was carried out in a cross sectional descriptive manner aimed to determine the prevalence of ESBL in *Escherichia coli* bacteria in layer and broiler chicken farms in Talun and Kademangan subdistricts, Blitar district. Samples of 76 cloacal swabs were taken from laying hens and broilers. Positive isolates of *Escherichia coli* were tested for antibiotic sensitivity on agar-resistant Mueller-Hinton agar and *Escherichia coli*-resistant beta lactam (ampicillin) groups and then confirmed using the Double Disc Synergy Test, to confirm as *Escherichia coli* producing ESBL. The results showed a high prevalence of MDR in commercial chicken farms in Blitar district. *Escherichia coli* has MDR resistance properties in Layer Chicken in Talun sub-district by 88.23%, and in Kademangan sub-district by 63.16%, while for Broiler chickens in Talun sub-district by 90% and in Kademangan sub-district by 100%. The prevalence of *Escherichia coli* producing ESBL in Kademangan Sub-district was 15.79% and in Talun sub-district by 5.88%, while in Broiler chickens in Kademangan sub-district was 85%, and in Talun sub-district the *Escherichia coli* producing ESBL was not detected.

**Keywords:** Broilers, *Escherichia coli*, Extended spectrum beta-lactamase, Layer, Multidrug resistance

## Introduction

The use of antimicrobials in the field of animal husbandry provides benefits for animals and breeders, but can pose a risk of antimicrobial resistance if the use is not appropriate to the dose or time of use. Animal husbandry is one source of antimicrobial resistance<sup>(1,2)</sup>. The consumption of antibiotics medically in the animal sector reaches around 80%, mostly to promote growth in healthy animals. The World Health Organization

(WHO) recommends that farmers stop using antibiotics regularly to promote the growth and prevention of diseases in healthy animals. WHO recommendations aim to help maintain the effectiveness of antibiotics that are important for human medicine by reducing unnecessary use in animals<sup>(3)</sup>. The use of antibiotics in animals can encourage antibiotic resistance, one of which is *Escherichia coli* which produces Extended Spectrum Beta-Lactamase (ESBL)<sup>(1,2,4)</sup>.

Extended Spectrum Beta-Lactamase (ESBL) is most commonly produced by *Enterobacteriaceae*, especially *Escherichia coli* and *Klebsiella pneumoniae*<sup>(5)</sup>. ESBL is derived from the mutated beta-lactamase enzyme. This mutation causes an increase in the enzymatic activity of beta-lactamase<sup>(6)</sup>. Beta-lactamase is an enzyme

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produced by bacteria that functions against beta-lactam antibiotics<sup>(7)</sup>.

The main bacteria that produce ESBL are *Escherichia coli* and *Klebsiella species*<sup>(5,8)</sup>. ESBL-producing bacteria can also be resistant to the antibiotics class of aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol, and sulfamethoxazole-trimethoprim<sup>(9)</sup>. The multidrug nature of resistance to third generation cephalosporin and other class antibiotics is often found in ESBL-producing bacteria<sup>(10)</sup>. Infections involving ESBL become an outbreak that exacerbates the infection of other diseases<sup>(1)</sup>. The existence of ESBL-producing *Escherichia coli* in poultry in Indonesia has been reported in broiler feces in chicken slaughterhouses in Bogor by molecular detection (genotypic) examination using a PCR of 6%<sup>(11)</sup> and clinical microbiology (phenotypic) examination by the double disc method of 25%<sup>(10)</sup>. Chicken livestock and egg production are important economic activities in Blitar District, with the second largest level in Indonesia involving thousands of workers<sup>(12)</sup>. Poultry is one of the important reservoirs of zoonotic bacterial agents<sup>(2)</sup>.

## Materials and Methods

### Research design

This research is a descriptive study conducted in the form of cross sectional observation with the aim to see the relationship of the prevalence of *Escherichia coli* producing ESBL and its relationship with laying hens and broilers as carrier birds in Kademangan and Talun subdistricts, Blitar District.

### Sampling and bacterial isolation

Samples of 76 cloacal swabs were taken from laying hens and broiler chickens in Talun and Kademangan subdistricts, Blitar. Random sampling of chicken cloaca swabs using sterile cotton swabs Amies Viscosa transport media (deltalab, Spain). And stored in a collar box before being taken to the laboratory<sup>(13)</sup>. Samples are stored at cold temperatures and taken to the laboratory for the isolation of *Escherichia coli* bacteria. Bacterial isolation on MacConkey selective media 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<sup>(14,15)</sup>.

### Antibiotic sensitivity test and ESBL-producing *Escherichia coli* confirmation test

Positive isolates of *Escherichia coli* were tested for antibiotic sensitivity tests on Mueller-Hinton agar (Merck, Germany),<sup>(16)</sup> using an antibiotic disk, namely the antibiotic ampicillin 10µg (Oxoid, England), streptomycin 10µg (Oxoid, England), erythromycin 15µg (Oxoid, England), tetracycline 30 µg (Oxoid, England), sulphamethoxazole-trimethoprim 25µg (Oxoid, England). Bacterial isolates were determined to be sensitive to antibiotics is determined through the size of the diameter of the light zone or inhibition formed based on CLSI standard recommendations<sup>(14,16,17)</sup>. *Escherichia coli* which is resistant to beta lactam group is then confirmed by Double Disc Synergy Test (DDST), to confirm it as an ESBL-producing bacteria. This confirmation test used the Amoxycillin-clavulanic 30µg antibiotic disc (Oxoid, England), Cefotaxime 30µg (Oxoid, England), Ceftazidime 30µg (Becton Dickinson, USA), and Aztreonam 30µg (Oxoid, England)<sup>(14,16)</sup>. The results of the evaluation after incubation showed that the inhibition zone that appeared in the cup was measured based on CLSI guidelines<sup>(16,18,19)</sup>.

## Results and Discussion

Results of Isolation identification of a total of 76 layer cloaca swabs and broiler chickens in Talun and Kademangan sub-districts, Blitar District, as many as 76 (95%) positive isolates of *Escherichia coli*. The existence of *Escherichia coli* in the chicken cloaca swab respectively 36 (90%) isolates in laying hens and 40 (100%) *Escherichia coli* isolates in broiler chicken cloaca swabs in Talun and Kademangan subdistricts, Blitar. This result was in line with research which states that the presence of *Escherichia coli* in broilers cloaca is 68.75%<sup>(20)</sup>.

The *Escherichia coli* resistance pattern (Figure 1) in the chicken cloacal swab in Talun sub-district (Table 1) showed the results in the laying hens, there is resistance to the antibiotic Ampicillin by 88.24%; in Streptomycin antibiotics resistance of 70.58%; Erithromycin antibiotic resistance was 88.24%; in Tetracyclin antibiotics resistance is 29.41%; in sulphamethoxazole-trimethoprim antibiotic resistance was 94.12%. *Escherichia coli* resistance patterns in broiler chickens, there was resistance to ampicillin antibiotics by 95%;

the antibiotic Streptomycin resistance is 80%; the antibiotic Erythromycin resistance is 80%; in Tetracyclin antibiotics resistance of 70%; in sulphamethoxazole-trimethoprim antibiotic resistance by 75%.

The resistance pattern of *Escherichia coli* in chicken cloaca swabs in Kademangan subdistrict (Table 1) showed that in laying hens, there is resistance to Ampicillin antibiotics at 68.43%; in Streptomycin antibiotics resistance of 68.43%; the antibiotic Erythromycin resistance was 73.68%; the Tetracyclin antibiotic resistance was 42.12%; in sulphamethoxazole-trimethoprim antibiotic resistance was 52.64%. The resistance pattern of *Escherichia coli* in broiler chickens, there was resistance to the antibiotic Ampicillin by 100%; the antibiotic Streptomycin resistance was 95%; the antibiotic Erythromycin resistance was 80%; in Tetracyclin antibiotics resistance of 15%; in sulphamethoxazole-trimethoprim antibiotic resistance by 90%.

The high level of resistance occurrence in laying hens in Blitar district is in line with the results of the sensitivity test in laying hens in Bogor, which shows the level of resistance in Amoxicillin (84.62%), Kanamycin (69.24%), Colistin (63.75%), Doxycycline (53.75%), Neomycin, and 100% in Tetracycline, Ciprofloxacin, Enrofloxacin (10). Other studies stated that the level of resistance of *Escherichia coli* bacteria in broilers in Bogor Regency to ampicillin (89.5%), streptomycin (84.2%), erythromycin (86.8%), tetracycline (89.5%), and trimethoprim-sulfamethoxazole (76.3%)<sup>(21)</sup>. High antibiotic resistance in broiler chickens was also reported in Jordan, where the highest resistance level in sulphamethoxazole-trimethoprim, was 95.5%<sup>(22)</sup>.

*Escherichia coli* which has MDR resistance (Figure 2) properties in laying hens on Talun sub-district is 88.23% (15/17), and in Kademangan sub-district is 63.16%. 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 kinds of drugs. Infection by *Escherichia coli* can be treated using sulfonamides, ampicillin, cephalosporins, chloramphenicol, tetracycline and aminoglycosides. Aminoglycosides are poorly absorbed by the gastrointestinal tract, and have toxic effects on the kidneys. The most commonly used type of antibiotic is

ampicillin<sup>(23)</sup>.

The prevalence of Multi-Drug Resistant (MDR) *Escherichia coli* bacteria in Broiler chickens in Blitar District is quite high (Figure 2), among others in Talun sub-district by 90% (18/20) and in Kademangan sub-district by 100% (20/20). Antibiotic resistance to *Escherichia coli* from broiler cloaca swabs on some antibiotics is a result of the continuous use of antibiotics by farmers to prevent and treat disease. Silbergerd et al. (2008) states that the addition of antibiotics in feed is a major factor in the increase in the prevalence of antibiotic resistance. Feed in the business of broiler chicken farming is a major component that reaches 60%, so that if the food circulating on the farm contains antibiotics can be a source of resistance<sup>(23)</sup>.

The prevalence of antibiotic resistance tends to increase, one of which is the Extended Spectrum Beta Lactamases (ESBL) producing bacteria a group of enzyme-producing bacteria that can hydrolyze beta lactam antibiotics containing oxymino groups such as cephalosporins one to third generation and aztreonam. This research was carried out in a cross sectional descriptive manner aimed to determine the prevalence of ESBL in *Escherichia coli* bacteria (Figure 3) in layer and broiler chicken farms in Talun sub-district and Kademangan sub-district, Blitar district.

The prevalence of ESBL-producing *Escherichia coli* (Table 1) in Kademangan District was 15.79% and in Talun sub-district it was 5.88%. The prevalence rate of *Escherichia coli* producing ESBL in the laying hens cloacal swab was the same as the results of the *Escherichia coli* study in the laying hens cloacal swab in Thailand by 6.7%<sup>(24)</sup>, but this result is much smaller than the rate of *Escherichia coli* producing layer ESBL in Thailand. in India in chicken layers around 42%<sup>(25)</sup>. The prevalence of *Escherichia coli* producing ESBL in Broiler chickens (Table 2) in Kademangan sub-district was quite high (Figure 3), including 85% (17/20), while in Talun sub-district *Escherichia coli* producing ESBL was not detected. The prevalence rate of *Escherichia coli* producing ESBL in cloaca swabs in broiler chickens is consistent with the prevalence of *Escherichia coli* producing ESBL in India in broiler chickens around 87%<sup>(25)</sup>.

**Table 1. Percentage of ESBL-producing *Escherichia***

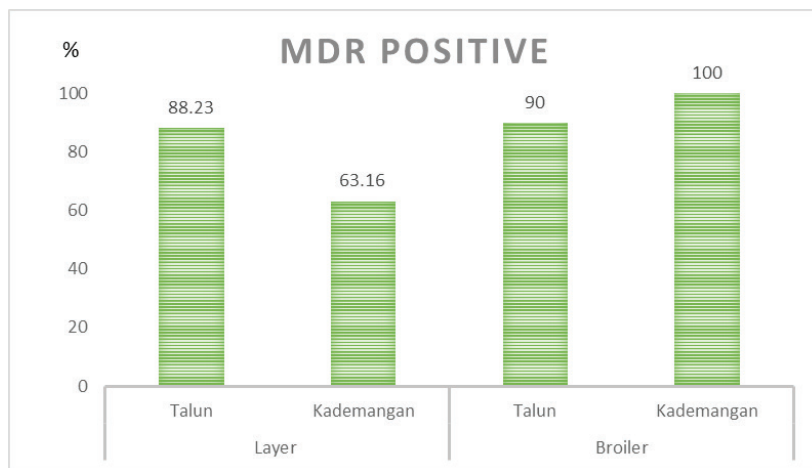
*coli* resistance from layer and broiler farms on Kademangan and Talun sub-districts, Blitar District.

| Location   | Poultry        | AMP           | S             | E             | TE           | SXT           | MDR positive | ESBL positive |
|------------|----------------|---------------|---------------|---------------|--------------|---------------|--------------|---------------|
| Talun      | Layer (n=17)   | 15<br>88.24 % | 12<br>70.58 % | 15<br>88.24 % | 5<br>29.41 % | 16<br>94.12 % | 15<br>88.23% | 1<br>5.88%    |
|            | Broiler (n=20) | 19<br>95 %    | 16<br>80 %    | 16<br>80 %    | 14<br>70 %   | 15<br>75 %    | 18<br>(90 %) | 0<br>0 %      |
| Kademangan | Layer (n=19)   | 13<br>68.43 % | 13<br>68.42 % | 14<br>73.68 % | 8<br>42.12 % | 10<br>52.64 % | 12<br>63.16% | 3<br>15.79%   |
|            | Broiler (n=20) | 20<br>100 %   | 19<br>95 %    | 16<br>80 %    | 3<br>15 %    | 18<br>90 %    | 20<br>100 %  | 17<br>85 %    |

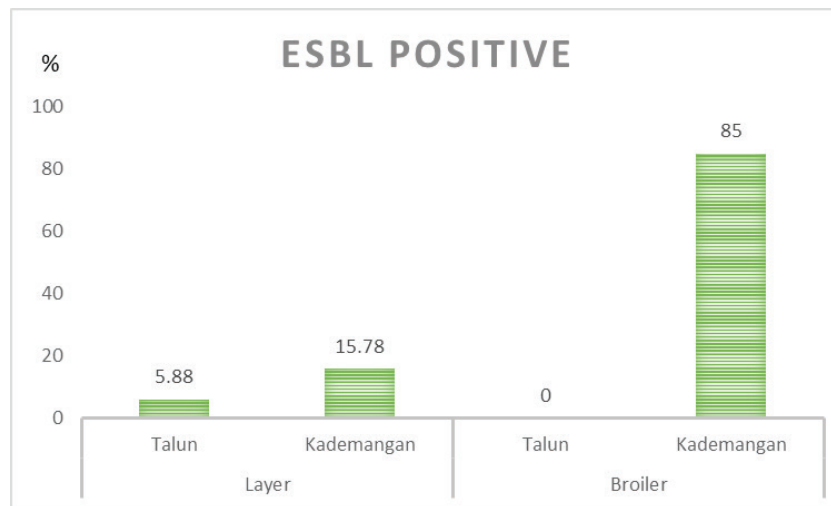
Note : AMP = ampicillin , S= Streptomycin , E= Erythromicin , TE= Tetracycline , SXT= Sulfamethoksazol-Trimetropin

**Figure 1. (A) Patterns of *Escherichia coli* resistance to several classes of antibiotics (1) Ampicillin, (2) Streptomycin, (3) Erythromicin, (4) Tetracycline, and (5) Sulfamethoxazole-Trimethoprim**

(B) *Escherichia coli* producing positive ESBL by the method Double Disc Synergy Test (DDST). Arrows: Synergy forms, ATM: Aztreonam, CAZ: Ceftasidime, AMC: Amoxycillin clavulanic, and CTX: Cefotaxime.



**Figure 2. Multidrug resistant (MDR) *Escherichia coli* from layer and broiler farms on Kademangan and Talun sub-districts, Blitar District**



**Figure 3. *Escherichia coli* producing ESBL in layer and broiler chickens in Kademangan and Talun sub-districts, Blitar District**

## Conclusion

*Escherichia coli* which has MDR resistance properties in layer and broiler chickens in Talun and Kademangan sub-districts is quite high, around 60-100%. The prevalence of *Escherichia coli* producing ESBL in laying hens in Kademangan Sub-district was 15.79% and in Talun sub-district by 5.88%, while the prevalence of *Escherichia coli* producing ESBL in Broiler chickens in Kademangan sub-district was 85% (17/20), and in Talun sub-district it was not detected. the prevalence of *Escherichia coli* producing ESBL.

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**Designation :** Freshinta Jellia Wibisono as a Doctoral candidate on Universitas Gadjah Mada, Bambang Sumiarto as a professor on Universitas Gadjah Mada, Tri Untari as an associate professor on Universitas Gadjah Mada, Mustofa Helmi Effendi as an associate professor on Universitas Airlangga, Dian Ayu Permatasari as a researcher on Universitas Airlangga, Adiana Mutamsari Witaningrum as a researcher on Universitas Airlangga

**Ethical Clearence:** Cloacal swabs were used in this study, hence ethical approval was not necessary. Cloacal swab samples were collected from two subdistrict on Blitar poultry farms in East Java province, Indonesia.

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