The presence of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli on layer chicken farms in Blitar Area, Indonesia

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Short Communication: The presence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* on layer chicken farms in Blitar Area, Indonesia

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Abstract. *Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. 2020. Short Communication: The presence of extended-spectrum beta-lactamase (ESBL) producing* Escherichia coli *on layer chicken farms in Blitar Area, Indonesia. Biodiversitas 21: 2667-2671.* This study was aimed to determine the incidence of Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* on layer chicken in Blitar area. This was a cross-sectional study with a total of 205 cloacal swabs of layer chicken taken randomly. The sample was in isolation identification on MacConkey media and ESBL confirmation test produced by *Escherichia coli* was then carried out by the Double Disc Synergy Test (DDST) method and the VITEK® 2 Compact Automated System method. This study showed that 185 (90.24%) isolates of positive *Escherichia coli* from a total of 205 samples of cloacal swabs of the layer chicken. The incidence of ESBL-producing *Escherichia coli* in cloacal swabs on layer chicken with the Double Disc Synergy Test (DDST) method and the VITEK® 2 compact automatic method was 13 (7.03%). Results in this study indicated that layer chicken has potential as reservoir for spreading ESBL to public health and needs strict hygienic measures to prevent their transmission to humans.

Keywords: Cloacal swab, *Escherichia coli*, ESBL, DDST, layer chicken, VITEK®2

INTRODUCTION

Use of antibiotics in the animal sector reaches around 80%, mostly to increase growth in healthy animals (WHO 2017). Antibiotic residue cases in poultry products in Indonesia are found in several regions with different types of antibiotics and ranged between 8 to 70% (Etikaningrum and Iwantoro 2017). Bacterial resistance to poultry antibiotics is a major problem in the Indonesian poultry industry (Niasono et al. 2019). The speed of discovery of new types of antibiotics is slower than the speed of increased antibiotic resistance which causes concern that one day there will be no effective antibiotics available for the treatment of resistant bacterial infections (Handayani et al. 2017). This potential is an important vigilance point for public animals as well as human health.

Beta-lactam, tetracycline, and thylosine are antibiotics that are often used in some poultry farms (Mehdi et al. 2018; Niasono et al. 2019). There is a general farmers' perception that the use of antibiotics as a preventive measure at a low cost, has no side effects and it increases the high use of penicillin preparations on farms (Memish et al. 2004). Increased bacterial resistance to antibiotics in general causes ineffective treatment of infectious diseases due to which infection continues and increases the risk of spreading infection to others (WHO 2017; Yusha'u and Umar 2016).

Infection involving the ESBL is an epidemic that worsens the infection of other diseases (Santos et al. 2013). The finding of an ESBL-producing bacterium of 14.84% in processed animal products is a matter of public health involving the environment as a source of spread of resistant bacteria (ESBL) for human and animal health (Niasono et al. 2019; Yusha'u and Umar 2016). The presence of ESBL-producing *Escherichia coli* in poultry in Indonesia has been reported in broiler feces in chicken slaughterhouses in Bogor with molecular detection (genotypic) examination using PCR of 6% (Lukman et al. 2016), and clinical microbiology (phenotypic) examination with an antibiotic sensitivity test method of 25% (Masruroh et al. 2016). In East Java, little is known about the ESBL cases from layer chicken farms and its distribution. Therefore, the aim of this study was to detect the ESBL producing *E. coli* isolated from cloacal swab of layer chicken samples in Blitar area in East Java, Indonesia and to understand the strains distribution.

MATERIALS AND METHODS

Research design, location, and sampling

This cross-sectional study was conducted between March and May 2019, using total sample of 205 cloacal swabs of layer chicken. Samples were taken randomly from layer chicken in Blitar District, East Java, Indonesia, and consisted of 5 sub-districts and 41 farms (Table 2). Samples were included in Amies Swab transport media and stored in a cooler box before being taken to the laboratory (Seni et al. 2016). Sample preparation and further examination were carried out as soon as possible after sampling.

Isolation and identification of *Escherichia coli*

The cloacal swab in the Viscosa (Deltalab, Spain) Amies Swab transport medium at cold temperatures were brought to the laboratory for the isolation of *E. coli* bacteria (Effendi 2018; Putra et al. 2019). Samples of cloacal swabs of layer chicken were cultured on selective MacConkey Agar media no. 3 (Oxoid, England) incubated at 35-37 ºC for 20-24 hrs. The pure colony of *E. coli* was identified by the Gram staining test, then biochemical identification of bacteria was carried out by the IMVIC test (Indol-Motility, Methyl Red, Voges Proskauer, Citrate) and TSIA (Triple Sugar Iron Agar).

Double Disc Synergy Test (DDST) for ESBL confirmation test

ESBL producing *E. coli* can be confirmed using a confirmation test by Double Disc Synergy Test (DDST). This confirmation test was carried out to evaluate the presence of an inhibitory zone of ESBL activity with clavulanic acid. This conventional method was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Merck, Germany). The DDST confirmation test used an antibiotic (Oxoid, England) disc Amoxicillin-clavulanate 30µg, Cefotaxim 30µg, Ceftazidime 30µg, and Aztreonam 30µg. The culture was incubated at temperatures of 35-37ºC for 18-24 hrs (CLSI 2017; Effendi et al. 2019). Evaluation results after incubation showed the presence of inhibitory zones that appeared in the cup were measured according to the Clinical and Laboratory Standards Institute (CLSI) guidelines as shown in Table 1.

VITEK®2 compact method of antibiotic sensitivity test

Inoculated isolates were selected from a single colony. Gram-negative isolates used the VITEK® 2 GN card. The isolates of this study used were pure *E. coli* isolates from cloacal swabs of layer chicken. The results were analyzed automatically by the system and interpreted as sensitive, intermediate, and resistant (Biomerieux 2017).

RESULTS AND DISCUSSION

Isolation and identification of *Escherichia coli*

The results of isolation and identification of 205 samples of cloacal swabs on layer chicken farms showed that 185 (90.24%) isolates were positive for *E. coli* (Table 2). Positive samples of *E. coli* on MacConkey Agar were identified with reddish-pink colonies (Figure 1), then confirmed by biochemical tests using IMVIC and TSIA. MacConkey media we used for rapid identification of enteric bacteria. The presence of crystal violet and bile salts in the MacConkey selective media in order to inhibit Gram-positive growth, so MacConkey media is used to grow Gram-negative bacteria (Anggraini et al. 2018; Estiningsih et al. 2016). The IMVIC test was used to distinguish *E. coli* in its biochemical activity with other coliform bacteria (Leboffe and Pierce 2011). IMVIC test showed a motile result, Indol positive red ring formed after Kovac's reagent was added, Methyl Red (MR) test was positively indicated by a red color change, Voges-Proskauer (VP) test was negative with no color change, Citrate negative test was confirmed with color the media remains green and no change (Figure 2). The TSIA test produced gas, the upright and sloping media were yellow and there was no H_2S at the bottom of the tube (Figure 2). The results of isolation and identification of positive *E. coli* isolates from this study were in accordance with the standard microbiological literature on morphology and biochemical tests of *E. coli* bacteria (Brooks et al. 2013).

Table 1. ESBL producing *Escherichia coli* on Recommendation disc CLSI (CLSI 2017)

Antibiotic disc		Inhibition zone on ESBL
Cefotaxime	CTX 30 µg	Inhibition Zone ≤ 27 mm
Ceftazidime	CAZ 30 µg	Inhibition Zone ≤ 22 mm
Cefpodoxime	PX 10 µg	Inhibition Zone ≤ 22 mm
Ceftriaxone	CRO 30 µg	Inhibition Zone ≤ 25 mm
Aztreonam	ATM 30 µg	Inhibition Zone ≤ 27 mm

Table 2. Data of ESBL producing *Escherichia coli* on this study

Location	Farms	Sample size	*Escherichia coli*		ESBL (DDST)		ESBL (VITEK)	
			Positive	Percentage	Positive	Percentage	Positive	Percentage
Ponggok	10	50	45	90%	1	2.22%	1	100%
Srengat	17	85	77	90.5%	8	10.39%	8	100%
Udanawu	6	30	27	90%	0	0	0	100%
Talun	4	20	17	85%	1	5.88%	1	100%
Kademangan	4	20	19	95%	3	15.79%	3	100%
Total in Blitar area	41	205	185	90.24% (185/205)	13	7.03% (13/185)	13	100% (13/13)

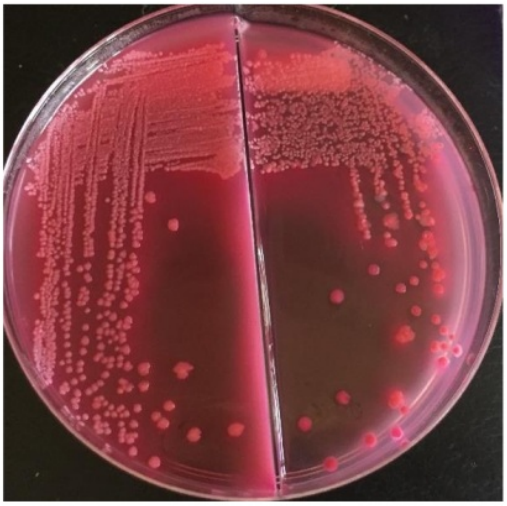

Figure 1. *Escherichia coli* on MacConkey Agar

Figure 2. Identification *Escherichia coli* by IMVIC and TSIA

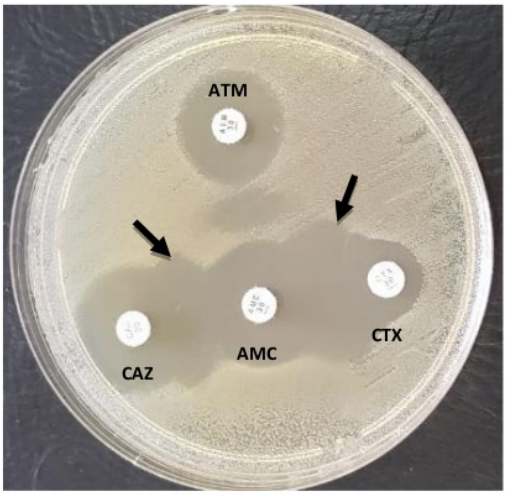

Figure 3. Confirmation ESBL producing *Escherichia coli* by Double Disc Synergy Test (DDST). Information: Cefotaxime (CTX); Ceftazidime (CAZ); Amoxicillin-clavulanate (AMC); Aztreonam (ATM); black arrow is synergy formed.

Double Disc Synergy Test (DDST) for ESBL confirmation test

The results of this study showed the incidence of ESBL producing *E. coli* in cloacal swabs in layer chicken with the Double Disc Synergy Test (DDST) method of 13 (7.03%) ESBL positive isolates (Table 2) and shown on Figure 3. The cefotaxime synergy with the combination of amoxicillin-clavulanate in the form of expansion of the barrier zone between the two disks showed that the bacteria were positive ESBL, this result was in accordance with Savira's statement ESBL. Positive results for ESBL-producing bacteria confirmed that there was an increase in the inhibition zone ≥ 5 mm between the diameter of the cephalosporin disk and the cephalosporin-clavulanate combination disk expressing positive ESBL germs (CLSI 2017; Savira 2014).

The VITEK®2 compact method of antibiotic sensitivity test

Positive isolate of ESBL-producing *E. coli* using the DDST method was then confirmed by the VITEK® compact 2 methods to identify and test antibiotic sensitivity to the third cephalosporin group. The results of the identification of the Vitek method showed 100% (13) positive isolates of *E. coli* and their sensitivity test showed 100% (13) ESBL producing isolates (Table 2). These results were consistent with the statement that the accuracy of VITEK® 2 compact automated system ranges from 97.8% (O'Hara 2005) to 98.02% (Duggal et al. 2012).

The results showed that the ESBL confirmation test with the DDST conventional method had the same incidence with the confirmation test using the VITEK® 2 compact method. The confirmation method of ESBL-producing *E. coli* in a conventional manner with the DDST method was more often used because it did not require analytical equipment at an expensive price and the cost of testing is relatively cheaper than the confirmation test using the VITEK® 2 compact method (Biomerieux 2017).

Discussion

In this study, ESBL-producing *E. coli* isolated from cloacal swab layer chicken samples from 41 layer chicken farms in Blitar area, Indonesia. Of the 41 layer chicken farms, almost of ESBL producing *E. coli* was detected in cloacal swab samples, which showed the possibility of the ESBL producer transmission route from food-producing animal farms (Gundogan and Avci 2013).

Escherichia coli as a commensal bacterium is commonly used as an indicator in surveillance and monitoring antibiotic resistance programs, because it is a gram-negative bacterium that is often found in animal feces and is associated with treatment in humans and often found conjugal plasmids that can move between enteric bacteria. *E. coli* as a reservoir of genes that have been resistant to antibiotics that can be transferred to other pathogenic bacteria (OIE 2015). The presence of commensal *E. coli* in the gut of livestock acts as a reservoir of resistant genes that can move horizontally to pathogenic *E. coli* and other bacteria in the food chain (Biutifasari 2018). This study was related with the incidence of ESBL producing *E. coli*

in accordance with its incidence in broiler chicken feces in Bogor by 6% (Lukman et al. 2016), but much smaller than the incidence of ESBL producing *E. coli* in India layer chicken which is around 42% (Brower et al. 2017). Infections involving ESBL become an outbreak that exacerbates the infection of other diseases (Santos et al. 2013).

The occurrence of antibiotic resistance in layer chickens with a fairly high prevalence rate causes large economic losses for farmers (Etikaningrum and Iwantoro 2017). Antibiotic resistance causes a high economic burden on the human and animal health sector. Rational therapy, government regulation, public education are important points in the strategy of handling resistance problems (Utami 2011; Putra et al. 2020). Approaches to deal with antibiotic resistance in the form of policies such as the development and dissemination of technical guidelines, but these guidelines are usually cut off from the context of animal husbandry without considering the complexity of the problems of day-to-day farming practices and are therefore ignored by farmers (Bellet 2018). ESBL-producing bacteria are increasingly reported to contaminate water and mud (Blaak et al. 2014; Laube et al. 2014). Farms that use contaminated water can be a possible route for ESBL-producing *E. coli* to enter the food chain (Reinthaler et al. 2010; Zheng et al. 2012). In the past, ESBL-producing *E. coli* was also isolated from river water and mud samples, which had the same resistance profile and ESBL gene as stool isolates in the same water. These results indicated the potential influence of chicken farming as a reservoir for spreading ESBL producing *E. coli* to the surrounding water environment (Laube et al. 2014; Overdevest et al. 2011).

In the production of food-producing animals, especially the chicken layer, high concentrations of microorganisms in the air often occur in the environment in cages (Shoaib et al. 2016). These microorganisms in such cages can survive in the form of aerosols for a long time in the air and transmitted via airflow (Gao et al. 2015). In this study, ESBL-producing *E. coli* was obtained from cloacal swab samples, the isolates from the samples showed high similarities, which showed *E. coli* transmission that produced in the environment at the backyard poultry farm (Kwoji et al. 2019). Previous research has shown the spread of ESBL-producing *E. coli* originating from surrounding area (Canton et al. 2008). The concentration of microorganisms is closely related to sanitation quality. Poor sanitation can benefit the spread of ESBL-producing *E. coli* (Saliu et al. 2012).

In conclusion, This study confirms the presence of ESBL products *E. coli* on layer chicken farms in the Blitar area. This isolate also showed high levels of antibiotic resistance to third-generation cephalosporins. These results are very important for public health awareness since layer chicken maintained close to the human population and can spread this resistant pathogen through the environment and farmers who are close to the cage for the maintenance of these layer chicken.

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