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Colibacillosis and Antibiotics Resistance Patterns in Broiler

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ABSTRACT

Colibacillosis is considered important in the poultry industry because it generates economic losses due to the disturbance of growth, the decline in production, an increase number of culled chicken, and reduced quality of carcasses and eggs. Study of colibacillosis and antibiotics resistance patterns in broiler was conducted at the Indonesian Research Centre for Veterinary Science. The aim of the study was to provide the latest information on the status of the presence of *Escherichia coli* in broiler farms in four districts in West Java Province as well as its antibiotics drug resistance patterns and their pathogenicity will be studied. A total of 196 samples of the intestine, liver, heart, egg yolk, air sac that showed gross abnormalities were sampled and feces were collected and used in this study. *Escherichia coli* was isolated for characterisation and antibiotics drug resistance patterns and their pathogenicity were investigated. *Escherichia coli* were recovered from 149 (76.02%) samples out of the total samples collected. In this present study, one (7.7%) *E. coli* isolate was serologically typed into O_{78} and 12 of them were untypable whereasthe other 7 (4.93%) isolates were serologycally typed into O_{157} and 135 were untypable. *In vitro* pathogenicity indicated that 91.55% of the isolates were positive for Congo red binding assay and 4.93% isolates were positive for haemolysine production. Antibiogram profiles indicated that 98.70, 79.30, 75.10, 61.40, and 57.70% isolates were resistance against to amphicillin, neomycin, streptomycin, sulfamethoksazol trimethropim, and kanamycin, respectively. A total of 149 (76,02%) histopathologically had consistent colibacillosis lesion with various degree of severity.

Key Words: Colibacillosis, Broiler, Resistance, Antibiotics

INTRODUCTION

Escherichia coli (*E. coli*) is one of the most economically important bacteria which is responsible for early chick mortality in poultry farms. *Escherichia coli* is normal microflora in the digestive tract of animals and human, but certain strains that are pathogenic in birds are called avian pathogenic *E. coli* (APEC). They are able to spread to various organs and cause systemic and fatal colibasillosis (Barnes et al. 2013). In general, pathogenic *E. coli* in poultry has specific serotypes, mostly consisted of serotype as follows: O_{78}, $O_{1,}$ and O_2, and under certain condition includes O_{15} and O_{55} (Barnes et al. 2013).

Colibacillosis in poultry industry is worldwide (Delicato et al. 2003; Ewers et al. 2004). The disease remains a major problem in the poultry industry because it generates economic losses due to the disturbance of growth, the decline in production, an increase in the number of culled chicken, and reduce quality of carcasses and eggs (Barnes et al. 2013).

Accordingly, APEC needs more attention as several studies have shown no association with extra intestinal pathogenic *E. coli* (ExPEC) in humans, especially uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC). This indicates that some APEC strains could be considered potential zoonosis as gen (Ewers et al. 2007; Moulin-Schouleur et al. 2007; Johnson et al. 2008).

Colibacillosis in Indonesia was first found at a farm in Bogor attacking 27.3% broiler aged 5-32 weeks (Poernomo 1988). Purnomo & Juarini (1996) have isolated 950 *E. coli* isolates, and have been classified into serotypes: O_1K_1: 85 cases (9.0%), O_2K_1: 489 cases (51.5%); $O_{78}K_{80}$;101 cases (10.6%) and other serotypes: 275 (28.9%). Recently, it has

have been reported that *E. coli* serotype O_1, O_2 and O_{78} were identified from colibacillosis cases in chickens farm in Yogyakarta (Wahyuwardani et al. 2014). Prompt diagnosis and treatment of colibacillosis are crucial to ensure optimal productivity in poultry farms.The aim of the study was to provide the latest status of colibacillosis in broiler farms in four districts in West Java Province as well as its antibiotics drug resistance patterns and their pathogenicity will be studied

MATERIAL AND METHODS

Sample collection

Samples were collected from some broiler farms heaving population range of 3,000 to 30,000, located in four districts in West Java Province, namely: Bogor, Sukabumi, Cianjur and West Bandung in 2014. The samples collected were as follows: intestine, liver, heart, air sac, egg yolk derived from sick or recently dead chickens. Any organs showing any gross lesion were sampled, as well as faecal samples were collected from suspected cages. Samples for *E. coli* isolation were placed in a cool ice box and transported to the laboratory whereas samples for histopathology were fixed in buffered neutral formalin 10%.

Isolation and identification

Isolation and identification of *E. coli* was conducted by standard methods with slight modification by using a specific medium according to Barrow & Feltham (2009). Each organ of the samples were diluted with buffer peptone water (0.5 g/4.5 ml) using a stomacher "80", then incubated at 37°C overnight. The day after, 0.5 ml of the suspension was grown in a solid medium Tryptone Bile salt X-glucuronide (TBX) and incubated at 37ºC for 24 hours. Suspected green colony of pure *E. coli* was stained with Gram and the morphology examined under the microscope. Biochemical characteristics were performed using identification device of API 20 E (Biomereux, France).

Serotyping

Each isolate of pure *E. coli* serotypes was determined by standard methods slide agglutination according to Murray (1984) using specific antisera of O_1, O_{78}, O_2 and produced by Indonesian Research Center for Veterinary Science (IRCVS). Serological test against specific O_{157} antisera was obtained by latex agglutination test using commercial antisera (Oxoid, England).

In vitro pathogenicity testing

Pathogenic or nonpathogenic *E. coli* can be distinguished *in vitro* by using invasive nature of the Congo Red dye binding test (Sharma et al. 2006) and the nature to produce haemolysin (Panigraphy & Ling 1990).

Haemolysis production test

Eschechia coli isolates were grown in 5% solid medium of sheep blood, and then incubated at 37°C for 24 hours. Colonies showing a clear zone (hemolysis) showed a positive haemolysin (Panigraphy & Ling 1990).

Congo reddye binding test

Eschechia coli isolates were grown in a solid medium of Congo Red Trypticase Soy agar added with 0.003% Congo Red dye (Sigma) and 0.15% bile salts (OXOID), incubated at 37°C for 24 hours, then stored at room temperature for 48 hours. Invasive isolates will indicated by the colony's ability to absorb Congo Red dye during 72 hours observation period. Red color indicates a positive whereas colorless means negative isolates (Sharma et al. 2006).

Antibiogram

Each *E. coli* isolates were tested its sensitivity to several kinds of antibiotics using Kirby-Bauer method disk diffusion susceptibility (Jorgensen & Turnidge 2007), modified with solid medium trypticase soy (TS) by placing antibiotics discs. A total of seven commercial antibiotics recommended for Gram-negative bacteria (Oxoid, England) was used in this test, namely: Gentamicin (CN) 10μg, Chloramphenicol (C) 30 μg, Ampicillin (Amp) 10 μg, Kanamycin (K) 30μg, Streptomycin (S) 10 μg, Sulfamethoksazol Trimethropim (SXT) 25 μg, Neomisin (N) 10 μg, Sulfamethoksazol Trimethropim (SXT) 25 μg and Streptomicin (S) 10 μg).

Clinicopathological examination

Clinical symptoms and gross lesions from sick and dead chicken were observed and recorded. Samples were fixed in neutral buffered formalin solution (BNF) 10%, then processed as paraffin blocks, cut at 3-4 μm thick and stained with H&E methods. Histopathological examination (HP) is performed using hematoxylin and eosin staining (H&E) according to standard procedures (Kumar & Kieman 2010). Examination of histopathological findings were assessed microscopically and lesions were analyzed descriptively.

RESULTS AND DISCUSSION

In this study, random sampling was collected from 36 broiler farms in four districts in West Java Province. Eighty-nine broiler consisting of 56 sick birds and 33 dead chicken were collected and necropsies were carried out. A total of 186 samples of the intestine, liver, heart, egg yolk, air sac that showed gross lesions consistent with colibacillosis were sampled and 10 faeces were collected for this study (Table 1).

Tabel 1. Total sample collected for detection of pathogenic *E. coli* isolates

Distric/total farms	Total of sample								
	Chicken		Gross lesion						Total
	Sick	Dead	Intestine	Liver	Heart	Egg yolk	Air sac	Feces	
Bogor/9	11	13	24	5	3	6	2	4	44
Sukabumi/13	13	9	22	14	4	6	4	4	54
Cianjur/3	4	4	8	7	6	0	1	2	24
West Bandung/11	28	7	35	25	1	13	0	0	74
Total/36	56	33	89	51	14	25	7	10	196

The characterization of *E. coli* isolated from septicemic sick and dead chickens collected from four districts in West Java was shown in Table 2. *Escherichia coli* was recovered from 149 (76,02%) samples out of the total 196 samples collected. In the present study, one (7.7%) *E. coli* isolate was typed serologycally into O_{78} and 12 were untypable. The other 7 (4.93%) isolates were typed serologycally into O_{157} and 135 were untypable.

While pathogenic or non pathogenic *E. coli* can be distinguished *in vitro* by using invasive nature of the Congo Reddye binding test and the nature to produce haemolysis, in terms of clinico-pathological findings, there were several pathognomonic lesions. The clinical evidence showed depression, weakness associated with respiratory distress and non-uniform growth rate (Khaton et al 2008). The results of the Congo red binding assay indicated that majority (91.55%) were positif and only 12 isolates were negative. Seven of 142 isolates recovered tested were positive for haemolysine production.

Tabel 2. Characterization of *E. coli* isolated from septicaemic sick and dead chickens collected from four districts in West Java Province

District	Total samples/chicken	Positive for *E. coli*	Characteristic *E. coli* (number positive/number tested)					
			α haemolysis	CR invasive	Serotype			
					O_1	O_2	O_{78}	O_{157}
Bogor	44/24	33	0/33	30/33	0/1	0/1	0/1	4/33
Sukabumi	54/22	31	0/25	18/25	0/2	0/2	0/2	1/25
Cianjur	24/8	22	4/22	22/22	0/5	0/5	1/5	1/22
West Bandung	74/35	63	3/62	60/62	0/5	0/5	0/5	1/62
Total	196/89	149	7/142	130/142	0/13	0/13	1/13	7/142

CR: Congo red

The sensitivity and resistance pattern of these isolates for various antibiotics are presented in Tabel 3. It was observed that 98.70, 79.30, 75.10, 61.40, and 57.70% isolates were resistance against amphicillin, neomycin, streptomycin, sulfamethoksazol trimethropim, and kanamycin, respectively. We also found multiple antibiotics resistance among several *E. coli* isolates from septicaemic sick and dead chikens collected from three districts in West Java can be seen in Figure 1.

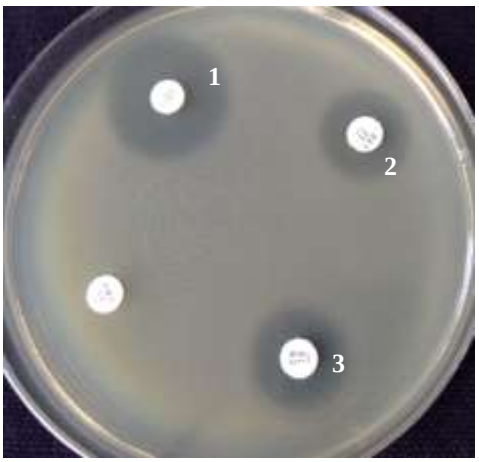


Figure 1. Antimicrobial sensitivity test for *E. coli* on Meuller Hinton agar *E. coli* zone to Cloramphenicol (1), Sulfamethoksazol Trimethropim (2), Neomycin (3)

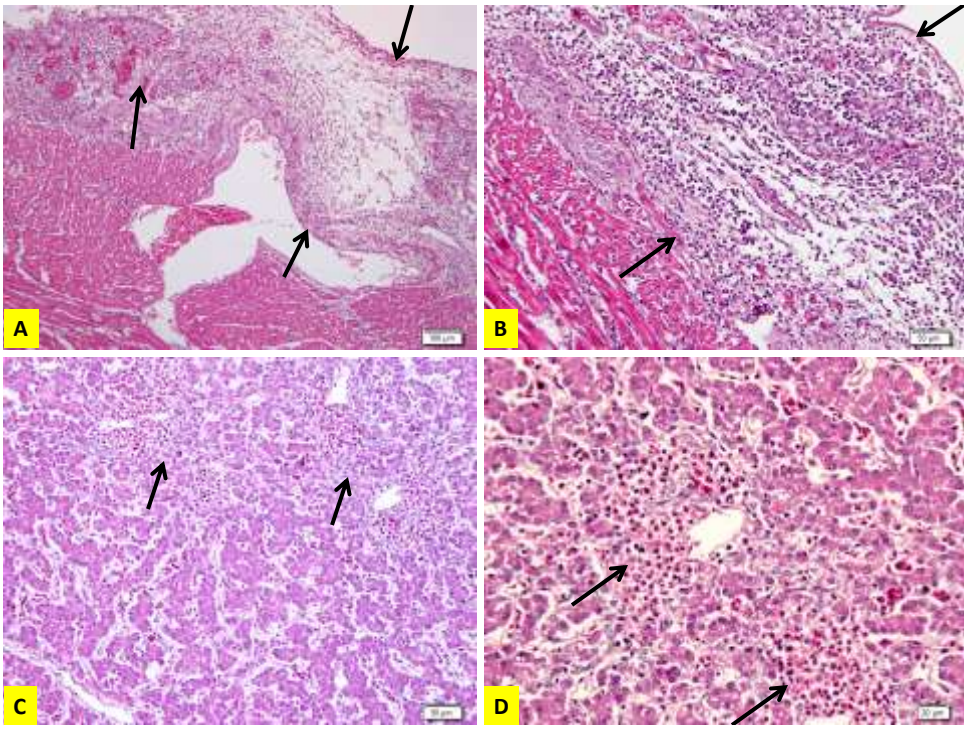
Tabel 3. Antibiotics sensitivity *E. Coli* isolates from septicaemic sick and dead chikens collected from three districts in West Java

District	No. of isolate	Average sensitivity test (%)	Antibiotics						
			A	C	G	K	N	ST	S
West Bandung	33	Resistant	100.0	24.2	39.4	72.7	84.9	81.8	78.80
		Intermediate	0.0	27.3	15.1	27.3	15.1	3.0	18.20
		Sensitive	0.0	48.5	45.5	0.0	0.0	15.2	3.00
Sukabumi	26	Resistant	96.2	88.5	30.8	38.5	76.9	69.2	84.60
		Intermediate	3.8	7.7	11.5	53.8	23.1	23.1	7.70
		Sensitive	0.0	3,8	57.7	7.7	0.0	7.7	7.70
Cianjur	21	Resistant	100.0	0.0	4.8	61.9	76.2	33.3	61.90
		Intermediate	0.0	0.0	4.8	38.1	23.8	38.1	19.05
		Sensitive	0.0	100.0	90.4	0.0	0.0	28.6	19.05
Total	80	Resistant	98.7	37.6	25.0	57.7	79.3	61.4	75.10
		Intermediate	1.3	11.6	10.5	39.6	20.7	21.4	15.00
		Sensitive	0.0	50.8	64.5	2.7	0.0	17.2	9.90

A: Amphicillin; C: Chloramphenicol; G: Gentamycin; K: Kanamycin; N: Neomycin; ST: Sulfamethoksazol trimethropim; S: Streptomycin

It shows that *E. coli* is sensitive to chloramphenicol and resistant to amphicillin, neomycin and sulfamethoksazol trimethropim. This resistance of *E. coli* was previously reported by Kabir (2010) that avian pathogenic *E. coli* strains were often resistant to antimicrobials approved for poultry including cephradine, tetracyclines, chloramphenicol, sulfonamides, amino-glycosides, β-lactam antibiotics as well as for fluoroquinolones.

Postmortem examination showed evidence of fibrinous hepatitis, pericarditis, peritonitis and air sacculitis as the major findings observed (Abu Daud et al. 2014). Associated histopathological lesions represented the degree of severity of the lesions, in correlation with the pathogenicity of the isolate. Figure 2 shows the most consistent lesions found in this study. This was in accordance with non-suppurative pericarditis, hepatitis and enteritis were pathognomonic lesions caused by Colibacillosis reported by Abu Daud et al. (2014).



A. Pericarditis, chicken AH2: edematous, heterophils infiltration and necrotizing pericarditis; B. Higher magnification of A; C. Hepatitis, chicken AH2: heterophil infiltration; D. Higher magnification of A

Figure 2. Colibacillosis

CONCLUSION

Most of isolated *E. coli* in this study were pathogenic and responsible for various types of leading to economic losses in poultry industry. The antibiotics sensitivity pattern revealed that isolated *E. coli* were resistance to several antibiotis. Therefore, a particular emphasis attention needs to be taken judiciously to select the antibiotics, especially after antibiotics sensitivity testing. Optimum antibiotic dose during sufficient time was needed to ensure effective treatment and control for colibacillosis in poultry. A better understanding addressed in this study will assist the poultry industry in reducing and eliminating avian colibacillosis from the poultry flocks, thereby reducing potential hazards to the public health posed by the bacterial diseases.

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