PREVALENCE OF *mcr-1* COLISTIN RESISTANT GENE IN *Escherichia coli* ALONG THE BROILER MEAT SUPPLY CHAIN IN INDONESIA

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ABSTRACT

Colistin is the last drug choice for dealing with the carbapenem-resistant *Enterobacteriaceae* bacteria; hence, this drug is very crucial to human health. The discovery of a plasmid-mediated colistin-resistant gene, the *mobilized colistin resistance-1* (*mcr-1*), signals a significant global health threat. Colistin sulfate is an antimicrobial agent which has been approved for use in broilers in Indonesia. Thus, this study aimed to measure the prevalence of colistin-resistant *Escherichia coli* and to detect the *mcr-1* colistin-resistant gene in *E. coli,* and *E. coli* O157:H7 in the entire supply chain of broilers in Bogor Regency, West Java Province, Indonesia. Samples were taken from 47 flocks that used colistin sulfate (47 pools of cloacal swabs, 47 pools of drinking water, and 47 pools of litters), seventy fresh meat samples and seven samples plucker swabs from seven small-scale poultry slaughterhouses, seventy fresh meat samples from seven traditional markets, and seventy cooked meat samples from seven small restaurants. The isolation of *E. coli* was done on each of the 358 samples, and 493 isolates were obtained. All the *E. coli* isolates were then tested for their susceptibility to colistin sulfate by using the agar dilution method. The detection of the *mcr*-1 gene from the colistin-resistant isolates (minimum inhibitory concentration > 2 μg/mL) was conducted using the polymerase chain reaction (PCR). The prevalence value of colistin-resistant *E. coli* in all the isolates was at 11.76% (CI 95%; CL 9.21–14.91%), and the prevalence of *mcr*-1 gene was at 10.55% CI 95%; CL 8.13–13.57%). A very good agreement correlation existed between the colistin-resistant phenotype and the *mcr-1* gene (κ = 0.939). The *mcr-1* gene was found in 89.66% colistin-resistant *E. coli* isolates. The two colistin-resistant and *mcr-1* carrying gene isolates were identified as *E. coli* O157:H7 serotype. This research was the first study attempt on the *mcr*-1 gene in Indonesia, covering the entire supply chain of broiler meat from farms to consumers. The results indicated the necessity to reduce the use of colistin sulfate in broiler management and to improve biosecurity measures, not only in farms but also in the entire supply chain of broiler meat production.

Keywords: broiler, colistin, *Escherichia coli*, *mcr-1*, supply chain

INTRODUCTION

Antimicrobial resistance is a serious threat to global public health and thus, needs attention from all sectors of the society. In India, the annual death rate of nearly 58,319 infants is attributed to antimicrobial-resistant infections, while in Europe, the annual estimated death rate of 25,000 is due to antimicrobial resistance to bacteria (Laxminarayan *et al.* 2013). Some experts estimate that by 2050 the antimicrobial-resistant infections will cause extra deaths of up to 10 million lives per year and inflict an economic loss of up to $ 100 trillion, mostly caused by *E. coli*, malaria, and tuberculosis (WHO 2014; Grace 2015). Among those microbes, only the *E. coli* resistance is linked to agricultural practices (Grace 2015). *Escherichia coli*

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is a commensal bacterium which is used as an indicator to monitor antimicrobial resistance in food animals and their products (Tadesse *et al.* 2012; OIE 2016). Food animals, along with their production environments, are considered as the reservoir of resistant bacteria and as the source of its introduction to humans (Schroeder *et al.* 2004; Marshall & Levy 2011).

The use of the same antimicrobials in humans and animals poses a global concern about the transmission of the same resistant bacteria from animals to humans. One antimicrobial agent used in animals and humans is the colistin sulfate. The discovery of the same colistin-resistant *E. coli* in the isolates from animals which are also found in humans further reinforces the possibility of transfer of the resistant *E. coli* colistin from animals to humans (Olaitan *et al.* 2015).

Colistin sulfate is a polymyxin antibiotic that was discovered in 1949, however, since the 1980s, the usage of colistin sulfate in humans has diminished because of its significant nephrotoxicity and neurotoxicity (Falagas & Kasiakou 2005; Morales *et al.* 2012). The increasing ability of the multidrug-resistant (MDR) gram-negative pathogenic bacteria to fight against the available antibiotics has required clinicians to reconsider the role of this old antibiotic as the last drug resource for fighting against lethal infections caused by MDR (Paterson & Harris 2016). Colistin sulfate has been proven effective against MDR *Acinetobacter species*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* MDR carbapenemase (Falagas & Kasiakou 2005; Catry *et al.* 2015; Nordmann *et al.* 2016). The World Health Organizations (2017) also categorized colistin as one of highest priority critically important antimicrobials for human medicine.

For more than 50 years, colistin sulfate has been used widely in food animals. The main indications are for the treatment of infectious diseases caused by *Enterobacteriaceae* in pigs, cattle, goat, sheep, rabbits, and poultry (Catry *et al.* 2015). Colistin sulfate can also be used as a growth promoter for broilers in the flocks that are infected with *Salmonella* (Fard 2004). However, since May 2017 Indonesia has banned the use of antibiotics as growth promoters, including the addition of colistin sulfate in the feed as growth promotor. Colistin sulfate can still be used in animals for therapy. In fact, there are more than 60 brands of commercial veterinary drugs already registered in Indonesia that contain colistin sulfate, either alone or combined with other antimicrobials (DGLAH 2016).

Before the discovery of the *mobilized colistin resistance (mcr)-1* gene in 2015 by Liu *et al.* (2015), the resistance of susceptible bacteria to colistin has been characterized by changes in the membrane or chromosomal mutations and theoretically is non-transferable by mobile genetic elements (Li *et al.* 2006; Landman *et al.* 2008; EMA 2016). The discovery of *mcr-1* gene in the plasmid of *E. coli* isolated from animals and humans raises global awareness of a new threat to the availability of antibiotics for MDR infection therapy. Less than a year after its discovery, 30 countries in 5 continents also reported that *mcr-1* gene was found in samples that were derived from animals or animal products (Schwarz & Johnson 2016). In Indonesia, however, very limited data is available on the colistin-resistant *E. coli* derived from food animals even though broiler is one food animal with the largest population in Bogor Regency, Indonesia (CSB 2015; DGLAH 2017). Hence, this study was conducted in Bogor Regency, West Java Province, Indonesia to measure (1) the prevalence of colistin-resistant *E. coli* in the supply chain pathway of broilers from flocks to cooked products, (2) the prevalence of *mcr-1* gene in *E. coli*, and (3) the prevalence of *mcr-1* gene in *E. coli* serotype O157:H7.

MATERIALS AND METHODS

Study Time

This study was carried out from February to December 2017. The isolation of *E. coli*, susceptibility test, Congo-red test, and detection of *mcr-1* gene was conducted at the National Veterinary Drug Assay Laboratory, the Directorate General of Livestock and Animal Health, Ministry of Agriculture, Republic of Indonesia.

Samples and Study Sites

Forty-seven flocks from five districts (Gunungsindur, Cibinong, Pamijahan, Cigudeg, dan Citeureup) in Bogor Regency were taken as a sample during approaching harvest time. The samples from each flock were cloacal swabs from ten birds per flocks, drinking water, and litter. All the sampled broiler flocks were taking colistin when being raised, the population per flock was less than 10,000 chickens, and the sampled flocks do not apply good biosecurity management in their flocks. The next broiler meat supply chain, which was sampled were small-scale poultry slaughterhouses, traditional markets, and small restaurant restaurants. The selection of districts to sampling in the broiler supply chain after from the flocks was based on information provided by the farmers or broiler collectors about their selling areas. Based on their information, seven districts were selected, i.e., Gunungsindur, Cibinong, Cigudeg, Citeureup, Ciawi, Leuwiliang, and Tanah Soreal. Seven fresh slices of meat and one pooled pluckers swab taken from the small-scale poultry slaughterhouse (SSPS), seven fresh slices of meat from the traditional market, and seven cooked slices of meat from the small restaurant were sampled from each district.

Isolation of *Escherichia coli*

The cloacal swab samples were obtained from ten chickens from each flock and pooled in 10 mL sterile 0.1% phosphate buffer saline (PBS) (Oxoid, UK). The drinking water (minimum 10 mL/flocks) and litter samples (minimum 100 g litter/flock) were taken from three different spots in each of the 47 flocks and pooled in sterile containers. Ten fresh broiler meat samples with minimum 100 g sample derived from ten chickens were taken from each of the SSPS and the traditional market. Samples of plucker swabs from SSPS were taken from three different spots inside the pluckers and pooled into 10 mL sterile 0.1% PBS (Oxoid-UK). Ten pieces of cooked broiler meat with a minimum 100 g/sample from 10 different chickens were taken from each small restaurant near the traditional market. Each of the raw and cooked meat samples came from different chickens from the flocks that have been sampled. This is to avoid duplication of samples that can affect the prevalence level.

The total number of samples to be tested consisted of 47 pools of cloacal swabs, 47 samples of drinking water, 47 pools of litter, 70 samples of fresh meat from SSPS, 7 pools of plucker swabs, 70 samples of fresh meat from traditional markets, and 70 samples of cooked meats. Each pool of cloacal and plucker swab samples was streaked directly onto MacConkey Agar (MCA) (Oxoid-UK) or Levine-Eosin Methylene Blue Agar (L-EMB) (Oxoid-UK) and incubated at 37 °C for 18–24 hours. Five colonies from each flock and three colonies from each plucker were taken. Samples of litter, fresh meat, and cooked meat, each weighing 10 grams, were put in a 90 mL of buffer phosphate saline (BPW) (Oxoid-UK) 0.1% and mixed with stomachers. One mL of drinking water was mixed with 9 mL of BPW 0.1%, then mix using vortex. Then, one mL of each BPW 0.1% solution was taken, put into 9 mL of Lauryl Sulphate Tryptose Broth (LSTB) (Oxoid–UK), and incubated at 35 ºC for 24–48 hours. One mL of LSTB solution was then poured into 9 mL EC medium (DB/Difco–FRA). The EC medium was then incubated at 45.5 °C for 24–48 hours. The growth of bacteria on LSTB and EC media was indicated by a change in the media, becoming turbid. One ose loop of the EC media was taken and then streaked in L-EMBA or MCA. One colony that was considered as *E. coli* was taken from each of those samples. The colony was then purified by streaking them again in L-EMBA or MCA. All *E. coli* isolates were confirmed using a biochemical test or IMViC Test, which consisted of sulfite indole motility (Oxoid-UK), methyl red-voges proskauer (MR-VP) (Oxoid-UK), and citrate (Oxoid-UK). The next tests only involved those colonies which generated these ImVic test results: Indole (+), MR (+), VP (-), and citrate (-) (INS 2008).

Colistin Sulfate Susceptibility and Pathogenic Testing of *Escherichia coli*

Susceptibility testing of *E. coli* was conducted using the agar dilution (AD) method to determine the minimum inhibitory concentration (MIC) value (Bahera *et al.* 2010; Morales *et al.* 2012; Dafopoulou *et al.* 2015). Mueller-Hinton agar (MHA) (Difco/DB-FRA) that contains colistin sulfate standard (Sigma-USA) with two-fold concentration dilution

ranging from 0.125 μg/mL to 16 μg/mL was used as the media. *Escherichia coli* ATCC 25922 was used as control isolate (CLSI 2016), while MHA without colistin sulfate was used as control media. The isolates were considered colistin-resistant when their MIC value > 2 μg/mL (Boyen *et al.* 2010; Morales *et al.* 2012; BSAC 2015; EUCAST 2017). The AD method tends to produce a higher MIC value than broth microdilution (BMD) method, yet it is a useful method to determine colistin resistance. When compared with the BMD method, as a reference method for susceptible test for colistin, the AD method showed a low rate of very major errors (false-susceptible result) which were 0.7–3.3 % and the rates of major errors (false-resistant result) were 2.4-4.9% (Bahera *et al.* 2010; Dafopoulou *et al.* 2015). Recently, the AD method was considered superior in terms of reproducibility, robustness, and ease compared to the broth dilution method for colistin susceptibility testing (Turlej-Rogacka *et al.* 2018).

To distinguish between normal and pathogenic *E. coli* isolates, all the samples were tested using the Congo-red test (Berkhoff & Vinal 1986). Susceptibility and pathogenic tests using the Congo-red method were replicated three times. The isolates with colistin resistant-pathogens were sent to the Indonesian Research Center for Veterinary Sciences, Ministry of Agriculture, Republic of Indonesia to determine which isolates are the O157:H7 serotype.

Detection of the *mcr-1* Gene

Detection of the *mcr-1* gene using polymerase chain reaction (PCR) was conducted as previously described by Liu *et al.* (2015) and Cavaco *et al.* (2016), with some modifications. The DNA extraction was performed using the boiling technique at 100 °C for 15 minutes using the preparation sample reagent PrepMan™ Ultra (Life-USA). The master mix for 25 μL reaction consisted of 12.5 μL Hotstart *master mix* (Qiagen-DEU), 1 μL (5 μM) primer *mcr-1* CLR-F (5'-CGGTCAGTCCGTTTGTTC-3'), 1 μL (5 μM) primer *mcr-1* CLR-R (5'-CTTGGTCGGTCTGTAGGG-3'), DNA *template* 5 μL (10x), and H_2O (Qiagen-DEU) up to 25 μL. The thermocycler PCR condition was at 94 °C 15 min + 25x (94 °C 30 sec + 57.5 °C 90 sec + 72 °C 60 sec) + 72 °C 10 min. *Escherichia coli* that carried *mcr-1* gene (code EC DI15) was used as the positive control, and *E. coli* ATCC 25922 was used as the negative control. Isolates with the *mcr-1* gene showed a band at 309 bp.

The kappa statistic (κ) was used to determine the agreement between the colistin-resistant phenotype and the presence of the *mcr-1* gene (Nguyen *et al.* 2016). κ value < 0 means poor agreement, 0-0.2 means slight, 0.21-0.40 means fair, 0.41-0.60 means moderate, 0.61-0.80 means substantial, and 0.80-1.00 means an almost perfect agreement (Thrusfield 2005).

RESULTS AND DISCUSSION

All the 47 flock samples matched the criteria for the sampling sites. The broiler flock samples were taken from five districts, namely Gunungsindur (2 flocks), Cibinong (7 flocks), Pamijahan (21 flocks), Cigudeg (12 flocks), and Citeureup (5 flocks). The other samples were taken from the small-scale poultry house (SSPS), traditional markets, and small restaurants which were located in the seven districts of Gunungsindur, Cibinong, Cigudeg, Citeureup, Ciawi, Leuwiliang, and Tanah Sareal. Not all samples showed the presence of *E. coli*. For instance, nine samples of drinking water were *E. coli* negative, while only twelve samples of cooked meat contained *E. coli*. The total number of *E. coli* isolates from the flock level to restaurant-level was 493 (Table 1).

Fifty-eight isolates of *E. coli* that have MIC values higher than 2 ug/mL were considered colistin-resistant (Table 2). The MIC values of *E. coli* colistin-resistant isolates from the samples was 4–8 μg/mL, except two colistin-resistant *E. coli* isolates taken from drinking water that has MIC value higher than 32 μg/mL (Table 2). The congo-red test was performed simultaneously with the susceptibility test. The results of the tests indicated that 15 isolates (3.04%) were considered colistin-resistant and pathogenic (Table 3).

Table 1 Number of *E. coli* isolates derived from the samples

Source	Number	Type of Samples	Sample Size	Number of Isolates
Flocks	47	Pools of cloacal swabs	47	235
		Drinking water	47	38
		Litter	47	47
		Fresh meat	70	70
SSPS	7	Pools of inside plucker swabs	7	21
Traditional markets	7	Fresh meat	70	70
Small restaurants	7	Cooked meat	70	12
Total	68		358	493

Table 2 Minimum inhibition concentration (MIC) values of colistin sulfate against *Escherichia coli*

Type of Samples	Number of isolates	MIC Value (μg/mL)								
		0.125	0.25	0.5	1	2	4*	8*	16*	>32*
Cloacal swabs	235	2	0	13	74	115	18	13	0	0
Drinking water	38	1	2	4	21	6	2	0	0	2
Litter	47	0	0	3	14	26	3	1	0	0
Fresh meat (SSPS)	70	0	0	11	31	21	6	1	0	0
Inside plucker swabs	21	1	0	9	5	4	2	0	0	0
Fresh meat (traditional markets)	70	0	1	8	29	22	6	4	0	0
Cooked meat	12	0	0	1	8	3	0	0	0	0
Total	493	4 (0.81%)	3 (0.61%)	49 (9.94%)	182 (36.92%)	197 (39.96%)	37 (7.51%)	19 (3.85%)	0 (0%)	2 (0.41%)

Notes: *Isolates with MIC > 2 μg/mL were considered colistin resistant

Based on the above results, the prevalence of colistin-resistant *E. coli* from flock level to restaurant-level varied from 0 to 14.29% (Table 3). The lowest prevalence value (0%) was found in cooked meat, while the highest prevalence value (14.29%) was found in fresh meat from traditional markets. The prevalence of colistin-resistant *E. coli* in the cloacal swabs from live broilers at flock level was 13.19% (CI 95%; CL 9.45–18.12%). This prevalence was slightly lower than the prevalence of colistin-resistant *E. coli* in the cloacal swabs of layer chickens from a previous study (Palupi *et al.* 2016), which was 14.94% (CI 95%; CL 8.95–23.90%). *Escherichia coli* colistin-resistant isolates were also found in the environment surrounding the flocks, especially the drinking water and litter, which were used in this research as part of the samples. Based on interviews, all flocks were provided with chlorinated drinking water, nine samples of which were *E. coli* negative. The purpose of chlorinating drinking water is to minimize microorganisms and inhibit the formation of biofilms (Amaral 2004). Four *E. coli* isolates (10.53%) out of 38 isolates from drinking water were found to be colistin-resistant. Colistin resistant *Escherichia coli* can be found in water sources near farms or water ponds (Ellem *et al.* 2017; Zhou *et al.* 2017). *Escherichia coli* was found in all litter samples, while the prevalence of colistin-resistant *E. coli* was 8.51% (CI 95%; CL 3.36–19.93%). The prevalence of *E. coli* in litter samples used in this study was higher than that in a previous study by Devendec *et al.* (2016).

The prevalence of colistin-resistant *E. coli* isolated from the interior of the pluckers was 9.52% (CI 95%; CL 2.65–28.91%). This result indicated a possibility that colistin-resistant *E. coli* was transferred from the SSPS through the pluckers. Live broilers at SSPS were generally kept in one cage and might have been brought from several farms depending on the supply of broiler collectors. Broilers were slaughtered when a customer comes in or based on 'off-shop' orders. Generally, SSPS had only one to two pluckers. The number of chickens put into

pluckers depended on the number of chickens purchased by consumers. Some chickens that had been slaughtered are generally put into the pluckers simultaneously, and this practice could have possibly transferred the colistin-resistant *E. coli* during the plucking and cleaning process. The prevalence of colistin-resistant *E. coli* in fresh meat taken from SSPS was lower than those taken from traditional markets (Table 3).

Only 12 out of 70 cooked meat samples contained *E. coli*, with a possibility that these were exposed to *E. coli* from the environment after the cooking process. Mostly Indonesian foods, like the study samples, are well cooked or even overcooked (>100 °C) and this could reduce the risk of *E. coli* in cooked meat. *E. coli* would die after being exposed to very high temperatures or over 100 °C (Lee & Kalentuç 2002). None of *E. coli* isolates from cooked meat was found to be colistin-resistant. Cooking temperatures that could kill bacteria would reduce the risk of having *E. coli* in meat. The sample from cooked meats that previously have colistin-resistant *E. coli* did not show any growth of *E. coli* after the meat was boiled at temperatures ≥ 100 °C for 30 minutes (Palupi *et al.* 2018).

Table 3 Prevalence of colistin-resistant *Escherichia coli* carrying *mcr-1 gene* and the results of the pathogenic test using Congo-Red test

Type of Samples	Number of Isolates					Prevalence of colistin-resistant *E. coli*	Prevalence of *E. coli* carrying *mcr-1* gene
	Tested	Colistin resistant	Carrying *mcr-1* gene	Colistin resistant and pathogenic	Colistin resistant carrying *mcr-1* gene – pathogenic		
Cloacal swabs	235	31	30	5	5*	13.19% (CI 95%; CL 9.45–18.12%)	12.77% (CI 95%; CL 9.09–17.64%)
Drinking water	38	4	1	2	0	10.53% (CI 95%; CL 4.17-24.13%)	2.63% (CI 95%; CL 0.47–13.49%)
Litter	47	4	4	2	2	8.51% (CI 95%; CL 3.36–19.93%)	8.51% (CI 95%; CL 3.36–19.93%)
Fresh meat (SSPS)	70	7	6	2	2*	10.00% (CI 95%; CL 4.93–19.23%)	8.57% (CI 95%; CL 3.99–17.47%)
Inside plucker swabs	21	2	1	1	1	9.52% (CI 95%; CL 2.65–28.91%)	4.76% (CI 95%; CL 0.85–22.67%)
Fresh meat (traditional markets)	70	10	10	3	3	14.29% (CI 95%; CL 7.95–24.34%)	14.29% (CI 95%; CL 7.95–24.34%)
Cooked meat	12	0	0	0	0	0.00% (CI 95%; CL 0.00–24.25%)	0.00% (CI 95%; CL 0.00–24.25%)
Total	493	58 (11.76%)	52 (10.55%)	15 (3.04%)	13 (2.64%)	11.76% (CI 95%; CL 9.21–14.91%)	10.55% (CI 95%; CL 8.13–13.57%)

Notes: *One isolate of these groups belongs to O157:H7 serotype and carried *mcr*-1 *gene* (Code K34d and D29).
CI = Confidence Interval, CL = Confidence Limit.

The prevalence of colistin-resistant *E. coli* among all the isolates in this study was 11.76% (CI 95%; CL 9.2–14.91%). Whereas, in some countries, the prevalence of colistin-resistant *E. coli* in broiler production chains varied between < 1% and 30% (Schrauwen *et al.* 2017; Irrgang *et al.* 2016; Malhotra-Kumar *et al.* 2016; Nguyen *et al.* 2016; Huang *et al.* 2017; Monte *et al.* 2017).

All the colistin-resistant *E. coli* isolates were then tested using PCR to detect the presence of the *mcr-1* gene (Table 3 and Fig. 1). The prevalence of colistin-resistant *E. coli* carrying *mcr-1* gene in the isolates was 10.55% (CI 955; CL 8.13–13.57%). In this study, the highest prevalence of *mcr-1* gene was at 14.29% (CI 95%; CL 7.95–24.34%) for colistin-resistant *E. coli* found in fresh meat samples from traditional markets, while the lowest prevalence of *mcr-1* gene, at 2.63% (CI 95%; CL 0.47–13.49%), was found in *E. coli* isolates from drinking water samples. The presence of colistin-resistant *E. coli* carrying *mcr-1* gene was also discovered in the isolates derived from cloacal swabs, drinking water, and litter obtained from a flock in Cibinong District with the code K18.

The results of Congo-red test and detection of *mcr-1* gene showed that 13 colistin resistant-pathogenic *E. coli* isolates were found to carry *mcr-1* gene (Table 2). Two of these isolates, i.e., one from a cloacal swab and one from SSPS fresh meat, were found to belong to *E. coli* O157:H7 serotype. *Escherichia coli* O157H7 serotype is an enterohemorrhagic *E. coli* (EHEC) and a zoonotic pathogen that is responsible for the majority of severe EHEC cases in humans (Ferens & Hovde 2011). Hence, EHEC serotype O157 must be included in the resistance surveillance and monitoring programs because this serotype is pathogenic to humans, but not to animals (OIE 2010). In this study, even if the prevalence of colistin-resistant *E. coli* carrying the *mcr-1* serotype O15:7H7 was very low at 0.41% (CI 95%; CL 0.11–1.47%), it still indicated a serious threat prevailing in the supply chain of broiler meat.

Table 4 The K values of *Escherichia coli* colistin-resistant phenotype and the genotype carrying *mcr-1* gene

Sample	Percentage of *mcr-1* gene in colistin-resistant *Escherichia coli* isolates	κ value
Cloacal swab	96.77%	0.981
Drinking water	25%	0.37
Litter	100%	1
Fresh meat from SSPS	85.71%	0.915
Plucker swab	50%	0.644
Fresh meat from traditional market	100%	1
Cooked meat form small restaurants	No colistin-resistant *E. coli*	0
Total	89.66%	0.939

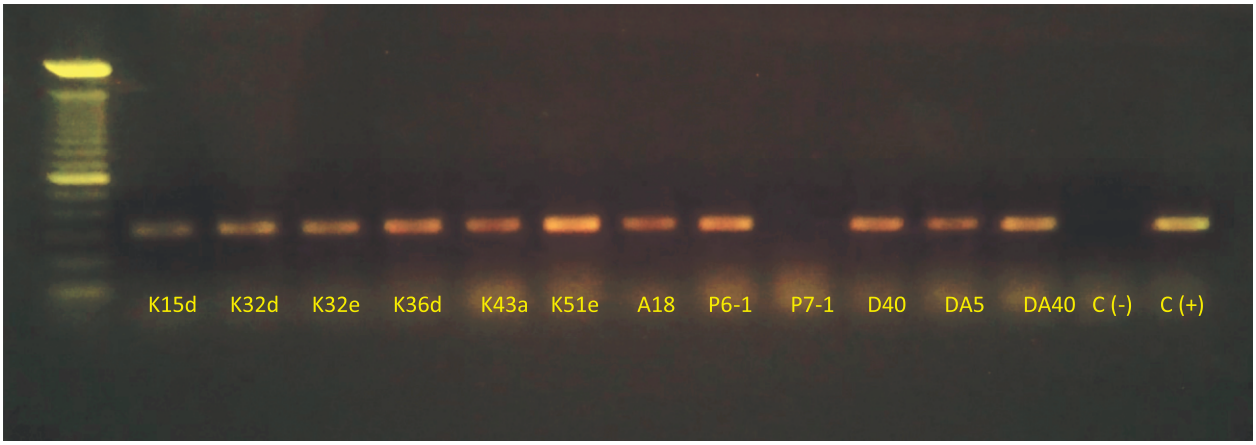

Figure 1 PCR result of the detection of *mcr-1* gene in colistin resistant *E. coli* isolates (target extend band 309 bp)
Notes: K15d, K32d, K32e, K36d, K43a, K51e are isolates from cloacal swabs; A18 is from drinking water; P6-1 and P7-1 are from plucker swabs; D40 is from fresh meat (SSPSH); DA5 and DA40 are from fresh meat (traditional markets); C(-) is *E. coli* ATCC 25922; and C (+) is *E. coli* carrying *mcr-1* gene (EC D15).

The discovery of the *mcr-1* gene elucidated enormously serious implications because this mediated plasmid transfer gene may threaten the availability of antimicrobials, which can reduce the infection of MDR gram-negative pathogens (Paterson & Harris 2016). The increasing number of cases of colistin-resistant bacterial infections in humans had been associated with increased mortality (Kontopoulou *et al.* 2010; Capone *et al.* 2013). Some studies already showed that *mcr-1* gene could be transferred from colistin-resistant *E. coli* to recipient susceptible bacteria such as *E. coli J53*, *Klebsiella pneumonia,* and *Pseudomonas aeruginosa* via conjugation (Liu *et al.* 2015; Nguyen *et al.* 2016; Shen *et al.* 2016). This study also showed that this gene could be transferred from *E. coli* to *Salmonella enteritidis* ATCC 13076 (Palupi *et al.* 2018). The spread of *mcr-1* gene was due to the diffusion of composite transposon rather than the diffusion of a specific plasmid or clone (Hadjadj *et al.* 2017). Hence, the gene could easily integrate into the various bacteria in animals or humans.

Results of this study provided some important information about the presence of *mcr-1* gene along the supply chain of broiler meat in Indonesia. The fact that *E. coli* O157:H7 carrying the *mcr-1* gene is found at several levels of the supply chain, served as a warning on the potential risk of using colistin sulfate in broilers and the importance of good handling techniques for broiler meat along the supply chain. However, the risk of human exposure to *mcr-1* gene was reduced when the broiler meat was already cooked. Therefore, it is highly recommended to cook meat at least or above the temperature that could kill the bacteria or that the cooking temperature must be at least at a level that can damage the bacteria DNA. The occurrence of resistance is facilitated through naked DNA, known as transformation. DNA remains stable at temperatures below 100°C and at 130 °C, DNA begins to undergo degradation and is completely degraded at 190°C, under dry condition (Karni *et al.* 2013).

The direct correlation between the use of colistin sulfate and the presence of a resistant gene was not easy to determine as this study only involved those flock samples taken near the harvest time. However, certain precautionary activities might prove useful; reducing the use of colistin sulfate as prophylaxis at farm level and using this antimicrobial substance only as a therapeutic agent, instead. Improper application of colistin sulfate would not only kill the normal bacteria in an animal's gut but would also multiply the colonies of resistant bacteria. Moreover, the observance of biosecurity protocols for the entire farm area, and the practice of good handling techniques for broiler meat along the supply chain could not be over-emphasized. The food animal products must be cooked at temperatures that could kill the bacteria and destroys its DNA.

CONCLUSION

The study results indicated the consequential distribution of *mcr*-1 gene along the supply chain of broilers. This means that the possibility, albeit small, of the spread of *E. coli* O157:H7 serotype that is colistin-resistant and that carries the *mcr-1* gene, is certain and bound to happen. Reducing the usage of colistin in food animals and proper handling of broilers and broiler products along the supply chain are then essential in reducing the risk of transfer of colistin-resistant *E. coli* to humans. Hence, the regular monitoring and surveillance of colistin resistance in other bacteria, especially those carrying other *mcr* genes, are strongly recommended.

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