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

Maria Fatima Palupi[1*], I Wayan Teguh Wibawan[2], Etih Sudarnika[2], Hera Maheshwari[3], Huda Shalahudin Darusman[3,4]

[1]National Veterinary Drug Assay Laboratory, Directorate General of Livestock and Animal Health, Ministry of Agriculture of the Republic of Indonesia, Jakarta 12550, Indonesia

[2]Department of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, Institut Pertanian Bogor, Bogor 16680, Indonesia

[3]Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, Institut Pertanian Bogor, Bogor 16680, Indonesia

[4]Primate Research Center, Institut Pertanian Bogor, Bogor 16151, Indonesia

[*]Corresponding author, e-mail: lupi_ima@yahoo.co.id

Running title: Prevalence of *mcr-1* gene in *Escherichia coli* along broiler meat supply chain

ABSTRACT

Colistin is the last drug choice for dealing with carbapenem-resistant *Enterobacteriaceae*; therefore, this drug is very crucial for human health. The discovery of a plasmid-mediated colistin resistance gene, *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. The purposes of this study were to measure the prevalence of colistin resistant *E. coli* and to detect the *mcr-1* colistin resistance 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 flocks that use colistin sulfate (cloacal swabs, drinking water, and litters), small-scale poultry slaughterhouses (fresh meats and plucker swabs), traditional markets (fresh meats), and small restaurants (cooked meats). Isolation of *E. coli* was done on each sample and 493 isolates were obtained. All *E. coli* isolates were then tested for their susceptibility to colistin sulfate by the agar dilution method. Detection of *mcr*-1 gene from colistin resistant isolates (minimum inhibitory concentration > 2 µg/mL) was conducted using polymerase chain reaction. The prevalence of colistin resistant *E. coli* from all isolates was 11.76% (CI 95%; CL 9.21–14.91%), and the prevalence of *mcr*-1 gene was 10.55% (CI 95%; CL 8.13–13.57%). There was a very good agreement between colistin resistance phenotype and *mcr-1* gene (κ = 0.939). The *mcr-1* gene was found in 89.66% colistin resistant *E. coli* isolates. Two colistin resistance and *mcr-1* carrying gene isolates were identified as *E. coli* O157:H7 serotype. This study was the first research on *mcr*-1 gene in Indonesia which covers the entire supply chain of broiler meat from farms to consumers. These results showed the necessity to emphasize a reduced use of colistin sulfate in broiler management and to improve biosecurity measure, not only in farms but also in the entire supply chain of broiler meat.

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

INTRODUCTION

Antimicrobial resistance is a serious threat to global public health that needs attention from many sectors. In India, nearly 58,319 infants die each year, which is attributable to antimicrobial resistant infections, and it is estimated that 25,000 people die a year in Europe from antimicrobial resistance to bacteria (Laxminarayan *et al.* 2013). Some experts estimate that by 2050 antimicrobial resistant infections will cause extra deaths 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). From those microbes, only *E. coli* resistance could be linked to agricultural practices (Grace 2015). *Escherichia coli* 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 the source of 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 of the antimicrobial agents that are used in animals and humans is 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 resistant *E. coli* colistin from animals to humans (Olaitan *et al.* 2015).

Colistin sulfate is a polymyxin antibiotic that was discovered in 1949, but since the 1980's 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 multidrug-resistant (MDR) gram-negative pathogen 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 (2011) also categorized colistin as 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 infection diseases caused by *Enterobacteriaceae* in pigs, cattle, goat, sheep, rabbits, and poultry (Catry *et al.* 2015). According to Fard (2004), colistin sulfate can be used as a growth promoter for broilers. Since May 2017 Indonesia has banned the use of antibiotics as growth promoters, including the usage colistin sulfate in feed. 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 *mobilized colistin resistance (mcr)-1* gene in 2015 by Liu *et al.* (2015), the resistance to colistin in susceptible bacteria has been characterized by the changing in membrane or chromosomal mutations and theoretically in non-transferable by mobile genetic elements (Li *et al.* 2006; Landman *et al.* 2008; EMA 2013). The discovery of *mcr-1* gene in plasmid of *E. coli* isolated from animals and humans raises a global awareness of the 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, the available data of colistin resistant *E. coli* derived from food animals are still very limited.

The purposes of this study were 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. Broiler is a food animal with the largest population in Indonesia. This study was conducted in Bogor Regency, West Java Province, Indonesia. West Java Province has the largest broiler population in Indonesia, while the largest broiler population in West Java can be found in Bogor Regency (CSB 2015; DGLAH 2017).

MATERIALS AND METHODS

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

Forty-seven flocks in five districts in Bogor Regency were sampled. Sampling on the flock was carried out when the harvest time was approaching. All flocks that have been sampled were used colistin when raising those broilers, population per flock was less than 10,000 chickens, and lack of biosecurity. The selection of seven districts in Bogor that provided the samples obtained from the entire supply chain of broiler meats was based on information provided by farmers or broiler collectors about their selling areas. One small-scale poultry slaughterhouse (SSPS), one traditional market, and one small restaurant were sampled in each district.

Isolation of *Escherichia coli*

Cloacal swab samples were obtained from ten chickens from each flock and pooled in sterile 0.1% buffer peptone water (BPW) (Oxoid, UK). Drinking water and litter samples were taken from three different spots in each flock and pooled in sterile containers. Ten fresh broiler meat samples derived from ten chickens were taken from each SSPS and traditional market. Samples of plucker swabs from SSPS were taken from three different spots inside the pluckers and pooled into sterile 0.1% PBS (Oxoid, UK). Ten pieces of cooked broiler meat from 10 different chickens were taken from each small restaurant near the traditional market. Each of 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 were 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 into 90 mL BPW 0.1% and mixed with stomachers. One mL of drinking waters was mixed with 9 mL of BPW 0.1%, then 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 taken and put into 9 mL of 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 media, which became turbid. One loop ose 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 would only involve 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 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 give higher MIC value than broth microdilution (BMD) method, but it 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, according to Turlec-Rogacka *et al.* (2018) study, the AD method was superior in terms of

reproducibility, robustness, and ease compared to the broth dilution methods for colistin susceptibility testing.

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

Detection of *mcr-1* Gene

Detection of *mcr-1* gene using polymerase chain reaction (PCR) was conducted as previously described (Liu *et al.* 2015; Cavaco *et al.* 2016) with some modifications. DNA extraction was performed using the boiling technique at 100 °C for 15 minutes using the preparation sample reagent PrepMan^{TM} Ultra (Life-USA). 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 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 *mcr-1* gene will show band in 309 bp.

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

RESULTS AND DISCUSSION

All sampled flocks matched the criteria for the sampling sites. Our 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 SSPS, traditional markets, and small restaurants which were located in seven districts consisting of Gunungsindur, Cibinong, Cigudeg, Citeureup, Ciawi, Leuwiliang, and Tanah Sereal. 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 flock level to restaurant level was 493 (Table 1).

Table 1 Number of *E. coli* isolates derived from 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
SSPS	7	Fresh meat	70	70
		Pools of inside plucker swabs	7	21
Traditional markets	7	Fresh meat	70	70
Small restaurants	7	Cooked meat	70	12
Total				493

Fifty-eight isolates of *E. coli* that have MIC value higher than 2 ug/mL were considered colistin resistant. The MIC value of *E. coli* colistin resistant isolates from these samples was 4–8 µg/mL, except two *E. coli* colistin resistant isolates from drinking water that has MIC value higher than 32 µg/mL (Table 2). 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 2 Minimum inhibition concentration value 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%)

Note: *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 was found in cooked meat, while the highest prevalence 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 chicken 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 drinking water and litter, which were used in this research as part of the samples. Based on interviews, all flocks were provided with chlorinate drinking water, nine samples of which were *E. coli* negative. The purpose of chlorinating drinking water is to minimize the prevalence of 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; Zhaou *et al.* 2017*). Escherichia coli* can be 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 (2016).

The prevalence of colistin resistant *E. coli* isolated from the inside of pluckers was 9.52% (CI 95%; CL 2.65–28.91%). This result provides us with important information that there is a possibility that colistin resistant *E. coli* is transferred through pluckers in SSPS. Live broilers at SSPS are generally kept in one cage and may have been brought from several farms depending on the supply of broiler collectors. Broilers were slaughtered when a customer comes in or based on order. In general, SSPS only has one to two pluckers. The number of chickens put into pluckers depends on the number of chickens purchased by consumers. Some chickens that have been slaughtered are generally put into the pluckers simultaneously, and this practice can lead to the possibility that colistin resistant *E. coli* is transferred during the plucking and cleaning process. The prevalence of colistin resistant *E. coli* in fresh meat taken from SSPS was lower than that taken from traditional markets (Table 3).

Only 12 out of 70 cooked meat samples contained *E. coli*, with a possibility that they were exposed to *E. coli* from the environment after the cooking process. Mostly Indonesian foods, like our samples, are well cooked or even overcooked (>100 °C) and this can reduce the risk of *E. coli* in cooked meat. According to Lee & Kalentuç (2002), *E. coli* will die after being exposed to very high temperatures or over 100 °C. None of *E. coli* isolates from cooked meat was found to be colistin resistant. Cooking temperatures that can kill bacteria will reduce the risk of the presence of *E. coli* in meat. We have studied the sample from cooked meats that have colistin resistance *E. coli* before, and it was not showing any growth of *E. coli* after the meat boiled at temperatures $\geq$ 100 °C for 30 minutes (forthcoming).

The prevalence of colistin resistant *E. coli* from all isolates was 11.76% (CI 95%; CL 9.2–14.91%). By comparison, the prevalence of colistin resistant *E. coli* in broiler production chains in

some countries varied between < 1% and 30% (Schrauwen *et al.* 2015; Irrgang *et al.* 2016; Malhotra-Kumar *et al.* 2016; Nguyen *et al.* 2016; Huang *et al.* 2017; Monte *et al.* 2017).

Table 3 Prevalence of colistin resistant *Escherichia coli* carrying *mcr-1 gene* and the results of 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%)

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

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

Data from our study show that there was a very strong agreement between all isolates *E. coli* that showed colistin resistance phenotype and genotypic carrying *mcr-1* gene. The κ value was 0.939 which κ value 0.81-1 was considered almost perfect agreement. The percentage of colistin resistant *E. coli* isolates were found to carry *mcr-1* gene was 89.66%. The lowest to the highest percentage of colistin resistance *E. coli* that carrying *mcr-1* gene were found in *E.coli* obtained from drinking water (25% with κ value was 0.37 which has fair agreement), pluckers (50% with κ value 0.644 which has substantial agreement), fresh meat from SSPS (85.71% with κ value 0.915), cloacal swabs from broilers at flock level (96.77% with κ value 0.981), litter (100% with κ value 1), and fresh meat in traditional markets (100% with κ value 1). With an exception of cooked meat samples, *mcr-1* gene was found in mostly colistin resistant *E. coli* isolates obtained from all levels of broiler meat supply chain. The strong agreement between colistin resistance phenotype and *mcr-1* gene is also reported in other studies (Irrgang *et al.* 2016; Nguyen *et al.* 2016; Huang *et al.* 2017; Monte *et al.* 2017).

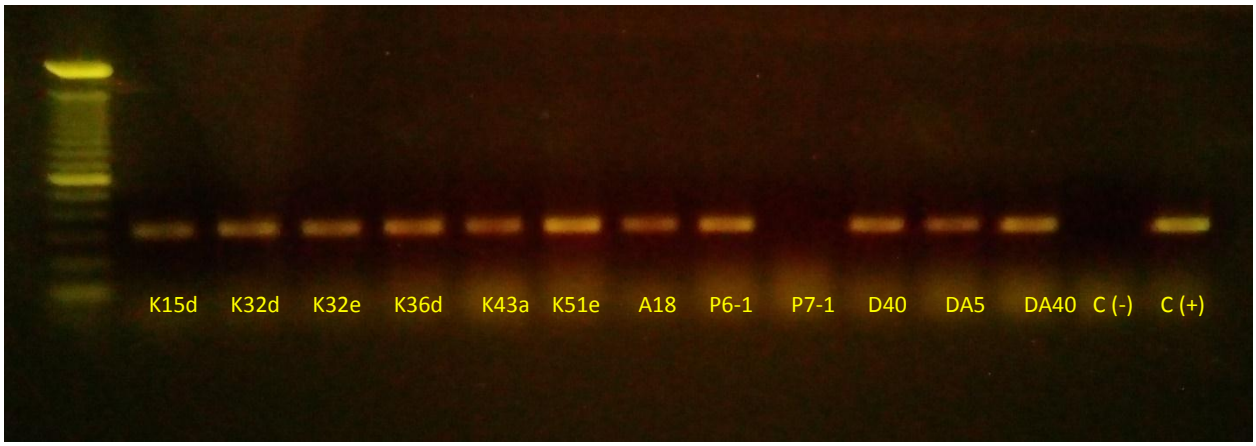

Figure 1 PCR result of the detection of *mcr-1* gene in colistin resistant *E. coli* isolates (target extend band 309 bp)
Note: K15d, K32d, K32e, K36d, K43a, K51e are isolates from cloacal swabs; A18 is from drinking water; P6-1 and P7-1 are from plucker swabs; D40 is from fresh meat

(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).

Based on the results of Congo-red test and detection of *mcr-1* gene, 13 colistin resistant-pathogenic *E. coli* isolates were found to carry *mcr-1* gene. 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 zoonotic pathogen that is responsible for the majority of severe EHEC cases in humans (Ferens & Hovde 2011). OIE (2010) suggested to include EHEC serotype O157 in resistance surveillance and monitoring programs because this serotype is pathogenic to humans, but not to animals. Even in this study the prevalence of colistin resistant *E. coli* carrying *mcr-1* serotype O157H7 was very low at 0.41% (CI 95%; CL 0.11–1.47%), but it indicated a serious threat along the supply chain of broiler meat.

The implication of the *mcr-1* gene discovery is enormous 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 bacteria infection in humans has been associated with increased mortality (Kantopoulou *et al.* 2010; Capone *et al.* 2013). Some studies already show that *mcr-1* gene can 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). Our study also shows that this gene can be transferred from *E. coli* to *Salmonella enteritidis* ATCC 13076 (forthcoming). According to Hadjadj *et al.* (2017), the spreading of *mcr-1* gene was due to the diffusion of composite transposon rather than the diffusion of a specific plasmid or clone. Because of this, the gene easily integrates into various bacteria in animals or humans.

This present study provides 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 *mcr-1* gene is found at several levels of the supply chain may serve as a warning about the potential risk of using colistin sulfate in broilers and the importance of good handling of broiler meat along the supply chain. However, the risk of human exposure to *mcr-1* gene was reduced when broiler meat has been cooked. Therefore, it is highly recommended to cook meat at least or above the temperature that can kill bacteria, in this case, *E. coli*. Another thing that is not less important is to ensure that the cooking temperature must be at least at temperature that can damage DNA. As we know that one of the occurrences of resistance is through naked DNA, known as transformation. DNA remains stable at temperatures below 100 °C and according to Karni *et al.* (2013), at 130 °C, DNA begins degradation and completely degraded at 190 °C under dry condition.

The direct correlation between the use of colistin sulfate and the presence of a resistant gene is not easy to determine as this study only involved flock samples taken near the harvest time. However, we continue to suggest reducing the use of colistin sulfate as prophylaxis at farm level and using this antimicrobial substance as therapeutic agent only instead. Improper application of colistin sulfate will kill normal bacteria in an animal's gut, but at the same time colonies of resistant bacteria will multiply. In addition to reducing the usage of colistin in flocks, it is also important to emphasize the biosecurity of the entire farm area and good handling of broiler meat along the supply chain. The food animal products must also be cooked in temperature that ensures killed the bacteria and destroys the DNA.

CONCLUSION

The results of our research provide an important indication of the distribution of *mcr*-1 gene along the supply chain of broilers. This means that there is a possibility, albeit small, of the spread of *E. coli* O157:H7 serotype that is colistin resistant and carries *mcr-1* gene. Based on this observation, reducing the usage of colistin in food animals and proper handling of broilers and broiler products along the supply chain are essential for reducing the risk of transfer of colistin resistant *E. coli* to humans. In addition to that, we also recommend regular monitoring and surveillance of colistin resistance in other bacteria, especially those carrying other *mcr* genes, such as *mcr*-2, *mcr*-3, and *mcr*-4.

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