**Risk Assessment of The Use of Colistin Sulfate In Broiler Due To *Escherichia coli* Resistance In Broiler Flocks**

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**ABSTRACT**

The risk assessment of antimicrobial resistance is very important to determine the risk of decreasing antimicrobial efficacy can be used as a basis for policymakers in allowing or prohibiting the use of an antimicrobial. This study aims to assess the risk of using colistin against E. coli resistance in the broiler flock. Risk assessment is carried out qualitatively using primary data, interviews, and secondary data. To obtain primary data various studies were carried out including monitoring the prevalence of colistin-resistant E. coli and mcr-1 also mcr-2 genes in broiler flocks, mcr-1 gene transfer from E. coli to Salmonella Enteritidis, mcr-1 gene sequencing, mutant selection windows of colistin against E. coli, and also multiresistant of E. coli colistin-resistant. Assessment of the risk of E. coli colistin-resistant in the broiler flocks through direct contact with live broiler flock environment with the resulting assessment is a medium risk with low uncertainty. Since colistin sulfate is very critically important for humans, the reduced use of colistin sulfate in animal production is necessary to reduce the risk of resistance. Reducing the use of colistin sulfate requires the collaboration of various sectors such as the government, veterinary drugs industries, farmers, and consumers.

Keywords: colistin sulfate, *mcr-1*, risk assessment, resistance, broiler

**INTRODUCTION**

The use of colistin sulfate in food production, which is the last drug choice for human as treatment of multiresistant gram-negative bacterial infections, raise a high interest in the world. Especially since the discovery of the *mobilized colistin resistant-1* (*mcr-1*) gene that can be transferred via plasmids in 2015 by Liu *et al.* (2015). This gene is transmissible to other bacteria and causes many countries to begin to reduce the use of colistin sulfate in animal production. Nevertheless, the prohibition on the use of an antimicrobial must go through scientific studies to produce valid results, such as by risk assessment.

  Risk assessment is part of a risk analysis that is very helpful for the government in making policies that are useful for assessing and managing risks to human and animal health regarding the increased antimicrobial resistance used in animals (OIE 2016). Antimicrobial risk assessment based on CODEX (2011) consists of hazard identification, hazard exposure, hazard characterization, and risk characterization. Until the time this research began, studies on the assessment of the risk of bacterial resistance due to the use of antimicrobials in production animals qualitatively and quantitatively have never been done in Indonesia.

We consider, along the supply chain of broiler meat, there are four main pathways of resistant-colistin E. coli exposes to humans. The first pathway is in broiler flocks, the second pathway is at a small slaughterhouse, the third pathway is in traditional markets through broiler fresh meat, and the last pathway is through broiler cooked meat products. In this paper, we only present an assessment of the risk of exposure to colistin-resistant E. coli in broiler flocks. The purpose of this study was to conduct a qualitative risk assessment of colistin sulfate on the emergence of colistin resistance in E. coli in broilers regarding the risk of human exposure to colistin-resistant *E. coli* in broiler flocks. It is hoped, this risk assessment can be used as consideration for determining the continued use of colistin sulfate in food animals, especially broilers in Indonesia.

**MATERIAL AND METHODS**

The study was conducted from November 2016 until January 2019 and principally generate primary and secondary data. Primary data collection was carried out by in vivo and in vitro effect of colistin sulfate exposure on E. coli resistance, conducting broiler supply chain modeling experiments in the laboratory, sampling in 47 flocks that use colistin sulfate (cloacal swabs, drinking water, and litters) to obtain the prevalence of colistin-resistant E. coli as well as mcr-1 and mcr-2 genes in 5 districts in Bogor-Indonesia, serotyping of E. coli O157:H7, questionnaires, expert opinions, gene transfer mcr-1 to S. Enteritidis, sequencing of the mcr-1 gene, multi-resistance of E. coli resistance colistin isolates, and mutant prevention concentration (MPC) research. The collection of secondary data was conducted thorough studies of scientific publications, unpublished data, and expert opinions. The risk assessment was made by using the data above to generate a qualitative risk assessment that developed from CODEX (2011) dan EMA (2018). Some parts of those researches have been published separately (Palupi et al. 2018a; Palupi et al. 2018b, and Palupi et al. 2019).

**Hazard Identification, Exposure Assessment, and Hazard Characterization**

To conduct hazard identification, various information about colistin sulfate and E. coli are needed, such as the prevalence of colistin-resistant E. coli, determination of resistance, the occurrence of cross-resistance or co-resistance, and minimum inhibitory concentration (MIC) data of colistin sulfate against E. coli. The information needed in the exposure assessment of colistin-resistant E. coli includes the description of colistin veterinary drug products circulating in Indonesia, the use of colistin in broilers, suppression of colistin resistance selection, the occurrence and rate of transfer of resistance, colistin concentration in the intestine lumen, selective windows of colistin sulfate, the prevalence of colistin-resistant E. coli from living and environmental broilers, and personnel characterization that can be directly exposed to colistin-resistant E. coli (Codex 2011, EMA 2018)

The information needed in assessing the hazard characterization of colistin-resistant E. coli from broilers to humans is the use of colistin sulfate for humans and the consequences if humans are exposed to colistin-resistant E. coli. The required details include the presence of colistin sulfate and its alternatives, the prevalence of colistin-resistant bacterial infection in humans, and the horizontal spread of resistance.

**The likelihood categorization of exposure assessment and Risk characterization**

Qualitative risk assessment was analyzed according to the stages in the CODEX CAC / GL 77-2011 document: Guidelines for Analysis of Foodborne Antimicrobial Resistance. Explanation or interpretation of hazard assessment is presented in Table 1

Table1 The qualitative interpretation of *likelihood* human exposure to colistin-resistant *Escherichia coli*

|  |  |
| --- | --- |
| Assessment | Interpretation |
| Negligible | The probability of exposure for susceptible people is extremely low so it can be negligible |
| Low | The probability of exposure for susceptible people is low but possible |
| Medium | The probability of exposure for susceptible people is likely |
| High | The probability of exposure for susceptible people is certain or very high |

Table 2 Likehood matrix combination of exposure assessment of colistin-resistant *E. coli*

|  |  |
| --- | --- |
| *Likelihood* 2 | *Likelihood* 1 |
| High | Medium | Low | Negligible |
| High | High | Medium | Low | Negligible |
| Medium | Medium | Low | Low | Negligible |
| Low | Low | Low | Negligible | Negligible |
| Negligible | Negligible | Negligible | Negligible | Negligible |

The results of the exposure assessment are obtained by making a likelihood assessment of each node in the pathway of exposure. The exposure assessment of each pathway was carried out using a combination of the likelihood combination matrix in Table 2 that developed from AFFA (2001).

 If multiple exposures are found in the pathway, the overall qualitative risk scoring for the exposure assessment is as follows: (1) if one of the partial risks is high, then the overall risk is also high; (2) if more than one partial risk is medium, overall risk is high; (3) if one partial risk is medium and the other partial risk (more than one) is low, the overall risk is high; (4) if there is one medium partial risk and the other partial risk is not medium, then the overall risk is medium; (5) if all partial risks are low, overall risk is medium; (6) if one or more partial risks is low, overall risk is low; and (7) if all partial risks can be neglected, the overall risk is negligible.

After conducting an exposure assessment, the next step is to conduct a hazard characterization assessment. Interpretations of hazard characterization assessments are listed in Table 3.

Tabel 3 Interpretation of likelihood assessment of hazard characterization colistin-resistant *Escherichia coli* qualitatively

|  |  |
| --- | --- |
| Categories | Interpretation |
| Negligible | Noadverse human health impact or consequnces  |
| Low | Symtoms are minimally bothersome and no therapy are needed |
| Medium | Symtoms are more pronounced than low categories but not life threatening. If an infection occurs, treatment is needed as indicated. |
| High or severe | Symtoms are potentially life threatening and requaire systemic treatment or hopitalization. Increase severity may occur due foodborne resistant bacteria.  |
| Very highor fatal | Directy or indirectly contributes to the death infected patient, treatment failure is likely expected due to foodborne resistant microorganism. No alternative treatment beside using colistin sulfate  |

In a qualitative risk assessment is important to understand the level of uncertainty of the information used. Categorization of information uncertainty using EFSA (2006), which are low, medium, and high uncertainty. The final step in assessing risk is to assess risk characterization. Risk characterization assessment is done by combining the results of exposure assessment and hazard characterization is presented in Table 4 (CODEX 2011).

Table 4 Matrix combination of risk characterization assessment colistin-resistant *Escherichia coli*

|  |  |
| --- | --- |
| Exposure assessment | Hazard characterization  |
| Negligible | Low | Medium | High | Very hIgh |
| Negligible | Negligible | Low | Low | Low | Low |
| Low | Negligible | Low | Low | Medium | High |
| Medium | Low | Medium | Medium | High | Very high |
| High | Low | Medium | High | Very high | Very high |

**RESULT AND DISCUSSION**

**Hazard Identification: Colistin sulfate and Colistin-resistant *Escherichia coli***

Colistin sulfate is a polymixin antibiotic used in animals and humans. Since 2017, polymyxin is categorized as the Highest Priority Critically Important Antimicrobials for Human Medicine (WHO 2017). Colistin sulfate has been used for decades in food animals as a therapy, prevention of infection, and as growth promotor. Colistin is very difficult to absorb by the digestive tract of broiler chickens (Lashev and Haritova 2003). Some commercial colistin sulfate veterinary drugs contain a single colistin sulfate and some are combined with other antimicrobials (EMA 2013; DGLAHS 2016).

Escherichia coli is a commensal bacterium that very important in monitoring and surveillance antimicrobial resistance in food animals and their products. The commensal bacterium is considered as a reservoir of antimicrobial resistance genes, which can transfer these genes to pathogenic bacteria (OIE 2016). Meanwhile, Escherichia coli serotype O157: H7 is a pathogenic zoonotic bacterium for humans (Riemann and Cliver 2006; Ferens and Hovde 2011). Food animals along with their products and their environments also considered a factor in the increasing or spreading of resistant bacteria.

The mechanism of resistance of E. coli to colistin is known through (1) mutations in specific regions, such as pmrA / B and phoP / Q, (2) mutations in the structure of lipopolysaccharide in the cytosol (ParR-ParS system), and (3) addition of phosphoethanolamine to lipid A that mediated by the mobilized colistin-resistant (mcr) genes (Fernández et al. 2010; Moskowitz et al. 2012; Liu et al. 2015). Co-resistance can occur with other polymyxin groups and several cationic peptides (Napier et al. 2013; Catry et al. 2015).

The spread of the mcr-1 gene can be through plasmids or conjugation, transposon composites, transformation, and chromosomes (Hadjaj et al. 2017; Lima Barbieri et al. 2017; Tada et al. 2017a; Sun et al. 2018). Our study also succeeded in transferring this gene from colistin-resistant E. coli to S. Enteritidis ATCC 13076 through conjugation (Palupi et al. 2018a). Escherichia coli is the most common bacteria found to have mcr-1 gene (Poirel et al. 2016). Our study showed that the prevalence of colistin-resistant E. coli along the broiler supply chain in Bogor was 11.76% (95% CI; CL 9.21-14.91%) with mcr-1 gene prevalence of 10.55% (95% CI; CL 8.13-13.57%) (Palupi et al. 2019). Our study also showed a very high agreement between colistin-resistant phenotype and mcr-1 genes genotype (89.66% conformity with a value of ĸ 0.939), we didn’t find mcr-2 gene, the MIC value of E. coli susceptible to colistin sulfate was 0.125-2 µg / mL, and the MIC value of colistin-resistant E. coli was 4 to > 32 µg/ mL.

**Exposure Assessment colistin-resistant Escherichia coli to Humans in Broiler Flocks**

Food animals and their environment are considered as one reservoir of resistant bacteria that can transfer directly or indirectly to humans (Marshall and Levy 2011; WHO 2016). We consider two major exposure branches pathway in the pathway exposure of colistin-resistant E. coli to humans in broiler flocks. First branches are through direct contact with live broilers and second branches are exposure through flock environment that contaminated with colistin-resistant E. coli (Fig. 1). Assessment of exposure through direct contact with live broilers involves two likelihood nodes, the first node is colistin-resistant E. coli in broilers due to the usage of colistin sulfate in the broiler (L1) and the likelihood of the process of human get exposed to colistin-resistant E. coli from live broilers (L3). The exposure pathway through the flock environment involved 3 likelihood nodes which are colistin-resistant E. coli derived from broilers (L1), the likelihood of flock environment get contaminated with colistin-resistant E. coli (L2), then the likelihood of colistin-resistant E. coli exposes humans through contact with the flock environment (L3).



Figure 1 Exposure pathway resistant-colistin E. coli to human in broiler flock. The first exposure is through direct contact with live broiler (L1 x L3) and the second is through the environment at flocks (L1 x L2 x L3)

**Likelihood Assessment Broiler with Colistin-Resistant *Escherichia coli* Due The Usage of Colistin Sulfate(L1)**

The use of colistin sulfate in Indonesia in food animals continues to increase, this is in line with the increased submission of imported raw materials and finished products of colistin sulfate which increased 50-58% from 2015-2017 (source information Sub Directorate for Veterinary Drug Supervision - DGLAHS). Based on our questionnaire in 47 broiler flocks that used colistin, the use of colistin sulfate combined with amoxicillin was the most used (87.43%). The drug is given through drinking water for 3 days and is used as therapy or flushing. No one uses a single colistin sulfate either as a therapy or as a growth promoter.

Our research results regarding mutant selection windows (MSW) of colistin (Palupi et al. 2018b) showed that 20x the recommended dose (75000 IU / kg BW) is still in the MSW range. Therefore, a colistin dose of 75000 IU / kg is estimated to fall within the MSW range or maybe below the MSW limit. If the concentration of drugs in the body of the chicken is still in the MSW range, it is very possible to emerge a single-step mutation resistance colony (Drlica 2003; Blondeau et al. 2004).

As mentioned in the Hazard Identification mechanism of resistance to colistin sulfate can occur due to spontaneous mutations due to antimicrobial exposure with a low concentration (Cavalieri et al. 2015). In our study (Palupi et al. 2018b), chickens that received an underdose of colistin (5 mg/kg in feed) for 40 days were found to have colistin-resistant E. coli higher than the group that received therapeutic doses for 3 days. This is supported by the results of our study of colistin resistance in vitro. Escherichia coli NIHJ within four days becomes resistant and E. coli ATCC 25922 within two days becomes resistant after continuous exposure to colistin with concentrations below its MIC value (Palupi, 2019).

The worrying mechanism of colistin sulfate resistance is through the mcr genes, especially mcr-1 gene. This gene is very easily propagated or transferred through conjugation of plasmids, chromosomes, transformation, and transposons (Hadjaj et al. 2017; Lima Barbieri et al. 2017; Tada et al. 2017a; Sun et al. 2018). Prevalence of colistin-resistant E. coli from live broilers in our study (Palupi et al. 2019) that taken from 47 broiler flocks was 13.19% (95% CI; CL 9.45-18.12%) and the prevalence of mcr-1 gene was 12.77% (95% CI; CL 9.09-17.64%). Refer to EMA (2018) both of these prevalence was medium. The association of colistin-resistant phenotype with mcr-1 gene was 96.77% or very high (ĸ 0.981). Based on the type of food animal, the drug administration route, difficult to absorb by the intestine, also MSW, we concluded the administration of colistin sulfate to broilers has a high risk of triggering resistance to colistin in commensal E. coli in broiler. Even so, the prevalence of colistin-resistant E. coli obtained from the cloacal swab was medium (13.19%) and the time between colistin sulfate administration and harvest time was relatively long. Therefore, based on the evaluation of data supported by direct research and various references, the likelihood of L1 was medium with low uncertainty.

***Likelihood*colistin-resistant*Escherichia coli* in broiler flock environment (L2)**

Environmental parameters used in this study are litter and drinking water. Litter is a potential environment and source for the transmission of pathogenic microorganisms to grow and move between animals or to humans (Chen and Jiang 2015; Soliman et al. 2018). One of them is colibacillosis caused by coliform. The majority of the flocks taken by the litter sample use brans as bedding, especially during the first and second week. Based on observations and interviews, during one production period, it is generally to cleans up the litter after all the chickens were harvest. The farmer prefers to add new brans to cover the formed litter and to keep the cage from getting damp.

Based on interviews with farmer or flock owners, all sampled flocks chlorinated drinking water before giving it to chickens. The purpose of chlorination in drinking water is to minimize the number of microorganisms and suppress the formation of biofilms (Amaral 2004). Respondents conveyed that the administration of colistin sulfate through drinking water was carefully calculated according to age, bodyweight estimate, and intake of drinking chicken to ensure there was no drinking water left to achieve the expected dose. Even if there is remaining drinking water, 19.15% of respondents said the remaining drinking water is discharged in the drainage system or land outside the cage. The rest of the drug is generally stored in a warehouse and will be used if needed. As many as 91.49% of respondents stated that the drug package was destroyed by burning, while 8.51% said it was discharged into a drainage. The possibility of colistin sulfate exposing the environment by with this practice can be said low.

Our research results (Palupi *et al.* 2019) show the prevalence of colistin-resistant *E. coli* and colistin-resistant *E. coli* *mcr-1* positive gene in litters were low, ie 8.51% (95% CI; CL 3.36-19.93%). The prevalence of colistin-resistant *E. coli* from drinking water samples was medium, ie 10.53% (95% CI; CL 4.17-24.13%). The prevalence of colistin-resistant *E. coli* positive *mcr-1* gene from drinking water samples was low, ie 2.63% (95% CI; CL 0.47-13.49%). On researchLe Devendec *et al.* (2016) found no colistin-resistant *E. coli* in litters in flocks using colistin sulfate. Based on the number of flocks sampled, 20 from 47 flocks had colistin-resistant *E. coli* from the cloaca swab, four flocks were found to have colistin-resistant *E. coli* in the litter, and four flocks were found to have colistin-resistant *E. coli* sulfate in drinking water. From these results, there was only one cage, found colistin-resistant *E. coli* isolates derived from the cloaca, litter, and drinking water. Therefore, based on the description above, the assessment of the possibility of exposure to colistin-resistant *E. coli* through the environment in broiler cages to humans was low with low uncertainty.

**Likelihood The Exposure Process of Colistin-Resistan*Escherichia coli* from Broilers to Humans in Broiler Cages (L3)**

Based on observations and questionnaires, personnel who can contact with chickens and the broiler cage environment are flock workers, health technical services (eg veterinarians), one-day-old chicken (DOC) senders, broiler collectors, broiler catchers, and litter collectors. Broiler direct contact with humans in the flock, intensive occurs when the chick in, weighing weight (once a week), and the harvest period. DOC is very rarely found in commensal E. coli. In our study (Palupi et al. 2018a), only 5.56% DOC was found with commensal E. coli and none were resistant to colistin. At the time of harvest, the personnel most frequently contacted with broilers are flock workers, bird collectors, and bird catchers. Other visitors who can contact the broiler in the flock are technical health service (25.5%) and DOC sender (25.5%).



Figure 2Flockworkers do not use proper PPE

Broiler farmworkers generally do not use personal protective equipment (PPE) when working, eg. gloves, boots, wear packs, or masks (Fig. 2). Visitors are also not provided with personal protective equipment. After working in a cage, 42.6% of workers simply cleaned themselves with water, 42.6% cleaned themselves with soap, and only 14.8% cleaned themselves with soap and with disinfectants, such as 70% alcohol.

The personnel most often in contact with litter and drinking water are flock workers. There is a litter collector that takes the litter when the broiler harvest is finished. When collecting litters, they do not use adequate PPE. Information on the use of PPE is crucial in taking litter because litter is a good medium for the development of pathogenic microbes. *Escherichia coli* O157: H7 can survive in the litter for 42-49 days at 37ºC, 46-56 days at 22ºC, and 63-70 days at 5ºC (Soliman*et al.* 2018).

Based on the assessment of the characteristics of E. coli, the prevalence of colistin-resistant E. coli with mcr-1 gene, resistance distribution patterns, raising management of broiler, and biosecurity practices in broiler flocks, colistin-resistant E. coli with mcr-1 gene may expose humans. Research conducted by Trung et al. (2017), showed that farmers who were exposed to broiler positive mcr-1 showed a higher risk of colonization of bacteria carrying mcr-1 than those that were not directly exposed to chickens or with farmers whose chickens did not have the mcr-1 gene.

Humans who are most likely to be exposed are flockworkers, then bird catchers, and litter collectors. Based on the evaluation of the information, the L3 exposure assessment is medium with low uncertainty. The uncertainty of information in making this exposure assessment is low because based on references, observations that give the same results, and interviews with experts also give the same opinion.

**Results of Likelihood Exposure Assessment of Colistin-resistant *Escherichia coli* to Humans in Broiler Flocks**

Calculation of likelihood for human exposure by colistin-resistant E. coli from broilers in a cage is obtained using a combination matrix of Table 2. The results of the likelihood exposure assessment of colistin-resistant E. coli to humans through direct contact with broilers (first branch pathway) are low with low uncertainty. This low assessment is obtained from the possibility of L1 (medium) x L3 (medium).

The likelihood of human exposure assessment by colistin-resistant *E. coli* through the flock environment is low, this likelihood exposure assessment involves L1 (medium) x L2 (low) x L3 (medium). The uncertainty through this pathway is also low. Low uncertainty assessment based on the majority of information sources obtained based on primary data, references that are very supportive and not contradictory, questionnaire results, and expert opinions that do not conflict with each other. The overall exposure assessment of colistin-resistant *E. coli* to humans at the broiler flock level uses the principle of multiple exposures in that the values are medium (low + low) with low uncertainty (Table 5).

Table 5 Recapitulation of exposure assessment colistin-resistant *Escherichia coli* to humans in broiler flock

|  |  |  |
| --- | --- | --- |
| Pathway description | Assessment | Uncertainty |
| Exposureof colistin-resistant *E. coli*to humans in broiler flock cages through direct contact with broilers | Low | Low |
| Exposureof colistin-resistant *E. coli*to humans in broiler flock through direct contact with the flock environment | Low | Low |
| Total exposure assessment(first + second branch pathway) | Medium | Medium |

**Hazard characterization of human exposure by colistin-resistant *Escherichia coli*in Broiler flock**

Information for hazard characterization is obtained through scientific publications and direct communication with Dr. Harry Parathon, SpOG (K). The use of colistin in Indonesia is very limited because it is used as the last drug choice for the treatment of pan-resistance. Colistin for the treatment of pan-resistant cases is difficult to obtain but in some large hospitals, it is provided as alternative medicine. Based on communication in July 2018, colistin methanosulfonate (injection) has not yet included in the health system and is likely to be held in the coming year. Based on communication with experts, it was mentioned that the prevalence of cases of pan-resistant human infection in Indonesia is still very low.

The prevalence of colistin-resistant E. coli-positive mcr-1 gene infections in hospitalized patients has been studied in several countries and ranges from 0% - 1.4% (Kim et al. 2017; Terveer et al. 2017; Principe et al. 2018). The higher prevalence is indicated by Eiamphungporn et al. (2018) in Thailand, reaching 27.7%. In the hazard characterization assessment of E. coli zoonosis, our study used E. coli serotype O157: H7. Colistin-resistant E. coli O157: H7 isolates with mcr-1 gene were only found in one isolate out of a total of 380 E. coli isolates from live broiler and flock environment (0.31%) or the prevalence was very low. Several studies have shown that colistin-resistant E. coli with mcr-1 from food animals and pets can move to humans (Olaitan et al. 2015; Zhang et al. 2016; Tada et al. 2017b).

In our study, 95.59% of colistin-resistant *E. coli* isolates were also found to be multiresistant (Palupi 2019). Colistin-resistant *E. coli* infections can still be treated with other antibiotics as long as the infecting bacteria are not multiresistant to the antimicrobial used. Therefore, based on the information obtained, the hazard characterization assessment of human exposure by colistin-resistant *E. coli* in broiler flocks is moderate with low uncertainty.

**Risk characterization of colistin-resistant *Escherichia coli* in broiler flock to Humans**

The final step in risk assessment is to carry out risk characterization based on exposure assessment pathway and hazard characterization assessment. The risk characterization assessment uses a combination of exposure assessment and hazard characterization matrices as in Table 4. The results of the risk characterization exposure assessment through the broiler flock are medium (medium x medium) with low uncertainty. The results of the risk characterization assessment are in Table 6.

**Table 6 Assessment of the risk characterization of colistine resistance *in Escherichia coli* in the broiler supply chain to humans**

|  |  |
| --- | --- |
| Descriptionexposure pathway | Likelihood |
| Exposure (E) | Hazard characterization (H) | Risk characterization(E x H) | Uncertainty |
| Exposureof colistin-resistant *E. coli*to human inbroiler flock | Medium | Medium | Medium | Medium |

**Risk Mitigation of Colistin-resistant *Escherichia coli* from broiler flocksto humans health**

The veterinary medicine industry plays an important role in the circulation of colistin sulfate. Fulfillment of pharmacological data and colistin sulfate resistance from animal drugs to be registered must be tightened based on scientific data per product. In 2016 the European Committee for Medicinal Products for Veterinary Use (CVMP) requested that all colistin sulfate combinations with other oral antimicrobials be withdrawn from the European Union (EMA 2016). Aproval for the registration of animal drug colistin sulfate combined with other antimicrobials must go through in-depth scientific evaluation.

Good farm management will reduce the risk of pathogenic bacterial infection. This will reduce the dependence of farmers on antimicrobials to prevent infection. The use of proper PPE to reduce the risk of direct contact with colistin-resistant *E. coli* also needs to be done when handling chickens, working, and taking litter in the farm. The use of PPE will greatly help reduce risks, especially for small scale broiler farms which are difficult to implement in three zones of livestock.

Education about the position of colistin sulfate to farmers or health managers on broiler farms is also very important. Farmers and health managers need to understand the importance of colistin for human health so as not to choose colistin sulfate as the first choice in handling cases of gram-negative bacterial infections in broilers.

**CONCLUSION**

Risk assessment of colistin-resistant E. coli risk in broiler flock through direct contact with live broilers and the enclosure environment to humans is medium with low uncertainty. This is due to the use of colistin sulfate which is the highest critically important antimicrobials for humans in broilers, the presence of the *mcr-1* gene that is easily transferred between bacteria, low biosecurity at the farm, contamination in the farm environment, and low use of PPE in the farm. Therefore, reducing the use of colistin sulfate in production animals is a necessity that cannot be avoided.

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**REFERENCES**

[AFFA] Agriculture, Fisheries and Forestry Australia. 2011. Guidelines for Import Analysis Draft September 2011. Biosecurity Development and Evaluation Biosecurity Australia

Amaral LA. 2004. Drinking water as a risk factor to poultry health. *Brazilian J Poult Sci.* 6(4):191–199.

Blondeau JM, Hansen G, Metzler K, Hedlin P. 2004. The role of PK/PD arameters to avoid selection and increase of resistance: mutant prevention concentration. *J Chemother*. 16(sup3):1-19. doi: 10.1080/1120009X.2004.11782371

[Catry B](https://www.ncbi.nlm.nih.gov/pubmed/?term=Catry%20B%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Cavaleri M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Cavaleri%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Baptiste K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Baptiste%20K%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Grave K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Grave%20K%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Grein K](https://www.ncbi.nlm.nih.gov/pubmed/?term=Grein%20K%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Holm A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Holm%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Jukes H](https://www.ncbi.nlm.nih.gov/pubmed/?term=Jukes%20H%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Liebana E](https://www.ncbi.nlm.nih.gov/pubmed/?term=Liebana%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Lopez Navas A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lopez%20Navas%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Mackay D](https://www.ncbi.nlm.nih.gov/pubmed/?term=Mackay%20D%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Magiorakos AP](https://www.ncbi.nlm.nih.gov/pubmed/?term=Magiorakos%20AP%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Moreno Romo MA](https://www.ncbi.nlm.nih.gov/pubmed/?term=Moreno%20Romo%20MA%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Moulin G](https://www.ncbi.nlm.nih.gov/pubmed/?term=Moulin%20G%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Muñoz Madero C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Mu%C3%B1oz%20Madero%20C%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Matias Ferreira Pomba MC](https://www.ncbi.nlm.nih.gov/pubmed/?term=Matias%20Ferreira%20Pomba%20MC%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Powell M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Powell%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Pyörälä S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Py%C3%B6r%C3%A4l%C3%A4%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Rantala M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Rantala%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Ružauskas M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Ru%C5%BEauskas%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Sanders P](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sanders%20P%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Teale C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Teale%20C%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Threlfall EJ](https://www.ncbi.nlm.nih.gov/pubmed/?term=Threlfall%20EJ%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Törneke K](https://www.ncbi.nlm.nih.gov/pubmed/?term=T%C3%B6rneke%20K%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [van Duijkeren E](https://www.ncbi.nlm.nih.gov/pubmed/?term=van%20Duijkeren%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26215780), [Torren Edo J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Torren%20Edo%20J%5BAuthor%5D&cauthor=true&cauthor_uid=26215780). 2015. Use of colistin-containing products within European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. *IntJAntimicrob Agents*. 46(3):297-306. doi: 10.1016/j.ijantimicag.2015.06.005.

Cavalieri SJ, Harbeck R, McCarter YS, Ortez JH, Rankin ID, Sautter RL, Sharp SE, Spiegel CA. 2005. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology

Chen Z, Jiang X. 2014. Microbiological safety of chicken litter or chicken litter-based organic fertilizers: A Review. *Agriculture.* 4: 1-29. doi:10.3390/agriculture4010001

[CODEX] Codex AlimentariousCommision. 2011. Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance Cac/Gl 77- 2011. [Internet] [Diunduhpada 13 Juli 2013]. Terdapatdalam www.fao.org.download>standards

[DGLAHS] Directorat General of Livestock and Animal Health Services. 2016. IndeksObatHewan Indonesia Ed. IX. Jakarta (ID): KementerianPertanianRepublik Indonesia. pp. 58–599.

Drlica K. 2003. The mutant selection window and antimicrobial resistance. *J AntimicrobChemother.*  August: 1-7.DOI: 10.1093/jac/dkg269

# [EFSA] European Food Safety Authority. 2006. Migratory birds and their possible role in the spread of highly pathogenic avian influenza. *EFSA J.* 357:1-46

[Eiamphungporn W,](https://www.sciencedirect.com/science/article/pii/S2213716518301176?via%3Dihub#!) [Yainoy S,](https://www.sciencedirect.com/science/article/pii/S2213716518301176?via%3Dihub" \l "!) [Jumderm C,](https://www.sciencedirect.com/science/article/pii/S2213716518301176?via%3Dihub" \l "!) [Tiengrim S](https://www.sciencedirect.com/science/article/pii/S2213716518301176?via%3Dihub" \l "!). [Thamlikitkul](https://www.sciencedirect.com/science/article/pii/S2213716518301176?via%3Dihub#!) V. 2018. Prevalence of the colistin resistance gene *mcr*-*1* in colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from humans in Thailand. [*J Global Antimicrob Resistance*](https://www.sciencedirect.com/science/journal/22137165)*.*[(15](https://www.sciencedirect.com/science/journal/22137165/15/supp/C%22%20%5Co%20%22Go%20to%20table%20of%20contents%20for%20this%20volume/issue)):32-35. ht[tps://doi.org/10.1016/j.jgar.2018.06.007](https://doi.org/10.1016/j.jgar.2018.06.007%22%20%5Ct%20%22_blank%22%20%5Co%20%22Persistent%20link%20using%20digital%20object%20identifier)

[EMA] European Medicine Agency. 2013. Use of colistin products in animals within The European Union: Development of resistance and possible impact on human and animal health. 7 Westferry Circus Canary Wharf London E14 4HB United Kingdom. [Internet] [Diunduh 24 Februari 2016]. Terdapatdalam[http://www.ema.europa.eu/docs/en\_GB/ document\_library/ Report/ 2013/07/WC500146813.pdf](http://www.ema.europa.eu/docs/en_GB/%20%20document_library/%20Report/%202013/07/WC500146813.pdf)

[EMA] European Medicine Agency. 2016. Advice on impacts of using antimicrobials in animals [Internet]. [Diunduh 7 September 2018]. Terdapatdalam[www.ema.europa.eu/ema/ index.jsp?curl=pages/ regulation/ general/ general\_content\_000639.jsp&mid=WC0b01ac058080a585](http://www.ema.europa.eu/ema/%20index.jsp?curl=pages/%20regulation/%20general/%20general_content_000639.jsp&mid=WC0b01ac058080a585)

[EMA] European Medicine Agency. 2018. Guideline on the assessment of the risk to public health from antimicrobial resistance due to the use of an antimicrobial veterinary medicinal product in food producing animals (Draft 2). [Internet] [Diunduh 01 Oktober 2018]. Terdapatdalam www.ema.europa.eu/docs/en\_gb/document\_library/scientific\_guideline/2018/07/ WC500252679.pdf

# [Fernández L](https://www.ncbi.nlm.nih.gov/pubmed/?term=Fern%C3%A1ndez%20L%5BAuthor%5D&cauthor=true&cauthor_uid=20547815), [Gooderham WJ](https://www.ncbi.nlm.nih.gov/pubmed/?term=Gooderham%20WJ%5BAuthor%5D&cauthor=true&cauthor_uid=20547815), [Bains M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bains%20M%5BAuthor%5D&cauthor=true&cauthor_uid=20547815), [McPhee JB](https://www.ncbi.nlm.nih.gov/pubmed/?term=McPhee%20JB%5BAuthor%5D&cauthor=true&cauthor_uid=20547815), [Wiegand I](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wiegand%20I%5BAuthor%5D&cauthor=true&cauthor_uid=20547815), [Hancock RE](https://www.ncbi.nlm.nih.gov/pubmed/?term=Hancock%20RE%5BAuthor%5D&cauthor=true&cauthor_uid=20547815). 2010. Adaptive resistance to the "last hope" antibiotics polymyxin B and colistin in *Pseudomonas aeruginosa* is mediated by the novel two-component regulatory system ParR-ParS. *A[ntimicrob Agents Chemother.](https://www.ncbi.nlm.nih.gov/pubmed/20547815%22%20%5Co%20%22Antimicrobial%20agents%20and%20chemotherapy.)*  54(8):3372-82. doi: 10.1128/AAC.00242-10.

Ferens WA, Hovde CJ. 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infections. *Foodborne Pathogens Dis.* 8(4):465-487

Hadjaj L, Riziki T, Zhu Y, Li J, Diene SM, Rolain JM. 2017. Study of *mcr-1* gene-mediated colistin resistance in *Enterobacteriaceae* isolated from humans and animals in different countries. *Genes.* 8:394. doi:10.3390/genes8120394

Kim YA, Yong D, Jeong SH, Lee K. 2017. Colistin resistance in *Escherichia coli* isolates from patients with bloodstream infection in Korea. *Ann Lab Med.* (37):172-173. https://doi.org/10.3343/alm.2017.37.2.172

Lashev L, Haritova A. 2003. Pharmacokinetics of colistin in broiler chickens. *Bulg J Vet Med.* 6(1):21-26.

Le Devendec L, Mourand G, Bougeard S, Léaustic J, Jouy E, Keita A, Couet W, Rousset N, Kempf. 2016. Impact of colistin sulfate treatment of broilers on the presence of resistant bacteria and resistance gens in stored or composted manure. *Vet Microbiol.* 194:98–106. doi: 10.1016/j.vetmic.2015.11.012.

Lima Barbieri N, Nielsen DW, Wannemuehler Y, Cavender T, Hussein A, Yan S-g, Nolan LK, Logue CM. 2017. *mcr-1* identified in avian pathogenic *Escherichia coli* (APEC). *PloS One.* 12(3):e0172997. doi: 10.1371/journal.pone.0172997

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R., Spencer J, Doi Y, Tian G, Domg B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lu L, He D, Zhou H, Liang Z, Liu JH, Shen J. 2015. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human being in china: a microbiological and molecular biology study. *Lancet Infect Dis.* 201. [http://dx.doi.org/10/1016/S1473-3099(15)00424-7](http://dx.doi.org/10/1016/S1473-3099%2815%2900424-7)

Marshall BM, Levy SB. 2011. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev.* 24(4):718-735

Moskowitz, S.M., M.K. Brannon, N. Dasgupta, M. Pier, N. Sgambati, A.K. Miller, S.E. Selgrade, S.I. Miller, M. Denton, S.P. Conway, H.K. Johansen, d N. Hoiby. 2012. Pmrb mutations promote polymyxin resistance of *Pseudomonas aeruginosa* isolated from colistin-treated cystic fibrosis patients. *Antimicrob Agents Chemother.* 56:1019-1030.

Napier BA, Burd EM, Satola SW, Cagle SM, Ray SM, McGann P, Pohl J, Lesho EP, Weiss DS. 2013. Clinical use of colistin induces cross-resistance to host antimicrobials in *Acinetobacter baumannii*. *mBio.* 4(3):e00021-13. doi:10.1128/mBio.00021-13.

[OIE] World Organization for Animal Health. 2016. Terrestrial Animal Health Code Ed. 25th. OIE. Paris, France. Chapter 6.7-6.8

Olaitan AO, Thongmalayvong B, Akkhavong K, Somphavong S, Paboriboune P, Khounsy S, Morand S, Rolain JM. 2015. Clonal transmission of a colistin-resistant *Escherichia coli* from a domesticated pig to a human in Laos. *J AntimicrobChemother*. 70:340–3404. doi:10.1016/j.ijantimicag. 2015.11.009

Palupi MF, Maheshwari H, Darusman HS, Sudarnika E, Wibawan IWT. 2018a. Resistansi *Escherichia coli* TerhadapKolistin dan Deteksi Gen *mcr-1* Pada BroilerAkibat Pemberian Kolistin Sulfat. *J Vet* Ed. Juni Vol. 19 No. 2: 167-207

Palupi MF, Darusman HS, Maheshwari H, Wibawan IWT, Sudarnika. 2018b. *Human & Veterinary Medicine International Journal of the Bioflux Society* Vol. 10 Issue 4: 163-168

Palupi MF. 2019. Dissertation: Risk Assessment of *Escherichia coli* Resistance and MutantPreventionConcentrationStudyDue to theUsage of Colistin Sulfate in Broiler. IPB University

Palupi MF, Wibawan IWT, Sudarnika E, Maheshwari H, Darusman HS. 2019. *Prevalence of mcr-1 Colistin Resistance Gene in Escherichia coli Along Broiler Meat Supply Chain in Indonesia. J Biotropia*Vol. 26 No. 2 2019. DOI:http://dx.doi.org/10.11589/btb.2019.26.2.1054

Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P. 2016. Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates in South Africa. *Antimicrob Agents Chemother.* 60:4394–4397. doi:10.1128/AAC.00444-16.

Principe L, Piazza A, Mauri C, Anesi A, Bracco S, Brigante G, Casari E, Agrappi C, Caltagirone M, Novazzi F, Migliavacca R, Pagani L, Luzzaro F. 2018. Multicenter prospective study on the prevalence of colistin resistance in *Escherichia coli*: relevance of *mcr-1*-positive clinical isolates in Lombardy, Northern Italy. *Infect Drug Resist*. 11:377–385

Riemann HP, Cliver DO. 2006. Foodborne Infections and Intoxicatios 3rd Ed. Academic Press Elsivier. pp 57-115, 719-720

Soliman ES, Sallam NH, Abouelhassan EM. 2018. Effectiveness og poultry litter amendments on bacterial survival and *Eimeria*oocyst sporulation. *Veterinary World.* 11(8):1064-1073

[Sun J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sun%20J%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [Li XP](https://www.ncbi.nlm.nih.gov/pubmed/?term=Li%20XP%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [Fang LX](https://www.ncbi.nlm.nih.gov/pubmed/?term=Fang%20LX%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [Sun RY](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sun%20RY%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [He YZ](https://www.ncbi.nlm.nih.gov/pubmed/?term=He%20YZ%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [Lin J](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lin%20J%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [Liao XP](https://www.ncbi.nlm.nih.gov/pubmed/?term=Liao%20XP%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [Feng Y](https://www.ncbi.nlm.nih.gov/pubmed/?term=Feng%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=29371103), [Liu YH](https://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20YH%5BAuthor%5D&cauthor=true&cauthor_uid=29371103). 2018. Co-occurrence of *mcr-1* in the chromosome and on an IncHI2 plasmid: persistence of colistin resistance in *Escherichia coli.* [*Int J Antimicrob Agents.*](https://www.ncbi.nlm.nih.gov/pubmed/29371103) 51(6):842-847. doi: 10.1016/j.ijantimicag.2018.01.007.

Tada T, Nhungc PH, Shimada K, Tsuchiya M, Phuong DM, Anh NQ, Ohmagari N, Kirikae T. 2017a. Emergence of colistin-resistant *Escherichia coli* clinical isolates harboring in Vietnam. *Int J Infect Dis.* 63:72–73. <http://dx.doi.org/10.1016/j.ijid.2017.07.003>

Tada T, Uechic K, Nakasone I, Shimada K, Nakamatsu M, Kirikae T, Fujita J. 2017b. Emergence of a colistin-resistant *Escherichia coli* clinical isolate harboring *mcr-1* in Japan. *Int J Infect Dis.* 63: 21–22. http://dx.doi.org/10.1016/j.ijid.2017.07.003

Terveer EM, Nijhuis RHT, Crobach MJT, Knetsch CW, Veldkamp KE, Gooskens J, *et al.* 2017. Prevalence of colistin resistance gene (*mcr-1*) containing *Enterobacteriaceae* in feces of patients attending a tertiary care hospital and detection of a *mcr-1* containing, colistin susceptible *E. coli*. *PLoS One.* 12(6):e0178598. <https://doi.org/10.1371/journal.pone.0178598>

Trung NV, Matamoros S, Carrique-Mas JJ, Nghia NH, Nhung NT, BichChieu TT, Mai HH, van Rooijen W, Campbell J, Wagenaar JA, Hardon A, Nhu Mai NT, Hieu TQ, Thwaites G, de Jong MD, Schultsz C, Hoa NT. 2017. Zoonotic transmission of *mcr-1*colistin resistance gene from small-scale poultry farms, Vietnam. *Emerg Infect Dis.* 23(3):529-532.

[WHO] World Health Organization. 2016. Antimicrobial resistance. [Internet] [Diunduh 23 Oktober 2016]. Terdapat dalam <http://www.who.int/mediacentre/factsheets/fs194/en/>

[WHO] World Health Organization. 2017. Critically important antimicrobials for human medicine – 5th rev. WHO. Switzerland. Licence: CC BY-NC-SA 3.0 IGO. Pp:12-37

Zhang XF, Doi Y, Huang X, Li HY, Zhong LL, Zeng KJ, Zhang YF, Patil S, Tian GB. 2016. Possible transmission of *mcr-1*–harboring *Escherichia coli* between companion animals and human. *Emerg Infect Dis*. 22(9):1679-1681. DOI: <http://dx.doi.org/10.3201/eid2209.160464>