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Antibiotic resistance of *Escherichia Coli* isolated from broiler farms in Medan and Deli Serdang Regency

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Abstract

Antimicrobial Resistance (AMR) is the ability of microbes to inhibit treatment effects triggered by overuse and inappropriate use of antibiotics in poultry farms. This study aims to get an overview of the risk factors of AMR. A total of 41 *E. coli* isolated from caecum in poultry slaughterhouses derived from broiler chicken farms in Medan and Deli Serdang. The sample was tested for AMR using the Microbroth Dilution technique (Sensititre® Microplate) with standards CLSI VET01S (2015) and CLSI M100. Information on risk factors was taken from farms using a questionnaire and analyzed with a logistic regression model (EPI Info 7 CDC). The result for sensitivity to 13 types of antibiotics showed that Trimethoprim had the highest resistance (95.1%), followed by ampicillin (90.2%), gentamicin (87.8%), nalidixide acid (85.4%), cefotaxime (82.9%), and sulfamethoxazole (82.9%). In contrast, colistin and meropenem have not shown resistance. As much as 92.7% of *E. coli* isolates had experienced Multi-Drug Resistance (MDR), which showed AMR to more than two antibiotic groups. The risk factor of farmer knowledge about AMR was associated with the MDR of *E. coli*. AMR requires the coordination of the stakeholders to carry out socialization and educate farmers in providing appropriate knowledge and using antibiotics wisely.

Keywords: Broiler, AMR, Medan and Deli Serdang, risk factors

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Introduction

Antibiotic administration in broiler farms is used as a treatment, feed additive to stimulate growth (growth promoter), increase poultry production, and the efficiency of food consumptions ratio (Masrianto, 2019). Inappropriate and excessive usage of antibiotics in the long term will cause Antimicrobial Resistance (AMR). AMR is the ability of microbes to resist the effects of treatments that have previously managed to overcome these microbes. Antibiotics groups of tetracycline, such as oxytetracycline and doxycycline, are the drugs most often used in poultry farms (Hintono, 2007). Its usage is for preventing or treating of infectious diseases of the respiratory tract, digestive tract, mycoplasmosis, and colibacillosis. Most farmers do not know AMR and most of antibiotics usage is aimed at increasing productivity and growth even though they know the dangers of using antibiotics on their farms if they don't comply with the rules (Sumambang et al., 2019).

Several *Eschericia coli* (*E. coli*) poultry-originated strains are potential sources of AMR-caused genes that can be transmitted to humans (Nhung et al., 2017). *E. coli* bacteria have genes that function to maintain resistance from the influence of antibiotics derived from plasmids. *E. coli* was detected to have plasmids for several drug resistance genes. Plasmids can carry resistance genes in bacteria that are sensitive to antibiotics (Rahmahani et al., 2020). The incidence of AMR in bacteria originating from animals such as broiler directly or indirectly impacts humans. Direct impacts can occur due to direct contact with bacteria of animal origin such as *E. coli* which is resistant to antibiotics. In contrast, indirect impacts include resistant bacteria that spread in environmental ecosystems such as contaminated water, air and soil and come into contact with humans. *E. coli* bacteria that contaminate poultry products can transmit resistance to the consumer and cause serious illness due to treatment failures (Putri et al., 2018).

Studies on antibiotics usage on farms in North Sumatera are relatively minimal, so studies are still needed to describe it. AMR testing uses *E. coli* isolates which are a common bacterium in the poultry digestive tract and is widely used to monitor AMR in animal products, including poultry. Until now, measurement of AMR has not been carried out in Medan City and Deli Serdang Regency, which have a high population of broiler farms in North Sumatra Province, so it is necessary to conduct a study to measure the incidence of AMR in these areas. The purpose of this study is to determine the incidence of AMR in *E. coli* isolates originating from broiler farms, to determine the types of antibiotics that have developed resistance, and to determine the risk factors that influence the incidence of AMR in *E. coli* isolates in broiler farms in Medan and Deli Serdang Regency.

Materials and Method

Sampling design and sampling sites

This research was conducted from July to September 2022. The research method used a cross-sectional study and the sampling technique was carried out on a budget purposed with a predetermined location and number of samples. The sampling sites included five poultry slaughterhouses in North Sumatra Province for chicken caecum collection, Disease Investigation Centre (DIC) Medan for isolation and identification of *E. coli* bacteria, 41 broiler chicken farms, and Quality Control Laboratory and Certification for Animal Products Bogor (QCLCAP) for AMR testing (Figure 1). The target sample for this research is E coli isolates. The standards for testing in the laboratory, and the interpretation of the AMR test results refer to the Clinical and Laboratory Standards Institute (CLSI VET01S 2015 and CLSI M100 2018).

Material and Method

McConkey agar (MCA/Merck, Germany), and Levine- Eosin Methylene Blue Agar (L-EMBA/Merck®, Germany), Nutrient Agar (NA/Merck®, Germany), Tryptic Soy Broth (TSB/Merck®, Germany), glycerol 20%, cryotube, Muller Hinton Agar (MHA/Thermo Scientific™, US), reference bacterial isolates (*E. coli* ATCC 25922/Culti-Loops™, USA), Muller Hinton Broth (MHB/Thermo Scientific™, US), sterile aquadest (DW), 70% alcohol, cooler box, sterile plastic, permanent markers, label paper, and ice packs, scissors and tweezers, 12 mm Petri dishes, Sensititre® microplate with 13 types of antibiotic, doseheads, nephelometer, microplate sealer, Sensititre® AIM, and Sensititre® Vision.

Caecum collection

The *E. coli* collected from the caecum, was collected during the evisceration (extraction of the innards) of the chicken at poultry slaughterhouse (Kesmavet, 2019). In the study, all of the caecum preparation in the veterinary public health laboratory DIC Medan. The caecum collected was then put into sterile plastic and data labeled such as sample code, sampling location, and the origin of the farm, and the cold chain is maintained at 4 ^{o}C during the trip to the laboratory.

Isolation and identification of *E. coli*

A total of three collected caecum was excreted and homogenized the faeces in a Petri dish and considered as one sample. One osse of faeces was taken and aseptically inoculated into MCA and incubated at 36 ^{o}C for 24 hours. Colonies of *E. coli* will turn reddish pink surrounded by cloudy zone on MCA media. Separated *E. coli* colonies were inoculated on L-EMBA medium and incubated at 36 ^{o}C for 24 hours. Colonies of *E. coli* will have a dark color in the center and flat with a green metallic metallic sheen (Feng et al., 2020). One colony of pure *E. coli* was then evenly inoculated on non-selective NA media evenly and incubated at 36 ^{o}C for 24 hours. Colonies in the NA medium was harvested by scraping all the colonies using osse and placing them in the cryotube storage medium. The *E. coli* isolates were then stored in the freezer at -20 ^{o}C.

Sensitivity test to antibiotics

The principle of the Antimicrobial Susceptibility Testing (AST) test is to test the sensitivity of *E. coli* bacteria to antibiotics using the Microbroth Dilution technique (Sensititre® microplate) (Table 1). A total of 13 types of antibiotics tested came from 10 antibiotic classes namely sulfonamides (sulfametoxazole and trimethoprim), quinolones (ciprofloxacine and nalidixic acid), tetracyclines, beta lactams (meropenem), macrolides (azithromycin), cephalosporins (cefotaxime and ceftazidime), phenicol (chloramphenicol), polypeptides (colistin), penicillins (ampicillin), and aminoglycosides (gentamycin). The results of the AST test are the Minimum Inhibitory Concentration (MIC) value of antibiotics against *E. coli*. MIC is measured and determined as the minimum concentration of an antibiotic that can inhibit bacterial growth. The lower the MIC value of the antibiotic, the greater the sensitivity of the bacteria (Greenwood, 1995).

E. coli isolates were inoculated on the NA media and incubated at 36 ^oC for 18-24 hours. One separate colony was isolated on MCA medium and then incubated at 36 ^oC for 18-24 hours. Colonies in MCA were taken one separate loop, and inoculated on MHA and then incubated at 37 ^oC for 18-24 hours. Isolate suspension 0.5 Mc Farland (≈1×108 CFU/ml) was prepared by taking several loops of bacterial culture on MHA and dissolving it in 5 mL of sterile DW in a glass test tube. Suspension is measured with a Nephelometer until the green light (0.5 Mc Farland) and homogenized with a vortex. Suspension taken 50 µL (for sample) and 10 µL (for control isolate) and dissolved in 11 mL MHB and homogenized. The suspension tube is mounted on a dosehead and placed in the Sensititre® AIM tool which has been set to pour 50 µL. The Sensititre® microplate is placed in the Sensititre® AIM. This tool will then automatically fill the plate with 50 uL suspension and cover it with a microplate sealer, and incubate at 37 ^oC for 18-24 hours. To have the results, a microplate is placed on the Sensititre® Vizion. After the tool has finished reading, then the MIC results are determined. Blocked bacteria are indicated by empty plate wells and if there are pellets and turbidity in the wells, it means that there is bacterial growth. The acceptability of the AMR test results is determined by the acceptability of the reference isolate (at least three bacteria) with the reference values shown in Table 1.

Data collection and analysis

Questionnaire data collection was carried out on broiler farms that slaughtered chickens at the poultry slaughterhouse. The variables observed included type of livestock business, farm system, herd population, poultry health management, network of veterinarians, farmer profiling, knowledge of antibiotic management, management of antibiotic usage, antibiotics program, antibiotic rotation program, treatment failure, antibiotics purpose usage, antibiotics combination, last usage of antibiotics administration. The incidence of AMR for *E. coli* isolates was measured at the farm level because each isolate was considered to represent one farm. The MIC value on the CLSI standard will determine that the isolate is resistant (R), intermediate (I), and susceptible (S). If there are resistant (R) and intermediate (I) farms are classified as resistant farms while susceptible (S) results are classified as negative or susceptible. The dependent variable in this study was the proportion of multidrug resistance (MDR) occurrences in broiler chicken farms in Medan City and Deli Serdang Regency. Data were analyzed using a multivariable logistic regression model (EPI Info 7 CDC). Initial univariable analysis was used to identify possible relationships between variables and outcomes. Each risk factor with a p-value ≤0.05 was considered as the factor most associated with AMR.

Results and Discussion

Isolation and identification of *E. coli*

The total pure *E. coli* isolates obtained were 41 isolates from 41 target farms. A total of 29 isolates came from Deliserdang Regency and 12 isolates came from Medan City.

Sensitivity test to antibiotics

The results of the AMR test showed that almost all of the *E. coli* isolates taken from 41 farms in Medan and Deli Serdang City 97,6% (40/41) experienced in AMR. AMR test againts 13 types of antibiotics showed that Trimethoprim had the highest resistance rate (95.1%) followed by ampicillin (90.2%), gentamicin (87.8%), nalidixide acid (85.4%), cefotaxim (82.9%), and sulfamethoxazole (82.9%), while the lowest resistance was the antibiotic chloramphenicol (36.6%), and for the antibiotics colistin and meropenem, the *E. coli* isolates in this study did not show resistance to these antibiotics (Graphic 1).

The incidence of AMR in this study came from five groups of antibiotics such as sulfonamides (trimethoprim and sulfamethoxazole), quinolones (nalidixic acid), cephalosporins (cefotaxime), penicillins (ampicillin), and aminoglycosides (gentamycin). According to Niasono et al., (2019), *E. coli* bacteria isolated from broiler farms in Subang Regency, have antibiotic resistance to 97.3% tetracycline, 87.8% sulfamethoxazole, 74.3% trimethoprim, and 68.9% ampicillin. The results of another study in Blitar Regency, the incidence of antibiotic resistance to *E. coli* bacteria in broilers showed a percentage of 88.75% (ampicillin), 78.75% (streptomycin), 76.87% (erithromycin), 50.63% (tetracyclin) and 75% (sulphamethoxazole-trimethoprim) (Wibisono et al., 2021). *E. coli* isolates from cloacal swabs taken from laying hens have a high percentage of antibiotic resistance to the antibiotic's ampicillin, ciprofloxacin, tetracycline, trimethoprim, and sulfamethoxazole of more than 50% (Witaningrum et al., 2020).

The resistance to trimethoprim and sulfonamides is high because these antibiotics have been widely used in the livestock sector for a long time. Resistance to sulfonamides and trimethoprim is caused by mutations in the gene encoding enzymes involved in the metabolic pathway for the synthesis of tetrahydrofolic acid, while the rare use of colistin has not caused resistance (Widayati & Subekti, 2018).

Factors that support the occurrence of AMR include the use of antibiotics that are inappropriate (irrational); for example, the use of antibiotics for a while is too short, the dose is too low, the diagnosis of the disease is wrong, and the potency is inadequate (Handayani 2017). The mechanism for the occurrence of AMR is that bacteria limit drug absorption by decreasing the number of porins and mutations that change the selectivity of the porin channel, modifying drug targets with ESBL enzymes, inactivating antibiotics by drug degradation or by transferring chemical groups to antibiotics, and efflux of active drugs through mutations that change the transport channel pump (Reygaert, 2018).

Treatment management in broilers is often carried out with several drugs such as antibiotics, vitamins and herbs which are usually given through drinking water. This aims to control disease, eradicate other organisms that inhibit growth and accelerate the growth of broilers (Rasyaf, 2009). Antibiotics that are often used in chicken farming include beta lactams, tetracyclines, sulfonamides, trimethoprim, bacitracin, erythromycine, neomycin, nystatin, oxytetracycline, and streptomycin (Wibisono et al., 2021). Other studies have shown that isolates and *E. coli* have high rates of antibiotic resistance, which is more than 80% for ampicillin, amoxicillin, and tetracyclines (Nhung et al., 2017). In other studies, *E. coli* isolates were also resistant to cefpodoxime, cefoxitin, sulfamethoxazole-trimethoprim, ceftizoxime, aztreonam, and nitrofurantoin. However, it did not show results on cholistin sulfate (Andriyani et al., 2020). According to Handayani et al., (2020) isolates of *E. coli* isolated from the caecum of chickens in the Bali, West Nusa Tenggara and East Nusa Tenggara regions also developed resistance to ampicillin, cephalothin and gentamicin respectively, while chloramphenicol was still susceptible but have a tendency to become resistant.

The incidence of AMR in bacteria originating from animals such as broiler chickens has a direct or indirect impact on humans. Direct impacts can occur due to direct contact with bacteria from animals that are resistant to antibiotics, while indirect impacts include resistant bacteria that spread in environmental ecosystems such as water, air and soil and come into contact with humans. *E. coli* bacteria that contaminate broiler chicken meat can transmit resistance to humans who consume it and can cause serious illness due to treatment failures (Putri et al., 2018).

The low incidence of AMR for the antibiotic chloramphenicol, and the absence of AMR for Colistin and Meropenem, may occur because the use of these three drugs is regulated in the Decree of the Minister of Agriculture No. 9736/2020 which prohibits its usage orally, parenterally and topically in the livestock industry broiler chickens (broilers), so that farmers no longer administrated these three types of antibiotics. Meropenem belongs to the carbapenem class, have the widest spectrum of activity and the greatest potential against Gram-positive and Gram-negative bacteria, so they are often called the last choice antibiotics given when patients have severe infections or are suspected of having resistant bacteria (Etebu & Arikekpar, 2016).

Based on Figure 3, trimethoprim, ampicillin, gentamicin, nalidixide acid, sulfamethoxazole, and ceftiofur are no longer recommended for use in broiler farms in Medan City and Deli Serdang Regency because there has been resistance of more than 80%. In addition, its use must be monitored and can be replaced with other antibiotics outside of the 13 types of antibiotics. This is consistent with the distribution of MDR cases of *E. coli* isolates in commercial chicken farms in Blitar Regency which shows an prevalence of 95.9% (Wibisono et al., 2021) and in Subang Regency of 93.2% (Niasono et al., 2019). Other studies have also shown that *E. coli* isolates have a strong correlation with resistance to various antibiotics, namely 93.3%. Most of the *E. coli* had strains that were multi-resistant to more than 10 antibiotics (Nhung et al., 2017).

Table 1. Antibiotic MIC standards and control isolates (CLSI M100, 2018)

No.	Antibiotic	Antibiotic Group	Kontrol isolat (µg/mL)			MIC (µg/mL)		
			S. aureus ATCC 29213	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	S	I	R
1	Sulfametoksazol	Sulfonamide	≤ 9.5	≤ 9.5	152-608	≤ 38	-	≥76
2	Trimetoprim		≤ 0.5	≤ 0.5	8-32	≤ 2	-	≥4
3	Kloramfenikol	Phenicol	2-16	2-8	-	≤ 8	16	≥32
4	Tetrasiklin	Tetrasiklin	0.12-1	0.5-2	8-32	≤ 4	8	≥16
5	Gentamisin	Aminoglikosida	0.12-1	0.25-1	0.5-2	≤ 4	8	≥16
6	Ampisilin	Penicillin	0.5-2	2-8	-	≤ 8	16	≥32
7	Asam nalidiksik	Quinolon	-	1-4	-	≤ 16	-	≥32
8	Siprofloksasin		0.12-0.5	0.004-0.015	0.12-1	≤ 1	2	≥4
9	Meropenem	Beta lactam	0.03-0.12	0.008-0.06	-	≤ 1	2	≥ 4
10	Azitromisin	Makrolide	0.5-2	-	-	≤ 16	-	≥ 32
11	Cefotaksim	Cephalosporin	1-4	0.03-0.25	-	≤ 1	2	≥ 4
12	Ceftadizime		4-16	0.06-0.5	-	≤ 4	8	≥ 16
13	Colistin	Polipeptide	-	0.25-2	-	≤ 4	-	≥8

Note: S: *Susceptible*; I: *Intermediate*; R: *Resistance*.

Multi drug resistance (MDR) *E. coli*

Based on the amount of drug resistance for each farm, the result was that 38 *E. coli* isolates (92.7%) taken from broiler farms in Medan City and Deli Serdang Regency had experienced MDR and is shown in Figure 2. Based on the graph below, the pattern of MDR resistance for each farm the highest was shown for MDR with the number of antibiotics of nine different types, namely 24.4% (10/41), followed by 10 and 11 types of antibiotics (22%), while there is 1 farm with no experience with AMR.

MDR bacterial develops resistance to at least 1 type of antibiotic from two or more groups of antibiotics (Aminah, 2016). MDR happened when bacteria have several different resistance genes that each confer resistance to different antibiotics. Accumulation of resistance genes often occurs in plasmids that can be transferred between bacteria in a single event. Another possibility that occurs is a single resistance mechanism that can give sensitivity to more than one antibiotic. The bacteria will pump the antibiotic out of the cell, so sometimes such pumps can recognize many different antibiotic molecules. In other words, bacteria use one pump to pump several different antibiotics (Nesta, 2014).

MDR events can be caused by bacteria that produce extended spectrum beta lactamase (ESBL). ESBL is an enzyme produced by bacteria Gram-negative bacteria such as *E. coli* and is produced in plasmids which already have resistance to β-lactam antibiotics. This enzyme hydrolyzes first, second, third generation cephalosporins. (Widodo et al, 2020). *E. coli* has experienced a lot of resistance to beta-lactam antibiotics, fosfomycin and quinolones (Handayani, 2020). The occurrence of MDR reduces the options available for antibiotic treatment and is a difficult problem to overcome in the treatment of infectious diseases (Wibisono et al., 2021).

Table 2. Details of antibiotics usage in animal husbandry

No	Antibiotic Group	Antibiotic	No. farm	%
1	Penicillin	Amoxicilline	37	90.2
		Ampicilin	4	9.8
2	Quinolon	Enrofloxacine	27	65.8
		Flumequin	4	9.7
3	Makrolida	Erytromicin	4	9.7
		Tilosin	3	7.3
5	Tetrasiklin	Doxisiklin	4	9.7
6	As fosfonat	Fosfomisin	3	7.3
7	Antikoksi	Toltrazuril	8	19.5
		Amprolium	7	17
8	Lincosamide	lincomisin	8	19.5
9	Aminoglikosida	Spectinomycin	8	19.5
10	Polipeptida	Colistin	5	12.1

Table 3. Analysis of risk factors for the incidence of MDR E coli

No	Factor		MDR (+)	MDR (-)	OR (95%CI)	π
1	AMR Knowledge	Yes	35	1	23.33(1.61- 338.42)	0.02*
		No	3	2		

Note: (*) have association if $\pi<0.05$

Farms profiling

In this study, the number of broiler farms analyzed was 41 farms originating from Medan City 12 farms and Deli Serdang Regency 29 farms. Based on the results of the questionnaire, the type of broiler farming business that was run mostly as partnerships with poultry companies (58.5%). Most of the chicken population (85.4%) in the range of 1000-5000 birds per cage with an average population of 2529 birds and has at least 500 to 10,000 birds at most.

Most of the broiler farms condition (82.9%) looked clean with lack of smell, flies and trash, although some of the cages still looked unclean. For the profile of livestock owners, the average age is 42 years with the youngest being 27 years old to the oldest 60 years old, with most of them having a high school level education (63.4%), although there are also those with a college education level of 29% and the rest still have elementary and junior high school education. Most of the experience of running a farm is over 5 years, namely 70% with an average of 8.6 years with a minimum of 2 years and a maximum of 20 years. There are only 36.6% of the broiler farms that been supported by veterinarians in monitoring poultry health poultry farmers who lack knowledge have poorer cage hygiene because knowledge can greatly influence people's behavior in the form of knowledge, attitudes and practices (Syafitri et al., 2021), including in handling broiler health and the use of antibiotics. The broiler farms in the study still needs to be improved in veterinarian supported. This is because the role of veterinarians in livestock is very influential on the treatment and health of chickens, especially in the use of antibiotics. In addition, in Indonesia there are still purchases of antibiotics without a prescription with insufficient knowledge about the use of antibiotics which underlies the use of antibiotics that are not suitable in animal husbandry (Niasono et al., 2019).

Antibiotic usage

According to the results of the study, antibiotic preparations used in broiler farms in Medan City and Deli Serdang Regency used penicillin class antibiotics such as amoxicillin (90.2%) and ampicillin (9.8%) and quinolones such as enrofloxacin (65.8%) and flumequin (9.7%). All sampled farms Detailed data on the percentage of antibiotic usage in broiler farms in Medan City and Deli Serdang Regency can be seen in Table 2. Many farms still use penicillin class antibiotics because farmers still state that these antibiotics have no side effects and low costs (Memish et al., 2004). Antibiotics that are often used in poultry farms in Thailand include amoxicillin, colistin, doxycycline, oxytetracycline (Wongsuvan et al., 2018). In addition, several countries in America also use types of antibiotics such as tetracycline and tylosin in poultry farms (Mehdi et al., 2018).

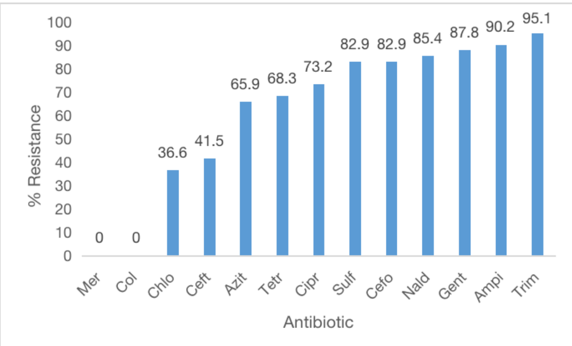

Figure 1. Percentage of AMR

Based on the results of the AMR test, the antibiotics trimethoprim, Ampicillin, gentamicin, nalidixide acid, cefotaxim, and sulfamethoxazole are antibiotics that have developed resistance in this farm. When compared with the detailed data on the use of antibiotics from the questionnaire, it can be concluded that the incidence of AMR in broiler chicken farms in Medan and Deli Serdang is resistance originating from the genotype. Antibiotic resistance coding genes in bacteria can be detected using polymerase chain reaction (PCR)-based detection methods. Several studies have detected genes encoding resistance for *E. coli* and *Staphylococcus* bacteria which are genotypically resistant to antibiotics (Khoirani et al., (2019) & Aziz et al., (2016)).

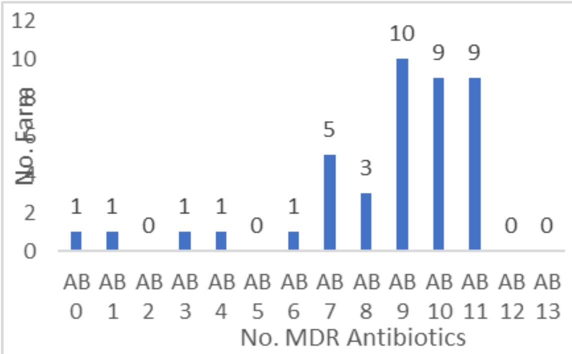

Figure 2. MDR incidence based on the number of antibiotics

Questionnaire analysis results

Complementary data collection in the form of farmer data, livestock management, and field observations around the farms was carried out during sampling through interviews and filling out questionnaires by farmers. Univariable analysis identified one variable that was potentially related to the incidence of MDR *E. coli*, namely the knowledge factor about antibiotic resistance of the farmer in broiler chicken farms (Table 4). This risk factor has a very strong influence on the incidence of MDR in *E. coli* isolates with a probability of 23 times opposite of farmers who does not have knowledge about AMR in broiler farms.

The strong level association between MDR and the farmer's level of knowledge about AMR showed that the problem of treatment failure with antibiotics and its effects on the health of chickens and humans who consume them is very important in the rational and appropriate use of antibiotics. Conversely, a lack of awareness can create uncertainty and poor judgment on the process of using antibiotics (Antao et al., 2018). Farmers obtain information on the use of antibiotics in broiler farms, which either come from the core of the partnership or share experiences from fellow breeders so that they have a better ability to manage livestock health. Good understanding, such as farmer education, is important to do in the use of antibiotics so that their use is appropriate and can prevent infection properly. Education also serves to correct misperceptions about the use of antibiotics in society (WHO, 2001).

Conclusion

Most of the *E. coli* isolates which isolated and identified from 41 caecum specimens derived from broiler farms in Medan City and Deli Serdang Regency (97,6%) experienced in AMR to trimethoprim (95.1%), ampicillin (90.2%), gentamicin (87.8%), nalidixide acid (85.4%), cefotaxim (82.9%), and sulfamethoxazole (82.9%), while it did not resistance to colistin and meropenem. It was also found that 92.7% of the *E. coli* samples experienced with multi drug resistant and the highest prevalence of 9 types of antibiotics at 24.4%. Farmers knowledge of AMR (definition of AMR and its dangers to broiler chicken farms) greatly influences the incidence of MDR resistance at risk of 23 times greater than farmers who do not know anything about it. Further research is needed to determine the *E. coli* gene resistance and ESBL enzyme using biotechnology methods (PCR).

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