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Resistance Of Antibiotic In Klebsiella Sp Isolate Of Chicken Origin And Feed Formulation

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Abstract – Antibiotics are one of the best achievements of modern medicine due to their importance in medical practice. However, antibiotics are currently considered less effective due to the emergence of antibiotic resistance worldwide. This study aims to identify Klebsiella sp resistance to 14 types of antibiotics and to produce feeds containing immunoglobulin Y(Ig Y) against Klebsiella sp. A total of 400 samples were isolated from Regencies, Cianjur, Sukabumi, and Bogor identified through a series of culturing, staining, and biochemical tests. Of the 400 isolated samples tested, 40 Klebsiella isolates were isolated. The result of the resistance test for these 40 Klebsiella sp isolates showed different results, with only 2 Klebsiella sp isolates showing no antibiotic resistance. Another result in the seventh week after vaccination was the presence of positive anti-Klebsiella sp. The provocation test after feeding the Ig Y formula did not cause death in chickens. The group of chickens fed either a 10% or 20% Ig Y diet, showed normal behavior, were healthy, and had a good appetite and dry stools. In comparison, the chickens that did not receive the Ig Y feed showed abnormal behavior, less agility, lack of appetite, and wet stools. The results of the clearance test showed that 8 treated chickens with resistant isolate samples obtained a good score, while in the group of sensitive isolate samples, 2 out of 8 treated chickens did not obtain a good score. The result of cross-reaction Ig Y against Klebsiella sp test to E. coli and Salmonella sp was negative.

Keywords – Antibiotics, Klebsiella sp, Resistance, Immunoglobulin Y, Feed

I. INTRODUCTION

Chicken farming, both laying and broiler breeds, is the largest livestock population and is one of the main sources of animal protein in Indonesia. The population of broiler chickens in Indonesia until 2019 was recorded at 3,149,382,220 tails, and is always increasing from year to year. West Java Province has the highest broiler population of 33 other provinces, namely 799,084,155 birds (Dirjenak and Keswan 2019). Broiler farms generally use antibiotics as *feed additives* growth promoters and prophylaxis (Murphy *et al.* 2016) to increase body weight and increase the efficiency of food converted to meat. Antibiotics are also used for disease prevention and treatment, especially for commercial chicken farmers. Antibiotics used in livestock production account for around two-thirds of global sales and consumption of antibiotics (Aarestrup 2012). The development of antibiotics is one of the best achievements in the field of modern medicine because their existence is important in medical practice, but antibiotics are currently considered less effective due to the emergence of resistance to antibiotics throughout the world. Many pathogenic bacteria are in the intestinal tract of animals which are transmitted to humans through feces or the food chain, thus becoming a source of public health problems (Manyi-Loh *et al.* 2018). Resistance to antibiotics is characterized by the increasing number of failures of antibiotics to prevent the growth or kill bacteria. Resistance to Antibiotics is one of the main challenges in the treatment of bacterial infectious diseases today (Logan and Weinstein 2017).

Klebsiella bacteria is one of the normal flora in poultry that is experiencing antibiotic resistance. This is reinforced by reports of the high incidence of resistance by *Klebsiella bacteria in various countries.* These bacteria can cause bacterial diseases that attack small livestock, large livestock, and poultry. The population and consumption of poultry meat in Indonesia, especially in West Java, is very high, so research on the resistance status of *Klebsiella* sp bacteria to various antibiotics needs to be done. Davis *et al.* (2018) explained that chicken meat is a potential reservoir for the transmission of virulent *Klebsiella* which is resistant to antibiotics from animals to humans. *Klebsiella* sp including Gram-negative bacteria which are normal flora in the skin,

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oral cavity, and intestines. However, this bacterium can become a pathogen in animals and humans in unhealthy conditions (immunosuppression). Murwani *et al.* (2017) stated that *Klebsiella* can be transmitted by inhalation, causing pneumonia, bacteremia, and nosocomial infections.

Immunoglobulin Y (IgY) is the lowest molecular weight immunoglobulin in the body of poultry, reptiles, and amphibians. IgY has biological properties which are a combination of the biological properties of IgG and IgE (Wibawan *et al* . 2003). Leslie and Clem (1969) mentioned several advantages of using poultry as a biological machine for producing antibodies, namely: having high affinity with persistent titers, high production of antibodies, collection of antibodies carried out non-invasively, and simple and economical isolation procedures. Another advantage of using IgY over IgG is that IgY is more resistant to temperature and pH changes than IgG, and does not cause cross-reactions with structural components of mammalian tissues and mammalian red blood cells (Larsson et al. *1993*). Research related to the application of IgY has been carried out on the H5N1 bird flu virus, diarrhea-causing bacteria *Escherichia coli* and *Salmonella enteritidis* (Wibawan *et al* ., 2009, Pasaribu *et al* ., 2008, Rawendra 2005), the use of IgY in fish pellets which are efficacious as vaccines anti- *Aeromonas hydrophila* (Hidayat 2006), anti- *Enterobacter sakazakii eggs* (Hidayat 2009), anti- *Koi Herpes Virus* (KHV) in goldfish and Koi goldfish (Pasaribu 2009), and egg production IgY-3A anti-bird flu and anti-diarrhea (Indrawati and Hidayat 2009).

This study aims to examine the resistance status *of Klebsiella* sp to various types of antibiotics that are often used in chicken farms in West Java. Producing anti-*Klebsiella* sp IgY and formula feed which is expected to protect chickens from diseases caused by *Klebsiella* sp infection.

II. Research Methods

2.1 Time and Place of Research

The research was carried out from August 2020 – August 2022 at the Medical Microbiology Laboratory, School of Veterinary Medicine and Biomedical Medicine, Peranian Institute, Bogor.

2.2 Isolate Preparation

Isolate *The Klebsiella* sp used was isolated and identified from 400 chicken samples, namely 150 samples from Cianjur Regency, 155 samples from Sukabumi Regency, and 95 samples from Bogor Regency.

2.3 Identification of *Klebsiella* sp

Before being re-identified, the isolates were cultured on *tryptic soy agar* (TSA) and incubated at 37 ºC, for 24 hours. Re-identification was carried out on *Mac Conkey agar* (MCA), *eosin methylene blue* (EMB) agar, *triple sugar iron agar* (TSIA), IMViC test (*indole, methyl red, Voges-Proskauer, citrate*), and urea. Incubation of bacteria on the test medium was carried out at 37 ºC for 24 hours. Samples were cultured in MCA medium, then the suspected colonies were subcultured in TSA medium. Gram Staining, IMViC Test, and Biochemistry and Carbohydrate Test. Incubation for 48 hours is reserved for the *methyl red* and *Voges-Proskauer tests* (Markey *et al* . 2013).

The read characteristics refer to Cowan's references, namely: Gram Negative properties, rod shape, aerobic facultative, Oxidase (positive), non-motile (anesthetized), carbohydrate fermentation, and nitrate reduction. Negative Motility (NM), Citrate Positive, Urease Dubius, TSIA (H_2S) Negative, Gas in Glucose Dubius, Lactose D u anesthetic, Maltose Positive, Mannitol Positive, and Indole Dubius.

2.4 Antibiotic Sensitivity Test

Klebsiella sp isolates were tested for sensitivity to 14 types of antibiotics, namely Ciprofloxacin (CIP) 5 µg, Tetracycline (TE) 30 mcg, Ampicillin (AMP) 10 µg, Erythromycin (E) 15 µg, Gentamycine (CN) 10 µg, Nalidixid acid (NA) 30 µg, Amoxyxillin (AML) 25 mcg, Oxytetracycline (OT) 30 µg, Doxycycline (DO) 30 mcg, Enrofloxacin (ENR) 5 µg, Streptomycin (S) 10 mcg, Chloramphenicol (C) 30 µg, Ceftazidime (CAZ) 30 µg, and Cefotaxime (CTX) 30 µg. Tests were carried out using the Kirby-Bauer *disk diffusion method* on *Mueller-Hinton agar* (MHA) media (CLSI 2020) in duplicate.

2.5 Production of IgY Anti *Klebsiella* sp

Production of IgY using bacterial culture isolates *Klebsiella* sp. resistance code 8.7 and *Klebsiella* sp sensitization code 30. The stage starts with bacterial culture and is rejuvenated for 24 hours at 37 ^{0}C, then he took 1 loop öse and put it in 100 mL of liquid medium (BHI Broth) for 24 hours at temperature 37 ^{0}C, disengage speed 3500 rpm for 15 minutes. Supernatant discarded _ Then added 10 ml of NaCl Physiol yogis, centrifuged at 3500-speed rpm for 15 minutes, then removed the supernatant and added 10 ml of Physiological NaCl (this was done 3 times until the pellet was clean). The pellets were made into a suspension until the Mc turbidity. Farland 1 (3.0 x 10 ^{8} cfu/mL) . This suspension was heated in *a water bath* for 1 hour at 60 ^{0}C. After the suspension is cold, take 1 ö se and scratch it on the medium TSA flat gar, then incubated for 24 hours at 37 ^{0}C ± 1 ^{0}C. If the bacterial colonies do not grow on the TSA medium, then a vaccine can be used to produce IgY.

2.6 IgY Formulation Feed and Challenge Test

The presence of IgY in egg yolk is checked weekly by test *Agar Gel Precipitation Test* (AGPT). The IgY product in chicken egg yolk is mixed into the chicken feed and given to the chickens according to the group.

2.7 Non-Specific Response Test (*Clearance Test*)

The Non-Specific Response Test *was* carried out by taking a *blood* sample using *the Clearance Test* method. This test is one of the *in vitro tests* to determine the body's response to chickens attacking foreign objects that enter the body. The results of this test are said to be good if the microbial mortality is ≥ 99.99%.

2.8. Cross Reaction Test

AGPT method to see whether IgY anti-*Klebsiella* sp affected *E. coli* and *Salmonella* sp.

2.9. Results Analysis

The results of *Klebsiella* sp identification, antibiotic sensitivity test, IgY production, challenge test, non-specific response test, and cross-reaction test are presented in figures and tables. All data were analyzed descriptively.

III. Results And Discussion

3.1 Identification of *Klebsiella* sp

Identification results obtained as many as 40 *Klebsiella* sp isolates from 400 samples examined the characteristics of the isolates.

3.2 Antibiotic Sensitivity Test

Total bacteria *Klebsiella* sp. 40 isolates after being tested for antibiotic resistance showed varying results where there were only 2 (two) isolates that did not show antibiotic resistance of *Klebsiella* sp bacteria (codes 4.10 and 8.17).

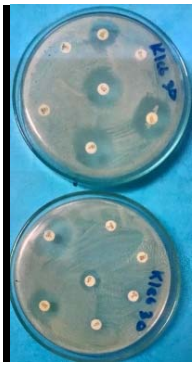

Figure 1. Clear zone showing *Klebsiella* sp. isolate not resistant yet

3.3 Production of IgY Anti *Klebsiella* sp

Week 3 after vaccination, *Klebsiella sp* bacteria with code 8.7 (Klebsiella *sp* which is already resistant) and *Klebsiella* sp bacteria with code 30 (Klebsiella *sp* which is still sensitive) has shown positive results in the AGPT test with the formation of a precipitation line. *Klebsiella sp* bacteria with code 8.7 and code 30 in the 7th week after vaccination, also showed positive results in the AGPT test with the formation of a precipitation line from egg yolks.

Figure 2. The presence of IgY in egg yolk is marked with a precipitation line

3.4 Challenge Test

The results of this test on a group of chickens that were given feed containing IgY in egg yolk (either 10% or 20%), showed normal behavior, was healthy, ate more, and had dry feces. The control group of chickens that were not given feed containing IgY showed abnormal behavior, less agile movements, decreased appetite, and wet feces. No chickens died from either the treatment or control groups.

Table 1 Results of Feeding IgY Formulations and Challenge Tests

Number of Chickens	Concentration of IgY	Information on IgY was obtained from *Klebsiella* sp.	Feed Description	Challenge Test Treatment (after 4 weeks given IgY	Information
3	10%	resistance	The feed contains IgY, given every day	2.5 mL *Klebsiella* sp.	Not dead
3	20%	resistance			
3	0% (Control)	-	Ordinary Feed		
3	10%	sensitive	The feed contains IgY, given every day		
3	20%	sensitive			
3	0% (Control)	-	Ordinary Feed		

3.5 Non-Specific Response Test (*Clearance Test*)

The results of the non-specific response test (Clearance *test*) showed that 8 treatment chickens (100%) of the resistant isolates (code 8.7) had good scores, while in the sensitive isolate group (code 30) there were 2 out of 8 treatment chickens that did not achieve good scores.

Table 2. Clearance test results for sensitive Klebsiella sp isolate (code 30)

No.	Sample Name	Concentration (bacteria) _	Contact Time (Minute)	Treatment	Examination Results (cfu/ml) (Bacterial *Klebsiella* sp, which is still alive)	Death (%)
1.	Suspension control of *Klebsiella* sp. (Sensitive bacteria)	1.9 x 10^{13}	30 minutes	-	1.9 x 10^{13}	0 %
2.	Chicken Blood (1)	1.9 x 10^{13}	30 minutes	not given Igy	2.7 x 10^{10}	99.79 %
3.	Chicken Blood (2)	1.9 x 10^{13}	30 minutes	not given Igy	3.4 x 10^{10}	99.77 %
4.	Chicken Blood (3)	1.9 x 10^{13}	30 minutes	not given Igy	3.2 x 10^{10}	99.75 %
5.	Chicken Blood (1)	1.9 x 10^{13}	30 minutes	given IGY 10%	2.2 x 10^{10}	**99.83 %**
6.	Chicken Blood (2)	1.9 x 10^{13}	30 minutes	given IGY 10%	2.3 x 10^{6}	99.99 %
7.	Chicken Blood (3)	1.9 x 10^{13}	30 minutes	given IGY 10%	3.2 x 10^{7}	99.99 %
8.	Chicken Blood (4)	1.9 x 10^{13}	30 minutes	given IGY 10%	2.5 x 10^{6}	99.99 %
9.	Chicken Blood (1)	1.9 x 10^{13}	30 minutes	given IGY 20%	3.2 x 10^{8}	99.99 %
10	Chicken Blood (2)	1.9 x 10^{13}	30 minutes	given IGY 20%	1.9 x 10^{7}	99.99 %
11	Chicken Blood (3)	1.9 x 10^{13}	30 minutes	given IGY 20%	2.9 x 10^{7}	**99.98 %**
12	Chicken Blood (4)	1.9 x 10^{13}	30 minutes	given IGY 20%	1.7 x 10^{7}	99.99 %

Table 3. Clearance test results for resistant Klebsiella sp. isolates (code 8.7)

No.	Sample Name	Concentration (bacteria)	Contact Time (Minute)	Treatment	Examination Results (cfu/ml) (Bacterial *Klebsiella* sp, which is still alive)	Death (%)
1.	Suspension	2.9 x 10^{13}	30	-	2.9 x 10^{13}	0 %

	control of *Klebsiella* sp. (resistant bacteria)		minutes			
2.	Chicken Blood (1)	2.9 x 10^{13}	30 minutes	not given Igy	2.3 x 10^{10}	99.92 %
3.	Chicken Blood (2)	2.9 x 10^{13}	30 minutes	not given Igy	2.2 x 10^{11}	99.24 %
4.	Chicken Blood (3)	2.9 x 10^{13}	30 minutes	not given Igy	3.2 x 10^{11}	98.90 %
5.	Chicken Blood (1)	2.9 x 10^{13}	30 minutes	given IGY 10%	2.1 x 10^{9}	99.99 %
6.	Chicken Blood (2)	2.9 x 10^{13}	30 minutes	given IGY 10%	3.3 x 10^{8}	99.99 %
7.	Chicken Blood (3)	2.9 x 10^{13}	30 minutes	given IGY 10%	1.9 x 10^{7}	99.99 %
8.	Chicken Blood (4)	2.9 x 10^{13}	30 minutes	given IGY 10%	2.2 x 10^{7}	99.99 %
9.	Chicken Blood (1)	2.9 x 10^{13}	30 minutes	given IGY 20%	1.1 x 10^{9}	99.99 %
10	Chicken Blood (2)	2.9 x 10^{13}	30 minutes	given IGY 20%	2.9 x 10^{8}	99.99 %
11	Chicken Blood (3)	2.9 x 10^{13}	30 minutes	given IGY 20%	2.3 x 10^{8}	99.99 %
12	Chicken Blood (4)	2.9 x 10^{13}	30 minutes	given IGY 20%	2.1 x 10^{8}	99.99 %

3.6 Cross Reaction Test

The results obtained from the anti-*Klebsiella sp IgY test against E. coli* and *Salmonella sp* bacteria were negative, which means they did not provide a protective effect.

Figure 3 Precipitation line is not formed (negative)

IV. Conclusions And Suggestions

4.1. Conclusion

The conclusions of this study were (1) the incidence of *Klebsiella* sp as much as 10% of the total sample was a number that could not be ignored, (2) 38 out of 40 *Klebsiella* sp isolates were resistant to one or several antibiotics, and (3) administration of IgY to feed cellularly helps the chicken's body response to ward off infecting *Klebsiella* sp bacteria.

4.2. Suggestion

Suggestions that can be given for further research are an examination of histo-pathological preparations in treated chickens.

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