Oral Presentation (MP-5)

Antibiotic Resistance of *Klebsiella* Species Isolated from Broiler Chickens in Sukabumi and Bogor Areas

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INTRODUCTION

Animal protein needs in Indonesia increase every year. One source of animal protein that is affordable for all levels of society is poultry. Poultry mostly farmed in several parts of Indonesia. The region that has the largest poultry population in Indonesia is the province of West Java. The regions in West Java that contribute the highest poultry populations are Sukabumi and Bogor. The largest number of poultry populations is broiler chicken population, which is 108,304,978 tails (3).

Antibiotics often used in livestock and poultry as a prevention of disease transmission and as a growth promoter (9). Routine use of antibiotics in the livestock industry, especially chickens, has a negative impact. One of them is the emergence of antibiotic resistance. One of the bacteria that has experienced antibiotic resistance is the *Klebsiella* species.

Klebsiella bacteria are Gram-negative bacteria are normal flora in the oral cavity, skin and intestines, but can become pathogens in animals and humans under certain conditions. This bacterium is one of the causes of bacterial diseases that attack large livestock and poultry. Many reports say that *Klebsiella* species have experienced resistance to antibiotics. *Klebsiella* had experienced resistance to several antibiotics, namely ampicillin as much as 66.7%, Nalidixic Acid 61.8%, tetracycline 59.8% and trimethropin 50% [5]. *Klebsiella* bacteria that are resistant to antibiotics are very dangerous for animal and human health. Diseases caused by *Klebsiella* bacteria that are resistant to antibiotics will be difficult to cure. Akova (2016) states that Gram-negative and Gram-positive bacteria that experience resistance will produce infections that are difficult to treat or cannot even be treated using antimicrobials [2].

MATERIALS AND METHODS

Sample Collection

The sample used in this study was a sample of cloaca swabs from broiler chickens taken from farms in Sukabumi and Bogor, West Java. The total sample studied amounted to 101 samples, 52 samples from Sukabumi and 49 samples from Bogor.

Isolation and Identification of Samples

Samples of cloaca swabs that have been taken are then cultured in Mac Conkey agar (MCA) medium. The culture was incubated at 37 °C for 24 hours. Bacterial colonies grown on MCA medium were observed. Species of *Klebsiella* have round colonies, convex, smooth, pink and mucoid. Single colonies identified as *Klebsiella* species then subcultured on TSA (Triptic Soy Agar) slant medium then incubated at 37 °C for 24 hours. Then microscopic observation to see the form of bacteria.

Biochemistry Test

Biochemical tests to ensure that bacteria isolated and microscopic and macroscopic identification are *Klebsiella*. The biochemical tests carried out were catalase test, Triple Sugar Iron agar (TSIA) test, IMViC test consisting of Sulfite Indol Motility (SIM) test, Methyl red-Voges Proskauer test, Simmon Citrate test, then glucose, lactose, maltose sucrose and mannitol fermentation test.

Antibiotics Susceptibility Test

Susceptibility testing for antibiotics following the disk diffusion method *Kirby-Bauer* used Mueller-Hinton agar based on Clinical and Laboratory Standards Institute guidelines (4). The antibiotics used were tetracycline, oxitetracycline, erythromycin, ciprofloxacin, nalidixic acid, gentamicin, and chloramphenicol. This test was carried with three repetitions.

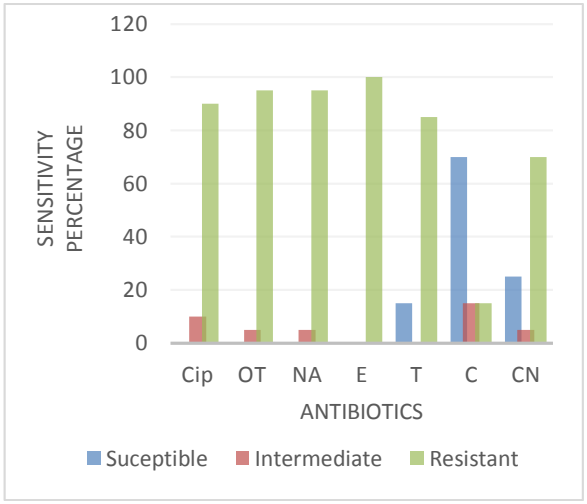
Klebsiella bacterial colonies growth at TSA media that were incubated at 37 °C for 24 hours were taken using ose then transferred to a test tube containing 5 ml of physiological NaCl. The mixture is in vortex until it is mixed, then it can be seen that the turbidity is the same as the turbidity in 0.5 McFarland soluble. The solution was taken as much

as 0.5 ml and put into a petri dish containing Muller Hinton agar (MHA) media and flattened using a cotton bud. Each paper disk containing antibiotics were then placed on the surface of the MHA media and incubated at 37 °Cfor 24 hours. After 24 hours the diameter of the inhibitory zone is measured. This susceptibility test for antibiotics was repeated three times at the same time.

RESULT AND DISCUSION

In this study, of the 101 samples that isolated using conventional selective media and biochemistry test, 20 samples were identified as *Klebsiella*. The 20 samples that have susceptibility test to antibiotics then measured for inhibitory zones. The inhibitory zone diameter measurement refers to CLSI 2018 [4]. The resistance of *Klebsiella* spp. to seven antibiotics was determined using the Kirby-Bauer disk diffusion assay. Isolates showed high levels of resistance; 90% were resistant to Ciprofloxacin, 95% to Oxytetracycline, 90% to Nalidixic Acid, 100% to Erythromycin, 85% to Tetracycline, 15% to Chloramphenicol and 70% to Gentamycin (Picture 1).

Some antibiotic classes in poultry production are penicillin, cephalosporins, aminoglycosides, glycopeptides, macrolides, sulfonamides, quinolones, fluoroquinolones, chloramphenicol and tetracyclines (1). Antibiotics such as bacitracin, chlortetracycline, erythromycin and penicillin, directly for the control and treatment of diseases in poultry. Currently given to poultry for a long time, especially at low levels, certain species of bacteria become resistant (8). Huddleston (2014) mentions the use of antibiotics continually causes stress can then trigger the nature of resistance in DNA so that mutations and genetic changes occur in bacterial cells [6].



Picture 1. Sensitivity of *Klebsiella* to ciprofloxacin (Cip), oxytetracycline (OT), nalidixic acid (NA), erythromycin (E), tetracycline (T), chloramphenicol (C) and Gentamycin (CN) antibiotics.

Table 1 Percentage of antibiotic resistance in *Klebsiella* spp. isolate code.

Code	% Isolates Suceptible		
	S	I	R
A.36	0	14,28	85,72
A.42	14,28	0	85,72
A.50	14,28	0	85,72
A.51	0	0	100
A.62	14,28	14,28	71,44
A.63	28,57	0	71,43
KA.8	14,28	0	85,72
KA.19	14,28	0	85,72
KA.22	28,57	0	71,43
CIA.14	0	14,28	85,72
CIA.22	28,57	0	71,43
CIA.23	14,28	0	85,72
CIA.27	28,57	14,28	57,15
CIA.28	14,28	0	85,72
CIA.29	0	0	100
G7.13	14,28	0	85,72
G7.20	14,28	0	85,72
G7.52	42,86	14,28	42,86
G7.59	14,28	14,28	71,44
C7.11	14,28	28,57	57,15

This study showed that *Klebsiella* bacteria resistant to more than one antibiotic. Even sample codes A.51 and CIA.29 were resistant to all antibiotics tested (table 1). This suggests that the isolated K*lebsiella* bacteria experienced multi-drug resistance. The report of cattle and chicken samples in Turkey were also positive for *Klebsiella* was multi-drug resistance resistant to ampicillin antibiotics, tetracycline, streptomycin, gentamicin and kanamycin (7). The *Klebsiella* species often found is *K. pneumonia*. *K. pneumonia* is an opportunistic bacterium that has a high diversity of antimicrobial resistance genes, even some new antimicrobial resistance (AMR) (10).

CONCLUSION

Klebsiella bacteria isolates from broiler chickens in the Bogor and Sukabumi regions obtained showed a high level of resistance to almost all antibiotics, except chloramphenicol type antibiotics still showed a good enough sensitivity to *Klebsiella* bacteria species.

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