REKAYASA
Journal of Science and Technology
https://journal.trunojoyo.ac.id/rekayasa

Rekayasa, 2023; 16(2): 117-121
ISSN: 0216-9495 (Print)
ISSN: 2502-5325 (Online)

Resistance of *Klebsiella Sp.* Isolated From Chicken and Cages to Chloramphenicol

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DOI: https://doi.org/10.21107/rekayasa.v16i2.20277

Abstrak

Bakteri Klebsiella merupakan salah satu flora normal pada unggas yang mengalami resistensi antibiotik. Hal ini diperkuat dengan laporan kejadian resistensi bakteri Klebsiella di berbagai negara cukup tinggi. Penelitian ini bertujuan untuk mengidentifikasi keberadaan Klebsiella sp. bakteri pada ayam dan kandang, serta status resistensinya terhadap kloramfenikol terkait Peraturan Menteri Pertanian No. 14 Tahun 2017. Rangkaian identifikasi meliputi kultur, pengamatan makroskopis dan mikroskopis, uji biokimia, dan uji fermentasi. Tes resistensi antibiotik menggunakan cakram kloramfenikol mengacu pada metode Kirby Bauer. Hasil identifikasi menunjukkan 6 sampel positif dari 60 sampel yang diambil dari ayam dan 2 sampel positif dari 21 sampel yang diambil dari kandang. Uji resistensi 8 Klebsiella sp. isolat menunjukkan 6 isolat sensitif, 1 intermediet dan 1 resisten terhadap kloramfenikol. Hal ini menjadi indikasi bahwa Peraturan Menteri Pertanian Nomor 14 Tahun 2017 cukup efektif melarang penggunaan kloramfenikol pada peternakan ayam.

Kata Kunci : klebsiella, unggas, antibiotic, kloramfenikol, resintensi

Abstract

Klebsiella bacteria is one of the normal flora in poultry that has developed antibiotic resistance. This is reinforced by reports of the incidence of resistance by *Klebsiella* bacteria in various countries which is quite high. This study aimed to identify the presence of *Klebsiella* sp. bacteria in chickens and cages, as well as their resistance status to chloramphenicol in relation to the Minister of Agriculture Regulation No. 14/2017. The series of identification included culture, macroscopic and microscopic observations, biochemical tests, and fermentation tests. Antibiotic resistance tests using chloramphenicol disks referring to the Kirby Bauer method. The identification results showed 6 positive samples from 60 samples taken from chickens and 2 positive samples from 21 samples taken from cages. Resistance testing of 8 *Klebsiella* sp. isolates showed 6 sensitive, 1 intermediate and 1 resistant isolates to chloramphenicol. This is an indication that the Minister of Agriculture Regulation No. 14/2017 is quite effective in prohibiting the use of chloramphenicol in chicken farms.

***Key words** : klebsiella, chicken, antibiotic, kloramphenicol, resistence*

INTRODUCTION

Broiler farms commonly use antibiotics as growth-inducing and prophylactic feed additives (Murphy *et al.* 2016) to increase body weight and improve the efficiency of food converted to meat. Antibiotics are also used for disease prevention and treatment, especially by commercial chicken farmers. Antibiotics used in livestock production account for about two-thirds of global antibiotic sales and consumption (Aarestrup 2012).

The environment is a determining factor for the development of microorganisms (Rini and Rochmah 2022). Environmental changes can significantly affect the physiology of microbial growth. Microorganisms such as pathogenic bacteria in the chicken coop environment can act as a medium for disease in both animals and humans. One of these pathogenic bacteria is *Klebsiella* sp. This bacterium belongs to the *Enterobactericeae* group with a natural distribution habitat in the digestive tract and respiration in living things (Orchue and Aliu 2015).

The population and consumption of poultry meat in Indonesia, especially the West Java region

Article History:
Received: October, 29th 2022; **Accepted**: July, 25th 2023
Cite this as :
Hidayat, R., Pasaribu, F.H.,Mulatsih, S & Amin, A.A. 2023. Resistence of Klebsiella Sp Isolated from Chicken and Cages to Chloramphenicol. Rekayasa. Vol 16(2). 117-121

is very high, so research on the presence and resistance status of *Klebsiella* sp bacteria to various antibiotics needs to be done, especially chloramphenicol which is banned by the Government of the Republic of Indonesia. Davis *et al.* (2018) explained that chicken meat is a potential reservoir for the transmission of antibiotic-resistant *Klebsiella* virulence from animals to humans. The level of resistance is influenced by the administration of antibiotics in efforts to prevent and treat microorganism infections. *Klebsiella* sp. in chickens can be identified through cloacal swab isolates and can be carried out through feces that pollute the environment. Gelgel and Sudipa (2020), found a population of 4% *Klebsiella* sp. in the air in chicken cages. *Klebsiella* sp. bacteria are naturally found in soil (Murwani *et al.* 2017).

Antibiotic residues in the environment affect the level of bacterial resistance to antibiotics. Antibiotics are metabolized in the body by 10% to 80%. Antibiotics are excreted through urine and feces which can pollute the environment and contain resistant microorganisms and antimicrobial resistant genes (FAO 2018). The resistance level of *Klebsiella* sp. according to Apriliani and Pinatih (2017) occurred in ampicillin antibiotics by 92% and tetracycline by 65%. Another study showed the level of resistance of *Klebsiella* sp. bacteria to ampicillin, oxytetracycline, erythromycin, and tetracycline antibiotics reached 100% and nalidixic acid as much as 71.4% (Naimmah 2019), Identification of *Klebsiella* sp. bacteria and their level of resistance in the chicken coop environment is still interesting to do because of the lack of information related to this topic.

METHODS

Location and Time

The research was conducted from August 2022 to April 2023 at the Laboratory of Medical Microbiology Division, School of Veterinary Medicine and Biomedical Science, and Close House Broiler Chicken Research, Faculty of Animal Husbandry, IPB University**.**

Sampling Procedures

Sampling was carried out on chickens, cage mats, feeders, drinkers, and air within the cage. *Klebsiella* sp isolates used were the result of isolation and identification of 60 samples from chickens and 21 samples from chicken coops. Samples of feeding and drinking bowls were taken using a sterile cotton swab which was inserted into a tube containing buffer peptone water and stored in a cool box. Sampling of litter mixed with feces was diluted with a sterile NaCl ratio of 1:9. The mixture of feces and sterile NaCl was homogenized using a vortex mixer. Samples of feeding, drinking, and litter were inoculated on Mac Conkey Agar media. Samples from each source were coded as samples 1, 2, 3 and 4 for air, feeding, drinking, and litter.

Air samples refer to Gelgel and Sudipa's (2020) research by looking at bacterial contamination on Blood Agar that opened in the cage. The use of Blood Agar media was modified with Mac Conkey Agar media. The broiler research close house has six partitions consisting of three sections. The media was placed for 30 minutes at three points representing each of the two partitions of the chicken coop.

Identification of *Klebsiella* sp.

Bacterial samples from all sources were inoculated on Mac Conkey Agar (MCA) and incubated for 24 hours at 37 ºC. Initial identification is done macroscopically by looking at the growth of bacterial colonies that have mucoid, pink to red characteristics that refer to the characteristics of *Klebsiella* sp. on Mac Conkey Agar (MCA) media. Colonies that characterized *Klebsiella* sp. were separated using an ose and cultured on Trypsin Soy Agar (TSA) slant media. Bacterial identification was carried out using Gram staining, biochemical tests, and fermentation tests. Biochemical tests performed include Triple Sugar Iron Agar (TSIA) test, indole, motility, urea, citrate, methyl red (MR), Voges-Proskauer (VP). Carbohydrate fermentation includes glucose, lactose, sucrose, mannitol and maltose. Interpretation of biochemical and sugar test results refers to Cowan and Steel's (2003), namely: Gram Negative nature, Rod shape, facultative aerobic, Oxidase (positive), non motile (anesthetized), carbohydrate fermentation, and nitrate reduction. Motility Negative (NM), Citrat Positive, Urease Dubius, TSIA (H2 S) Negative, Gas in Glucose Dubius, Lactose Dubius, Maltose Positive, Mannitol Positive, and Indol Dubius.

Antibiotic resistance test

Antibiotic resistance test using Kirby-Bauer Disk Diffusion Test method with 24 hours old bacterial

culture. Isolates were made into a suspension solution with a concentration of 1.5x108 CFU/ml or 0.5 M McFarland 1 (CLSI 2021). The test uses Mueller Hinton Agar media by placing an antibiotic disk on the bacterial suspension inoculation in the media. The media is then incubated at 37 °C for 24 hours.

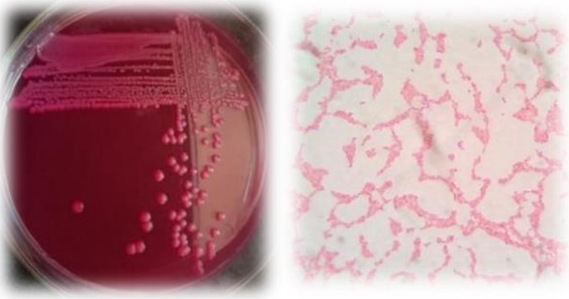
Data Analysis

The results of *Klebsiella* sp identification and antibiotic resistance testing are presented in figures and tables. All data were analyzed descriptively. Analysis of the resistance test was carried out by interpreting the standards of the Clinical and Laboratory Standards Institute (CLSI 2021).

RESULTS AND DISCUSSION

Bacterial Identification of *Klebsiella* sp.

The results of isolation and identification obtained as many as 8 *Klebsiella* sp isolates from a total of 81 samples examined for isolate characteristics characterized by large round colonies, mucoid, and pink to red in color. Of the 8 isolates that were characterized, 6 isolates (5c1, 5c2, 5c4, 8c26, 8c29, and 8c30) taken from chickens and 2 isolates (3k1 and 3k2) taken from cages. Colonies of *Klebsiella* sp. macroscopically and microscopically are presented in Figure 1.



(A) (B)

Figure 1. *Klebsiella* sp. colonies macroscopically on Mac Conkey Agar media (A), *Klebsiella* sp. colonies microscopically on Gram stain (B)

MacConkey Agar medium is the primary selective medium for Klebseilla sp. bacteria with mucoid and pink colony growth (Rawy *et al.* 2020). *Klebsiella* sp. on MCA has characteristics that are almost similar to *Escherichia coli* bacteria. *Escherichia coli* colonies are small round semi-mucoid, unlike the *Klebsiella* sp. colonies with a large round shape and mucoid elevation (Widianingsih and De Jesus 2018).

According to Bulele *et al.* (2019), Gram-positive bacteria have a purple color, while Gram-negative bacteria are red. The difference in color is due to differences in the constituents of the bacterial cell wall. *Klebsiella* sp. is a Gram- negative bacterium of the *Enterobacteriaceae* group that has a thin peptidoglycan, so that the color of crystal violet fades when exposed to alcohol (Aristyawan *et al.* 2017). Gram-negative bacteria showed positive results in the 3% KOH test and negative in the oxidase test. Positive results in the 3% KOH test are characterized by the presence of mucus formed and a negative reaction if no mucus is formed (Hardiansyah *et al.* 2020). A positive oxidase test is characterized by a change in color to violet blue after giving oxidase reagent and no color change occurs in negative results (Antriana 2014). Biochemical tests in the form of sugar test and carbohydrate fermentation test are presented in Table 1. The discovery of *Klebsiella* sp. bacterial isolates not only from chickens but also from cages is in accordance with the statement of Bola *et al.* (2021), that *Klebsiella* sp. can be found naturally in soil and water.

Table 1 Identification results of *Klebsiella* sp. bacteria in chickens and cages

Sample Origin	Sample Code	Total Sample	Positive	Negative
Chicken	5c and 8c	60	6 (10%)	54 (90%)
Cage	3k	21	2 (9,52%)	19 (90,48%)

n: number of samples; %: percentage of results

Antibiotic resistance test

A total of 8 isolates of *Klebsiella* sp. bacteria that have been identified with isolate codes 5c1, 5c2, 5c4, 8c26, 8c29, 8c30, 3k1 and 3k2 were tested for resistance to chloramphenicol antibiotics. Antibiotic resistance test results showed 6 *Klebsiella* sp. isolates (5c1, 5c2, 8c29, 8c30, 3k1 and 3k2) were sensitive, 1 isolate (5c4) was resistant, and 1 isolate (8c26) was intermediate to chloramphenicol antibiotics. The zone of inhibition formed on the media indicates the presence of bacterial growth that can be inhibited by antibiotics. The test results using this method are divided into three category interpretations namely sensitive, intermediate and resistant which are presented in Table 2.

Table 2. Resistance test results of 8 *Klebsiella* sp. isolates to chloramphenicol

Group	Antibiotic	Zone of inhibiton* (mm)			Isolates code		
		S	I	R	5c1,5c2,8c298c30,3k1,3k2	8c26	5c4
Phenicol	C (30 µg)	>18	13-17	<12	S	I	R

*Source: CLSI 2021; C: Chloramphenicol; S: Sensitive; I: intermediate; R: Resistant

Chloramphenicol has a mechanism of action that is able to bind to the 50s ribosomal subunit, resulting in inhibition of protein synthesis. According to Rahman and Prihartini (2021), *Klebsiella* sp. showed sensitivity to chloramphenicol antibiotics with an inhibition zone of 23 mm. The research of Naimmah (2019), showed sensitive results of 85% in *Klebsiella* sp. isolates against chloramphenicol antibiotics. The use of this antibiotic is prohibited both orally, parenterally and topically in the Minister of Agriculture Regulation number 14 of 2017 (DITJENNAK 2017). The prohibition of using chloramphenicol type antibiotics is indicated to be quite effective based on the results of 6 out of 8 *Klebsiella* sp isolates getting sensitive results (75%). However, the discovery of 1 resistant *Klebsiella* sp isolate (12.5%) is an important finding as a comprehensive evaluation of the implementation of the Minister of Agriculture Regulation number 14 of 2017.

Bacteria in the environment with resistant properties are able to increase the spread of higher resistance properties through various media in the environment such as water, soil, and air (Frieri *et al.* 2017). The presence of resistant *Klebsiella* sp. bacteria in the cage environment has the potential for resistant gene transfer activity to other microorganisms. This can have an impact on increasing bacterial resistance to antibiotics. Inappropriate use of antibiotics can also support the increase in antibiotic resistance in humans, animals, and the environment (Wibisono *et al.* 2020). According to Greesoon *et al.* (2013), antibiotic resistance occurs due to continuous and inappropriate use of antibiotics. Antibiotic residues can spread to the environment such as soil, water, and air. These bacteria can be transmitted from the host to the environment carrying resistant genes through water, plants, and sewage lines. Resistance genes are received and transferred from plasmids through horizontal gene transfer with various bacterial species in the environment. This is one of the pathways for the transfer of resistance genes from microbes in the environment. (Wyres and Holt 2018).

CONCLUSIONS

The results of the study of 81 samples taken from chicken farms obtained 6 samples from chickens and 2 samples from cages positive for *Klebsiella* sp. The resistance status is as many as 6 sensitive *Klebsiella* sp isolates (75%), 1 intermediate isolate (12.5%) and 1 resistant isolate (12.5%). The prohibition of using chloramphenicol antibiotics is indicated to be quite effective.The study was conducted in a chicken farm environment with various types of cages and different seasons or climates. In addition, resistance testing to other types of antibiotics is needed to determine the possibility of Multidrug Resistance (MDR).

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