Gentamicin Resistance on *Escherichia coli* Isolated from Cats

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ABSTRACT
Antibiotic resistance is currently an important problem in the field of animal and human health. The main triggering factor for the incidence of bacterial resistance is wisdom in the use of antibiotics. Handling cases of bacterial infections in cats still make antibiotics be the main choice. One of the antibiotics that is still used in cases of bacterial infection in cats is gentamicin. The use of this antibiotic is oriented towards treating ear infections (otitis). Bacteria that are often found as the cause of otitis cases come from Gram-positive and Gram-negative bacteria. Resistance to bacteria in the ear cavity can occur through uncontrolled use of antibiotics or interactions with other bacteria that are resistant. Interactions between bacteria that cause the transfer of genetic material carrying resistant traits have been widely reported in Gram-negative bacteria through plasmid intermediaries. Body licking behavior in cats allows interactions between bacteria from different habitats, especially *Escherichia coli* from the digestive tract. *Escherichia coli* has been reported to have developed resistance in various animals and humans. The study aimed to measure the resistance level of *Escherichia coli* isolated from cats to gentamicin. The method used in this research is *disk diffusion Kirby-Bauer.* The results of this study show that 56 samples of cat rectal swab are isolated from *Escherichia coli* and confirmed by biochemical tests. There are 11 samples (19.64%) showing resistance and 45 (80.36%) susceptible samples. These results indicate that gentamicin can still be one of the antibiotics choice in the treatment of cases of *Escherichia coli* infection in both the ear cavity and digestive tract..

Keywords: *Escherichia coli, gentamicin, antibiotic resistance*

1. INTRODUCTION

The use of antibiotics in the treatment of bacterial infections is still the main choice for both humans and animals. Antibiotics are used on animals both in farm animals, veterinary clinics, and wildlife with a treatment orientation that is tailored to the purpose. Gentamicin is one of the antibiotics in handling infectious cases in pets in animal clinics. The orientation of its use is to treat infections of the urinary tract as well as infections of open wounds on the skin and causes infections of the ear (otitis). Gentamicin works by inhibiting protein synthesis in bacteria.

Bacterial resistance due to the irrational use of antibiotics in animals has been widely reported. The most resistant bacteria are the normal flora of the digestive tract. *Escherichia coli* is a group of Gram-negative enterobacteria that has been reported to be resistant to both in poultry farming [1], in cattle farm [2], in Zoo [3], and in poultry farm [4].

Escherichia coli resistance in cats to gentamicin can be influenced by the pattern of fur licking behavior. Topical treatment in cats allows entry of low doses of antibiotics into the digestive tract which causes exposure to several bacteria including *Escherichia coli* [5]. Continuous exposure causes resistance to bacteria in the digestive tract. This study aims to measure the level

of resistance of *Escherichia coli* isolated from cats to gentamicin as an antibiotic that is often used in the treatment of infectious cases in veterinary clinics.

2. MATERIALS AND METHODS

This study was conducted from September 2019 to September 2020. The type of sample used was rectal swabs from cat inpatients from several veterinary clinics. The criteria for the cat as the source of the sample were limited to patients with complaints of bacterial infection. The number of samples used were 64 samples collected from 10 veterinary clinics in the city of Bogor. Sample transport media used *buffer peptone water* (BPW) 0,1% at temperatures 4°C.

2.1 Isolation and Identification of Bacteria

The collected samples were then isolated on the media Eosin Methylene Blue Agar (EMBA) which was incubated at 37°C for 18–24 hours [20]. *Escherichia coli* colonies growing on EMBA media were then confirmed microscopically and macroscopically. Microscopic confirmation was carried out by Gram staining which included morphology and chemical properties and bacterial cell walls. Macroscopically, confirmation is done by looking at the shape of the *Escherichia coli* colonies on the EMBA and the biochemical properties of the bacteria through the test IMViC (Indole, Methyl Red, Voges Proskauer, Citrate), Urea test, carbohydrate fermentation and Triple Sugar Iron Agar (TSIA).

2.2 Antibiotic Resistance Test

Antibiotic resistance test followed the method disk diffusion Kirby-Bauer by using Muller-Hinton Agar (MHA) which refered to Clinical and Laboratory Standards Institute guidelines year 2018 and work procedures [19]. The antibiotic used in the test was gentamicin (Macrolide) because it is one of the antibiotics of choice used for the treatment of bacterial infections. Samples that had been confirmed both microscopically and macroscopically were cultured again on the media Tryptic Soya Agar (TSA) to obtain pure cultures in large quantities. The initial stage in the resistance test was by making a suspension. The bacterial suspension obtained from TSA was diluted with NaCl physiological up to standard 0,5 Mcfarland or equivalent to $1,5 \times 10^8$ CFU ml^{-1}. A total of 1 ml of suspension was poured and then leveled on the MHA. Diffusion disks containing antibiotics were placed on the MHA surface with the same distance and then incubated at a temperature of 35°C for 16–18 hours and the inhibition zone was measured. Gentamicin inhibition zone standard based on standard (CLSI 2018) which is greater than 15 mm (≥ 15 mm) for susceptible, 13–14 mm to intermediate, and ≤ 12 mm for resistance criteria. This resistance test is repeated 3 times (triplo) at the same time.

3. RESULTS AND DISCUSSION

The results of the isolation and identification of the media Eosin Methylene Blue Agar and Gram staining show that 100% (64/64) of the sample were confirmed as *Escherichia coli.* Confirmation of samples by biochemical test found 87.5% (56/64) of samples showing biochemical characteristics *Escherichia coli.* A total of 12.5% (8/64) show the presence of bacterial contamination where there are 9.37% (6/64) of the sample showing the formation of gas H_2S and 3,12% (2/64) the sample shows a negative result on the test Methyl Red (Table 1). The results of isolation and identification obtain 56 isolates that are confirmed as *Escherichia coli.* The biochemical test of *Escherichia coli* show the ability to ferment carbohydrates (glucose, lactose, sucrose, maltose, and mannitol) and to produce acid and gas, positive results on the indole test and methyl red test, and negative results on urea and citrate tests, while other tests has varies positive results [6].

Eosin Methylene Blue Agar is a selective medium for isolating *Escherichia coli* bacteria. These bacteria are identified by the presence of a distinctive color of the colony, which is metallic green. *Escherichia coli* grows rapidly in EMBA after 24 hours of incubation (colony growth begins in the first 5 hours), especially at the ideal pH [7]. EMBA is used to distinguish non-fecal coliform and fecal coliform bacteria. In this medium, *Escherichia coli* fermented lactose and produced citric acid to produce a metallic green complex. This color is an indicator of bacteria that can ferment lactose strongly and/or bacteria that ferment sucrose (typical of fecal coliform bacteria).

Isolation of *Escherichia coli* in cats has been widely carried out to observe pathogenic activity in the habitat and outside the habitat [8] and the relationship between disease incidence and humans [9]. *Escherichia coli* is a normal flora in the digestive tract in both animals and humans. This bacterium is opportunistic so it is closely related to infections that cause disease. In cats, these bacteria have been reported to be associated with the incidence of infectious diseases in the gastrointestinal tract [10], urinary tract infection (UTI) [11] and cats as pets that have the potential to serve as reservoirs for the release of pathogenic *Escherichia coli* to humans.

A resistance test was carried out on 56 samples which were confirmed as *Escherichia coli.* The test results show that not all *Escherichia coli* collected from 10 veterinary clinics show resistance to gentamicin (Table 2). Four veterinary clinics become the source of samples that show *Escherichia coli* that is resistant to gentamicin. Overall, the test results show that there are 80, 36% (45/56) susceptible samples and 19.64%

Table 1. Isolation and Identification of *Escherichia coli*

Test			Test Result	
Eosin Methylene Blue Agar				
	Metallic Green Colony		100% (64/64)	
Gram Staining				
	Baccil/Red		100% (64/64)	
	Coocus/Blue		0,00% (0/64)	
Biochemical Test			Positive	Negative
	IMViC			
		Indole	100% (64/64)	0,00% (0/64)
		MR	96,87% (62/64)	3,13% (2/64)
		VP	0,00% (0/64)	100% (64/64)
		Citrate	7,81% (5/64)	92,19% (59/64)
	Triple Sugar Iron Agar Test			
		Carbohydrate		
		Glucose	95,31% (61/64)	4,69% (3/64)
		Lactose	95,31% (61/64)	4,69% (3/64)
		Sucrose	95,31% (61/64)	4,69% (3/64)
		Gas	87,50% (56/64)	12,50% (8/64)
		H_2S	4,69% (3/64)	95,31% (61/64)
	Carbohydrate Fermentation Test			
		Glucose	89,06% (57/64)	10,94% (7/64)
		Lactose	93,75% (60/64)	6,25% (4/64)
		Sucrore	85,94% (55/64)	14,06% (9/64)
		Maltose	93,75% (60/64)	6,25% (4/64)
		Mannitol	89,06% (57/64)	10,94% (7/64)
	Urea		0.00% (0/64)	100% (64/64)

(11/56) samples show resistance and no samples show intermediate criteria. The highest *Escherichia coli* resistance is found in clinic B, which are six out of seven samples.

The use of gentamicin in cats is oriented towards the treatment of bacterial infections of the urinary tract [12] and topical treatment of external wounds [13]. Gentamicin has a narrow therapeutic dose with a peak concentration ranging from 8-10 mg/L and a valley concentration of 0.5-2 mg/L where changes in small amounts of drug dose can cause toxic effects [14]. Gentamicin works by binding to the 30s ribosomal subunit of bacteria, thereby it inhibits the translocation of peptidyl tRNA from the A site to the P site and disrupts the mRNA code [15]. Bacteria will fail to synthesize proteins vital for their growth. This causes the bacteria to experience changes in cell permeability so that antibiotics will more easily enter the cell and then interfere with cell activity and the cell will die.

Gentamicin is a broad-spectrum aminoglycoside antibiotic that is nephrotoxic to humans and animals. Aminoglycoside antibiotics have two or more amino groups attached to the benzene group and are bactericidal [21] Aminoglycoside antibiotics work effectively against aerobic Gram-negative bacteria. However, aminoglycosides can also be used for Gram-positive bacteria, namely *Staphylococcus* spp. Aminoglycosides interfere with protein synthesis by irreversibly binding to the 30s ribosomal subunit in bacterial cells. This irreversible nature causes antibiotics to be bactericidal because the initiation of protein synthesis is inhibited [16]. [22] states that the aminoglycoside bond with the 30s ribosomal subunit causing errors in reading the genetic code in mRNA, amino acid pairs will form the wrong polypeptide chain so that the protein formed is different (disrupted protein synthesis).

The resistance of Gram-negative bacteria to aminoglycosides is due to the modification of antibiotics

Table 2. Resistance test results

Clinic Code	∑ Sample	Test results		
		S	I	R
Clinic A	7	71,43 (5/7)	-	28,57 (2/7)
Clinic B	7	85,71 (6/7)	-	14,29 (1/7)
Clinic C	11	72,73 (8/11)	-	27,27 (3/11)
Clinic D	1	100 (1/1)	-	-
Clinic E	4	100 (4/4)	-	-
Clinic F	5	80 (4/5)	-	20,00 (1/5)
Clinic G	8	50 (4/8)	-	-
Clinic H	3	100 (3/3)	-	-
Clinic I	4	100 (4/4)	-	-
Clinic J	6	100 (6/6	-	-

Information: Susceptible (S), Intermediate (I), Resistant (R)

by enzymatic activity. These enzymes are aminoglycoside *N-acetyltransferases*, aminoglycoside *O-nucleotidyltransferases*, and aminoglycoside *O-phosphotransferase* [17]. These three enzymes are usually located in the periplasmic space [16]. Each enzyme is encoded by a gene, namely aac, ant, and aph [17]. Enzymes acetylate free amino groups and phosphorylate or adenylate hydroxyl groups so that antibiotics are inactive. Resistance can also occur due to modification of the permeability of the bacterial outer membrane so that the aminoglycoside process entering the bacteria is disrupted [18], as well as changing the aminoglycoside receptor on the 30s ribosomal subunit [16].

4. CONCLUSION

Gentamicin is an aminoglycoside antibiotic that can still be an alternative in the treatment of bacterial infections in cats. The results of this study show that 80.36% of *Escherichia coli* the tested isolates are still susceptible to gentamicin.

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