

Isolation, identification, and antimicrobial sensitivity test of bacteria isolated from the rectal swab of african pygmy hedgehog (*Atelerix albiventris*) and sunda porcupine (*Hystrix javanica*)

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Abstract. *Atelerix albiventris* and *Hystrix javanica* were widely known as pets or livestock in Indonesia, but there has been no study about bacteria from the rectal swab before. This study aims to isolate, identify, and analyze the antibiotic sensitivity of the isolated bacteria from the rectal swab of *Atelerix albiventris* and *Hystrix javanica*. Rectal swab samples were cultured on blood agar plate and identified by selective media and biochemical tests. Kirby Bauer's disk diffusion method was used for the antimicrobial sensitivity test. The result shows that the bacteria identified from the rectal swab samples of *Atelerix albiventris* are *Escherichia coli* (75%) and *Proteus mirabilis* (25%), meanwhile the bacteria identified from *Hystrix javanica* are *Escherichia coli* (100%). The identified *Escherichia coli* found from the sample is sensitive to Amikacin, Amoxicillin, Ampicillin, Enrofloxacin, Fosfomicin, Kanamycin, Chloramphenicol, Streptomycin, Tetracycline, and Trimethoprim; and resistant to Erythromycin and Penicillin G. The identified *Proteus mirabilis* is sensitive to Amikacin, Amoxicillin, Ampicillin, Kanamycin, and Trimethoprim; intermediate to Enrofloxacin; and resistant to Erythromycin, Fosfomicin, Chloramphenicol, Penicillin G, Streptomycin, and Tetracycline. This research concludes that the bacteria found from the rectal swab of *Atelerix albiventris* and *Hystrix javanica* are bacteria with similar species and characters.

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1 Introduction

In certain mammals, some of the hair is modified into spines [1] for example in the hedgehog and porcupine. Both species have spines on their bodies, but taxonomically, the two animals are not related to each other. *Atelerix albiventris* is a hedgehog that is popular as a pet in Indonesia but is not endemic to Indonesia, while *Hystrix javanica* is a porcupine which is endemic to Indonesia [2].

Atelerix albiventris belongs to the family *Erinaceidae* and the order *Eulipotyphla* [3]. Common characteristics of the *Atelerix albiventris* are black eyes and large dark ears [4], a body with black and white spots without dorsal stripes [5], a brown muzzle, and a face, legs, and abdominal surface covered with white hair [4]. Body weight 250-600 grams [6]. *Atelerix albiventris* is a monogastric omnivore that feeds on a wide variety of invertebrates, frogs, lizards, snakes, rats, eggs, fruit and fungi in the wild. Generally, *Atelerix albiventris* are solitary and nocturnal animals [7].

Hystrix javanica belongs to the family *Hystricidae* and the order *Rodentia* [8]. The conservation status of *Hystrix javanica* in the International Union for Conservation of Nature (IUCN) [8] is the least concern or has a low risk of extinction. However, according to the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia Number P.106 / MENLHK / SETJEN / KUM.1 / 12/2018 [9] *Hystrix javanica* is a protected animal. Unfortunately, in some areas in Indonesia, especially Central Java and East Java, the Sunda porcupine is hunted to be sold and consumed for meat because some people believe that consuming porcupine meat can cure asthma and increase body vitality [10]. *Hystrix javanica* generally weigh between 5-16 kg with a round, well-built body, and a small head and ears. Based on the type of food, *Hystrix javanica* is a monogastric herbivore that feeds on fruit, plant roots and tubers in the wild [11].

Atelerix albiventris and *Hystrix javanica*, which are known as pets and animals that are consumed, certainly have a close relationship with human life. Owners of *Atelerix albiventris* or breeders and slaughterers of *Hystrix javanica* have a great opportunity to be exposed to bacteria and other microorganisms through direct contact either with these animals or their feces. In the southern white-breasted hedgehog (*Erinaceus concolor*) which belongs to the same family as *Atelerix albiventris*, it has been reported that bacteria identified from the gastrointestinal tract are *Escherichia coli* (34.5%), *Proteus sp.* (27.5%), *Pseudomonas sp.* (12%), *Shigella sp.* (8.7%), *Yersinia sp.* (6.9%), *Salmonella sp.* (6.9%), and *Klebsiella sp.* (3.4%) [12]. Research [13] stated that the most identified bacteria from the North American hedgehog (*Erethizon dorsatum*) originating from the same suborder as *Hystrix javanica* were bacteria from the order Bacteroidales (52.1%), Clostridiales (30.9%), Burkholderiales (0.1%), Coriobacteriales (0.1%), Pseudomonadales (1.5%), Erysipelotrichiales (0.8%), Flavobacteriales (0.9%), Enterobacteriales (0.3%), Lactobacillales (0.2%), and Verrucomicrobiales (0.9%). Although there has been research that discusses bacteria from the animal gastrointestinal tract which are taxonomically closely related to *Atelerix albiventris* and *Hystrix javanica*, there is no information regarding bacteria from the gastrointestinal tract of *Atelerix albiventris* and *Hystrix javanica*, especially bacteria from rectal swabs and their sensitivity to antibiotics.

This study aims to isolate, identify, and test the antibiotic sensitivity of bacteria from the rectal swabs of *Atelerix albiventris* and *Hystrix javanica*.

2 Materials and Method

2.1 Ethical approval

Ethical clearance (Number: 0129 / EC-FKH / Int. / 2019) was obtained through the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta for this research.

2.2 Sample collection

The samples used in this study were rectal swabs from two female *Atelerix albiventris* (S1 and S2) from the Animal and Ornamental Plant Market in Yogyakarta, Bantul and one female *Hystrix javanica* (S3) from community-owned farms in the Tawangmangu area, Karanganyar. The animals were in good health and there was no indication of digestive system disturbance when the samples were taken. Samples were taken using small cotton swabs. Before use, cotton swabs are moistened with physiological NaCl solution first. The samples were then stored in the Brain Heart Infusion (BHI, Merck™) broth and taken to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta to carry out the isolation and identification process.

2.3 Isolation and identification of bacteria

Each sample was inoculated on two Blood agar plate (BAP) media. One BAP was incubated aerobically (a), and the other BAP was incubated anaerobically (an) for 18-24 hours at 37°C. After the incubation was completed, the colony formed from the sample culture was observed for its morphology in terms of shape, elevation, edge, color, and hemolytic characteristics. Furthermore, Gram staining was carried out to observe the morphology of bacteria for each sample culture and to determine the characteristics of Gram from bacteria in the sample culture. Then, the sample culture was inoculated on selective media such as Eosin Methylene Blue Agar (EMBA, Merck™) and Mac Conkey Agar (MCA, Merck™). The media was incubated aerobically and anaerobically for 18-24 hours at 37°C. After the incubation process was completed, colony morphology and media color changes were observed, then Gram stain was done again to observe the morphology of the bacteria and as a confirmation step that the sample culture was pure. The sample culture was then identified by biochemical tests, such as the catalase test, oxidase test, urease test, carbohydrate fermentation test with glucose, sucrose, lactose, galactose, sorbitol, mannitol and maltose, indole test, methyl red test, Voges Proskauer test, citrate test, and also inoculation of sample cultures on Triple Sugar Iron Agar (TSIA). Then, the bacteria were identified by adjusting the biochemical characters from the literature [14].

2.4 Antibiotic sensitivity test

Antibiotic sensitivity test was carried out using the Kirby Bauer disk diffusion method. Culture of bacterial samples were re-cultured on BHI broth media (Merck™) to obtain stock cultures, then centrifuged at 5000 rpm. The supernatant from the bacterial culture was removed, while the pellet portion was dissolved with Phosphate Buffered Saline (PBS, pH 7.4, Sigma™) to make a suspension. The turbidity of the suspension was equated with McFarland 0.5 which was equivalent to a concentration of 1.5×10^8 CFU / ml [15]. The suspension was homogenized using a vortex mixer and evenly inoculated using a cotton swab on Mueller Hinton Agar (MHA, Merck™) media which had been given an antibiotic disk.

The antibiotic disks used in this study were Amikacin (AK, 30µg), Amoxicillin (AML, 25µg), Ampicillin (AMP, 10µg), Enrofloxacin (ENR, 5µg), Erythromycin (E, 15µg), Fosfomycin (FOS, 50µg), Kanamycin (K, 30µg), Chloramphenicol (C, 30µg), Penicillin G (P, 10IU), Streptomycin (S, 30µg), Tetracycline (TE, 30µg), and Trimethoprim (W, 5µg) (Oxoid™). One plate of MHA media was filled with six antibiotic disks with uniformly spaced between the antibiotic disks. MHA media were incubated aerobically and anaerobically at 37°C for 18-24 hours. After the incubation process, the inhibition zone formed around the antibiotic disk was viewed and measured in diameter, then adjusted by the Clinical and Laboratory Standards Institute [16] to determine the interpretation of bacterial sensitivity to antibiotics.

3 Results

A total of 6 sample cultures from *Aterlix albiventris* and *Hystrix javanica* rectal swabs were collected for this study. Four sample cultures were obtained from *Aterlix albiventris* rectal swabs (S1a, S2a, S1an, S2an), while two sample cultures were obtained from *Hystrix javanica* rectal swabs (S3a, S3an). The results of biochemical tests on all sample cultures were listed in Table 1.

Table 1. Results on selective media, biochemical tests, and Gram staining of isolates from *Aterlix albiventris* and *Hystrix javanica*

Media/ Test	<i>Aterlix albiventris</i>				<i>Hystrix javanica</i>	
	S1a	S2a	S1an	S2an	S3a	S3an
MCA	Pink colonies	Colorless colonies	Pink colonies	Pink colonies	Pink colonies	Pink colonies
EMB	Green metallic sheen colonies	Colorless colonies	Green metallic sheen colonies			
Catalase	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-
Urease	-	+	-	w+	-	w+
Glucose	+	+	+	+	+	+
Sucrose	-	-	-	-	-	-
Lactose	+	-	+	+	+	+
Galactose	+	+	+	+	+	+
Sorbitol	+	-	+	+	+	+
Mannitol	+	-	+	+	+	+
Maltose	+	-	+	+	+	+
Indole	+	-	+	+	+	+
Methyl red	+	+	+	+	+	+
Voges proskauer	-	-	-	-	-	-
Citrate	-	-	-	-	-	-
TSIA	Y/Y	R/Y	Y/Y	Y/Y	Y/Y	Y/Y
H ₂ S production	-	+	-	-	-	-

Gram-staining	Rod, Gram -					
S1: first <i>Atelerix albiventris</i> ; S2: second <i>Atelerix albiventris</i> ; S3: <i>Hystrix javanica</i> ; a: aerobic; an: anaerobic; +: positive results, -: negative results, w+: partially pink, Y/Y: yellow on slant and butt agar of TSIA, Y/R: yellow on slant and butt agar of TSIA.						

Based on the comparison of the biochemical characteristics of the sample culture with the literature [14], *Escherichia coli* was identified in 3 out of 4 (75%) *Atelerix albiventris* rectal swab samples, while *Proteus mirabilis* was identified in 1 out of 4 (25%) *Atelerix albiventris* rectal swab samples. The results of identification in two samples from the rectum swab of *Hystrix javanica* were *Escherichia coli* (100%) (Table 2).

Table 2. Identified bacteria from all isolates

Isolates	Identified Bacteria
<i>Atelerix albiventris</i>	
S1a	<i>Escherichia coli</i>
S2a	<i>Proteus mirabilis</i>
S1an	<i>Escherichia coli</i>
S2an	<i>Escherichia coli</i>
<i>Hystrix javanica</i>	
S3a	<i>Escherichia coli</i>
S3an	<i>Escherichia coli</i>

S1: first *Atelerix albiventris*; S2: second *Atelerix albiventris*; S3: *Hystrix javanica*; a: aerobic; an: anaerobic

Five *E. coli* isolates identified from 3 cultures of *Atelerix albiventris* rectal swab samples and 2 cultures of *Hystrix javanica* rectal swab samples showed sensitivity to the antibiotics Amikacin (100%), Amoxicillin (100%), Ampicillin (100%), Enrofloxacin (100%), Fosfomycin (100%), Kanamycin (100%), Chloramphenicol (100%), Streptomycin (100%), Tetracycline (100%) and Trimethoprim (100%); and resistance to the antibiotics Erythromycin (100%) and Penicillin G (100%) (Table 3). One *P. mirabilis* isolate identified from the *Atelerix albiventris* rectal swab sample culture showed sensitivity to the antibiotics Amikacin, Amoxicillin, Ampicillin, Kanamycin, and Trimethoprim; intermediate to the antibiotic Enrofloxacin and resistant to the antibiotics Erythromycin, Fosfomycin, Chloramphenicol, Penicillin G, Streptomycin, and Tetracycline (Table 4).

Table 3. Results on antibiotic sensitivity test of *E. coli* isolates

Antibiotic	Number of <i>E. coli</i> isolate, n=5 (%)		
	Resistant	Intermediate	Susceptible
AK	-	-	5 (100%)
AML	-	-	5 (100%)
AMP	-	-	5 (100%)
ENR	-	-	5 (100%)
E	5 (100%)	-	-
FOS	-	-	5 (100%)
K	-	-	5 (100%)
C	-	-	5 (100%)
P	5 (100%)	-	-
S	-	-	5 (100%)
TE	-	-	5 (100%)
W	-	-	5 (100%)

AK:Amikacin, AML: Amoxycillin, AMP: Ampicillin, ENR: Enrofloxacin, E: Erythromycin, FOS: Fosfomycin, K: Kanamycin, C: Chloramphenicol, P: Penicillin G, S: Streptomycin, TE: Tetracycline, W: Trimethoprim.

Table 4. Results on antibiotic sensitivity test of *P. mirabilis* isolates

Antibiotic	Number of <i>P. mirabilis</i> isolate, n=1 (%)		
	Resistant	Intermediate	Susceptible
AK			1 (100%)
AML			1 (100%)
AMP			1 (100%)
ENR		1 (100%)	
E	1 (100%)		
FOS	1 (100%)		
K			1 (100%)
C	1 (100%)		
P	1 (100%)		
S	1 (100%)		
TE	1 (100%)		
W			1 (100%)

AK:Amikacin, AML: Amoxycillin, AMP: Ampicillin, ENR: Enrofloxacin, E: Erythromycin, FOS: Fosfomycin, K: Kanamycin, C: Chloramphenicol, P: Penicillin G, S: Streptomycin, TE: Tetracycline, W: Trimethoprim.

4 Discussion

Atelerix albiventris and *Hystrix javanica*, which are known as pets and livestock in Indonesia, both have a close relationship with human life. Owners of African pygmy hedgehogs or porcupine breeders and slaughterers have a great chance of being exposed to bacteria and other microorganisms through direct contact with either the animal or its feces. In another study [12], bacteria have been identified in the gastrointestinal tract of the southern white chest hedgehog (*Erinaceus concolor*), which comes from the same family as the African pygmy hedgehog, such as *Escherichia coli*, *Proteus sp.*, *Pseudomonas sp.*, *Shigella sp.*, *Yersinia sp.*, *Salmonella sp.*, and *Klebsiella sp.* Research [13] stated that the most identified bacteria from the North American porcupine (*Erethizon dorsatum*) originating from the same suborder as the Sunda porcupine, namely the suborder Hystricomorpha, are bacteria originating from the order Bacteroidales, Clostridiales, Burkholderiales, Coriobacteriales, Pseudomonadales, Erysipelotales, Flavobacteriales, Enterobacteriales, Lactobacillales, and Verrucomicrobiales. In this study, the results obtained are in accordance with the two literature [12,13] because the bacteria identified from the rectal swab of *Atelerix albiventris* are *Escherichia coli* and *Proteus mirabilis*, while the bacteria identified from the rectal swab of *Hystrix javanica* are *Escherichia coli*. *Escherichia coli* and *Proteus mirabilis* are bacteria belonging to the *Enterobacteriaceae* family. Bacteria belonging to the *Enterobacteriaceae* are widely distributed in the environment in soil, water, plants, and in the intestines of animals and humans [14].

Based on these results, it can be seen that there are differences in bacterial species identified from *Atelerix albiventris* and *Hystrix javanica* rectal swabs. Differences in the bacteria identified can be influenced by differences in the environment in which the two species live. The *Atelerix albiventris* used in this study came from the Animal and Ornamental Plant Market in Yogyakarta, Bantul. Meanwhile, the *Hystrix javanica* used in the study came from farms in Tawangmangu, Karanganyar. The difference in land elevation, environmental temperature, environmental sanitation, and also the diversity of microbes in

the environment can be factors that cause differences in the bacterial species identified from the two animals.

The next differentiating factor for bacterial species identified is the difference in feed given to these animals. This statement is supported by literature [17] which states that the microflora in the digestive tract (intestinal) is strongly influenced by feed. Based on the type of food, the two species belong to different groups. *Atelerix albiventris* is actually an omnivore which in the wild often feeds on various types of invertebrates, frogs, lizards, fruits, and fungi [7]. In domesticated *Atelerix albiventris*, the usual feed can be crickets, Hong Kong caterpillars (mealworms), or special pellets for mini hedgehogs. Meanwhile, *Hystrix javanica* is a herbivore that in the wild often eats fruit, plant roots and tubers [11]. On farms, *Hystrix javanica* is usually fed in the form of vegetables, fruits and grass.

The identified resistance of *E. coli* and *P. mirabilis* to several types of antibiotics may occur due to inaccurate use of antibiotics, and also because of the transfer of resistant factors through plasmids from bacteria in the environment to bacteria which are normal microflora in hedgehogs and porcupines. Inaccurate use of antibiotics, for example, excessive use of antibiotics in animals, use of antibiotics below the recommended dosage, or use in cases without indications requiring antibiotics. This can result in bacteria becoming resistant to antibiotics. Continuous exposure to antibiotics will result in bacteria forming a self-defense mechanism so that later bacterial growth can no longer be inhibited by antibiotics or called resistance. This is supported by the literature [17] which states that any form of exposure to antimicrobial agents in bacteria will increase the prevalence of bacterial resistance to these antimicrobial agents. Antibiotic exposure can occur through medication in animals or feeding containing feed additives in the form of antibiotics.

The factor that allows bacterial resistance to antibiotics is the transfer of resistant factors through plasmids from bacteria in the environment to bacteria which are normal microflora in an animal. Bacteria can acquire resistant genes through genetic transfer by the conjugation method [18]. Gene exchange occurs between adjacent bacterial cells. A spontaneous DNA mutation can occur in a plasmid in a bacterial cell. These mutations can occur from genes that are resistant to antibiotics. First the plasmid will replicate in bacteria that have a gene that is resistant to an antibiotic, then the resistant gene will be transferred to other bacteria. Plasmids can transfer genetic information between different bacteria. Bacteria in the environment, for example from water, soil, or plants that have genes resistant to antibiotics will also digested into the digestive tract of hedgehogs, for example, so that they can transfer resistant genes through plasmids to bacteria which become normal microflora in the digestive tract of both types of hedgehogs. This causes the normal microflora in the digestive tract, especially the rectum, which was studied in this study to become resistant to antibiotics.

One of the genetic information transferred through plasmids is the enzymatic regulation of protein synthesis which can inactivate antibiotics. Plasmids will control the formation of enzymes in bacteria, for example the synthesis of the enzyme penicillinase (β -lactamase) in *E. coli*. The enzymes produced work in the periplasmic space to degrade the antibiotics, so that the antibiotic components that have penetrated the bacterial outer membrane can be destroyed before the antibiotics arrive at their workplaces [19]. The final impact is that the growth of bacteria can no longer be inhibited by antibiotics so that it can be said that there is bacterial resistance to these antibiotics.

5 Conclusion

This study concluded that the bacteria in the *Atelerix albiventris* rectal swab were *Escherichia coli* and *Proteus mirabilis*. Meanwhile, the bacteria on the rectal swab *Hystrix javanica* is *Escherichia coli*. *Escherichia coli* and *Proteus mirabilis* identified in this study were known to be resistant to several antibiotics. Humans who have a chance of direct contact

with these animals and their feces should maintain sanitation and hygiene, for example by washing their hands after direct contact with animals and their feces, although the pathogenicity of isolated bacterial strains is not known with certainty.

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