Biochemical characteristics and antibiotic resistance of bacterial isolate from *Ctenocephalides felis*

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**Abstract.** Cats are the most domesticated animals kept by humans in the world. Cat fleas are ectoparasites that have the potential to transmit disease caused by microbes in humans. Biochemical identification research and antibiotic resistance tests have been carried out against bacterial isolates from cat fleas. Cat fleas were isolated from cats in Manado City, North Sulawesi, Indonesia. Isolation of bacteria was conducted using the scratch method on nutrient agar media. The pure bacterial culture is then used for biochemical analysis and antibiotic resistance testing. Biochemical analysis and resistance tests were performed using the Vitec 2 Compact instrument with a standardized automatic analysis model. The results obtained three species of bacteria from cat fleas based on biochemical identification, namely *S. equorum*, *C. freundii*, and *Pantoea* spp. Antibiotic resistance test on *S. equorum* showed that of the 60 types of antibiotics used, 55 were sensitive and 7 were resistant. Furthermore, *C. freundii*, of the 18 types of drugs, 7 were sensitive and 11 were resistant. Meanwhile, in *Pantoea* spp., Sensitive and resistant drugs were not found. However, the results of this study prove that bacteria from cat fleas have the potential to infect humans with relatively high antibiotic resistance.

1. **Introduction**

The spread of infectious diseases transmitted by insects is a major public health issue in tropical countries. Species in the insect class are the largest contributor to the arthropod phylum, which act as disease vectors in humans and animals. Several insect species related to public health include *Aedes aegypti* (dengue virus vector), *Anopheles* sp. (malaria vector), *Culex* sp. (vector of filariasis), and fleas (vector of parasites and vector of pathogenic microbes) [1,2]. However, parasite insects in pets such as cats and dogs are potential vectors of pathogenic microbes in humans as well. A very little exploration of pathogenic bacteria found in human-pet parasite insects is still being explored. In fact, pets interact closely with humans, so that they have the potential to be a vector of transmission of various infectious diseases caused by pathogenic viruses and bacteria [3-6].

High population growth rates, uneven population distribution, low educational, and socio-economic levels are factors in the development of diseases transmitted by arthropods to communities in tropical countries [6]. The characteristics of pathogenic microbes that are genetically easy to change have the potential to raise new health problems in the future. One of the pathogenic microbial vectors in pets and humans is cat fleas. As domestic animals, the microbes carried by cat fleas have the potential to infect humans. On the other hand, cat fleas are also ectoparasites [7,8].
C. felis and C. canis are competent vectors for zoonotic pathogens such as Rickettsia and Bartonella spp. Increased knowledge of cat flea diversity and phylogenetics is important for understanding the pathogen transmission cycle sustained by cat fleas. Cat fleas not only cause health problems in animals but also in humans. Reported by Eisen et. al. [3]; The cat flea causes an epidemic of disease caused by Y. pestis in Uganda. Furthermore, cat flea bites can cause flea allergic dermatitis (FAD), for example the case of C. felis cat flea infestation in six male students in Kuala Lumpur with clinical symptoms of Pruritus and Macula papular rashes [9,10]. When sucking blood, cat fleas also inject saliva so that it irritates the host. The indirect impact of cat flea bites is its role as a vector of plague disease [3-5]. Another disease that can be transmitted by cat fleas is parasitic worms in humans because it is the host between the Diphylidium caninum tapeworms [11]. Dipylidiasis cases through oral transmission have been reported by Adam, et al., [12] in a 41-year-old male in Sudan. Thus the identification and test of antibiotic resistance of bacterial isolates from cat fleas are very necessary.

2. Materials and methods

2.1. Sample

The study was started by conducting a location survey as a sampling site for cat fleas (Ctenocephalides felis) on cats in Manado City, North Sulawesi, Indonesia (Figure 1). Determination of location based on the largest number of cats in Manado City. The survey results obtained four locations (four districts) as sampling sites, namely: Karombasan, Malalayang, Paal dua, and Ranotana. The four locations found many cat populations that interact with humans. Each location was taken 10 individual cats as a source of cat fleas. Cat fleas were isolated directly from the body parts of cat samples, namely: head, neck, chest, groin, and back. Each location was taken 10 adult cat fleas without differentiating gender. Caught C. felis insects were included in the sample bottles. The sample was taken to the Parasitology Laboratory of the Medical Faculty, Sam Ratulangi University.
Figure 1. Map of C. felis sample locations from Manado City, North Sulawesi Indonesia (Red circles are sample locations for C. felisi). (Map source: https://www.google.com/maps/place/Sulawesi).
2.2. Isolation of bacteria
The bacteria were isolated from the body surface and sliced from the posterior to the anterior direction of C. felis. Isolation of bacteria was carried out through the scratch method. The bacteria were cultured on nutrient agar media (Merck). After incubation for 3 x 24 hours, the morphology of the bacteria was observed. Incubation was carried out at 37°C in an incubator. The purified isolates were used for biochemical analysis and antibiotic sensitivity testing [13].

2.3. Biochemical analysis of bacterial isolates using Vitek 2 Compact.
Biochemical identification was performed using the Vitek 2 Compact automatic identification instrument, which is available at the Laboratory of the Regional General Hospital Dr. Kandouw Manado (Figure 3). Vitek 2 Compact is an automatic identification system for microorganisms. The latest technology using Vitek 2 Compact makes it easy to use, namely with only 3 stages of examination that will easily obtain the results of identification and sensitivity) of antibiotics that have been validated and interpreted by international standards Clinical Laboratory Standard International (CLSI) [14,15].
The three stages are preparation or standardization of the inoculum turbidity, entering data with a barcode system, and inserting a card into the instrument. Furthermore, the whole process of inoculation, incubation, reading, validation, and interpretation of the results will be carried out automatically by the instrument. Furthermore, the completed examination will automatically produce a printout, while the ID / AST (Identification / Antimicroba Sensitivity Test) card by the system will automatically be discarded.

The principle of automatic identification is to use an identification card, on the card, there is a well or like a biochemical test medium that is modified in such a way that it can be used for rapid identification of bacteria. The test procedure with the Vitek2 Compact tool starts from the gram test, selecting the card, and making a bacterial suspension according to the McFarland standard and identification using the tool until an identification result sheet comes out. Based on the theory that the results obtained in identification with Vitek 2 Compact are expressed as a percentage for the correctness of the identified organisms (Table 1) [14,16].

| Confidence Level | Choice | % Probability |
|------------------|--------|---------------|
| Excellent        | 1      | 96 to 99      |
| Very Good        | 1      | 93 to 95      |
| Good             | 1      | 89 to 92      |
| Acceptable       | 1      | 85 to 88      |

2.4. Analysis of sensitive drugs and resistance to bacteria
Analysis of antibiotics that are sensitive and resistant to bacterial isolates found in cat fleas was carried out together with the identification of bacteria. In this study using an automatic biochemical method (Vitek 2 Compact).

3. Results and discussion
Bacterial isolates from C. felis were identified using the Vitek2 Compact automatic bacterial identification instrument. Vitek 2 Compact is used in bacterial identification and antibiotic sensitivity testing. Validation of analysis results according to clinical laboratory international standards (CLSI). Bacterial isolates were isolated from the body surface and sliced from the posterior to the anterior direction of C. felis. Bacterial isolation which was carried out by using the scratch method resulted in three bacterial isolates with the morphological characteristics of the elevation and colour.
The results of identification by Vitek 2 Compact showed that the three bacterial isolates were different species. Based on the confidence level of the three isolates, it shows that isolate C1 has an acceptable confidence level with a probability of 88%, isolate C2 has a very good confidence level (very good) with a probability of 93%, and isolate C3 has a very good confidence level (very good) with a probability of 95% (Table 2).

Table 2. Results of biochemical identification of isolates with Vitek 2 compact.

| No | Sample Code | Confidence Level | % Probability | Identification Results |
|----|-------------|------------------|---------------|------------------------|
| 1  | C1          | Acceptable       | 88            | Staphylococcus equorum |
| 2  | C2          | Very Good        | 93            | Citrobacter freundii   |
| 3  | C3          | Very Good        | 95            | Pantoea spp            |

The biochemical profile of the analysis results for each isolate is shown in the Vitek 2 Compact output (Tables 3 to 5). S. equorum is a gram-positive bacterium. A total of 43 biochemical test parameters, 14 parameters showed positive results, while the rest were negative. Citrobacter freundii and Pantoea spp are gram-negative bacteria.

Table 3. Biochemical composition of S. Equorum.

| No | Symbol | Chemistry name      | No | Symbol | Chemistry name      |
|----|--------|---------------------|----|--------|---------------------|
| 2  | AMY    | D-Xylene            | 13 | APPA   | L-Proline Arylamidase |
| 13 | CDEX   | Beta-Galactosidase  | 20 | LeuA   | Tyrosin Arylamidase  |
|    |        | Beta-Glucuronidase  | 28 | AlaA   | L-Pyrrolidonyl-Arylamidase |
|    |        | L-Pyrrolidonyl-Arylamidase | 38 | dRIB   | Lactose            |
|    |        | Urease              | 47 | NOVO   | Novobiocin Resistance |
|    |        | dSor                | 57 | dRAF   | Arginine Dihydrolase i |
|    |        | dMAL                | 64 | OPTO   | Saccharose/Sucrose  |

Table 4. Biochemical parameters of Staphylococcus equorum.

| No | Symbol | Chemistry name      | No | Symbol | Chemistry name      |
|----|--------|---------------------|----|--------|---------------------|
| 1  | dXYL   | D-Xylitol           | 23 | ProA   | Beta-Galactosidase  |
| 2  | BGAL   | Beta-Glucuronidase  | 24 | TryA   | Beta-Glucuronidase  |
| 3  | BGURr  | L-Galactosidase     | 25 | ILATk  | L-Glucuronidase     |
| 4  | PyrA   | L-Galactosidase     | 26 | O129R  | L-Glucuronidase     |
| 5  | URE    | Urease              | 27 | ASPA   | L-Glucuronidase     |
| 6  | LAC    | Lactose             | 28 | dSor   | L-Glucuronidase     |
| 7  | NOVO   | Novobiocin Resistance | 29 | SAL    | L-Glucuronidase     |
| 8  | NC6.5  | Growth in 65% NaCl  | 30 | ADHI   | Arginine Dihydrolase i |
| 9  | MBdG   | Methyl B-D Glucopyranoside | 31 | BGAR   | Beta-Galactopyranosidase |
| 10 | dRAF   | D-Fructose          | 32 | AGAL   | Beta-Galactopyranosidase |
| 11 | SAC    | Saccharose          | 33 | NAG    | Beta-Galactopyranosidase |
| 12 | dTRE   | D-Trehalose         | 34 | dMNE   | Beta-Galactopyranosidase |
| 13 | OPTO   | Optochin Resistance | 35 | AMAN   | Beta-Galactopyranosidase |
| 14 | dMAN   | D-Mannitol          | 36 | POLYB  | Beta-Galactopyranosidase |
| 15 | Ure    | Urease              | 37 | dMAL   | Beta-Galactopyranosidase |
| 16 | AMY    | D-Amygdalin         | 38 | AGLU   | Beta-Galactopyranosidase |
| 17 | APPA   | Ala-Phe-Pro Arylamidase | 39 | PHOS   | Beta-Galactopyranosidase |
| 18 | LeuA   | Leucin Arylamidase  | 40 | D GAL  | Beta-Galactopyranosidase |
| 19 | ALA    | Alanine Arylamidase | 41 | BACI   | Beta-Galactopyranosidase |
| 20 | dRIB   | D-Ribose           | 42 | PUL    | Beta-Galactopyranosidase |
| 21 | PIPLC  | Phosphatidylinositol Phospholipase C | 43 | ADH2S  | Beta-Galactopyranosidase |
| 22 | CDEX   | Cyclodextrin       |    |        | Beta-Galactopyranosidase |
### Table 5. Biochemical composition of *Pantoea* spp.

| No | Symbol | Chemistry name | No | Symbol | Chemistry name |
|----|--------|----------------|----|--------|----------------|
| 2  | APPA   | 3              | ADO | 4      | PyrA           | 5              | IARL | 7      | dCEL | 9      | BGAL |
| 10 | H2S    | 11             | BNLG | 12     | AGLtp         | 13             | dGLU | 14     | GGT  | 15     | CFF  |
| 17 | BGLU   | 18             | dMAL | 19     | dMAN          | 20             | dMANE | 21     | BXYL | 22     | BAlap |
| 23 | ProA   | 26             | LIP  | 27     | PLE           | 29             | TyrA  | 31     | URE  | 32     | dSOR |
| 33 | SAC    | 34             | dTAG | 35     | dTRE          | 36             | CIT   | 37     | MNT  | 39     | 5KG  |
| 40 | IALTk  | 41             | AGLU | 42     | SUCT          | 43             | NAGA  | 44     | AGAL | 45     | PHOS |
| 46 | GlyA   | 47             | ODC  | 48     | LDC           | 53             | IHISa | 56     | CMT  | 57     | BGUR |
| 58 | O129R  | 3              | ADO  | 61     | IMLTα         | 62             | ELLM  | 64     | ILATa|        |      |

### Table 6. Biochemical parameters of *Pantoea* spp.

| No | Symbol | Chemistry name | No | Symbol | Chemistry name |
|----|--------|----------------|----|--------|----------------|
| 1  | H2S    | Produksi H2S  | 25 | ProA   | L-Prolin Arylamidase |
| 2  | BGLU   | Beta-Glucose  | 26 | GGAA   | Glu-Gly-Arg-Arylamidase |
| 3  | BGURr  | Beta-Glucuronidase | 27 | PLE    | Palatinose |
| 4  | PyrA   | L-Pyrrolidonyl-Arylamidase | 28 | AGLtp  | Glutamyl Arylamidase Pna |
| 5  | SAC    | Saccharose/Sucrose | 29 | SUCT   | Succinate alkalinization |
| 6  | dTRE   | D-Trehalose    | 30 | ELLM   | Ellman |
| 7  | dMAN   | D-Mannitol     | 31 | BGAL   | Beta-Galactosidase |
| 8  | APPA   | Ala-Phe-Pro Arylamidase | 32 | OFF    | Fermentation Glucose |
| 9  | IALTk  | L-Lactate alkalinization | 33 | LDC    | Lysine Decarboxylase |
| 10 | GlyA   | Glycine Arylamidase | 34 | IMTLα  | L-Malate assimilation |
| 11 | O129r  | O129 Resistance | 35 | IARL   | L-Arabitol |
| 12 | dMAL   | D-Maltose      | 36 | NAGA   | Beta-N-Acetyl |
| 13 | LIP    | Lipase         | 37 | IHISA  | Histidine assimilation |
| 14 | dTAG   | D-Tagatosa     | 38 | BAlap  | Beta-Alanine Arylamidase |
| 15 | AGLU   | Alpha-Glucosidase | 39 | dSOR   | D-Sorbitol |
| 16 | ODC    | Ornithine Decarboxylase | 40 | SKG    | 5-Keto-D-Glconate |
| 17 | dGLU   | d-Glucose      | 41 | PHOS   | Phosphatase |
| 18 | dMNE   | d-Mannose      | 42 | ADO    | Adonitol |
| 19 | TyrA   | Tyrosine Arylamidase | 43 | BNAG   | Beta-N-Acetyl-Glucosaminidase |
| 20 | CIT    | Citrate/Sodium | 44 | ILATa  | L-Lactate assimilation |
| 21 | dCEL   | D-Cellobiose   | 45 | MNT    | Malonate |
| 22 | GGT    | Gamma-Glutamyl-Transferase | 46 | AGAL   | Alpha-Galactosidase |
| 23 | BXYL   | B-Xylose       | 47 | CMT    | Coumarate |
| 24 | URE    | Urease         |        |        |                |

### Table 7. Biochemical Parameters of *Citrobacter freundii*.

| No | Symbol | Chemistry name | No | Symbol | Chemistry name |
|----|--------|----------------|----|--------|----------------|
| 1  | H2S    | Produksi H2S  | 25 | ProA   | L-Prolin Arylamidase |
| 2  | BGLU   | Beta-Glucose  | 26 | GGAA   | Glu-Gly-Arg-Arylamidase |
| 3  | BGURr  | Beta-Glucuronidase | 27 | PLE    | Palatinose |
| 4  | PyrA   | L-Pyrrolidonyl-Arylamidase | 28 | AGLtp  | Glutamyl Arylamidase Pna |
| 5  | SAC    | Saccharose/Sucrose | 29 | SUCT   | Succinate alkalinization |
| 6  | dTRE   | D-Trehalose    | 30 | ELLM   | Ellman |
| 7  | dMAN   | D-Mannitol     | 31 | BGAL   | Beta-Galactosidase |
| 8  | APPA   | Ala-Phe-Pro Arylamidase | 32 | OFF    | Fermentation Glucose |
| 9  | IALTk  | L-Lactate alkalinization | 33 | LDC    | Lysine Decarboxylase |
| 10 | GlyA   | Glycine Arylamidase | 34 | IMTLα  | L-Malate assimilation |
| 11 | O129r  | O129 Resistance | 35 | IARL   | L-Arabitol |
| 12 | dMAL   | D-Maltose      | 36 | NAGA   | Beta-N-Acetyl |
| 13 | LIP    | Lipase         | 37 | IHISA  | Histidine assimilation |

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Table 7. Cont.

| No | Code  | Enzyme Name         | No | Code  | Enzyme Name         |
|----|-------|---------------------|----|-------|---------------------|
| 14 | dTAG  | D-Tagatosa          | 38 | BAAlap| Beta-Alanine Arylamidase |
| 15 | AGLU  | Alpha-Glucosidase   | 39 | dSOR  | D-Sorbitol          |
| 16 | ODC   | Ornithine Decarboxylase | 40 | SKG   | 5-Keto-D-Gloconate  |
| 17 | dGLU  | d-Glucose           | 41 | PHOS  | Phosphatase         |
| 18 | dMNE  | d-Mannose           | 42 | ADO   | Adonitol            |
| 19 | TyrA  | Tyrosine Arylamidase| 43 | BNAG  | Beta-N-Acetyl-Glucosaminidase |
| 20 | CIT   | Citrate/Sodium      | 44 | ILATa | L-Lactate assimilation |
| 21 | dCEL  | D-Cellobiose        | 45 | MNT   | Malonate            |
| 22 | GGT   | Gamma-Glutamyl-Transferase | 46 | AGAL  | Alpha-Galactosidase  |
| 23 | BXYL  | B-Xylose            | 47 | CMT   | Coumarate           |
| 24 | URE   | Urease              |    |       |                     |

The results of the identification of bacteria by biochemical analysis methods showed that three bacterial isolates from C. felis belonged to different species. Furthermore, the three isolates had different biochemical profiles. *Citrobacter freundii* has also been reported to be found on the body surface of cockroaches [17]. Meanwhile, *Staphylococcus* sp is a common pathogenic bacterium [18]. *Pantotea* sp. many are reported to be associated with plants and not with animals [19]. The existence of *Pantotea* sp from the identification results in this study is interesting to study more deeply.

3.1. Antibiotic sensitivity and resistance

The results of the sensitivity and resistance analysis of *C. freundii* showed that seven types of antibiotics were sensitive and 11 types of antibiotics were resistant. A total of 18 drugs are circulating regularly in the market, only seven are sensitive and 11 are resistant. This shows that antibiotics against these bacteria have often been used. Meanwhile, the results of the analysis of drug sensitivity and resistance to *S. equorum*, there were 45 sensitive and only 5 resistances.

The results of the sensitivity analysis and drug resistance of the isolate *Pantotea* spp showed that of the 60 types of antibiotics circulating regularly in the community, generally, 55 were sensitive, while only 5 were resistant. This is certainly very encouraging for the community, indicating that the use of antibiotics against these bacteria is still very little. Many cases of antibiotic resistance *Citrobacter freundii* have been reported. *Citrobacter freundii* has even become multidrug resistance [20]. Report from Indonesia, *Citrobacter freundii* is 100% resistant to cefadroxyl, cefuroxime, cephalexin, clindamycin, doxycycline, erythromycin, lincomycin, oxacillin, colistin sulfate, sulfonamides and metronidazole [21]. Isolated from cockroaches, *Staphylococcus equorum* has been reported to have moderate antibiotic resistance [22]. *Pantotea* sp, has been reported very little in animals. Generally, found in plants.

There are still few isolation studies and analyzes of bacterial antibiotic resistance in insects, especially parasite insects in human pets. Research in this field is important because pathogenic bacteria in pet parasite insects have the potential to infect humans and cause disease in the future.

4. Conclusion

There are three isolates of pathogenic bacteria that were isolated from *C. felis* from Manado City, North Sulawesi, Indonesia. The results of sensitivity and antibiotic resistance tests of the three isolates showed that the level of antibiotic resistance was moderate.

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