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Identification of bacteria on Cockroach feet from Hospital area in Palu city and test of sensitivity to antibiotic

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Abstract. One of the mechanical vectors that co-exist with humans is the Cockroach (*Periplaneta americana*). Cockroaches act as a vector because they are closely related to the presence of bacteria carried on their feet. Cockroaches usually live mainly in homes, restaurants, hotels, hospitals, warehouses, offices, libraries, and elsewhere. These animals are thought to transmit various diseases and act as a vector or as a hospes. This research aims to identify the bacteria in Cockroach feet from Hospital area and test sensitivity to antibiotics. This research is laboratory exploratory, using the standard methods of bacterial isolation and identification, Gram staining, Microbact system KIT and antibiotic sensitivity test using “The Bauer-Kirby standard procedure”. Data obtained from the isolation, Gram staining, biochemical test, and analyzed with microbact system 2000 software. The results of bacterial identification on Cockroach legs, which were obtained from three hospitals area in Palu, *Klebsiella ozaenae*, *Salmoella arizona*, *Salmonella sp.*, *Escherichia coli*, *Salmonella choleraesuis* and *Salmonella simultans*. The results of sensitivity test to six of these bacteria against five kinds of antibiotics found all of these bacteria resistant to Vancomycin, Chloramphenicol, and Amoxicillin, but still sensitive to Ciprofloxacin and Ofloxacin.

1. Introduction

Cockroaches (*Periplaneta americana*) can be found around hospital buildings, such as in-care rooms, intensive therapy zones, surgical sections, kitchens, and medicine room. The hospital area provides a suitable area for breeding Cockroaches [1]. Cockroaches are potential as agents of nosocomial infections such as viruses, bacteria, fungi, and other important pathogenic agents [2], [3]. Cockroaches play an important role as mechanical vectors for pathogenic microorganisms, such as bacteria that cause diarrhea, dysentery, cholera, and polio. Transmission of the disease can occur through the feet or other parts of the Cockroaches that have been contaminated with pathogenic microbes and living habits. They are referred to as an ideal mechanical vector for pathogenic microorganisms [3].

Other researchers report that Cockroaches and flies carry several species of bacteria on their external surfaces. Some identified pathogenic bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus*, *Escherichia coli*, *Enterobacter spp.*, *Klebsiella spp.*, *Serratia spp.*, *Proteus vulgaris*, *Proteus spp.*, *Shigella spp.*, and *Salmonella spp.*, [4], [5], [6], [7]. Bacteria isolated from hospital area in Iran have experienced multi-drug resistant (MDR), against Ampicillin, Chloramphenicol, and Tetracycline [8], [9]. Cockroaches living in the hospital area are very vulnerable to exposure pathogenic bacteria that have been resistant to antibiotics and carried on the leg organs so that potential as a vector causing nosocomial infection [10].



This study is very important because it is novel research as providing information about the dangers of Cockroaches as a vector of pathogenic bacteria and related to bacterial resistance to antibiotics in the hospital and the cause of the nosocomial infection that has not been reported in Indonesia.

2. Materials and methods

This research was conducted in Biology Laboratory, Faculty of Teacher Training and Education of Tadulako University with an explorative and descriptive method. The tools used in this research Photomicroscope, autoclave, incubator, micropipette, Canon-brand camera, Laminar Air Flow (LAF), Microbact system software and digital colony counter. The research materials were bacterial samples from Cockroaches, Nutrient Agar (NA), Triple Sugar Iron Agar (TSIA) (Oxoid Ltd), Eosin Methylene Blue Agar (EMBA) (Oxoid Ltd), Mannitol Salt Agar (MSA) (Oxoid Ltd), Salmonella Shigella Agar (SSA)-(Oxoid Ltd), MacConkey Agar (Oxoid Ltd), Blood Agar (Oxoid Ltd), Gram dye, 70% alcohol, and pepper disc antibiotic.

2.1. Bacterial identification and antibiotic sensitivity test

The sample of this research was the bacteria isolated from Cockroach's feet caught in the hospital area in Palu City, then taken to Laboratory for bacterial identification.

2.1.1. Isolation and culture of bacteria. The process of bacterial isolation and culture following the work procedures are undertaken by Madigan [11], [12].

2.1.2. Identification of bacteria. For bacterial isolation performed in accordance with standard procedure, through culture on the specific medium, Gram staining [13], biochemical test using Kit and Microbact system software [14].

2.1.3. Test antibiotic sensitivity. For bacterial sensitivity analysis of antibiotics using standard procedure "The Bauer-Kirby procedure" [15], [16].

3. Results and discussion

3.1. Characteristics of bacterial colonies grown on general medium

The result of the isolation of bacteria on Cockroach feet that was grown on NA medium, obtained mixed colony showing on figure 1.

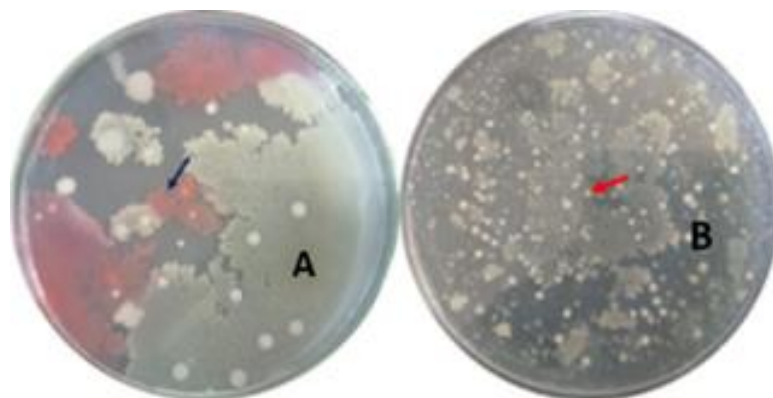


Figure 1. Samples of bacterial colonies in Medium NA isolates, Anutapura Hospital (A), and Madani Hospital (B).

The results of this study showed there were five different bacterial colonies growing on NA medium (Figure 1). Then these colonies was planted on selected medium and found that colonies grown on

EMBA medium characterized by green to metallic blue with irregular colonic surfaces, therefore it was believed as *Escherichia coli* bacteria.

The next bacterial colonies were round, slightly turbid, with a flat surface. This colony was suitable for bacteria *Klebsiella ozaenae*. As we know that *Klebsiella spp.*, grow easily in common media commonly used to isolate Enterobacteriaceae. Therefore, in this study used NA media. *Klebsiella* strains grow as yellow and slimy colonies, while members of Enterobacteriaceae produce smaller colonies with pink, red or orange colonies.

Other bacteria have characteristic round colonies, opaque and white with flat surface colonies, growing on specific SSA medium, the isolated bacteria was *Salmonella sp.* *Salmonella* bacteria are generally fermented glucose, reducing nitrate to nitrite with a negative oxidase test [17], [18]. The use of BSA medium contains bismuth sulfite and brilliant green that can inhibit the growth of Gram-negative bacteria and Gram-positive, and ferrous sulfate as an indicator when the bacteria produce H_2S . Colonies of bacteria that grow in a jet black color called black jet colony [16].

3.2. Results of biochemical test in TSIA media and microbact system

After subsequent colony observations, biochemical tests were performed on the TSIA medium and Microbact system. Biochemical test results are then displayed on the Table 1 of Microbact system test results.

Table 1. Microbact system software analysis results.

Sample	Species of bacteria	Score Kit Microbact system	Level of validity
A	<i>Klebsiella ozaenae</i>	4702	90,14%
B	<i>Salmonella arizona</i>	5741	87,15%
C	<i>Salmonella sp</i>	7713	95,21%
D	<i>Escherichia coli</i>	4720	86,38%
E	<i>Salmonella choleraesuis</i>	7701	88,83%
F	<i>Salmonella sp</i>	7713	95,25%
G	<i>Salmonella simultans</i>	6711	98,65%
H	<i>Escherichia coli</i>	4622	97,08%

Note: A to H is the sample code in Microbact system software.

The results of Microbact system software analysis obtained (Table.1) the percentage of validity level of bacteria species name identified with 86.38% to 98.65% validation range of six types of bacteria found. Biochemical tests aimed to identify the biochemical activity of bacteria in TSIA medium, and continued testing of Microbact system because the TSIA test results have not been able to determine the genus name and type of bacteria obtained. The test result with Microbact system showed that from 8 (eight) samples tested, there were 6 types of bacteria with the following validity: *Klebsiella ozaenae* (90,14%), *Escherichia coli* (86,38%), *Salmonella sp.* (95.25%), *Salmonella choleraesuis* (88.83%) and *Salmonella arizonae* (87.74%), *Salmonella simultaneous* and *E.coli*. This result was in accordance with Microbact system software analysis.

3.3. Gram staining result of bacterial cell morphology

The results of morphology observation of bacterial cells found on Cockroach feet in the area of hospitals in the Palu city as follows:

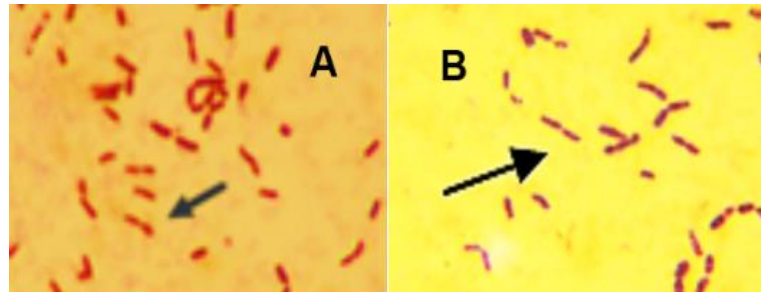


Figure 2. *Salmonella sp* cells, (A) isolates of Madani hospital and *Escherichia coli* (B) isolate Wirabuana hospital.

The results of bacterial staining proved that the bacterial isolates obtained were classified as Gram-negative bacteria. Gram staining is a method of differential staining to distinguish species of bacteria into two large groups (Gram-positive and Gram-negative), primarily based on the chemical and physical properties of their cell walls [19], [20]. Some isolated bacteria showed a high prevalence (92.3%) of microbes on the *P.americana* exoskeleton.

Several bacteria isolated from several samples showed a high prevalence (92.3%) of microbes on *P.americana* exoskeleton. These pathogenic bacteria can lead to gastroenteritis along with other internal or external infections throughout the body, especially in areas with open wounds [21]. Other evidence supports that was found 11 types of bacteria in the intensive Care Room of Children and concluded that Gram-negative bacteria that predominate in the intensive care space of children such as *Staphylococcus sp.*, and *Pseudomonas sp.*, which is an important bacterial cause of nosocomial infections [22].

3.4. Antibiotic sensitivity test results on bacterial isolates

The following is a picture of the diameter antibiotic inhibition zone against the bacteria *Klebsiella* and *Salmonella arizonae*.

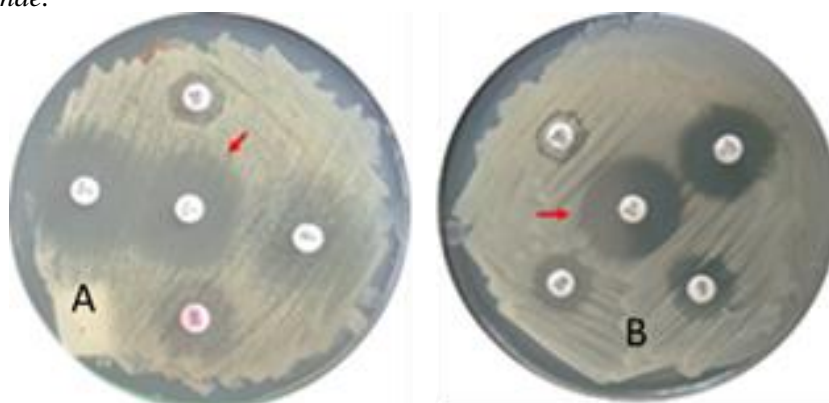


Figure 3. The antibiotic inhibition zone against bacterial isolates *Klebsiella ozaenae* (A) and *Salmonella arizonae* (B).

According to the observation, bacteria have different sensitivity to five antibiotics namely Chloramphenicol, Amoxicillin, Vancomycin, Ciprofloxacin, and Ofloxacin. Based on the results of bacterial isolation of Cockroach feet as many as five types of bacteria were known as Gram negative bacteria namely *Escherichia coli*, *Salmonella arizonae*, *Salmonella sp*, *Klebsiella ozaenae*, *Salmonella simultaneous* and *Salmonella choleraesuis*. The results of this antibiotic sensitivity test have found that

Escherichia coli, *Salmonella arizonae*, *Salmonella sp*, *Klebsiella ozaenae*, *Salmonella simultaneous* and *Salmonella choleraesuis* are resistant to Amoxicillin.

E.coli bacteria, *Klebsiella ozaenae*, *Salmonella choleraesuis* were resistant to Chloramphenicol, whereas *Salmonella sp* bacteria, and *Salmonella* was simultaneously resistant to Amoxicillin and Chloramphenicol, so consideration should be given. The reason for the selection of antibiotics is because these antibiotics, Ciprofloxacin, Ofloxacin, Vancomycin, Amoxicillin, and Chloramphenicol, in the city of Palu and in Indonesia, still be the selected medicine generally for the treatment of infectious diseases.

Quinolone class antibiotics such as Ciprofloxacin have become Drug of Choice in India, replacing Chloramphenicol, Amoxicillin, and Cotrimoxazole which are only used for certain cases. Further research results prove that *Escherichia coli* bacteria resistant to Amoxicillin and Chloramphenicol, but Vancomycin is intermediate [23].

On the other hand, Ciprofloxacin and Ofloxacin were still sensitive to *E.coli* bacteria, so it needs to be considered as the preferred antibiotic. For groups of *Salmonella sp* bacteria, *Salmonella arizona*, *Salmonella choleraesuis*, and *Salmonella simultaneous*, all have been resistant to antibiotics Amoxicillin and Chloramphenicol. However, Ciprofloxacin, Ofloxacin, and Vancomycin were still recommended for using in cases of diseases associated with infection by the *Salmonella* group of bacteria. Therefore, it is necessary to consider using this antibiotic as part of treatment therapy for cases associated with the infection of the *Salmonella* group of bacteria while antibiotics that still have a good enough level of sensitivity. Ciprofloxacin is still recommended to use against the five bacteria because it has a good enough level of sensitivity.

Other researchers reported two Gram-positive bacteria and five Gram-negative bacteria resistant to Ampicillin (13.7% -100%), Chloramphenicol (14.3% -71.4%), Tetracycline (14.3%-73.3%) and Sulfamethoxazole trimethoprim (14.3% - 57.1%) [24]. Similarly, the Enterobacteria group 96% resistant to Gentamicin, 84% against Ampicillin, 75.3% for Cephalothin, 66.7% against Ampicillin-sulbactam, 50% for aztreonam, 30% against Chloramphenicol [25]. This proves that cockroaches living in the hospital area carry resistant bacteria from their environment.

Changes in bacterial resistance to an antibiotic can be caused by several things, such as exposure often exposed to antibiotics, exposure to inadequate antibiotics, and not preceded by a sensitivity test. Likewise, for patients with longer hospitalization may also affect increased resistance because of the risk of infection with resistant strains of higher bacteria. The results of this study can also provide information and description of patterns of bacterial resistance that exist around the Hospital in the city of Palu, and the presence of Cockroaches as a vector causing nosocomial infections. This information and knowledge need to be improved in order to support the use of antibiotics rationally and appropriately and can be used as a guide to empirical therapy in the prevention of nosocomial infections in hospitals.

4. Conclusion

Based on the results of the research it can be concluded that the bacteria identified on Cockroach feet from the hospital area in the Palu city are namely; *Escherichia coli* bacteria, *Klebsiella ozaenae*, *Salmonella arizonae*, *Salmonella choleraesuis*, *Salmonella sp*, and *Salmonella simultaneous*. In addition, three types of antibiotics Vancomycin, Chloramphenicol and Amoxicillin have been resistant to all these bacteria, while Ciprofloxacin and Ofloxacin antibiotics still have a good level of sensitivity.

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