41032-123334-1-SM.doc

by alkauniyahbiologi@gmail.com 1

Submission date: 23-Aug-2024 03:39AM (UTC-0700)
Submission ID: 2436670278
File name: 41032-123334-1-SM.doc (1.28M)
Word count: 5936
Character count: 32357

AL-KAUNIYAH JOURNAL TEMPLATE

DETEKSI DAN IDENTIFIKASI BAKTERI PATOGEN PADA SEMUT *Monomorium* sp. DI LINGKUNGAN RUMAH SAKIT UMUM KOTA KENDARI SULAWESI TENGGARA

DETECTION AND IDENTIFICATION OF PATHOGENIC BACTERIA IN *Monomorium* sp. ANT IN THE ENVIRONMENT OF KENDARI CITY HOSPITAL SOUTHEAST SULAWESI

Amirullah[1], Nur Arfa Yanti[1]*, Wahyuni Nurul Surachma[1], Suriana[1]

[1]Department of Biology, Faculty of Mathematics and Natural Science, Halu Oleo University
Kampus Hijau Bumi Tridharma, Jl. HEA Mokodompit, Anduonohu Kendari Sulawesi Tenggara, 93232

* Corresponding author, e-mail: nur.yanti@uho.ac.id

Abstrak

Semut *Monomorium* sp. merupakan salah satu serangga yang dianggap sebagai hama di lingkungan rumah sakit dan berpotensi menjadi vektor mekanis yang membawa bakteri patogen dan menyebabkan penyebaran penyakit. Penelitian ini bertujuan untuk mendeteksi dan mengidentifikasi bakteri patogen yang ditemukan pada semut *Monomorium* yang berasal dari lingkungan Rumah Sakit Umum Daerah (RSUD) kota Kendari, Sulawesi Tenggara. Pengambilan sampel *Monomorium* sp. dilakukan dengan metode *bait/sugar trap* di 3 lokasi RSUD Kendari, yaitu ruang rawat inap internal, instalasi gizi dan instalasi laboratorium. Deteksi bakteri patogen dilakukan dengan metode tuang pada media selektif, yaitu *Mac Conkey Agar* dan *Mannitol Salt Agar*. Identifikasi bakteri dilakukan dengan analisis numerik-fenetik berdasarkan karakter fenotipik menggunakan aplikasi MVSP 3.1. Hasil penelitian mengidentifikasi 5 spesies bakteri patogen yang ditemukan pada *semut Monomorium* dari lingkungan Rumah Sakit Kota Kendari. Tiga spesies bakteri ditemukan pada semut dari ruang rawat inap internal, yaitu *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* dan *Brevundimonas vesicularis* dan dua spesies ditemukan pada semut dari instalasi gizi, yaitu *Pseudomonas luteola* dan *Staphylococcus auricularis*. Bakteri patogen tidak ditemukan pada semut dari instalasi laboratorium.

Kata kunci*:* Bakteri patogen; infeksi nosokomial; vektor mekanik; semut *Monomorium*

Abstract

Monomorium sp. ant is one of the insects that are considered pests in the hospital environment and potential to be mechanical vectors that can carry pathogenic bacteria and cause the spread of disease. This study aims to detect and identify pathogenic bacteria found in Monomorium sp. ants originating from the environment of Kendari City Hospital, Southeast Sulawesi. Sampling of Monomorium sp. was carried out by the bait/sugar trap method in 3 locations of Kendari City Hospital, namely internal inpatient rooms, nutrition installations and laboratory installations. Detection of pathogenic bacteria was carried out by the pour plate method on selective media, namely Mac Conkey Agar and Mannitol Salt Agar. The identification of bacteria was carried out by numerical-phenetic analysis based on phenotypic characters using the MVSP 3.1 application. The results of the study identified 5 species of pathogenic bacteria found in Monomorium sp. ants from the Kendari City Hospital environment. Three species of bacteria were found in ants from the internal inpatient room, namely Pseudomonas aeruginosa, Stenotrophomonas maltophilia and Brevundimonas vesicularis and two species found in ants from nutrition installations, namely

Pseudomonas luteola and Staphylococcus auricularis. No pathogenic bacteria were found in ants from the laboratory installation.

***Keywords**: Mechanical vector; Monomorium ant; Nosocomial infection; Pathogenic bacteria*

INTRODUCTION

The presence of ants in residential environments and public facilities is known to often cause considerable inconvenience and losses (Klimeš and Okrouhlík, 2015). Ants can transmit disease agents through food contamination and spread diseases due to association with several pathogenic microorganisms (Koehler *et al*., 2017). Generally, people are not too worried if there are ants in their food and continue to consume food that has been infested with ants, even though ants are known to be vectors of disease through their movements. Foodborne diseases will generally cause diseases such as enteric infections and urinary tract infections (UTIs) in consumers who consume these contaminated foods.

One of the public facilities that cannot be separated from the existence of ants is health service places such as hospitals (Lestari *et al*., 2019). Several types of ants, apart from being pests, are also reported to have the potential to be mechanical vectors for various human diseases. Ants can also penetrate wound bandages and other sterile equipment found in hospitals, so ants can be referred to as mechanical vectors that cause the spread of disease (Wetterer, 2010).

Several species of insects can be vectors of pathogenic bacteria, one of which is *the Monomorium* sp. (Setianingsih et al., 2017; Alharbi et al., 2019). *Monomorium* sp. ants are one of the types of insects that are widely found in residential environments, including hospitals (Lestari et al., 2019; Alharbi et al., 2019). The ant that has been known to be the most famous hospital pest in the United Kingdom is *the Monomorium* sp. Based on research also conducted by Alharbi et al. (2019), found that ants and bacterial associations have been detected in many hospitals, raising concerns about the role of ants as vectors of disease and the spread of microbes. Several studies conducted in hospitals have also shown a mutualistic relationship between ants and the presence of bacteria found on the ant's exoskeleton (Lestari et al., 2019). *Monomorium* sp. ants can cause major common problems, such as skin irritation and skin lesions that develop into infections. The infection is due to the presence of pathogenic microorganisms found in *Monomorium* sp. ants, such as *Escherichia coli*, *Pseudomonas* sp., *Salmonella* sp., *Clostridium* sp., *Proteus vulgaris* and *Micrococcus pyogenes* (do-Nascimento et al., 2020). Infection is caused by pathogenic bacteria that can enter the patient's body, healthcare workers or other non-compounding objects and materials that are in the hospital environment such as contaminated devices or food and humid environments through *Monomorium* sp. ants as an intermediate (vector).

Alharbi *et al*. (2019), reported that the bacteria found from ants were 68.8% *of Bacillus* spp. and *Listeria* spp., as well as 16.4% of *Streptococcus* spp. and *Staphylococcus aureus*, while Lestari *et al*. (2019), reported that *Monomorium* sp. ants found in postpartum patient inpatient positive carried *Escherichia coli* and *Bacillus* sp. bacteria, while *Monomorium* sp. found in internal medicine hospitalization and children tested positive carrying *Escherichia coli*, *Bacillus* sp. and *Staphylococcus* sp. bacteria.

This study aims to detect and identify pathogenic bacteria carried by *Monomorium* sp. ants in the Kendari City Hospital environment. Information about pathogenic bacteria that cause nosocomial infections obtained from this study is expected to be a reference in efforts to anticipate disease transmission through *Monomorium* sp. ants as disease vectors.

MATERIALS AND METHODS

This research was carried out from January to March 2023 at Kendari City Hospital and at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari. *Monomorium* sp. ants were collected from Kendari City Hospital. The specific media were used for the detection of pathogenic bacteria namely Mc Conkey Agar (MCA) and Mannitol Salt Agar (MSA) media. The media and reagents were used for the

characterization of bacteria are Nutrient Agar media, Blood Agar media, Gram painting reagents, API (Analytical Profile Index) 20E Kit (characterization kit for the Enterobacteriaceae group), API 20NE Kit (characterization kit for the non-Enterobacteriaceae group) and API 20 Staph Kit (characterization kit for the Staphylococcus group) (Biomerieux, 2015).

Sampling of *Monomorium* sp. Ant

Ant sampling was carried out in the Kendari city hospital environment, including the Internal inpatient Room, Laboratory Installation and Nutrition Installation. Sampling was carried out using *the bait/sugar trap* method by making a bait trap in the form of *a microtube* containing cotton soaked in sugar solution and sterilized. Bait traps (bait/*sugar* traps) are placed open at certain corners on the path that ants often pass and the floor is cleaned first using *alcohol swabs*. If a number of ants have entered the trap, the trap is immediately closed and then stored in the refrigerator before the detection and isolation of bacteria is carried out, so that the temperature is maintained (Setianingsih *et al*., 2017 and Lestari *et al*., 2019).

Detection of Pathogenic Bacteria from *Monomorium* sp.

Detection of pathogenic bacteria from *Monomorium* sp. ants was carried out by the pour plate method on a specific medium. A sample of 5 *Monomorium* sp. ants was put into a bottle containing 90 mL of sterile and suspended aquadest solvent solution. The suspended sample was taken as much as 1 mL using a micropipette and *blue tip* and placed in a vial containing 9 mL of diluent solution and suspended to obtain a 10^{-1} dilution. Then 1 mL of dilution 10^{-1} is taken and placed in a vial containing 9 ml of diluent solution and suspended to obtain dilution 10^{-2}. The dilution stage is carried out until dilution 10^{-3}, then 1 mL is taken from each dilution 10^{-1}, 10^{-2}, 10^{-3} and put into a petri dish, then the selective media MCA (Mac Conkey Agar), and MSA (Mannitol Salt Agar) is poured into each Petri dish that has contained the sample suspension. After that, the petri dish is homogenized and incubated at room temperature for 24 hours. The detection of pathogenic bacteria is then carried out by observing the color of the colony and the shape of the colony that grows on a specific medium, and compared with the literature (Soedarto, 2015; Riski *et. al*., 2017).

Purification of Bacterial Isolate from selective media

Bacterial colonies that grew on a specific medium and exhibit pathogenic bacterial characteristics, are isolated and purified. Bacterial isolates are selected based on differences in colony morphology which include shape, edges, elevation, color and structure in bacteria on a selective medium. Colonies that have different characteristics are isolated by scratching method on Nutrient Agar (NA) medium in petri dishes. Bacterial isolates that have been purified are stored using inclined NA on test tubes for characterization.

Characterization of Bacteria

The characterization of pathogenic bacteria is carried out based on the morphological, physiological and biochemical characterization of bacteria. Cell morphological characterization is carried out by Gram staining. Physiological characterization includes oxygen demand test using Nutrient broth media and biochemical characterization includes catalytic test using H_2O_2 reagent, hemolysis test using Blood Agar media and biochemical tests using API 20E kit, API 20NE kit and API Staph kit.

Identification of Bacteria by Numerical-Phenetic Analysis

This identification was carried out using character data from bacterial isolates and compared with the character of the reference strain The data on the character of bacterial isolate was analyzed using a numerical-phenetic analysis method with the MVSP (*Multi Variate Statistical Package*) program version 3.1. The similarity of the phenotypic character of bacteria is determined based on *the Simple Matching Coefficient* (SSM) value. The grouping was carried out using the UPGMA (*Unweighted Pair Group Method with Arithmatic Averages) algorithm*. The result of the analysis was presented as dendrogram. The resulting dendrogram was used as a basis to determine the similarities between bacterial isolates and reference strains (Yanti et al., 2019).

RESULTS

The detection of pathogenic bacteria in *Monomorium* sp. ants was carried out based on the characteristics of bacterial colonies that grew on the selective media of Mac Conkey Agar (MCA) and Mannitol Salt Agar (MSA). The characteristics of bacterial colonies growing on selective media are shown in Table 1.

Table 1. Detection of bacteria from the *Monomorium* sp. ant on selective media

Sample Location	Isolate code	Media	Colony Characteristics				Suspected bacteria
			Color	Shape	Edge	Media discoloration	
Internal inpatient Room	I.1	MCA	Colorless	round	Flat	Transparent Media	Pseudomonas
	I.2	MCA	Colorless black dot in the middle	round	Flat	Transparent Media	Pseudomonas
	I.3	MCA	Colorless	irregular	Uneven	Transparent Media	Pseudomonas
Nutrition Installation	G.1		Colorless black spot in the middle	irregular	Uneven	Transparent Media	Pseudomonas
	G.2	MSA	Yellowish-white with yellow zones	round	Flat	Media changes from red to yellow	Staphylococcus
Laboratory Installation	There is no growth in MCA and MSA media						

Note : MCA = Mac Conkey Agar, MSA = Mannitol Salt Agar

The cell characteristics of the five bacteria isolates isolated from the selective media were observed using Gram painting to determine the cell shape and Gram reaction shown in Figure 1.

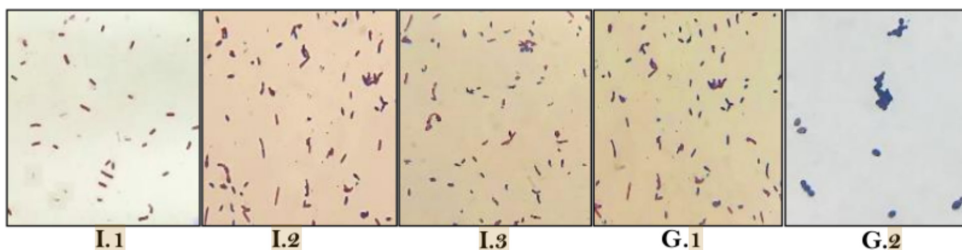

Figure 1. Visualization of bacterial isolate cells from Monomorium sp. observed under a microscope with 1000x magnification

The phenotypic characters analyzed totaled 62 characters including cell morphological characters, biochemical characters and characters using the API Kit, as listed in Table 2. The references species used at the identification stage amounted to 5 species, namely *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Pseudomonas luteola*, *Brevundimonas vesicularis* and

Staphylococcus auricularis. The selection of references species was carried out based on the results of preliminary identification using API software apiweb™ (*https://apiweb.biomerieux.com*).

Table 2. Phenotypic characters of the pathogenic bacteria from *Monomorium* sp. ant and reference species

No.	Characteristics	Bacteria Isolates and references species									
		I.1	***Pseudomonas aeruginosa***	**I.2**	***Stenotrophomonas maltophilia***	**G.1**	***Pseudomonas luteola***	**I.3**	***Brevundimonas vesicularis***	**G.2**	***Staphylococcus auricularis***
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
1.	Bacil Cell Shape	+	+	+	+	+	+	+	+	-	-
2.	Coccus Cell Shape	-	-	-	-	-	-	-	-	+	+
3.	Gram Properties	-	-	-	-	-	-	-	-	+	+
4.	Motility	+	+	+	+	+	+	+	+	-	-
5.	Aerob	+	+	+	+	+	+	+	+	-	-
6.	Anaerob facultative	-	-	-	-	-	-	-	-	+	+
7.	Catalase	+	+	+	+	+	+	+	+	+	+
8.	Fermenting Carbohydrates	-	-	-	-	-	-	-	-	+	+
9.	α-Hemolysis	-	-	-	-	-	-	-	-	-	-
10.	β –Hemolysis	+	+	-	-	-	-	-	-	+	+
11.	γ -Hemolysis	-	-	+	+	+	+	+	+	-	-
12.	Oksidase	-	+	-	-	-	-	+	+	-	-
13.	Ortho nitrophenyl βD Galactopyranosidase (ONPG)	-	-	-	-	+	+	-	-	-	-
14.	Arginine Dihydrolase (ADH)	+	+	-	-	-	-	-	-	-	-
15.	Lysin Decarboxilase (LDC)	-	-	-	-	-	-	-	-	-	-
16.	Ornithin Decarboxilase (ODC)	-	-	-	-	-	-	-	-	-	-
17.	Citrate utilization (CIT)	+	+	+	+	-	-	-	-	-	-
18.	H_2S	-	-	-	-	-	-	-	-	-	-
19.	Urease (URE)	-	-	-	-	-	-	-	-	+	+
20.	Tryptophan deaminase (TDA)	-	-	-	-	-	-	-	-	-	-
21.	Indol production (IND)	-	+	-	-	-	-	-	-	-	-
22.	Voges Proskauer (VP)	+	-	+	-	+	+	-	-	+	+
23.	Gelatinase (GEL)	+	+	-	-	-	-	-	-	-	-
24.	D-glucosa fermentation (GLU)	+	+	-	+	+	+	-	-	+	+
25.	D-mannitol (MAN)	+	+	+	+	+	+	-	-	-	-
26.	Inositol (INO)	-	-	-	-	-	-	-	-	-	-
27.	D-sorbitol (SOR)	-	-	-	-	-	-	-	-	-	-
28.	L-Rhamnose (RHA)	-	-	-	-	-	-	-	-	-	-
29.	D-saccharose (SAC)	-	-	-	-	-	-	-	-	+	+
30.	D-melibiosa (MEL)	-	-	-	-	-	-	-	-	-	-
31.	Amygdalin (AMY)	-	-	-	-	-	+	-	-	-	-

Table 2. Continued

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
32.	Arabinose (ARA)	-	-	-	-	-	+	-	-	-	-
33.	NO3 Reduction	-	-	-	-	-	-	-	-	-	-
34.	L-tryptophan (TRP)	-	-	-	-	-	-	-	-	-	-
35.	Esculin hydrolysis (ESC)	-	-	-	-	-	-	+	+	-	-
36.	4-Nitrophenyl βD galactopyranosidase (PNPG)	-	-	-	-	-	-	-	-	-	-
37.	D-glucose [GLU]	-	-	-	-	-	-	+	-	-	-
38.	L-Arabinose [ARA]	-	-	-	-	-	-	-	-	-	-
39.	D-Mannose [MNE]	-	-	-	-	-	-	-	-	-	-
40.	D-mannitol [MAN]	-	-	-	-	-	-	-	-	-	-
41.	N acetyl glucosamine [NAG]	-	-	-	-	-	-	-	-	-	-
42.	D-maltose [MAL]	-	-	-	-	-	-	-	+	-	-
43.	Potassium gluconate [GNT]	-	-	-	-	-	-	-	-	-	-
44.	Capric acid [CAP]	-	-	-	-	-	-	-	-	-	-
45.	Adiptic acid [ADI]	-	-	-	-	-	-	-	-	-	-
46.	Malic acid [MLT]	-	-	-	-	-	-	-	-	-	-
47.	Trisodium citrate [CIT]	-	-	-	-	-	-	-	-	-	-
48.	Phenylacetic acid [PAC]	-	-	-	-	-	-	-	-	-	-
49.	Fructose (FRU)	-	-	-	-	-	-	-	-	+	+
50.	D-Mannose (MNE)	-	-	-	-	-	-	-	-	+	+
51.	D-maltose (MAL)	-	-	-	-	-	-	-	-	+	+
52.	D-lactose (LAC)	-	-	-	-	-	-	-	-	-	-
53.	D-trehalose (TRE)	-	-	-	-	-	-	-	-	+	+
54.	D-mannitol (MAN)	-	-	-	-	-	-	-	-	-	-
55.	Xylitol (XLT)	-	-	-	-	-	-	-	-	-	-
56.	D-melibiose (MEL)	-	-	-	-	-	-	-	-	-	-
57.	Nitrat reduction (NIT)	-	-	-	-	-	-	-	-	-	-
58.	β-Naphthyl phosphate (PAL)	-	-	-	-	-	-	-	-	-	-
59.	D-Raffinose (RAF)	-	-	-	-	-	-	-	-	-	-
60.	D-xylose (XYL)	-	-	-	-	-	-	-	-	-	-
61.	Methyl αD-glucopyranoside (MDG)	-	-	-	-	-	-	-	-	-	-
62.	N acetyl glucosamine (NAG)	-	-	-	-	-	-	-	-	-	-

Note : Characters 13-62 using the API kit
Characters for reference species based on *Bergey's Manual of Determinative Bacteriology*

The dendrogram results of numerical-phenetic analysis of phenotypic characters between pathogenic bacterial isolates that were successfully detected from *Monomorium* sp. ants at Kendari City Hospital and 5 reference species using the MVSP version 3.1 program are listed in Figure 2.

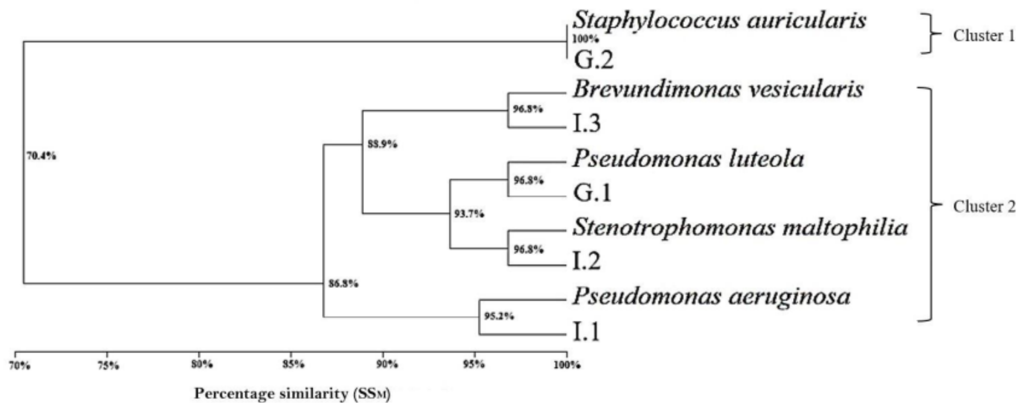

Figure 2. The dendrogram showing the level of similarity between the pathogenic bacterial isolates were detected from *Monomorium* sp. ant at Kendari City Hospital and the references species was based on the analysis of *Simple Matching Coefficient* (SSM) and the *Unweighted Pair-Group Method with Arithmetic Average* (UPGMA) algorithm based on phenotypic characteristics

The results of the identification of pathogenic bacterial isolates detected from *Monomorium* sp. ants at Kendari City Hospital based on numerical-phenetic analysis are listed in Table 3.

Table 3. Pathogenic bacterial isolate species detected from *Monomorium* sp. ants at Kendari City Hospital based on numerical-phenetic analysis

No.	Species	Isolate code	Ant location
1.	*Pseudomonas aeruginosa*	I.1	Internal inpatient Room
2.	*Stenotrophomonas maltophilia*	I.2	Internal inpatient Room
3.	*Brevundimonas vesicularis*	I.3	Internal inpatient Room
4.	*Pseudomonas luteola*	G.1	Nutrition Installation
5.	*Staphylococcus auricularis*	G.2	Nutrition Installation

DISCUSSION

Based on Table 1, it is known that several isolates of pathogenic bacteria detected from *Monomorium* sp. ants were obtained from internal inpatient rooms (3 isolates) and nutrition installations (2 isolates). In the laboratory installation, no bacterial colony growth was found on MCA and MSA media. This is caused in laboratory installations the room is cleaned periodically using antiseptic compounds. In addition to that actions or activities in the laboratory are carried out aseptic, so that the room conditions are always sterile.

Table 1 shows that the characteristics of bacterial colonies in *Mac Conkey Agar* (MCA) media are generally colorless and the media around the transparent colony however there are two isolates (isolate I.2 and G.1) that form black spots in the center of the colony. The characters of this colony is one of the characteristics of bacteria that does not have the ability to ferment lactose and it is suspected that the bacteria belong to the genus *Pseudomonas*. This is in accordance with the statement of Darna *et al*. (2018), which reported that a group of bacteria that had colorless colonies and black spots in the middle of the colony on *Mac Conkey agar* medium showed that the bacteria were not able to ferment lactose, but could break down sulfur-containing amino acids and produce H2S, resulting in the formation of black spots in the middle of the colony. do-Nascimento et al. (2020) stated that Pseudomonas is bacteria do not ferment lactose, and the character of the colony

on the MCA medium is colorless and so that the surrounding bacterial colony is transparent. Mac Conkey agar is a selective medium used to detect rod-form Gram-negative bacteria, especially members of the *Enterobacteriaceae* family and the genus *Pseudomonas* and a differentiation medium between Gram-negative bacilli bacteria that ferment lactose by not fermenting lactose (do-Nascimento et al., 2020).

Bacterial colonies that grow on *Mannitol Salt Agar* (MSA) media (isolate G.2) show a yellowish-white colony character with a yellow zone around it, a rounded shape and flat edges and a color change around the colony from red to yellow (Table 1). This is causes by the bacteria have the ability to ferment mannitol. Bacteria with colony characteristics like this are thought to be a group of bacteria of the genus *Staphylococcus*. This is in accordance with the statement of Dewi, (2013), that the bacterial group of the genus *Staphylococcus* in the Mannitol *Salt Agar* (MSA) medium shows the growth of yellowish-white colonies surrounded by yellow zones due to the ability to ferment mannitol. Bacteria that are unable to ferment mannitol appear to be red or pink zones. The yellow zone indicates the fermentation of mannitol and produces acid, causing a change in the medium from red to yellow (Dewi, 2013; Mamay, 2022). Mannitol salt agar media is a selective and differential medium for detecting *Staphylococcus* bacteria and distinguishing *Staphylococcus* bacteria from other species from the fermentation ability of mannitol which changes the color of the phenol red indicator from red to yellow (Mamay, 2022).

Based on the results of observation of the morphology of bacterial isolate cells isolated from *Monomorium* sp. ants, it is known that four bacterial isolates, namely isolates I.1, I.2, I.3 and G.1, have a bacil (rod) cell shape and the cells are red which indicates Gram negative bacteria, while 1 isolate, namely isolate G.2, has a coccus (round) cell shape with bluish-purple cells indicating Gram positive bacteria (Figure 1). Gram-negative traits in bacteria are characterized by red cells, while Gram-positive traits in bacteria are characterized by cells that are purple when observed under a microscope (Brabb *et al*., 2012). Four bacterial isolates (isolates I.1, I.2, I.3 and G.1) have bacilli-shaped cells and are Gram-negative, indicate that the bacterial isolates belong to the genus *Pseudomonas*. This is in accordance with the statement of Suyono and Farid (2011), state that bacteria of the genus *Pseudomonas* have the characteristics of basil-shaped cells and are Gram-negative. The isolate of G.2 have coccus-shaped cells with a clustered arrangement cell and Gram-positive, is indicated as member of the genus *Staphylococcus*. This statement is supported by Brabb *et al*. (2012), that the genus *Staphylococcus* group has the main characteristics with morphological features in the form of coccus cells and Gram-positive.

Dendrogram based on the similarity of 5 bacterial isolates and 5 references species shown in Figure 2, forming 2 main clusters. Cluster 1 consists of G.2 isolates and the reference species *Staphylococcus auricularis* with a similarity value of 100% while cluster 2 consists of pathogenic bacterial isolates and reference species which are in the genus *Pseudomonas* group with two species having undergone reclassification of the bacterial genus, namely the genus *Brevundimonas* and *Stenotrophomonas* which were previously included in the genus *Pseudomonas*.

Cluster 2, which is a group of the genus *Pseudomonas* with a cluster similarity value of 86.8%, forms 4 subclusters. Subcluster 1 consists of bacterial isolate I.3 and the reference species *Brevundimonas vesicularis* with a similarity value of 96.8% (Figure 2). According to Gupta *et al*., (2014), *Brevundimonas vesicularis* is a Gram-negative bacillus bacterium that is aerobic, non-spore and does not ferment glucose. *Brevundimonas vesicularis* was formerly known as *Pseudomonas vesicularis* and was reclassified as a new genus *Brevundimonas* by Segers *et al*. (1994). Subcluster 2 consists of G.1 and *Pseudomonas luteola bacterial isolates* with a similarity value of 96.8% (Figure 2). Sub cluster 3 consists of isolate bacteria I.2 and *Stenotrophomonas maltophilia* with a similarity value of 96.8% (Figure 2). *Stenotrophomonas maltophilia* was first isolated in 1943 and identified as *Pseudomonas maltophilia* (Hugh and Leifson, 1963). However, based on the results of the analysis of the 16SRNA gene, it is known that *P. maltophila* is more appropriately named *Xanthomonas malthophilia* (Swings *et al*., 1983) and based on further analysis it is proven that this organism has its own genus, so the classification and naming of *Xanthomonas malthophilia* is named *Stenotrophomonas maltophilia* (Palleroni and Bradbury, 1993). Subcluster 4 consists of I.1

and *Pseudomonas aeruginosa* bacteria isolates with a similarity value of 95.2% (Figure 2). Based on the similarity value between bacterial isolate and the reference species shown in Figure 2, it is known that G.2 bacterial isolate is identical to *S. auricularis*, isolate I.3 is identical to *B. vesicularis, isolate* G.1 is identical to *P. luteola,* isolate I.2 is identical to *S. maltophilia* and isolate I.1 is identical to *P. aeruginosa*. Priest & Austin (1993) stated that bacterial isolates are considered identical to a particular species if the value of their phenotypic character similarity ≥ 80%.

Based on Table 3 shows that the pathogenic bacteria detected in *Monomorium* sp. ants from the internal inpatient room consist of 3 species of bacteria, all of which belong to the group of Gram-negative bacil bacteria, namely *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Brevundimonas vesicularis* while the pathogenic bacteria detected in ants from the nutrition plant consisted of a group of Gram-negative bacil bacteria, namely *Pseudomonas luteola* bacteria and a group of Gram-positive coccus *bacteria Staphylococcus auricularis*. These results are in line with a study reported by Setianingsih *et al*., (2017), which found Gram-negative bacilli bacteria in the ants *Monomorium* sp. and Lestari *et al*. (2019), also reported that *Monomorium* sp. ants as the causative vector of nosocomial infections found in hospitals carry the pathogenic bacteria *Staphylococcus* sp.

Pathogenic bacteria detected in *Monomorium* sp. ants as mechanical vectors originating from internal dormitories and nutrient institutions, as shown in Table 3 can cause nosocomial infections. Nosocomial infections or what is referred to as *Health care Associated Infections* (HAIs) are infections that are acquired in the hospital from patients who have been hospitalized for at least 72 hours and the patient does not show symptoms of infection when admitted to the hospital (Baharutan *et al*., 2015).

Disease transmission through mechanical vectors such as insects can come from feces, urine or sputum of the sufferer which are only attached to the body part of the vector and can then be transferred to food or drinks at the time of landing/absorbing the food, thus causing many cases of nosocomial infections (Wijayanti, 2008). According to Sardi, (2021), pathogenic bacteria that have a high virulence level and are often found in humid areas in the hospital environment from the Gram-negative bacteria group, namely *Pseudomonas* spp., and Gram-positive bacteria, namely the genus *Staphylococcus*.

Pseudomonas aeruginosa is one of the bacteria that causes nosocomial infections in humans (Darmadi, 2008). *P. aeruginosa* as an opportunistic pathogenic bacteria can cause invasive conditions in patients with critical illnesses as well as patients with very low immunity levels (Putri *et al*., 2015).

Stenotrophomonas maltophilia is a Gram-negative, aerobic, non-fermenting glucose bacteria, which is a nosocomial pathogenic (Fujita *et al*., 1996). *S. maltophilia* is known as multi-drug-resistant organism pathogenic bacteria (MDROs) Gram-negative causative agents of nosocomial infections that cause respiratory tract infections (pneumonia) in hospitalized patients, bacteremia, endocarditis, meningitis, gastrointestinal infections, as well as urinary tract infections (Pien *et al*., 2015).

Brevundimonas vesicularis is a nonfermented, oxidase and catalase-positive Gram-negative bacil (rod) form bacteria that is one of the human opportunistic pathogenic bacteria that can be found in abundance in the environment and causes several serious diseases in immunocompromised patients (Ryan & Pembroke, 2018). Nosocomial infectious diseases related to *B. vesicularis,* one of which is in dialysis patients who have resistance to quinolones, which is a class of broad-spectrum antibiotics used in the treatment of various bacterial infections (Paramasivam *et al*., 2021). *Pseudomonas luteola* is a Gram-negative bacteria in the form of bacilli and catalase-positive which is the causative agent of respiratory tract infections (pneumonia), urinary tract infections, skin infections, eye infections, ear infections, and *septicemia* in humans that causes sepsis, which is a serious condition because inflammation occurs that extends throughout the body and can cause death (Ali and Aljanaby, 2022).

Staphylococcus auricularis, which is a member of the genus *Staphylococcus*, is a group of opportunistic pathogenic bacteria that can cause infections in humans who have a weakened

immune system (Wahyuni *et al*., 2017). *S. auricularis* is a normal flora on human skin and mucous membranes. This bacteria can be pathogenic because it can hemolyze the blood (Table 2), coagulate plasma and produce enzymes and toxins that are stable at hot temperatures in the intensive care room, so they can cause nosocomial infections due to food poisoning and *toxic shock syndrome* (Baharutan *et al*., 2015).

CONCLUSION AND SUGGESTION

Five pathogenic bacteria were detected in the ants *Monomorium* sp. obtained in the Kendari City Hospital, namely 3 species from the internal inpatient room and 2 species from nutrition installation. Pathogenic bacteria from ants originating from the internal inpatient room were identified as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Brevundimonas vesicularis* and 2 species of bacteria from the nutrition installation, namely *Pseudomonas luteola* and *Staphylococcus auricularis*. Ant *Monomorium* sp. which came from the laboratory installation in the Kendari City Hospital, no pathogenic bacteria were found.

Based on the results of this study, it is known that *Monomorium* sp. ants are a potential vector for the spread of disease-causing bacteria. Therefore, Attention should be focused on the hygiene of food storage, which is considered to be the main source of uncontrolled re-infestation. Hospitals are advised to adopt pest prevention management through licensed professionals.

ACKNOWLEDMENTS

The author would like to thank the management of Kendari City Hospital for providing permits and facilities for the implementation of this research.

REFERENCES

Alharbi, J. S., Alawadhi, Q., & Leather, S. R. (2019). *Monomorium* Ant is a Carrier for Pathogenic and Potentially Pathogenic Bacteria. *BMC Research Notes*, *12*(1), 1–5. https://doi.org/10.1186/s13104-019-4266-4

Ali, M. A., & Aljanaby, A. A. J. (2022). First Case Report of *Pseudomonas Luteola* Isolated From Urinary Tract Infection in Babylon City, Iraq. *Aquaculture*, *381*, 1–6. https://doi.org/10.1051/e3sconf/202338101102

Baharutan, A., Rares, F. E. S., & Soeliongan, S. (2015). Pola Bakteri Penyebab Infeksi Nosokomial pada Ruang Perawatan Intensif Anak di BLU RSUP Prof. Dr. R. D. Kandou Manado. *Jurnal E-Biomedik*, *3*(1), 412–419. https://doi.org/10.35790/ebm.3.1.2015.7417

Biomerieux. (2015). *Api & Id 32 Identification Databases Booklet* (Lyon (ed.)). BioMerieux SA. https://www.biomerieux-diagnostics.com/sites/clinic/files/9308960-002-gb-b-apiweb-booklet.pdf

Brabb, T., Denise, N., Andrew, B., & dan Martha, H. (2012). *Infectious Diseases* (Chapter 23). Elsevier Inc. https://doi.org/10.5694/j.1326-5377.2002.tb04256.x

Darmadi. (2008). *Infeksi Nosokomial Problematika dan Pengendaliannya*. Jakarta: Salemba Medika,

Darna, Turnip, M., & Rahmawati. (2018). Identifikasi Bakteri Anggota Enterobacteriaceae pada Makanan Tradisional Sotong Pangkong. *Jurnal Labora Medika*, *2*(2), 6–12. http://jurnal.unimus.ac.id/index.php/JLabMed

Dewi, A. K. (2013). Isolasi, Identifikasi dan Uji Sensitivitas *Staphylococcus aureus* terhadap Amoxicillin dari Sampel Susu Kambing Peranakan Ettawa (PE) Penderita Mastitis Di Wilayah Girimulyo, Kulonprogo, Yogyakarta. *Jurnal Sain Veteriner*, *31*(2), 138–150.

do-Nascimento, L. E., Amaral, R.R., Ferreira, R. M. A., Trindade, D. V. S., do-Nascimento, R. E., da-Costa, T. S., & Souto, R. N. P. (2020). Ants (Hymenoptera: Formicidae) as Potential Mechanical Vectors of Pathogenic Bacteria in a Public Hospital in the Eastern Amazon, Brazil. *Journal of Medical Entomology*, 20(10), 1–8. doi: 10.1093/jme/tjaa062

Fujita, J., Yamadori, I., Xu, G., Hojo, S., Negayama, K., Miyawaki, H., Yamaji, Y., & Takahara, J. (1996). Clinical Features of *Stenotrophomonas maltophilia* Pneumonia in

357 Immunocompromised Patients. *Respiratory Medicine*, *90*(1), 35–38.
358 https://doi.org/10.1016/S0954-6111(96)90242-5
359 Gupta, P. K., Appannanavar, S. B., Kaur, H., Gupta, V., Mohan, B., & Taneja, N. (2014). Hospital
360 Acquired Urinary Tract Infection by Multidrug-Resistant *Brevundimonas vesicularis*. *Indian*
361 *Journal of Pathology and Microbiology*, *57*(3), 486–488. https://doi.org/10.4103/0377-
362 4929.138789
363 Hugh, R., & Leifson, E. (1963). A Description of The Type Strain of *Pseudomonas maltophilia*.
364 *International Bulletin of Bac Teriological Nomenclature and Taxonomy*, *13*(5), 133–138.
365 Klimeš, P., & Okrouhlík, J. (2015). Invasive Ant *Tapinoma melanocephalum* (Hymenoptera:
366 Formicidae): A Rare Guest or Increasingly Common Indoor Pest in Europe? *European*
367 *Journal of Entomology*, *112*(4), 705–712. https://doi.org/10.14411/eje.2015.089
368 Koehler, P. G., Vazquez, R. J., & Pereira, R. M. (2017). Ants 1. *Department of Entomology and*
369 *Nematology, UF/IFAS Extension*, *1*(1), 1–7. http://edis.ifas.ufl.edu
370 Lestari, D.N., & Ginandjar, R. H. (2019). Kontaminasi Bakteri pada Semut *Monomorium* sp.
371 (Hymenoptera: Formicidae) yang Ditemukan di Ruang Rawat Inap Kelas III Rumah Sakit
372 "X" Kabupaten Kendal. *Jurnal Kesehatan Masyarakat*, *7*(1), 246–251
373 Mamay (2022). Penggunaan ekstrak kayu Secang dan kol ungu pada media Manitol Salt Agar
374 untuk menumbuhkan *Staphylococcus*. *Jurnal Analis Kesehatan Klinikal Sains*, 10(1),62-72
375 Palleroni, N. J., & Bradbury, J. F. (1993). *Stenotrophomonas*, a New Bacterial Genus for
376 *Xanthornonas maltophilia* (Hugh 1980) Swings *et al*. 1983. *International Journal of*
377 *Systematic Bacteriology*, *43*(3), 606–609. https://doi.org/10.1099/00207713-43-3-606
378 Paramasivam, V., Paez, A., Verma, A., Landry, D., & Braden, G. L. (2021). *Brevundimonas*
379 *vesicularis* Peritonitis in a Chronic Peritoneal Dialysis Patient. *Case Reports in Nephrology*
380 *and Dialysis*, *11*(3), 314–320. https://doi.org/10.1159/000517140
381 Pien, C. J., Kuo, H. Y., Chang, S. W., Chen, P. R., Yeh, H. W., Liu, C. C., & Liou, M. L. (2015).
382 Risk Factors for Levofloxacin Resistance in *Stenotrophomonas Maltophilia* From
383 Respiratory Tract in a Regional Hospital. *Journal of Microbiology, Immunology and*
384 *Infection*, *48*(3), 291–295. https://doi.org/10.1016/j.jmii.2013.09.005
385 Priest, F. G., & Austin, B. (1993). *Modern Bacterial Taxonomy* (Second Edi). Chapman & Hall.
386 https://books.google.com/books?id=g2tRKA-_O6UC&pgis=1
387 Putri, A. A., Rasyid, R., & Rahmatini, R. (2014). Perbedaan Sensitivitas Kuman *Pseudomonas*
388 *Aeruginosa* Penyebab Infeksi Nosokomial terhadap Beberapa Antibiotika Generik dan Paten.
389 *Jurnal Kesehatan Andalas*, *3*(3), 327–331. https://doi.org/10.25077/jka.v3i3.112
390 Riski, K, Fakhrurazi dan Mahdi, A. (2017). Isolasi Bakteri *Staphylococcus aureus* pada Ikan Asin
391 Talang-Talang (*Scomberoides commersonnianus*) di Kecamatan Leupung Kabupaten Aceh
392 Besar. *Jurnal JIMVET*, *01*(3), 366–374.
393 Ryan, M. P., & Pembroke, J. T. (2018). *Brevundimonas* spp: Emerging Global Opportunistic
394 Pathogens. *Virulence*, *9*(1), 480–493. https://doi.org/10.1080/21505594.2017.1419116
395 Sardi, A. (2021). Infeksi Nosokomial: Jenis Infeksi dan Patogen Penyebabnya. *Seminar Nasional*
396 *Riset Kedokteran*, *2*(1), 117–125.
397 Setianingsih, I., Ridha, M. R., Hidayat, S., & Andiarsa, D. (2017). Semut sebagai Vektor Mekanik
398 Bakteri di Dalam Gedung Balai Litbang P2B2 Tanah Bumbu, Kalimantan Selatan: Studi
399 Pendahuluan. *Journal of Health Epidemiology and Communicable Diseases*, *3*(2), 42–49.
400 https://doi.org/10.22435/jhecds.v3i2.1791
401 Segers, P., Vancanneyt, M., Pot, B., Torck, U., Hoste, B., Dewettinck, D., Falsen, E., Kersters, K.,
402 & De Vos, P. (1994). Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and
403 *Pseudomonas vesicularis* Busing, Doll, and Freytag 1953 In *Brevundimonas* gen. nov. As
404 *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov.,
405 Respectively. *International Journal of Systematic Bacteriology*, *44*(3), 499–510.
406 https://doi.org/10.1099/00207713-44-3-499
407 Simothy, L., Mahomoodally, F., & Neeto, H. (2018). A study on the potential of ants to act as
408 vectors of foodborne pathogens. *AIMS Microbiology*, 4(2): 319–333. DOI:

409 10.3934/microbiol.2018.2.31
410 Soedarto. (2015). *Mikrobiologi Kedokteran*. 5th edition. Sagung Seto. Hal. 193-384.
411 Suyono, Y., & Farid, S. (2011). Identifikasi dan karakterisasi bakteri Pseudomonas pada tanah yang
412 terindikasi terkontaminasi logam. *Jurnal Biopropal Industri*, *02*(01), 8–13
413 Swings, J., De Vos, P., Van den Mooter, M., & De Ley, J. (1983). Transfer of *Pseudomonas*
414 *maltophilia* Hugh 1981 to the genus Xanthomonas as X*anthomonas maltophilia* (Hugh
415 1981) comb. nov. *International Journal of Systematic Bacteriology*, *33*(2), 409–413.
416 https://doi.org/10.1099/00207713-33-2-409
417 Wahyuni, Y., Jamilah, & Dwi, S. (2017). Isolasi Bakteri Patogen Oportunistik dari Tambak Udang
418 Sumatera. *Agrohita*, *1*(2), 71–75.
419 http://www.tjyybjb.ac.cn/CN/article/downloadArticleFile.do?attachType=PDF&id=9987
420 Wetterer, J. K. (2010). Worldwide Spread of The Pharaoh Ant, *Monomorium pharaonis*
421 (Hymenoptera: Formicidae). *Myrmecological News*, *13*(1), 115–129
422 Wijayanti, T. (2008). Vektor dan Reservoir. *Balaba: Jurnal Litbang Pengendalian Penyakit*
423 *Bersumber Binatang Banjarnegara*, *02*, 18–19.
424 http://ejournal2.litbang.kemkes.go.id:81/index.php/blb/article/view/742
425 Yanti, N. A., Sembiring, L., Margino, S., Muhiddin, N.H., Ahmad, S. W., & Ardiansyah. (2019).
426 Polyphasic Identification of Amylolytic Bacteria Producing Bioplastic Poly-β-
427 hydroxybutyrate (PHB). *Sains Malaysiana* 48(12), 2663–2673.
428 http://dx.doi.org/10.17576/jsm-2019-4812-07.

41032-123334-1-SM.doc

ORIGINALITY REPORT

12%	11%	6%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

#	Source	Type	%
1	bmcresnotes.biomedcentral.com	Internet Source	1%
2	garuda.kemdikbud.go.id	Internet Source	1%
3	journalarticle.ukm.my	Internet Source	1%
4	docplayer.info	Internet Source	1%
5	journal.frontiersin.org	Internet Source	1%
6	sciencedocbox.com	Internet Source	1%
7	Submitted to Universiti Sains Malaysia	Student Paper	1%
8	repository.unimal.ac.id	Internet Source	<1%
9	Rifka A. N. Safitri, Sarsa A. Nisa, Nurul Inayah, Taufiq P. Nugraha et al. "KAJIAN AWAL POTENSI OPOSUM LAYANG (Petaurus		<1%

breviceps) SEBAGAI RESERVOIR BAKTERI ZOONOTIK DAN RESISTENSI ANTIMIKROBA", BERITA BIOLOGI, 2021
Publication

10	doaj.org Internet Source	<1 %
11	www.scilit.net Internet Source	<1 %
12	Saher Islam, Devarajan Thangadurai, Jeyabalan Sangeetha, Zaira Zaman Chowdhury. "Food Microbial and Molecular Biology - From Fundamentals to Applications", Apple Academic Press, 2023 Publication	<1 %
13	www.neliti.com Internet Source	<1 %
14	ojs.uho.ac.id Internet Source	<1 %
15	journal.unnes.ac.id Internet Source	<1 %
16	microbiologynote.com Internet Source	<1 %
17	NINA TURNIP. "Effect of nira (Arenga pinnata Merr) tapping tools on the abudance and identification of microorganisms", Journal of Engineering and Scientific Research, 2023	<1 %

Publication

	Source	
18	www.researchgate.net Internet Source	<1 %
19	ejmcm.com Internet Source	<1 %
20	Theresia Mindarsih, Rosina Kardina Kidi Hurek. "Corelation Between Worm Infection and Anemia in Pregnant Women at Sikumana Health Center, Kupang", Jurnal Aisyah : Jurnal Ilmu Kesehatan, 2021 Publication	<1 %
21	e-journal.unair.ac.id Internet Source	<1 %
22	ijcspub.org Internet Source	<1 %
23	V. V. Kulkarni, P. S. Girish, S. B. Barbuddhe, B. M. Naveena, M. Muthukumar. "Analytical Techniques in Meat Science", CRC Press, 2021 Publication	<1 %
24	Y. Anzai. "Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 07/01/2000 Publication	<1 %
25	journal.biotrop.org Internet Source	

		<1%
26	jppipa.unram.ac.id Internet Source	<1%
27	kneeactive.info Internet Source	<1%
28	repository.upnjatim.ac.id Internet Source	<1%
29	www.ajol.info Internet Source	<1%
30	www.biorxiv.org Internet Source	<1%
31	Busse, H.J.. "Classification and identification of bacteria: current approaches to an old problem. Overview of methods used in bacterial systematics", Journal of Biotechnology, 19960606 Publication	<1%
32	epdf.pub Internet Source	<1%
33	repository.lppm.unila.ac.id Internet Source	<1%
34	repository.ub.ac.id Internet Source	<1%
	www.jeeng.net	

35	Internet Source	<1 %
36	www.scielo.br Internet Source	<1 %
37	"Bioplastics for Sustainable Development", Springer Science and Business Media LLC, 2021 Publication	<1 %
38	Mariam Ahmed Ali, Ahmed Abduljabbar Jaloob Aljanaby. "First case report of Pseudomonas Luteola isolated from urinary tract infection in Babylon City, Iraq", E3S Web of Conferences, 2023 Publication	<1 %

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

41032-123334-1-SM.doc

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12