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

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

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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 and spread pathogenic bacteria and cause nosocomial infections. 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 MacConkey 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

Abstrak

Semut Monomorium sp. merupakan salah satu serangga yang dianggap sebagai hama di lingkungan rumah sakit dan berpotensi sebagai vektor mekanis yang membawa dan menyebarkan bakteri patogen penyebab infeksi nosokomial. 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 MacConkey 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, 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; Semut Monomorium; Vektor mekanik*

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INTRODUCTION

The presence of ants in residential environments and public facilities is known to often cause considerable inconvenience and losses (Picanco et al., 2023). Ants can transmit disease agents through food contamination and spread diseases due to association with several pathogenic microorganisms (Alharbi et al., 2019). 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 (Sharaf et al., 2021).

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% *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 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 used for the characterization of bacteria are buffered peptone water (BPW), Brain Heart Infusion Agar, Nutrient Agar media, Blood Agar media, Gram painting reagents, Analytical Profile Index (API) 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 several 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; 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 selective medium, namely MacConkey Agar (MCA) and Mannitol Salt Agar (MSA). MacConkey agar is a selective medium for growing and differentiating a group of rod-shaped Gram-negative bacteria based on lactose metabolism (Jung & Hoilat, 2024) whereas Mannitol salt agar is a selective and differential medium for isolation and identification of *Staphylococcus* bacteria (Mamay, 2022). A sample of 5 *Monomorium* sp. ants was put into a bottle containing 90 mL of buffered peptone water and a suspended solvent solution. Serial dilutions out to 10^{-3} were prepared in buffered peptone water. 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 and MSA were poured into each petri dish that has contained the sample suspension, and the plate was homogenized. After that, the plates were incubated at 37 °C for 24 hours to detect expected colonies. The detection of pathogenic bacteria was then carried out by observing the color of the colony and the shape of the colony that grows on a selective medium (MCA and MSA media), and compared with the literature. The presumptive colonies of the target bacteria were selected and sub-cultured on brain heart infusion agar, followed by plating on nutrient agar (NA), prior to further identification (StobnickaKupiec et al., 2024).

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 (MacConkey Agar and Mannitol Salt Agar media). Colonies that have different characteristics are isolated by the scratching method on 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 the bacterial isolate was analyzed using a numerical-phenetic analysis method with the Multi-Variate Statistical Package (MVSP) 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

Unweighted Pair Group Method with the Arithmetic Averages (UPGMA) algorithm. The result of the analysis was presented as a 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 MCA and 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	MCA	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= MacConkey 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. The cell morphology of the five bacterial isolates is shown in Figure 1.

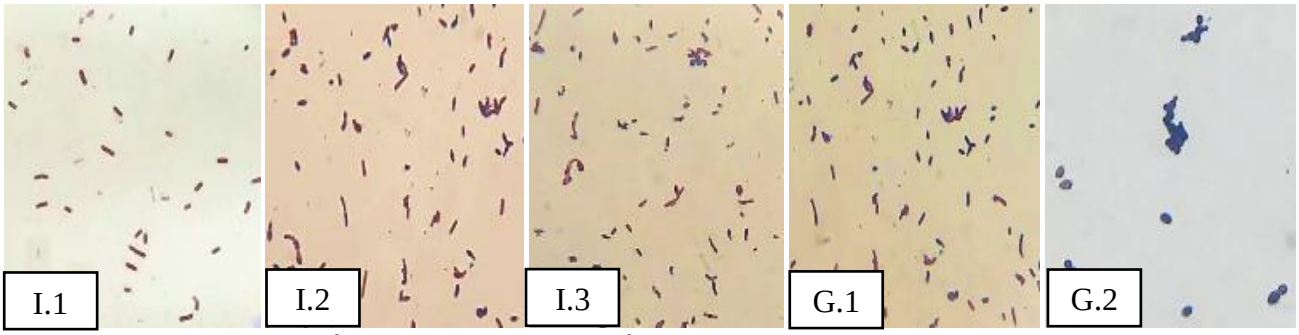

Figure 1. Visualization of bacterial isolate cells from *Monomorium* sp. observed under a microscope with 1000× magnification. Note: I.1, I.2, and I.3 are bacterial isolates isolated from ants from the internal inpatient room; G.1 and G.2 are bacterial isolates isolated from ants from nutrition installation

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 reference 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 reference 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

Characteristics	Bacteria isolates and reference species									
	I.1	*Pseudomonas aeruginosa*	I.2	*Stenotrophomonas maltophilia*	G.1	*Pseudomonas luteola*	I.3	*Brevundimonas vesicularis*	G.2	*Staphylococcus auricularia*
Bacil cell shape	+	+	+	+	+	+	+	+	-	-
Coccus cell shape	-	-	-	-	-	-	-	-	+	+
Gram properties	-	-	-	-	-	-	-	-	+	+
Motility	+	+	+	+	+	+	+	+	-	-
Aerob	+	+	+	+	+	+	+	+	-	-
Anaerobic facultative	-	-	-	-	-	-	-	-	+	+
Catalase	+	+	+	+	+	+	+	+	+	+
Fermenting carbohydrates	-	-	-	-	-	-	-	-	+	+
α-hemolysis	-	-	-	-	-	-	-	-	-	-
β-hemolysis	+	+	-	-	-	-	-	-	+	+
γ-Hemolysis	-	-	+	+	+	+	+	+	-	-
Oksidase	-	+	-	-	-	-	+	+	-	-
Ortho nitrophenyl βD galactopyranosidase (ONPG)	-	-	-	-	+	+	-	-	-	-
Arginine dihydrolase (ADH)	+	+	-	-	-	-	-	-	-	-
Lysin decarboxylase (LDC)	-	-	-	-	-	-	-	-	-	-
Ornithine decarboxylase (ODC)	-	-	-	-	-	-	-	-	-	-
Citrate utilization (CIT)	+	+	+	+	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-
Urease (URE)	-	-	-	-	-	-	-	-	+	+
Tryptophan deaminase (TDA)	-	-	-	-	-	-	-	-	-	-
Indol production (IND)	-	+	-	-	-	-	-	-	-	-
Voges Proskauer (VP)	+	-	+	-	+	+	-	-	+	+
Gelatinase (GEL)	+	+	-	-	-	-	-	-	-	-
D-glucose fermentation (GLU)	+	+	-	+	+	+	-	-	+	+
D-mannitol (MAN)	+	+	+	+	+	+	-	-	-	-
Inositol (INO)	-	-	-	-	-	-	-	-	-	-
D-sorbitol (SOR)	-	-	-	-	-	-	-	-	-	-
L-rhamnose (RHA)	-	-	-	-	-	-	-	-	-	-
D-saccharose (SAC)	-	-	-	-	-	-	-	-	+	+
D-melibiose (MEL)	-	-	-	-	-	-	-	-	-	-
Amygdalin (AMY)	-	-	-	-	-	+	-	-	-	-
Arabinose (ARA)	-	-	-	-	-	+	-	-	-	-
NO_3 reduction	-	-	-	-	-	-	-	-	-	-
L-tryptophan (TRP)	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis (ESC)	-	-	-	-	-	-	+	+	-	-
4-nitrophenyl βD galactopyranosidase (PNPG)	-	-	-	-	-	-	-	-	-	-
D-glucose (GLU)	-	-	-	-	-	-	+	-	-	-
L-arabinose (ARA)	-	-	-	-	-	-	-	-	-	-
D-mannose (MNE)	-	-	-	-	-	-	-	-	-	-
D-mannitol (MAN)	-	-	-	-	-	-	-	-	-	-
N acetyl glucosamine (NAG)	-	-	-	-	-	-	-	-	-	-
D-maltose (MAL)	-	-	-	-	-	-	-	+	-	-
Potassium gluconate (GNT)	-	-	-	-	-	-	-	-	-	-
Capric acid (CAP)	-	-	-	-	-	-	-	-	-	-
Adiptic acid (ADI)	-	-	-	-	-	-	-	-	-	-
Malic acid (MLT)	-	-	-	-	-	-	-	-	-	-
Trisodium citrate (CIT)	-	-	-	-	-	-	-	-	-	-
Phenylacetic acid (PAC)	-	-	-	-	-	-	-	-	-	-
Fructose (FRU)	-	-	-	-	-	-	-	-	+	+
D-mannose (MNE)	-	-	-	-	-	-	-	-	+	+
D-maltose (MAL)	-	-	-	-	-	-	-	-	+	+
D-lactose (LAC)	-	-	-	-	-	-	-	-	-	-

Characteristics	Bacteria isolates and reference species									
	I.1	*Pseudomonas aeruginosa*	I.2	*Stenotrophomonas maltophilia*	G.1	*Pseudomonas luteola*	I.3	*Brevundimonas vesicularis*	G.2	*Staphylococcus auricularia*
D-trehalose (TRE)	-	-	-	-	-	-	-	-	+	+
D-mannitol (MAN)	-	-	-	-	-	-	-	-	-	-
Xylitol (XLT)	-	-	-	-	-	-	-	-	-	-
D-melibiose (MEL)	-	-	-	-	-	-	-	-	-	-
Nitrate reduction (NIT)	-	-	-	-	-	-	-	-	-	-
β-naphthyl phosphate (PAL)	-	-	-	-	-	-	-	-	-	-
D-raffinose (RAF)	-	-	-	-	-	-	-	-	-	-
D-xylose (XYL)	-	-	-	-	-	-	-	-	-	-
Methyl αD-glucopyranoside (MDG)	-	-	-	-	-	-	-	-	-	-
N acetyl glucosamine (NAG)	-	-	-	-	-	-	-	-	-	-

Note: Characters Ortho nitrophenyl βD to N acetyl glucosamine (NAG) using the API kit. Characters for reference species based on Bergey's Manual of Determinative Bacteriology (Holt et al., 2000)

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. The dendrogram in Figure 2 shows the percentage similarity between the five bacterial isolates and the reference species.

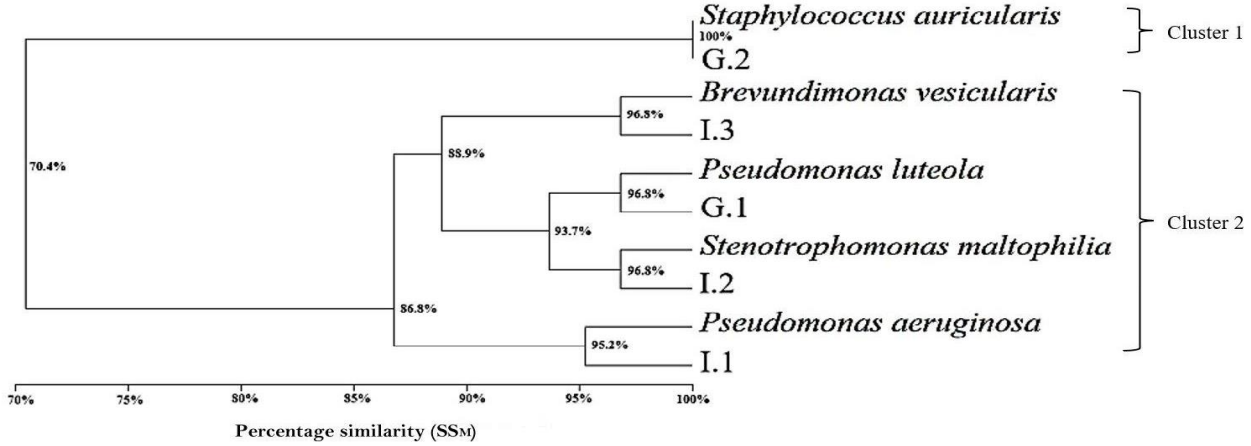

Figure 2. The dendrogram showing the level of similarity between the pathogenic bacterial isolates was detected from *Monomorium* sp. ants at Kendari City Hospital and the reference species was based on the analysis of Simple Matching Coefficient (SSM) and the Unweighted Pair-Group Method with Arithmetic 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. Five bacterial isolates were identified in 5 different species and belonged to 4 genera namely *Pseudomonas, Stenotrophomonas, Brevundimonas,* and *Staphylococcus*.

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

Species	Isolate code	Ant location
Pseudomonas aeruginosa	I.1	Internal inpatient Room
Stenotrophomonas maltophilia	I.2	Internal inpatient Room
Brevundimonas vesicularis	I.3	Internal inpatient Room
Pseudomonas luteola	G.1	Nutrition Installation
Staphylococcus auricularia	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 where 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 MacConkey 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 characteristics of this colony is one of the characteristics of bacteria that do 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 Jung and Holiat (2024), which reported that a group of bacteria that had colorless colonies and black spots in the middle of the colony on MacConkey agar medium showed that the bacteria were not able to ferment lactose, but could break down sulfur-containing amino acids and produce H_2S, resulting in the formation of black spots in the middle of the colony. Do Nascimento et al. (2020) stated that *Pseudomonas* is a bacteria that does not ferment lactose, and the character of the colony on the MCA medium is colorless so that the surrounding bacterial colony is transparent. MacConkey 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; Al-Saffar & Jarallah, 2019; Jung & Holiat, 2022).

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 caused by the bacteria having 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 by the statement of Widianingrum and Salasia (2021), 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 (Widianingrum & Salasia, 2021; 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 basil (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 purple cells when observed under a microscope (Tian et al., 2022). Four bacterial isolates (isolates I.1, I.2, I.3, and G.1) have bacilli-shaped cells and are Gram-negative, indicating that the bacterial isolates belong to the genus *Pseudomonas*. This is to the statement of Sekhi (2022), states that bacteria of the genus *Pseudomonas* have the characteristics of basil-shaped cells and are Gram-negative. The isolate of G.2 has coccus-shaped cells with a clustered arrangement cell and is Gram-positive, is indicated as a member of the genus *Staphylococcus*. This statement is supported by Tian et al. (2022), 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 reference 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 Stabler et al. (2018), *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 isolating 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 & 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 genus, so the classification and naming of *Xanthomonas malthophilia* is named *Stenotrophomonas maltophilia* (Palleroni & 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.* Yanti et al. (2019) 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 installation 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 installations, 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 (Raoofi et al., 2023).

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 (Carrasquilla et al., 2023). 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 (Shi et al., 2023). *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 (Qin et al., 2022).

Stenotrophomonas maltophilia is a Gram-negative, aerobic, non-fermenting glucose bacteria, which is a nosocomial pathogenic (Raad et al., 2023). *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 (Hafiz et al., 2022; Said et al., 2021).

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., 2022).

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 & Aljanaby, 2023).

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 (Ha & Heitner, 2021). *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* (Williford et al., 2018).

CONCLUSION

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*, *Brevundimonas vesicularis*, and 2 species of bacteria from the nutrition installation, namely *Pseudomonas luteola* and *Staphylococcus auricularis*. *Monomorium* sp. ant 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.

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