Journal of Public Health and Pharmacy
2775-4952 (Online - Elektronik)
Research Articles Open Access

Pathogenic Bacteria on Musca domestica: Identification and Antibiotic Sensitivity via MALDI-TOF

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ARTICLE INFO

Manuscript Received: 20 Oct 2024
Revised: 21 Jan, 2025
Accepted: 25 Jan, 2025
Date of publication: 10 Feb, 2025
Volume: 5
Issue: 1
DOI: 10.56338/jphp.v5i1.6268

KEYWORDS

MALDI-TOF;
Mass Spectrometry;
Pathogenic Bacteria;
Antibiotic Sensitivity;
Flies

ABSTRACT

Introduction: Flies are mechanical vectors that can transmit a variety of pathogenic bacteria, potentially causing human infections, especially in hospital settings. Rapid and accurate identification of these pathogenic bacteria, as well as assessment of their sensitivity to antibiotics, is essential for effective infection control.
Methods: Sampling of flies in temporary rubbish bins in the hospital environment. Isolation of pathogenic bacteria from fly body surface by culture method, identification of bacteria using MALDI-TOF Mass Spectrometry technology. Antibiotic sensitivity test was conducted using Viteks 2 Compact to assess the effectiveness of therapy against the isolated bacteria. Ethical approval was obtained from Hasanuddin University Makassar.
Results: Four species of pathogenic bacteria, namely Staphylococcus sciuri, Staphylococcus xylosus, Klebsiella pneumoniae, and Proteus mirabilis, were successfully identified quickly and accurately using the MALDI-TOF Mass Spectrometry method. Staphylococcus sciuri and Staphylococcus xylosus are sensitive to Benzylpenicillin, Oxacillin, and Ciprofloxacin, although Staphylococcus sciuri shows moderate resistance to Moxifloxacin. Klebsiella pneumoniae is resistant to Ampicillin, but is effective when combined with Sulbactam, while Proteus mirabilis is generally sensitive, except for Tigecycline. These findings are relevant for infection prevention strategies in hospitals, providing more appropriate antibiotic use guidance and supporting antimicrobial resistance control programs.
Conclusion: MALDI-TOF Mass Spectrometry method proved effective in the rapid and accurate identification of pathogenic bacteria from flies. Antibiotic sensitivity testing is essential to determine the most effective therapy and avoid the use of less effective antibiotics.

Publisher: Pusat Pengembangan Teknologi Informasi dan Jurnal Universitas Muhammadiyah Palu

INTRODUCTION

Research on pathogenic bacteria found in flies has become an important topic in microbiology, given the role of flies as vectors in the spread of infectious diseases. Flies are often found to carry a variety of pathogenic bacteria that can cause disease in humans, especially in environments with poor sanitation (1),(2),(3)(4). Flies, particularly Musca domestica species, have long been recognised as mechanical vectors that can transmit more than 100 types of pathogens, including bacteria, viruses and parasites. (5)(6), (7). Houseflies have an important role in transmitting bacteria such as Escherichia coli and Salmonella spp. from contaminated sources to human food (8). Research (9) successfully isolated various types of bacteria from the body surface of flies, but their sensitivity to various types of antibiotics is not yet known.

Although various studies have explored pathogenic bacteria in flies, most of the previous studies used culture-based identification methods, which are time-consuming and have limitations in detecting non-culture bacteria (10). These traditional identification methods are also often insensitive to bacterial strains that have different phenotypes. Recent research has shown that molecular, such as mass spectrometry, can provide faster and more accurate results compared to conventional culture methods (11). However, studies that inoculated the rinsed suspensions on Mac-Conkey Agar media, EMBA (Eosin methylene Blue Agar) for Gram-negative bacteria, and Media MSA (Mannitol Salt Agar) for gram positive bacteria. The application of technologies such as VITEK®MS FLEXPREP with MALDI-TOF Mass Spectrometry method for identification of pathogenic bacteria from flies is still limited (12), (13) (14).

This study used VITEK®MS FLEXPREP technology MALDI-TOF Mass Spectrometry method for rapid and effective identification of pathogenic bacteria from flies. A comparative study reported that MALDI-TOF technology reduced the time to obtain results from 24 to 48 hours to less than 30 minutes. This technology has not been widely used in the context of fly-borne bacteria research, which makes this study unique and has the potential to make a significant contribution to understanding fly-borne microbial profiles (13)(14),(15). On the other hand, increasing antibiotic resistance in fly-borne pathogenic bacteria is also a major concern. Previous studies have shown an increase in resistance to common antibiotics in bacteria isolated from flies in urban environments (16), (17).

Antibiotic resistance remains one of the biggest threats to public health (18), (19). Despite several years of efforts to reduce the selection and transfer of resistance through the rational use of antibiotics. In recent years there has been an increase in the rate of resistance to antibiotics. Various types of bacteria have become antibiotic resistant such as Staphylococcus aureus (MRSA), multidrug-resistant (MDR) Mycobacterium tuberculosis, Acinetobacter sp. which is resistant to most antimicrobial agents (20), (21). There are 20 bacterial isolates from flies showing a presentation of resistance to antibiotics, namely erythromycin 75%, tetracycline 50%, chloramphenicol 25%, amoxycillin 25%, but the type is not yet known (22).

The use of VITEK2 Compact is an automated system that can test the sensitivity of bacteria to various antibiotics with high accuracy. The use of this tool in research on bacteria found on flies has not been widely reported, thus making a new contribution to microbiological testing methods. Substantially, this study is also relevant in efforts to prevent nosocomial infections in hospitals, where flies are often found and can act as agents of pathogen transmission. The results of this study may assist in the development of more effective infection control strategies (23).

Previous research by Anderson (24)(25) showed that the level of environmental sanitation has a direct correlation with the number of pathogenic bacteria found on flies. This study will extend those findings by adding the dimension of rapid identification and antibiotic sensitivity testing, which has not been widely explored. Although many studies have been conducted to identify bacteria in flies, challenges in detecting antibiotic resistance with conventional methods remain. This study overcomes these obstacles by using the latest technology that can generate faster and more accurate data.

The use of VITEK MS® FLEXPREP in this study will not only identify bacteria quickly but also allow the detection of a broader spectrum of bacteria, including bacteria that are difficult to identify with ordinary culture methods. This technology has been proven effective in clinical studies, but has not been widely applied in environmental studies such as this. This study is expected to make a significant contribution to the microbiological and pharmaceutical literature by providing new data on the microbial profile of flies and their antibiotic resistance patterns. This will help in the development of more effective public health policies (26).

Given the significant role of flies as disease vectors, the results of this study are expected to contribute to more effective disease prevention efforts, particularly in the context of infection control in urban and hospital environments.

The main objective of this study was to identify pathogenic bacteria carried by flies and determine their sensitivity to various antibiotics, with the hope of providing information that can be used in the development of more effective antibiotic therapies (27), (28).

This study fills the gap by providing specific data on pathogenic bacteria carried by flies, which have been underpaid as an alternative to overcoming antibiotic resistance. By analysing the sensitivity of bacteria to various antibiotics, this study provides important information about existing resistance patterns. The results can be used to identify alternative antibiotics that are still effective, help overcome infections that are resistant to standard therapy, and support the development of more targeted antibiotic resistance control policies and programs.

Studying antibiotic sensitivity has important significance in facing the ever-increasing challenge of antimicrobial resistance. Antibiotic resistance is a serious threat to global health, as it reduces the effectiveness of treatment against bacterial infections, increases morbidity, mortality, and treatment costs. In this context, antibiotic sensitivity analysis to isolated bacteria, such as from flies, can provide critical information about existing resistance patterns. This knowledge allows for the identification of alternative antibiotics that are still effective, so that they can be used as a solution to address infections that are resistant to standard therapy. In addition, these findings also contribute to the development of policies for the wise use of antibiotics, support antimicrobial resistance control programs, and prevent the spread of resistant bacteria in the health environment and society.

METHOD

Research Design

This study is an experimental study, to test the sensitivity of bacteria isolated from flies to antibiotics. The selection of antibiotics for sensitivity tests in this study, such as Benzylpenicillin, Oxacillin, Ciprofloxacin, Moxifloxacin, and Ampicillin, was based on their clinical relevance as well as the spectrum of activity against the pathogenic bacteria tested. Benzylpenicillin and Oxacillin represent β-lactam antibiotics that are often used to treat infections caused by gram-positive bacteria, including Staphylococcus spp. Ciprofloxacin and Moxifloxacin, which belong to the fluoroquinolone class, were chosen for their effectiveness in inhibiting DNA gyrase in bacteria, making them a mainstay in the therapy of both gram-negative and gram-positive bacterial infections. Ampicillin, as a broad-spectrum β-lactam, is used to evaluate the sensitivity of gram-negative bacteria such as Klebsiella pneumoniae. The selection of these antibiotics includes a variety of mechanisms of action, thus providing comprehensive sensitivity data to support the appropriate treatment of bacterial infections and prevent the spread of antimicrobial resistance

Collection of M. domestica

Flies were collected in temporary waste bins in the hospital environment using sterile plastic traps. The sampling technique was done by random sampling, where about 30 flies were caught using fly traps in the form of sterile plastic bags. Fly sample containers were handled sterile. Fly samples are immediately taken to the laboratory for isolation of pathogenic bacteria. The house fly sample was confirmed as M. domestica in the laboratory using a determination method based on morphology with entomologists (Figure 1).

The sample in this study was flies taken from the BRUD Luwuk garbage collection location. The number of samples taken was 10 mosquitoes that were caught using sterile nets. All flies that are netted are used as samples without specifying a special catch point assuming that the sampling location has the same characteristics.

Culture and isolation of bacteria from house flies

Prior to bacterial isolation, external sterilization is performed to remove contaminants on the fly surface. This procedure involves washing the flies in a 70% ethanol solution for 1 minute, followed by rinsing using sterile water to avoid the effects of alcohol residues that may affect the results of bacterial isolation (29).

The method of isolating bacteria from the body surface of flies begins with washing the externally sterilized flies in physiological NaCl solution to release bacteria attached to the body. The rinsed suspension was inoculated on Mac-Conkey Agar media, EMBA (Eosin methylene Blue Agar) for Gram-negative bacteria, and Media MSA (Mannitol Salt Agar) for bacteria Gram positive. After incubation at 37°C for 24-48 hours, morphological observations of growing bacterial colonies were made including color, shape, size, edge, surface, and elevation. Furthermore, purification of each growing bacterial colony was carried out until a pure culture was obtained.

Bacterial Identification by MALDI-TOF Mass Spectrometry method

The process of identifying pathogenic bacteria using MALDI-TOF MS technology begins with the isolation of bacterial colonies from culture media, which are then taken and mixed with a special matrix (usually sinapinic acid) on a MALDI target plate. Laser light is emitted to ionize proteins, mainly ribosomal proteins, from the bacterial sample. These ionized proteins are then accelerated in an electric field and detected based on their time of flight to the detector. Each bacterium has a unique protein spectrum profile resulting from the passage of proteins that vary based on their molecular weight. These spectra are compared to a MALDI-TOF database that already has spectral profiles of various known bacteria. Identification is performed by matching the protein spectrum of the sample to the reference spectrum in the database, thus enabling rapid and accurate identification down to the species level (12)

Bacterial Sensitivity Test to Antibiotics

The process of bacterial sensitivity testing using the VITEK 2 Compact system begins with preparing a suspension of bacteria that have been identified, according to a certain concentration (0.5 McFarland). This bacterial suspension is then inserted into a VITEK 2 test card that already contains various types of antibiotics in different concentrations. This card is inserted into the VITEK 2 Compact device which automatically reads and measures the bacterial growth in each well through the principle of turbidimetry. The resulting data is compared to an internal database to determine the Minimum Inhibitory Concentration (MIC) value. This MIC indicates the lowest antibiotic concentration that can still inhibit bacterial growth, thus providing an antibiotic sensitivity profile of the tested bacteria. Subsequently, the data were analysed descriptively.

RESULTS

Fly Identification

One type of flies were collected from the hospital's temporary rubbish bins. Based on the results of morphological identification, type of flies were obtained, namely house flies (Musca domestica).

A B C

Morphology of Musca domestica flies; A. Dorsal Thorax B. Abdomen; C. Wings

The house fly is greyish black incolour with four longitudinal dark lines on the dorsal thorax. Thewings of the housefly refer to the shape of the housefly (*Musca domestica* Linn.)venation, namely the4th vein (M1+2) curves sharply towards the costa approaching the 3rd vein (R4+5).

Bacterial isolation from flies

Based on the results of bacterial isolation from the body surface of *Musca domestica* flies, the same bacterial colonies were obtained as in table 1 below.

Table 1. Morphology of bacterial colonies on various types of culture media

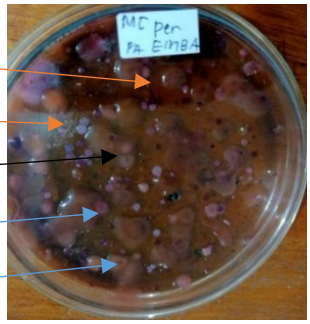
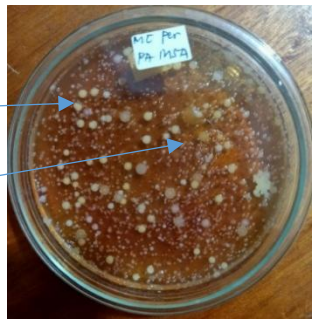
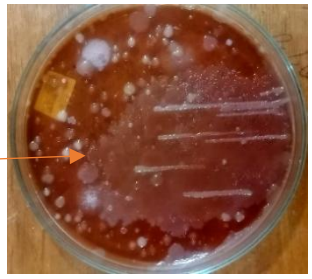
No.	Isolat Code	Sample Origin	Colony Morphology
1	Col.1/EMBA Col.2/EMBA Col.3/EMBA Col.4/EMBA Col.5/EMBA	Body surface of *M. domestica* flies	
2	Col.2/MSA Col.7/MSA	Body surface of flies *M. domestica*	
3	Col 3/MaC	Body surface of *M. domestica* flies	

Table 2. Colony Characteristics on Various Culture Media

Sample	Color	Shae	Size	Consistency	Edge	Surface	Elevation
C1/EMBA	Purple green gloss	Round	Great	mucoid	entire	Smooth and glossy	Convex
C2/EMBA	Pink	Round	Medium	mucoid	entire	Smooth and glossy	Convex
C3/EMBA	Light purple	Round	Small	Dry	Flat	Smooth	Convex
C4/EMBA	Pink	Large round	Great	Mucoid	Flat	Smooth and glossy	Convex
C5/EMBA	Deep purple	Small round	Small	Dry	Flat	Smooth	Convex
C2/MSA	Putih	round	Medium	Mucoid	Flat	Smooth	Convex
C7/MSA	Light yellow	round	Medium	Dry	Flat	Smooth	Convex
C3/MaC	Colourless	Irregular	Large	Dry	Uneven	Smooth	Flat

Table 2 shows the characteristics of bacterial colonies from various samples on different culture media, namely Eosin Methylene Blue Agar (EMBA), Mannitol Salt Agar (MSA), and MacConkey Agar (MaC). Bacterial colonies on EMBA media showed color variations ranging from purple to glossy green to pink, with round shapes and sizes varying from small to large. Most colonies had a mucoid consistency, flat edges (entire), smooth and shiny surfaces, and convex elevations, except for samples C3/EMBA and C5/EMBA which were dry and small in size. On MSA media, sample C2 has white colonies with a slimy consistency, while sample C7 is light yellow with a dry consistency; both have a round shape, flat edges, smooth surface, and convex elevation. Meanwhile, bacterial colonies on MaC media showed more varied characteristics, where sample C3 was colorless, irregularly shaped, large, dry consistency, uneven edges, smooth surface, and flat elevation. This variation shows that different culture media can affect the growth and morphological characteristics of the resulting bacterial colonies.

Table 3. Types of Pathogenic Bacteria Identified Results

Sample	Type of Bacteria
C1/EMBA	Klebsiella pneumoniae
C/EMBA	Klebsiella pneumoniae
C3/EMBA	Klebsiella pneumoniae/Klebsiella variculata
C4/EMBA	Klebsiella pneumoniae
C5/EMBA	Klebsiella pneumoniae
C2/MSA	Staphylococcus sciuri
C7/MSA	Staphylococcus xylosus
C3/MaC	Proteus mirabilis

Table 3 shows the results of identifying the types of pathogenic bacteria from the samples tested on various culture media. On Eosin Methylene Blue Agar (EMBA) media, the dominant bacteria found was Klebsiella pneumoniae, which was found in samples C1, C2, C4, and C5, while sample C3 contained a mixture of Klebsiella pneumoniae and Klebsiella variculata. On Mannitol Salt Agar (MSA) media, two types of pathogenic bacteria were found, namely Staphylococcus sciuri in sample C2 and Staphylococcus xylosus in sample C7. Meanwhile, MacConkey Agar (MaC) media showed the presence of Proteus mirabilis in C3. These identification results show the diversity of pathogenic bacteria types that can grow on certain culture media, where Klebsiella pneumoniae dominates on EMBA media, while Staphylococcus and Proteus are more suitable for identification on MSA and MaC media. Bacterial identification using the MALDI-TOF Mass Spectrometry method provided highly accurate results, with a confidence level of 99.9%.

Table 4. Sensitivity Test Results of Staphylococcus sciuri Against Various Antibiotics

No.	Antomicrobial	MIC	Interpretation
	Cefoxitin	**NEG**	**-**
1.	Benzylpenicillin	0.12	S
2.	Ampicillin		
3.	Oxacillin	0.5	S
4.	Gentamicin	≤ 0.5	S
5.	Ciprofloxacin	≤ 0.5	S
6.	Levofloxacin	0.5	S
7.	Moxifloxacin	1	I
	Inducible Clindamycin Resistance	NEG	-
8.	Erythromycin	≤ 0.25	S
9.	Clindamycin	≤ 0.25	S
10	Quinupristin/Dalfopristin	0.5	S
11.	Linezolid	2	S
12.	Vancomycin	1	S
13.	Tetracycline	≤ 1	S

No.	Antomicrobial	MIC	Interpretation
	Cefoxitin	**NEG**	-
14.	Tigecycline	≤ 0.12	S
15	Trimethoprim/Sulfamethoxazole	≤ 10	S

Antibiotic sensitivity testing in Table 4 above shows that S. sciuri is sensitive to several antibiotics, including Benzylpenicillin, Oxacillin, Gentamicin, Ciprofloxacin, and Levofloxacin. However, S. sciuri showed an intermediate response to Moxifloxacin, indicating that this antibiotic may be less effective in the treatment of infections caused by this bacterium. In addition, the high sensitivity to other antibiotics such as Quinupristin/Dalfopristin, Linezolid, Vancomycin, and Tetracycline suggests that antibiotic therapy is reliable for controlling infections caused byS. sciuri. However, AES results showed variability, emphasizing the need for additional testing to confirm antibiotic sensitivity results.

Table 5. Results of Staphylococcus xylosus Sensitivity Test to Various Antibiotics

No.	Antomicrobial	MIC	Interpretation
	Cefoxitin	NEG	-
1.	+ Cloxacillin		S
2.	+ Dicloxacillin		S
3.	Oxacillin	0.5	S
4.	Gentamicin	≤ 0.5	S
5.	Ciprofloxacin	≤ 0.5	S
6.	Levofloxacin	0.5	S
7.	Moxifloxacin	≤ 0.25	S
	Inducible Clindamycin Resistance	NEG	-
8.	Erythromycin	≤ 0.25	S
9.	Clindamycin	≤ 0.25	S
10	Quinupristin/Dalfopristin	1	S
11.	Linezolid	2	S
12.	Vancomycin	1	S
13.	Tetracycline	≤ 1	S
14.	Tigecycline	≤ 0.12	S
15	Trimethoprim/Sulfamethoxazole	≤ 10	S

Staphylococcus xylosus bacteria in Table 5 above showed high sensitivity to various antibiotics, including Cloxacillin, Dicloxacillin, Oxacillin, Gentamicin, and fluoroquinolones such as Ciprofloxacin and Moxifloxacin. The absence of clindamycin-induced resistance strengthens the potential use of Clindamycin in the treatment of infections caused by S. xylosus. Good sensitivity to other antibiotics such as Quinupristin/Dalfopristin, Linezolid, Vancomycin, and Tetracycline confirms the therapeutic effectiveness of the antibiotics that can be used. Overall, the AES results showed consistency in the sensitivity of S. xylosus to antibiotics, supporting the reliability of available treatments.

Table 6. Sensitivity Test Results of Proteus mirabilis Against Various Antibiotics

No.	Antimicrobial	MIC	Interpretation
1.	Ampicillin	<=2	S
2.	Ampicillin/Sulbactam	<=2	S
3.	Piperacillin/Tazobactam	<=4	S
4.	Urine	<=4	S
5.	Ceftazidime	<= 1	S
6.	Ceftriaxone	<= 1	S
7.	Cefepime	<= 1	S
8.	Astreonam	<= 1	S
9.	Ertapenem	1*	I
10	Meropenem	<= 0.25	S

No.	Antimicrobial	MIC	Interpretation
11.	Amikacin	<=2	S
12.	Gentamicin	<=1	S
13.	Ciprofloxacin	≤ 0.25	S
14.	Tigecycline	4	R*
15	Trimethoprim/Sulfamethoxazole	<=20	S

P. mirabilis bacteria in Table 6 above showed good sensitivity to Ampicillin and Ampicillin/Sulbactam combination, as well as other beta-lactam antibiotics such as Piperacillin/Tazobactam and cephalosporins. However, resistance to Tigecycline was identified, which suggests that this antibiotic may not be effective against P. mirabilis. The high sensitivity to Meropenem and fluoroquinolones such as Ciprofloxacin suggests that available antibiotic therapies are still highly effective in treating infections caused by P. mirabilis. The AES results showed consistency in the sensitivity of P. mirabilis to the antibiotics tested, supporting the use of existing therapies.

Table 7. Results of Klepsiella pneumoniae Sensitivity Tests to Various Antibiotics

No.	Antimicrobial	MIC	Interpretation
1.	Ampicillin	>= 32	R
2.	Ampicillin/Sulbactam	4	S
3.	Piperacillin/Tazobactam	<=4	S
4.	Urine	<=4	S
5.	Ceftazidime	<= 1	S
6.	Ceftriaxone	<= 1	S
7.	Cefepime	<= 1	S
8.	Astreonam	<= 1	S
9.	Ertapenem	<= 0.5	S
10	Meropenem	<= 0.25	S
11.	Amikacin	<=2	S
12.	Gentamicin	<=1	S
13.	Ciprofloxacin	≤ 0.25	S
14.	Tigecycline	<= 0.5	S
15	Trimethoprim/Sulfamethoxazole	<=20	S

K. pneumoniae bacteria in Table 7 above showed significant resistance to Ampicillin, but the combination with Sulbactam was effective in overcoming the resistance. Piperacillin/Tazobactam and other beta-lactam antibiotics such as Ceftazidime, Ceftriaxone, and Cefepime showed high sensitivity, making them effective choices in the treatment of K. pneumoniae infections. Carbapenem antibiotics such as Ertapenem and Meropenem were also highly effective, with high sensitivity corroborating their role as first-line therapy for infections caused by these bacteria. The AES results showed consistency in the sensitivity.

Based on the results of the sensitivity test, Staphylococcus sciuri showed high sensitivity to almost all antibiotics tested, especially Tigecycline (MIC ≤ 0.12 μg/mL) and Moxifloxacin (MIC ≤ 0.25 μg/mL). Proteus mirabilis is also sensitive to most antibiotics, such as Ampicillin, Gentamicin, and Ciprofloxacin, but is resistant to Tigecycline and has intermediate sensitivity to Ertapenem. Meanwhile, Klebsiella pneumoniae is resistant to Ampicillin but sensitive to other antibiotics, including Tigecycline (MIC ≤ 0.5 μg/mL) and Meropenem (MIC ≤ 0.25 μg/mL). Thus, Staphylococcus sciuri is the bacteria most sensitive to different types of antibiotics in this testf K. pneumoniae to the antibiotics tested, indicating the reliability of the available therapies.

DISCUSSION

The morphology of the housefly (Musca domestica) can be identified through specific characteristics on its thorax, abdomen and wings. The thorax of the housefly is greyish black with four dark longitudinal stripes on the dorsal side that help distinguish this species from other flies. The wings of this fly have distinctive venation, with the 4th vein (M1+2) curving sharply towards the costa approaching the 3rd vein (R4+5), which is used as an important diagnostic feature in entomological classification. Recent studies have shown that venation on the wings of flies,

including Musca domestica, not only plays a role in identification, but also affects flight performance and adaptation to environmental conditions

Other studies have also found that housefly wing morphology shows variation depending on geographical factors and environmental conditions. For example, studies of morphometric variation in Musca domestica across different regions have shown adaptations in wing size and shape based on geographic location, reflecting adaptive responses to local selection pressures such as temperature and food availability. This study shows that flies from different regions have variations in wing size, which can have an effect on their flying ability and dispersion, making them an important tool in the study of fly evolution and ecology.

Discussion of the results in Table 2 shows that the characteristics of bacterial colonies on various culture media vary in terms of colour, shape, size, consistency, edges, surface, and elevation. For example, on EMBA media, Klebsiella pneumoniae colonies displayed green, pink, and dark purple glossy colours, with slimy consistency in large and medium-sized colonies, and dry in smaller colonies. This is in line with previous studies showing that Klebsiella pneumoniae generally produces purple or pink coloured colonies on selective media such as EMBA, with a smooth and shiny surface due to the significant polysaccharide capsule content of the bacterial cell wall, which contributes to the slimy nature and shiny surface (30), (31).

The difference in characteristics on Mannitol Salt Agar (MSA) media indicates the influence of the medium on the growth of Staphylococcus spp., where in the table, sample C2 / MSA forms white and slimy colonies of (28), (32), (33), while C7 / MSA forms light yellow colonies of Staphylococcus xylosus and dry consistency.

This is in accordance with research which states that Staphylococcus spp. produce colour variations on MSA depending on the ability to ferment mannitol, which can change the colour of the media to yellow due to acid production, as found in S. xylosus. Meanwhile, Mac Conkey (MaC) media showed that Proteus mirabilis colonies produced by samples C3 had characteristics with irregular shapes and flat elevations, as well as non-pigmented colours, which are commonly found in Proteus colonies on selective media such as MaC. This is in accordance with previous reports stating that Proteus mirabilis produces colourless colonies due to its inability to ferment lactose, as well as the tendency to form colonies with uneven edges (34-36). This variation in colony characteristics reflects the adaptation of bacteria to different growth environments and demonstrates the importance of proper media selection for identification and isolation of pathogenic bacteria.

Staphylococcus sciuri identified from flies using the MALDI-TOF Mass Spectrometry method with a confidence level of 99.9%, demonstrating that this technique is highly accurate and reliable in identifying bacterial species associated with human infections. S. sciuri is known as a coagulase-negative bacterium that is often found in animals and the environment, and can cause opportunistic infections in humans. (37), especially in individuals with weakened immune systems.

Antibiotic sensitivity testing of S. sciuri showed that it was sensitive to a number of antibiotics, including Benzylpenicillin, Oxacillin, and Gentamicin (38). These results suggest that antibiotic therapy using Benzylpenicillin and Oxacillin could be an effective option in the treatment of infections caused by S. sciuri (37), (39). However, resistance to Moxifloxacin with an intermediate interpretation emphasises the need for caution in antibiotic selection, especially in clinical settings where antibiotic resistance is a significant problem. Overall, these results reinforce the importance of proper identification and antibiotic sensitivity testing to ensure effective therapy (40).

Staphylococcus xylosus, which was also identified with a confidence level of 99.9% through MALDI-TOF Mass Spectrometry, is known as a coagulase-negative bacterium commonly found on animal and human skin. [41], (42). Although usually considered non-pathogenic, S. xylosus can cause infections especially in immunocompromised patients or those using implanted medical devices. The sensitivity test results showed that S. xylosus was sensitive to various antibiotics such as Cloxacillin, Dicloxacillin, and Oxacillin. The high sensitivity to these antibiotics indicates that coagulase-negative staphylococci, including S. xylosus, can be effectively treated with beta-lactam antibiotics. In addition, sensitivity to aminoglycosides such as Gentamicin and fluoroquinolones such as Ciprofloxacin adds options in antibiotic therapy. The absence of induced clindamycin resistance (NEG) also provides additional options for the use of Clindamycin without the risk of resistance developing during therapy (43). These results confirm that S. xylosus remains susceptible to a range of antibiotics, although antibiotic resistance should be continuously monitored.

Proteus mirabilis, identified with a confidence level of 99.9% using MALDI-TOF Mass Spectrometry, is a bacterium commonly found in the urinary tract and is frequently involved in recurrent or prolonged urinary tract

infections (UTIs) (22), (23). P. mirabilis has the ability to form biofilms and produce urease, making it more challenging to treat (24). Sensitivity test results indicate that P. mirabilis is susceptible to Ampicillin and the Ampicillin/Sulbactam combination, as well as other beta-lactam antibiotics such as Piperacillin/Tazobactam and cephalosporins (25) (27). However, the sensitivity to Ertapenem, which only showed an intermediate interpretation, underscores the need for more careful consideration in its use, especially as resistance to carbapenems is increasing (26). An interesting finding is the resistance to Tigecycline, indicating that this antibiotic is not effective against P. mirabilis in this context, which may be associated with specific resistance mechanisms such as efflux pump overproduction. Overall, these results confirm that although P. mirabilis remains susceptible to many antibiotics, therapeutic choices should be made cautiously based on the local resistance profile (36).

Klebsiella pneumoniae, identified in this study with a confidence level of 99.9%, is an important pathogen associated with nosocomial infections, particularly in patients with weakened immune systems or those using medical devices. K. pneumoniae is known for its ability to develop resistance to various antibiotics (28), (29), including beta-lactam antibiotics, through the production of beta-lactamase enzymes (30). Sensitivity test results showed that K. pneumoniae is resistant to Ampicillin, which is consistent with the knowledge that this bacterium is naturally resistant to many beta-lactam antibiotics. However, the combination of Ampicillin with Sulbactam demonstrated effectiveness, indicating that beta-lactamase inhibitors can overcome this resistance mechanism (27)(31). Other antibiotics, such as Piperacillin/Tazobactam, Ceftazidime, Ceftriaxone, and carbapenems like Meropenem, also showed high efficacy, indicating that they remain the primary treatment options for K. pneumoniae infections. However, the global increase in carbapenem resistance emphasizes the need for prudent use of these antibiotics. These findings support the use of combination antibiotics and carbapenems in the treatment of K. pneumoniae infections, while continuously monitoring resistance development.

CONCLUSION

This study successfully identified four types of pathogenic bacteria isolated from flies using the MALDI-TOF Mass Spectrometry method with a very high confidence level of 99.9%. The identified bacteria include Staphylococcus sciuri, Staphylococcus xylosus, Proteus mirabilis, and Klebsiella pneumoniae. The identification method used has proven to be highly accurate and reliable in determining pathogenic bacterial species that have the potential to cause infections in humans.

Antibiotic sensitivity test results showed that Staphylococcus sciuri and Staphylococcus xylosus are generally sensitive to various antibiotics, including Benzylpenicillin, Oxacillin, Gentamicin, and fluoroquinolones such as Ciprofloxacin. However, Staphylococcus sciuri exhibited intermediate resistance to Moxifloxacin, highlighting the importance of selecting the appropriate antibiotics in clinical therapy. Meanwhile, Klebsiella pneumoniae demonstrated resistance to Ampicillin, but the combination with Sulbactam enhanced treatment effectiveness. Other beta-lactam antibiotics and carbapenems such as Ertapenem and Meropenem showed high efficacy, making them the primary choices in the treatment of infections caused by this bacterium. Proteus mirabilis exhibited good sensitivity to most of the tested antibiotics, but resistance to Tigecycline indicated a limitation in using this antibiotic to treat infections caused by this bacterium.

AUTHOR'S CONTRIBUTION STATEMENT

Maria Kanan developed the design and implementation of the research, collected data, analysed and interpreted the data, wrote and revised the draft article, and gave final approval of the version to be published. Herawati contributed to the implementation of the research, collected data, revised the draft article, and gave final approval of the version to be published. Sandy N. Sakati contributed to the implementation of the research and collected data. Inda Hafid and Muhammad Syahrir contributed to the analysis and interpretation of the data and gave final approval of the version to be published.

CONFLICTS OF INTEREST

The authors have stated that they do not have any conflicts of interest, including personal relationship or financial matters, that could potentially influence the results of this study.

SOURCE OF FUNDING STATEMENTS

The authors received funding from Directorate of Research, Technology, and Community Service for this research.

ACKNOWLEDGMENTS

The authors express their deepest gratitude and highest appreciation for the support and assistance from all parties involved in this research. Special thanks go to the Directorate of Research, Technology, and Community Service management for providing funding for the implementation of this research.

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