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Bacterial Culture Streak Method for Inventory of Enterobacteriaceae Causes Diarrhea in the Body of House *Fly (Musca Domestica)*

Urip[1*], Titin Emilia Nirmala Sari[1], Maruni Wiwin Diarti[1], Erlin Yustin Tatontos[1]

[1] Department of Health Analyst, Poltekkes Kemenkes Mataram, West Nusa Tenggara, Indonesia

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Corresponding Author:
Urip
uriprama64@gmail.com

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Abstract: Diarrhea is an endemic disease in Indonesia and is a potential disease Extraordinary Events (KLB) which are often accompanied by death. Based on data on diarrhea cases at the Narmada Community Health Center in 2017 there were 1.679 cases of diarrhea from 11 villages in Narmada District. One of the causes of these cases of diarrhea is the presence of house flies as a vector carrying disease. The purpose of this study was to determine the bacterial culture of the streak method for inventory of *Enterobacteriaceae* of diarrhea in the body of the house fly (*Musca domestica*). This research is descriptive observational. The sample in this study were 30 Musca domestica which were captured in the Kramajaya village environment in *Nutrienth Agar Plate* (NAP) and *Mac Conkey Agar* (MCA) media as well as the INVIC MUTSI Biochemical Test media on bacterial colonies that needed further identification to determine species. The analysis of the results was carried out descriptively. The identification results were found in the family *Enterobacteriaceae* genus *Enterobacter agglomerans* in the body of the house fly (Musca domestica) samples taken from the landfill and the family Moraxellaceae family genus *Acinetobacter baumannii* taken from the kitchen and home terrace.

Keywords: Bacteria; Diarrhea; Flies; Streak method

Introduction

Microorganisms are the cause of most diseases. This can be seen from the ability of microorganisms to infect humans, animals and plants and can cause diseases that range from mild infections to death. One of the microorganisms that can cause disease is bacteria. One of the families of bacteria that often cause disease in humans is the *Enterobacteriaceae family* (Chandra, 2007) .

Enterobacteriaceae is a group of gram-negative bacteria in the form of bacilli, move with flagelperitrics and decompose glucose by producing gas. The *Enterobacteriaceae* family is the cause of most food-borne illnesses. Several families of *Enterobacteriaceae* are pathogenic, including members of the genera *Enterobacter* , *Serratia* , *Escherichia* , *Proteus* , *Salmonella* , *Shigella* , and *Klebsiella* (Darna et al., 2018) .

One disease that comes from food is diarrhea. Diarrhea is caused by several families of bacteria including the *Enterobacteririaceae family*. The bacterial group includes the genera *Escherichia*, *Proteus*, *Salmonella*, and *Shigella* (Darna et al., 2018).

In order to identify the types of *Enterobacteririaceae* which cause diarrhea, a method for culturing them is needed. One of these methods is the streak method. The streak method is a technique for cultivating bacteria to obtain separate single colonies by streaking them on the media (Nudyanto & Zubaidah, 2015) .

One of the vectors for the spread of *Enterobacteririaceae bacteria*. Flies have the ability to fly one to two miles so that they can carry microbes from various places where they have visited (Prabowo, 1992). The presence of a foul odor coming from a dirty place can attract flies to that place in search of something to eat. Usually, these places are places that have a lot to do with human activity. When flies eat in dirty places, all parts of the fly's body such as the body, wings and legs will be filled with disease germs (Safitri et al., 2017) .

Kramajaya Village is one of the villages in Narmada District, West Nusa Tenggara. This village has an area of

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1.01 km ^{2} with a population density of 4,597 people and is the village with the most densely populated area among other villages in Narmada District (BPS Lombok Barat, 2020). Environmental conditions in the village that do not meet health requirements which include water sources, latrines, garbage disposal, sewage drainage and unhealthy housing conditions with temperature, lighting, humidity, type of floor and ventilation are factors that influence the density of fly populations that can be cause diarrheal disease (Depkes RI, 1999). Based on Narmada Health Center data, Total of Diarrhea cases at the Narmada Health Center in 2017, 1,679 cases of diarrhea, were found from 11 villages in Narmada District. From these data, Kramajaya Village is the village that has the highest incidence of diarrhea in Narmada District.

Based on the description above, the *Enterobacteriaceae family* in the body of the house fly (*Musca domestica*) has an important role in the occurrence of diarrhea. Therefore, research is needed to determine exactly the type of bacteria that causes diarrhea from house flies (*Musca domestica*) taken in the Kramajaya environment. The importance of this research is due to the absence of data and research conducted on this matter.

Method

Based on figure 1, type of research is descriptive observational, that is, the researcher directly observes the object to be examined, then describes it descriptively to determine whether or not bacteria are present in the bodies of flies taken in the Kramajaya village environment.

Sampling technique in this study was using the *Non-Random Purposive Sampling technique*, namely sampling based on criteria made by the researchers themselves. The sample criteria used in this study were *Muscadomestica flies* found in the Kramajaya village environment. The samples used in the study were 30 Musca domestica house flies caught in the Kramajaya village environment which were taken from three places (TPS, Kitchen and Home Terrace) 10 flies were caught in each place.

House flies were collected by catching them using insect nets (sweep nets) in the Kramajaya village environment according to the criteria. The flies that were caught were identified so that only house flies (*Musca domestica*) were collected. Then the house flies were put into the bottle, crushed with a sterile glass stirrer, then given 2 ml of sterile distilled water and labeled with information on the number of flies and the time of capture.

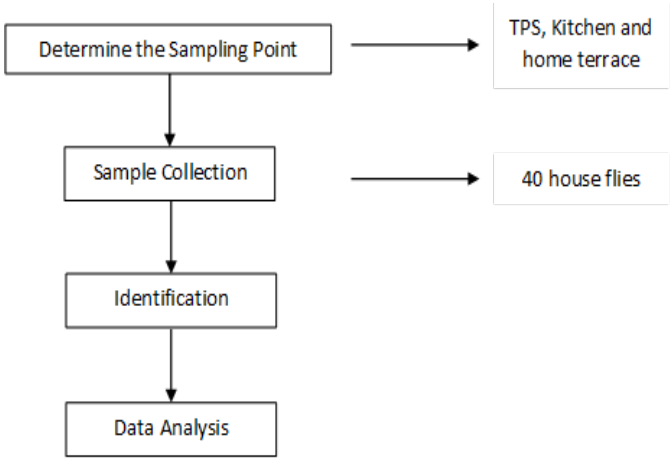

Figure 1. Stage of this research

Identification of gram-negative bacilli by culturing in nutrient agar media, namely 20 g of NA suspended in 1 liter of distilled water, heated while stirring until boiling. Then sterilized using an autoclave at 121°C for 15', PH ± 7.1 then poured into the plate at ± 45°C. Furthermore, cultures were carried out with Mac Conkey Agar (MCA) media and IMVIC MUTSI Biochemical Test media on bacterial colonies that needed further identification to determine species. Descriptive analysis of results.

Results and Discussion

Examination of the morphology of bacterial cells identified from kitchens, landfills and terraces of houses isolated on NAP and MCA media can be seen in the Table 1.

Table 1. Characteristics of colonies on NAP and MCA media on the bodies of house flies taken from kitchens, garbage dumps and terraces of houses

Sampling Location	Colony	
	NAP	MCAs
Kitchen	Colony Size: Small Round shape Consistency: Soft White color Surface: Smooth Edge Colonies: Flat	Colony Size: Round shape Consistency: Soft Surface: Smooth Edge Colonies: Flat
TPS	Shape: Irregular Consistency: Soft Color: Golden Yellow Surface: Smooth	Round shape Consistency: Soft Surface: Smooth Edge Colonies: Flat
House Terrace	Colony Size: Small Round shape Consistency: Soft White color Surface: Smooth Edge Colonies: Flat	Colony Size: Small Round shape Consistency: Soft Surface: Smooth Edge Colonies: Flat

Table 1 Shows that the results of the characteristics of the colonies on the body samples of house flies isolated on NAP media were small, round in shape, soft in consistency, golden yellow in color, with a smooth surface and flat colony edges in media A and media C, while those in media B had the following characteristics: Colonies are golden yellow, soft consistency and smooth surface. After making Gram preparations, they were isolated on MCA media. The results showed that the characteristics of the colonies on media A, B and C were spherical in shape, luna consistency, smooth surface and flat colony edges.

Table 2. Gram staining results on NAP and MCA media

Colony Code	Cat Gram results	
	NAP	MCAs
Colony A	Shape: Cocoacil Red Properties: Gram negative	Shape: Cocoacil Red Properties: Gram negative
Colony B	Shape: Basil Red Properties: Gram negative	Shape: Basil Red Properties: Gram negative
Colony C	Shape: Cocoacil Red Properties: Gram negative	Shape: Cocoacil Red Properties: Gram negative

Information:
Colony A : Sample from the galley
Colony B : Sample from TPS
Colony C : Sample from the terrace of the house

Table 2 shows the results of the growth of colonies A and C on NAP and MCA media in the form of coccobacillus, red in color and Gram negative. Then in the B colony in the form of bacilli, red and Gram negative. The results of the colony biochemical tests taken on MCA media can be seen in the Table 3.

Table 3. Biochemical Test

Colony Code	Mot	Ur	AP	SCA	TSI		
					2S _ _	Gas	Slope Base
Colony A	-	-	+	+	-	-	K/K
Colony B	+	-	+	+	-	+	A A
Colony C	-	-	+	+	-	-	K/K

Information:
AP : Peptone Water
SCA : *Simon's Citrate Agar*
TSI : *Triple Sugar Iron*
A/A : Fermenting Carbohydrates
K/K : Not Fermenting Carbohydrates

A and C, which showed positive results on AP and SCA media on TSI media, indicating that the bacteria did not produce H2S or gas and could not Fermenting carbohydrates are marked with a red base color (alkaline) and a red slope (alkaline). Then for colony B, positive results were obtained in the motility, AP and SCA tests on TSI media indicating that the bacteria can ferment all carbohydrates (glucose, lactose and sucrose) is characterized by a yellow base color (acidic) and a yellow slope (acidic).

Table 4. Sugar Test

Colony Code	Confectionery Media					
	Gluk	Suk	Lacquer	Man	Mall	Sor
Colony A	-	-	-	-	-	-
Colony B	+	+	-	+	+	+
Colony C	-	-	-	-	-	-

Information:
Colony A : Sample from the galley
Colony B : Sample from TPS
Colony C : Sample from the terrace of the house

Table 4 shows that the results of the sugar test in colonies A and C were unable to ferment sugar, marked by no change in color to yellow in the media, while in colony B, positive results were obtained in all media marked by a color change from purple to yellow except for lactose. no color change occurs.

Test results in Tables 1, 2, 3, 4 and 5 found *Acinetobacter baumanii bacteria* in isolate A and isolate C with the characteristics of bacteria in the form of coccobacilli, gram negative, with sugar results: glucose negative, sucrosan negative, lactose negative, mannitol negative, maltosan negative, negative sorbitol, and biochemical results: negative motility, negative urea, positive indole, positive SCA, negative H_2S, negative Gas, Bottom Slope (K/K). While the results on isolate B found a type of bacteria with the genus *Enterobacter agglomerans* with the characteristics of bacteria in the form of bacilli, gram negative, with sugar results: positive glucose, positive sucrose, negative lactose, positive mannitol, positive maltose, positive sorbitol, and biochemical results : positive motility, negative urea, positive indole, positive SCA, negative H_2S, positive gas, bottom slope (A/A).

Table 5. Identification result data *Enterobacteririaceae* on the body of the *Musca domestica fly*

Colony Code	Types of Bacteria
Colony A	*Acinetobacter baumaniii*
Colony B	*Enterobacter agglomerans*
Colony C	*Acinetobacter baumaniii*

In this study, a sample of 30 house flies (*Musca domestica*) was taken from three sampling locations, namely 10 samples of house flies from the kitchen, 10 samples of house flies from the terrace and 10 samples from landfills. The results obtained from 1 sample of house flies were found to be a bacterial genus belonging to *Enterobacteriaceae* which was taken from landfills. Based on the results of observations on the morphological characters of the cells, it was shown that

the genus of bacteria which had a red staining result indicated that the bacteria were gram-negative bacteria, bacillus-shaped cells, which belong to the genus *Enterobacter bacteria.*

The results of 2 samples of other house flies found a bacterial genus belonging to *Moraxellaceae* taken from the kitchen and terrace of the house, the results of observing the morphological characters of the cells showed that the bacterial genus which had a red staining result indicated that the bacteria were gram-negative bacteria, coco bacilli-shaped cells, which includes bacteria belonging to the genus *Acinetobacter* .

The results of this study were supported by research by Tatontos et al (2015) which found gram-negative bacteria, namely *Klebsiella pneumonia* and *Proteus vulgaris* on the bodies of house flies at the abianbuh market, as well as research conducted by Setiyorini (2017) which stated that *Salmonella bacteria were found* in house flies and green fly at Legi Citra Niaga market, Jombang. Another study conducted by Putri (2018) also succeeded in identifying 4 bacterial isolates from the genera *salmonella*, *providencia*, *Escherichia* and *vibrio* in the bodies of house flies at TPA Sukawinatan and Palembang Main Market.

House flies (*Musca domestica*) are often found because house flies like bad smells, usually these flies eat liquid materials such as milk and syrup like fruits, vegetables that are wet and smelly. This causes flies to be carriers of disease vectors and can spread disease. Transmission of diseases caused by flies due to contact of flies with humans and food. There are several diseases that can be transmitted through contamination of water, air, food, hands and person-to-person contact, including diseases such as myiasis, diarrhea, cholera and others (Chandra, 2007) .

Members of the family *Enterobacteriaceae* are gram-negative facultative anaerobic rods that can be motile or non-motile; motile bacterial strains have peritrichous flagella. All species reproduce on artificial media and convert glucose, where they form acid or acids and gases. These bacteria also produce catalase enzymes with their natural habitat in the digestive tract of humans and animals. Most *Enterobacteriaceae* are normal flora in the digestive tract, although some are widely distributed in the surrounding environment (Tham, 2012) .

Enterobacter sp. It is a Gram-negative bacterium, facultative anaerobic, rod-shaped and can move (motile), the means of movement is in the form of peritrichous flagella, namely flagella which are evenly spread over the entire surface of the cell. If *Enterobacter sp.* cultivated on artificial media then it shows the activity of converting glucose, then forming acid and gas. These bacteria reduce nitrate to nitrite. These bacteria can form capsules, citrate and acetate which can be used as the sole carbon source (Jawetz et al., 2007) .

Bacteria *Enterobacter sp.* consists of 14 types of subgroups but the most frequently found species are *Enterobacter aerogenes, Enterobacter cloacae*, *Enterobacter agglomerans* and *Enterobacter sakazakii*. *Enterobacter* is a motile organism that easily grows on the media used for the isolation of enteric bacteria. Most of the isolates ferment lactose quickly and give color to the colonies except for *E. agglomerans* (Purwoko, 2007).

E.agglomerans is a gram-negative bacillus in the form of bacilli, does not form spores, is facultatively anaerobic, has a width of 0.6-1 um and a length of 1.2-3.0 um, cells are motile, can grow at temperatures of 20-30 º C (Mardaneh & Dallal, 2013). *E.agglomerans* is an opportunistic pathogenic bacterium, causing wound infections, bacteremia and urinary tract infections and can generally be found in soil, water and vegetables as well as in some animals or insects such as rats and flies. *E.agglomerans* is often involved in infection than *E.aerogenes* and *E.cloacae* (Sharma, 2012).

E.agglomerans biochemical test showed positive results except for urea media which was marked by no color change from pink to purplish red indicating that this bacterium is unable to produce the enzyme urease which can decompose urea so that it is alkaline. The indole test obtained positive results indicated by the formation of a red ring after adding covac's reagent, the red color was produced from resindol which is the result of the reaction of the amino acid tryptophan to become indole with the addition of covac's.

The positive result for indole indicated that *E.agglomerans* used the amino acid tryptophan as a carbon source. On motility media, positive results were obtained as seen from the spread of white bands on the media, this indicates that this bacterium has a means of locomotion in its growth process. *The Simon's Citrate Agar (SCA)* media showed positive results with a change in the color of the media from green to blue, this indicates that these bacteria are capable of forming citrate as the main carbon source. *Triple Sugar Iron (TSI)* media is a medium used to see the ability of bacteria to ferment sugar and to see the ability of bacteria to produce gas and H_2S, in this study the results showed that bacteria could not produce H_2S and were in the form of gas at the bottom. tube.

E.agglomerans sugar test showed a positive reaction on glucose, sucrose, mannitol, maltose and sorbitol media marked by a color change from purple to yellow, indicating that *E.agglomerans is* capable of fermenting carbohydrates. Except for lactose there is no color change. The bacterium *Acinetobacter baumannii* belongs to the genus *Acinetobacter*, The bacterium *A.baumannii* is an opportunistic or nosocomial pathogen, naturally found in the environment, soil, water and sewage (Scerpella et al., 1995).

A.baumannii bacteria are gram-negative coco bacilli or diplococci, do not have flagella, do not have spores,

have fimbriae, are aerobic, give negative oxidase and positive catalase tests. On medium for MacConkey easy to grow well. Bacteria *Acinetobacter sp.* can grow well at temperatures of 25ºC - 37ºC. *A.baumannii* species can also grow well at 42°C, while other species at this temperature do not grow well or cannot grow, even *A.baumannii bacteria* can grow at 44°C (Noorhamdani, 2004) .

The biochemical test for *Acinetobacter baumannii* showed negative results except for Indole and SCA media indicated by the presence of a red ring formed after adding covac's reagent to indole media and a color change from green to blue on SCA media. From these results it can be concluded that *A.baumannii* uses the amino acid tryptophan as a source carbon and is able to produce citrate as the main carbon source. The results of negative motility and urea media indicate that these bacteria do not have a means of locomotion in the growth process and are unable to produce the enzyme urease which can decompose urea so that it is alkaline or alkaline because the media does not change from pink to purplish red. The sugar test yielded negative results on all media indicating that *A.baumannii* could not ferment carbohydrates.

A.baumannii is *commonly* found as a bacterium that causes nosocomial infections in the urinary tract, surgical wound infections, blood vessel infections, ventilator-associated pneumonia (VAP) and meningitis, especially in patients with low immune systems who are in intensive care units (Dharmawan & Layanto, 2018). *A.baumannii* bacteria ranks third as the most common cause of hospital-acquired pneumonia in 10 countries in the Asian continent and is most often found in the use of ventilators in pneumonia infections. The mortality rate for infectious disease cases caused by *A.baumannii bacteria* reaches 23% in hospitalized patients and 43% in patients in intensive care (Pertiwi & Budayanti, 2018) .

Conclusion

The conclusion of this study is that it found bacteria in the *Enterobacteriaceae* family namely, *Enterobacteragglomerans* and bacteria from the *Moraxellaceae family*, namely, *Acinetobacter baumannii* on the body of the house fly (*Musca domestica*).

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