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Identification of Pathogenic Bacteria in Liquid Waste from "X" Hospital in Kediri City

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ABSTRACT

Introdiction*: Hospitals can not only be a place for people to seek treatment, but they can also get diseases, especially infectious diseases. One source of the spread of infectious diseases comes from hospital waste, so proper waste processing is needed. Hospital wastewater contains many pathogenic bacteria such as Enterobacter cloacae, Bacillus sp., Kluyvera sp., Enterobacter sakazaki, Klebsiella oxytoca and E.coli. This makes it necessary to manage hospital wastewater to prevent the spread of disease, especially by pathogenic bacteria. On the other hand, research is still needed on the specific types of bacteria found in wastewater, including hospitals in Kediri City.* ***Aims****: to determine the types of pathogenic bacteria at the "X" hospital in Kediri City.* ***Result:*** *the density of bacteria in the bottom waste was greater than in the top and middle parts, with an average of 1,830 bacteria/100 ml. The results of the identification test showed that pathogenic bacteria contained in the waste from hospital "X" in Kediri City included Klebsiella (25%), E.coli (50%) and Pseudomonas (25%).* ***Conclusion:*** *The waste from "X" Hospital Kediri City contains three types of pathogenic bacteria: Klebsiella, E. coli, and Pseudomonas.*

KEYWORDS*: Bacteria, waste, hospital, Kediri*

INTRODUCTION

A hospital is a health installation to care for and treat various diseases. On the other hand, hospitals are also places where people get different kinds of diseases, especially infectious diseases. One source of the spread of infectious diseases comes from hospital waste. Waste is the remainder of the activities of living creatures originating from various activities and can be in solid, liquid, or gas form (Singh, 2023). Hospital waste is all the waste generated from hospital activities that can pollute the environment and potentially transmit infections (Ningrum et al., 2017). Generally, hospital waste is divided into medical and non-medical waste. Based on its form, hospital waste can be categorized into three types: solid waste, liquid waste, and gas waste (Chand et al., 2020). One type of dangerous hospital waste is hospital liquid waste.

Hospital liquid waste is the remaining waste product or all wastewater, including feces originating from hospital activities, which

contains pathogenic bacteria such as *Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, and *Klebsiella pneumonia*. Hospital liquid waste that is not managed correctly can cause the spread of infectious diseases. Therefore, hospitals must manage liquid waste according to applicable requirements and standards (Bhandari et al., 2023). Liquid waste quality is the condition of liquid waste, expressed in terms of discharge, content, and pollutant materials. The maximum discharge by Ministry of Environment Regulation Number Kep//51/MENLH/10/1995 is the highest discharge that is still permitted to be discharged into the environment. Important liquid waste quality parameters to know are suspended solids, dissolved solids, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), coliform organisms, pH, Dissolved Oxygen (DO), chlorine requirements, nutrients, heavy metals, and other parameters (Ranti et al., 2020).

The increased number of healthcare facilities in hospitals causes increased environmental pollution. This also causes an increased risk of spreading infectious diseases. The research results of Astawa & Tarini (2017) show that wastewater at Sanglah Hospital contains several pathogenic bacteria such as *Enterobacter cloacae, Bacillus sp., Enterobacter Okazaki, Klebsiella oxytoca,* and *E.coli*. Similar results were also reported by Winarti (2020), who reported that the wastewater at RSKIA Sadewa contained Total Coliform bacteria of $2400x10^3$ MPN/100ml. This shows that it is necessary to manage hospital wastewater to prevent the spread of diseases, especially those caused by pathogenic bacteria.

Based on the results of this research, it is necessary to identify bacteria in hospital waste, including hospital "X" in Kediri City. This study aims to identify the types of bacteria still present in the waste from hospital "X" in Kediri City. This research is essential to prevent the transmission of infectious diseases due to hospital waste contamination, control nosocomial infections and antibiotic resistance, support better health policies, and improve preparedness and response to potential disease outbreaks.

METHODS

Materials

The materials needed in this study are Lakosa Broth 1 (LB I), Lakosa Broth 3 (LB III), Brilian Green Lactose Broth(BGLB), Eosin Methylene Blue (EMB), Centrimid, Mannitol Slat agar (MSA), Salmonella Shigella Agar (SSA), Tripel Suger Iron Agar (TSIA) and Indol, Methil red, Voges Prouskover, Citrat (IMVC).

Preparation and Sampling

Sterile glass bottles are opened at 120ºC for 1 hour. Waste collection is carried out at the inlet and outlet of the IPAL (outlet) at the "X" hospital in Kediri City, with the number of samples in this study being 12 samples (2

IPALs, each IPAL is taken at 3 points, namely the top, middle, bottom points and repeated two times). The samples were 100ml of outlet liquid waste per bottle; samples were taken within a day using sterile bottles coded and taken to the laboratory with a dry ice box for examination, with standardized parameters (Yatini, et. al., 2021).

Bacterial Identification

Preliminary test

A total of 5 tubes containing LB III were added, each with a sample of hospital waste "X" 10 ml. Five LB I tubes were prepared and filled with 1ml of sample each. Each tube was then taken in 0.1 ml (100 ul), and all treatments were carried out sterilely in the LAF. The tubes filled with water samples were homogenized so that the waste samples were evenly mixed with the LB III and LB I media; LB III and LB I media were incubated in an incubator at 37 ° C for 24 hours (Yatini, et. al., 2021).

Confirmation test

Observing changes in each LB III and LB I tube that had been incubated to see the turbidity and the emergence of gas. If Media LB III and I are vivacious, the confirmation test is continued by inoculating the sample into the BGLB media in a sterile manner in LAF. The results are incubated in an incubator for 24 hours at a temperature of 37°C (Yatini, et. al., 2021).

Complementary test

We are observing changes in each BGLB tube incubated for 24 hours. Positive results

Identification of Pathogenic bacteria

are indicated by samples that turn cloudy and form gas. The results in the confirmation test tube are adjusted to the MPN (Most Probable Number) table of variety 555. Positive BGLB results are then inoculated into EMB, Centrimtde, SSA, MSA, TSIA, and IMVC media and then incubated for 24 hours at a temperature of 37°C to identify bacteria in the waste of the hospital "X" Kediri City (Yatini, et. al., 2021).

RESULTS AND DISCUSSION

This study identified pathogenic bacteria in the waste of hospital "X" Kediri City. The study identified bacteria at 3 points: the top, middle, and bottom. The results of bacterial density at various sampling points are shown in Table. 1. The results of bacterial identification in the waste of hospital "X" Kediri City are shown in Table 2.

Table 1 shows that the waste of hospital "X" Kediri City at the bottom has more bacteria than at the top and bottom. The density of the bacteria in the lower waste shows an average of 1,830 bacteria/100 ml. Several things can affect the higher density of bacteria in the upper part: environmental conditions, nutrients, and bacterial sedimentation (Fletcher, 2015). At the bottom of the waste reservoir, it generally has a lower temperature and high humidity so that bacteria can grow more easily. In addition, at the bottom of the waste container, there is a possibility of sedimentation of organic materials from hospital waste (such as blood and tissue) so

Table 1. Bacterial Density at Various Sampling Points in The Waste of Hospital "X" Kediri City

No	Sample code	Positive Combination	Bacterial Density/100 ml
1	A1.1	5-5-2	540
2	A1.2	5-5-2	540
3	A2.1	5-5-5	>2400
4	A2.2	5-5-4	1600
5	A3.1	5-5-5	>2400
6	A3.2	5-5-5	>2400
7	B1.1	5-4-2	220
8	B1.2	5-4-4	350
9	B2.1	5-5-2	540
10	B2.2	5-5-2	540
11	B3.1	5-5-4	1600
12	B3.2	5-5-3	920

A shelter, 1 top dot, 2 middle dots, 3 bottom dots, repeat 2x
B shelter, 1 top dot, 2 middle dots, 3 bottom dots, repeat 2x

that it becomes a source of nutrition for bacterial growth (Mahdi & Gomes, 2019). The density of bacteria also tends to be higher because piles of solid particles generally settle at the bottom of the waste (Maina & Susan Muthoni, 2020).

Table 2 shows where the waste from the hospital "X" in Kediri City contains 3 types of pathogenic bacteria, namely *Klebsiella* bacteria 25%, *E. coli* 50%, and *Pseudomonas* 25%. These results are by the research of Cuetero-Martínez et al. (2023) which identified the presence of pathogens in hospital waste: *Escherichia coli*, Enterobacter *spp*., *Pseudomonas aeruginosa*, and *Bacillus spp*. Zhang et al. (2021) also reported similar results and identified various pathogenic bacteria, including *Escherichia coli*, *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in hospital waste. The presence of bacteria in hospital waste is due to the physiological properties of pathogenic bacteria and resistance to disinfectants and antibiotics, contributing to their presence in hospital waste Gönder et al. (2021).

Table 2 shows that the type of pathogenic bacteria most often found in hospital waste "X" in Kediri City is E. coli. *E. coli* is the main cause of hospital urinary tract infections (UTIs) and gastrointestinal infections. Many hospital patients are treated for this condition, so hospital wastewater generally contains pathogenic *E. coli* bacteria from patients with this infection (Osińska et al., 2017). In addition, *E. coli* can survive in various environmental conditions, including in-hospital wastewater, which often has varying pH and changing nutrient concentrations, and can also utilize various organic substrates in hospital waste, including nutrients from food waste and metabolic products of the body (Akther et al., 2018; Paulshus et al., 2019). These results cause *E. coli* in hospital wastewater, including Hospital "X" in Kediri City.

Table 2. Bacterial Identification in The Waste of Hospital "X" Kediri City

Sample code	Colour of Colony Selective Media	TSIA	Indol	MR	VP	Citrat	Bacterial identification
A1.1	*Metalic sheen*	L= acid, D=acid, H2S = -, Gas = +	+	+	-	-	*E. coli*
A1.2	*Pink Mucoid*	L= acid, D=acid, H2S = -, Gas = +	-	_	+	+	*Klebsiella*
A2.1	*Metalic sheen*	L= acid, D=acid, H2S = -, Gas = +	+	+	-	-	*E. coli*
A2.2	*Pink Mucoid*	L= acid, D=acid, H2S = -, Gas = +	-	_	+	+	*Klebsiella*
A3.1	*Metalic sheen*	L= acid, D=acid, H2S = -, Gas = +	+	+	-	-	*E. coli*
A3.2	*Pink Mucoid*	L= acid, D=acid, H2S = -, Gas = +	-	_	+	+	*Klebsiella*
B1.1	*Metalic sheen*	L= acid, D=acid, H2S = -, Gas = +	+	+	-	-	*E. coli*
B1.2	*Phyocianin*	L= alkalis, D=alkalis, H2S = -, Gas = -	-	_	-	+	*Pseudomonas*
B2.1	*Metalic sheen*	L= acid, D=acid, H2S = -, Gas +	+	+	-	-	*E. coli*
B2.2	*Phyocianin*	L= alkalis, D=alkalis, H2S = -, Gas = -	-	_	-	+	*Pseudomonas*
B3.1	*Metalic sheen*	L= acid, D=acid, H2S = -, Gas +	+	+	-	-	*E. coli*
B3.2	*Phyocianin*	L= alkalis, D=alkalis, H2S = -, Gas = -	-	_	-	+	*Pseudomonas*

Selective media used:
Eosin Methylene Blue (EMB) for E.coli and *Klebsiella* bacteria.
Centrimed for *Pseudomonas* bacteria

Table 2 also shows that besides E. coli, two other pathogenic bacteria are found in Hospital "X" in Kediri City, namely *Klebsiella* and *Pseudomonas*. *Klebsiella spp*. is a gram-negative bacteria known as an opportunistic pathogen. *Klebsiella spp*. has a polysaccharide capsule that helps evade the immune system and increases its virulence. This capsule also helps bacteria survive in hostile environments, such as hospital waste (Gomes et al., 2021). *Klebsiella spp*. can also utilize various carbon sources, including glucose and lactose, which are common in hospital waste and survive in low oxygen conditions. This causes *Klebsiella*

spp. to be often found in hospital waste, including in the waste of hospital "X" in Kediri City (Sakkas et al., 2019).

Another type of pathogenic bacteria found in the waste of hospital "X" in Kediri City is *Pseudomonas*. *Pseudomonas aeruginosa* is a highly adaptive bacteria and can survive in various environmental conditions, including environments with high humidity and inconsistent nutrients, such as hospital waste (Khan et al., 2024). One of the main reasons why *Pseudomonas aeruginosa* is often found in hospital waste is its ability to form biofilms (Divyashree et al., 2022). Biofilms are communities of bacteria that adhere to surfaces and are protected by an extracellular matrix, which makes them more resistant to cleaning and disinfection. Hospital waste often has a variety of surfaces that can support biofilm formation (Suleyman et al., 2018).

CONCLUSION

Based on the research results, it can be concluded that the density of the number of bacteria in the lower part of the waste is greater than in the upper and middle parts, with an average of 1,830 bacteria / 100 ml. For identification results, *Klebsiella* bacteria were obtained from 3 samples (25%), *E. coli* from 6 samples (50%), and *Pseudomonas* from 3 samples (25%).

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