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# IDENTIFICATION OF BACTERIA IN POST-WASTEWATER TREATMENT AT RATATOTOK HOSPITAL IN SOUTHEAST MINAHASA

Environmental Science

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## ABSTRACT

The hospital is a facility where numerous diseases are treated. However, the hospital is also a site where numerous diseases, such as infection, can be contracted. Large quantities of liquid waste are produced by hospitals in Indonesia. This liquid waste is contagious, contains pathogenic bacteria and toxic compounds that are hazardous to human health and the environment. Antibiotics were detected as a component of hospital wastewater in previous research, and the majority of bacteria isolated from this wastewater were shown to be resistant to routinely used antibiotics. By isolating and identifying bacteria, this study intends to detect antibiotic contamination in the liquid waste disposal system at Ratatotok General Hospital, Southeast Minahasa. The methodology employed is quantitative descriptive research. The sample consists of liquid waste from the site of discharge. Bacterial isolation and identification according to Bergey's Manual of Determinative Bacteriology. For the sensitivity test employing the Kirby Bauer method and antibiotic, Amoxicillin and Gentamicin is employed, and the results are interpreted according to CLSI guidelines. Based on the results of the study, the bacteria Streptococcus spp, Escherichia spp and Staphylococcus spp were identified in the wastewater at Ratatotok General Hospital, Southeast Minahasa. The bacteria identified showed an interpretation of being resistant to the antibiotic Amoxicillin and some bacteria is still sensitive for Gentamicin.

## KEYWORDS

Hospital wastewater, identification of bacteria, sensitivity test.

## INTRODUCTION

The hospital is a facility for treating a variety of societal disorders. However, it is indisputable that the hospital is also a site where many ailments, such as infections, can be contracted. This hospital-acquired infection can be classified as an infection related to health services, which, according to the World Health Organization, is an infection acquired by a patient in a hospital or other health facility that has not manifested or is not in the incubation period when the patient is admitted. These include hospital-acquired illnesses that persist after discharge and occupational infections among hospital and health facility personnel.[1]

This health care-related infection may be caused by bacteria, viruses, or fungus. These pathogens may originate from the normal flora of the patient's body or from the patient's care environment, such as the water, air, food, and beverages provided to the patient. These bacteria can cause a variety of illnesses, including diarrhea, typhoid fever, pneumonia, and hemorrhagic colitis.[2]

Hospitals are one of the sources of garbage in a region. Solid, liquid, and gaseous hospital waste can be contagious, include pathogenic bacteria and toxic substances, and be detrimental to human health and the environment. Hospitals in Indonesia generate vast quantities of trash, which can be hazardous to health and contaminate the environment. The study of hospital wastewater treatment in Indonesia revealed that only 53.4% of hospitals treat their wastewater. Hospitals only examine the quality of liquid waste 57.5 percent of the time. This demonstrates how great the potential for hospitals to contaminate the environment and cause additional issues including disease spread is. The standard for the maximum amount of liquid waste that can be discharged into the environment is known as the liquid waste quality standard. The quality standard of liquid waste for hospitals refers to the maximum amount of liquid waste that may be discharged into the environment from a hospital activity.[3]

In the WHO guidebook for the prevention of hospital-acquired infectious diseases, gram-negative bacteria such as *Pseudomonas aeruginosa, Aeromonas hydrophilia, Burkholderia cepacia, Stenotrophomonas maltophilia, Serratia ma - rcescens, Flavobacterium meningo-septicum, Acinetobacter calcoa-ceticus, Legionella*.[1.]

The usage of antibiotics to eliminate the previously indicated microorganisms may have prevented the patient's sickness. Nonetheless, it is evident that this makes these germs resistant to the antibiotics used to treat them. This may be owing to the irresponsible use of antibiotics, as many bacteria are now resistant to antibiotics. In addition to the above-mentioned bacteria, many other resistant bacteria can cause health care-associated infections, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) / Vancomycin-resistant *Staphylococcus aureus* (VRSA), bacteria that produce extended-spectrum beta-lactamases (ESBL), multi-drug resistant organisms (MDRO), Vancomycin Resistant *Enterococcus* (VRE), and *Clostridium difficile*.[4]

Ratatotok Hospital in Southeast Minahasa is said to have an existing Waste Water Treatment Plant (IPAL/WWTP). This system serves to remove biological and chemical waste from the water so that it may be utilized for other reasons. Effective WWTPs will produce effluent that conforms to environmental quality criteria.[5] Antibiotics have been identified as a component of hospital liquid waste in a number of earlier studies.[6] Resistant and persistent, the antibiotics found in this waste pose a significant risk when used repeatedly. Hospital effluents contain a broader range and higher concentrations of pharmaceuticals, but are typically released into sewers without treatment. Due to the fact that urban wastewater treatment plants are not designed to treat HWW, treated effluent may still contain pollutants that can negatively impact the receiving aquatic ecosystem.[7]

The sewage treatment procedure can lower the amount of bacteria in wastewater, however effluent often still contains a substantial number of resistant and vulnerable bacteria. Several studies have also studied the microbiological composition of hospital wastewater and identified pathogens comparable to those found in household waste. 5 to 10 percent of the microorganisms found in hospital wastewater are *Bacillus spp, Staphylococcus spp, and Streptococcus spp. Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Proteus*, and *Enterobacter spp*. have all been documented as nosocomial infections. It has also been observed that the majority of these microorganisms are resistant to commonly used antibiotics, resulting in the spread of several diseases and infections.[8]

## METHODS

Quantitative descriptive research method is a research that has the aim to describe a phenomenon, events, symptoms and events that occur factually, systematically, and accurately. Phenomena can be in the form of forms, activities, relationships, characteristics, as well as similarities and differences between phenomena. This study used a cross-sectional descriptive method to determine the types of bacteria found in the water waste disposal outlet of the Ratatotok General Hospital, Southeast Minahasa. For examination of pH, Biological Oxygen Demand, Chemical Oxygen Demand, Total Suspended Solids, and Total Coliform, were carried out at the Manado SGS-WLN Laboratory using the standard reference APHA-4500H+.B (2017) and

APHA 5210-B(2017).[9] For the identification of bacteria, the examination is carried out in the Lab. Microbiology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University.

The sample in this study came from the wastewater treatment plant (WWTP) in Ratatotok Hospital, Southeast Minahasa. Sampling was carried out following the Indonesian National Standard on Water and Wastewater Section 59 using different sterile bottles. Sampling was carried out on water samples from the WWTP outlet of Ratatotok Hospital, Southeast Minahasa.

Primary data obtained from water samples that have been identified will then be presented in tabular form.

## RESULTS AND DISCUSSION

In table 1, it shows the results examination of pH, Biological Oxygen Demand, Chemical Oxygen Demand, Total Suspended Solids, and Total Coliform.

**TABLE – 1**

| No | Test Description | UoM | Result | GRL | Method Reference9 |
|---|---|---|---|---|---|
| 1 | pH | n/a | 7.92 | 6-9 | APHA-4500H+.B (2017) |
| 2 | Biologycal Oxygen Demand | mg/L | 10 | 30 | APHA 5210-B (2017) |
| 3 | Chemical Oxygen Demand | mg/L | 25 | 100 | APHA-5220-D (2017) |
| 4 | Total Suspended Solids | mg/L | 6 | 30 | APHA-2540-D (2017) |
| 5 | Total Coliform | MPN/100mL | >24200 | 3000 | APHA-9223-B (2017) |

In water chemistry, pH measurement is one of the most significant and commonly used assays. Almost every aspect of water supply and wastewater treatment is pH-dependent, including acid-base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control. pH is utilized in alkalinity and carbon dioxide measurements, as well as in numerous acid-base equilibriums. At a particular temperature, pH or hydrogen ion activity indicates the degree of a solution's acidic or basic nature.

The biochemical oxygen demand (BOD) determination is an empirical test that uses established laboratory processes to evaluate the relative oxygen demands of wastewaters, effluents, and polluted waterways. Measuring waste loads to treatment plants and evaluating the BOD-removal performance of such treatment systems are the most common applications of this test. The test analyzes the molecular oxygen utilized during a predetermined incubation period for the biochemical breakdown of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferric iron. It may also quantify the oxygen required to oxidize reduced forms of nitrogen (nitrogenous demand), unless their oxidation is inhibited by an inhibitor. The seeding and dilution processes estimate the BOD at a pH range of 6.5 to 7.5.

The chemical oxygen demand (COD) is the quantity of a certain oxidant that reacts with a sample under controlled conditions. The amount of consumed oxidant is given as its oxygen equivalent.

A sample is filtered through a weighted standard glass-fiber filter, and the residue retained on the filter is dried at 103 to 105 degrees Celsius to a constant weight. The rise in filter weight shows the total amount of suspended particles. If the suspended material clogs the filter and prolongs filtration, it may be essential to raise the filter's diameter or reduce the volume of the sample. Calculate the difference between total dissolved solids and total solids to obtain an estimate of total suspended solids.

The enzyme substrate test uses hydrolyzable substrates to detect both total coliform bacteria and Escherichia coli enzymes simultaneously. When using the enzyme approach, the total coliform group is defined as all bacteria containing the enzyme -D-galactosidase, which cleaves the chromogenic substrate, releasing the chromogen. Escherichia coli are bacteria that produce a positive total coliform response and contain the enzyme -glucuronidase, which cleaves a fluorogenic substrate, releasing the fluorogen. The test may be administered in multiple-tube, multi-well, or presence-absence (100 mL sample) formats.

For this results pH, Biological Oxygen Demand, Chemical Oxygen Demand, Total Suspended Solids, it can be seen that the WWTP outlet in Ratatotok Hospital still shows results that are in accordance with the given standard.

The results for total coliform indicate a high score relative to the given standard.

**Tabel 2** The results of bacterial identification by morphological tests, bacterial physiological tests and biochemical tests at the outlet of liquid waste.

| | Isolate | | | |
|---|---|---|---|---|
| | 10-1 | 10-2 | 10-3 | 10-4 |
| Morphology Test | | | | |
| Gram staining | - | + | + | + |
| Cell shape | Basil | Coccus | Coccus | Coccus |
| Physiology & biochemistry Test | | | | |
| Motilities | - | + | - | - |
| Indole | - | - | - | - |
| H2S | - | - | - | - |
| Gas | + | + | + | + |
| Glucose | + | + | + | + |
| Maltose/lactose | + | - | + | + |
| Lysine | + | + | + | + |
| Citrate | - | + | + | + |
| Catalase | + | - | + | - |
| Identification results | Escherichia spp | Streptococcus spp | Staphylococcus spp | Staphylococcus spp |

Three distinct bacterial species, namely Escherichia spp, Streptococcus spp, and Staphylococcus spp, have been discovered at the wastewater system's output (Table 2). The categorization of bacteria were modified in accordance with the ninth edition of Bergey's Manual of Determinative Bacteriology.The bacteria detected from the wastewater outlet, Escherichia spp, exhibit the features of a motile bacillus/rod cell shape because they have peritrichous flagella and can be non-motile, according to Bergey's Manual of Determinative Bacteriology, 9th Edition. Fermentation of glucose and lactose results in the generation of acids and gases that can be observed within 24 hours. Due to the fact that these bacteria consume less nitrate as an energy source, the citrate test results were negative.

The bacteria observed at the effluent outlet were Streptococcus spp, which had round or ovoid cell morphologies, were gram-positive, and could be motile in certain strains, but they were more frequently non-motile. On the inclined media, these bacteria are able to ferment carbohydrates or glucose, causing the bottom of the Triple Sugar Iron Agar (TSIA) to turn yellow, while the slant remains red. Negative results were also observed in the catalase test, which was characterized by the absence of bubbles after the addition of 3% hydrogen peroxide (H2O2), as the bacteria were unable to produce catalase enzymes, which directly catalyze the conversion of toxic hydrogen peroxide into water and oxygen.

The following bacteria, *Staphylococcus spp*., has a single round cell or pairs arranged in tetrads and irregular groupings. It is a non-motile gram-positive bacteria. Most strains create acetoin from glucose and ferment diverse carbohydrates by reducing nitrate. Additionally, Staphylococcus spp utilizes citrate as its primary carbon source and ammonium ion as its sole nitrogen source. This occurs when the addition of citrate raises the pH of the media, causing the color of Simmons Citrate media to change from green to blue. This study's findings are consistent with research conducted on the University of Benin Teaching Hospital's wastewater (UBTH). Eight bacterial isolates, including *Bacillus spp, Streptococcus spp, Escherichia spp,* and *Staphylococcus spp*, were discovered.[11] *Escherichia spp* and *Bacillus spp* bacteria were discovered in the wastewater treatment plant (WWTP) of Sanglah Hospital in Bali, Indonesia, according to research on the detection of bacteria in residential wastewater.

**Table 3.** Antibiotic sensitivity test against bacteria

| No. | Antibiotics | Isolate code | | |
|---|---|---|---|---|
| | | RRT A.1 | RRT A.2 | RRT A3 |
| 1. | Amoxicillin | 9,5 | 9 | 7 |
| 2. | Gentamicin | 8,5 | 30 | 27 |

After identifying the bacterial isolates, a sensitivity test to antibiotics is conducted using the disc diffusion method or the Kirby Bauer method. Calculated using the zone of inhibition of Amoxicillin and Gentamicin antibiotic discs. Table 3 presents the results of measurements and interpretations.

The antibiotic sensitivity test results for Amoxicillin and Gentamicin against bacteria are shown in Table 3, and the diameter is measured with a caliper. All bacteria with a generated diameter 15 mm were resistant to the average diameter of the inhibitory zone for Amoxicillin antibiotics. In the meantime, RRT A.2 and RRT A.3 isolates exhibited an inhibition zone of >24 mm, indicating that the antibiotic was still sensitive.

Three species of *Streptococcus spp, Escherichia spp*, and *Staphylococcus spp* were resistant to the antibiotic Amoxicillin, according to the results of a sensitivity test conducted on bacterial isolates from the wastewater disposal system of Ratatotok Buyat General Hospital in Southeast Minahasa.

Based on statements from the Clinical & Laboratory Standards Institute (CLSI) regarding several groups of organisms regarding zone diameter and MIC breakpoints, other groups of organisms do not yet have standard methods for assessing sensitivity or susceptibility to antibiotics due to insufficient research to develop reproducible, definitive standards. These organisms may require different media or incubation conditions, or their growth rates may vary significantly between strains. A consultation with an infectious disease specialist is indicated for these bacteria in order to determine the need for susceptibility testing and the interpretation of results. 13

Such as studies conducted on wastewater from Mater Misericordiae Hospital, Afikpo Medical Center, and His Grace Hospital in the city of Afikpo, Nigeria. The research has the conclusion that all bacterial isolates were resistant to more than three drugs. *Staphylococcus spp* and *Escherichia spp* were the identified isolates. Afikpo Medical Center isolates exhibited resistance to the medications cefixime and ceftriaxone, but erythromycin was the most effective drug. Then, His Grace hospital isolates demonstrated that the most effective antibiotics were fluoroquinolones (levofloxacin and ciprofloxacin), but the isolates were resistant to cefixime, ceftriaxone, and clindamycin. At the Mater Misercordiae hospital, the isolated strain exhibited the same pattern of resistance reported at the other two hospitals, with levofloxacin being the most effective antibiotic against the isolates.14 The results of an antibiotic sensitivity test on 24 samples of hospital wastewater (influent and effluent) from Hawassa University Referral Hospital (HURH) and 8 samples of hospital wastewater from Yirgalem Hospital (YAH) in Southern Ethiopia revealed that Escherichia spp (E.coli strain) is resistant to ceftriaxone.15 In a study of wastewater from the University of Calabar Teaching Hospital (UCTH) and General Hospital Calabar (GHC), *Streptococcus bacterium spp* were among the isolates. Both hospitals are public health centers in Calabar, Cross River State, Nigeria. *Streptococcus spp* was resistant to ceftriaxone and all other antibiotics utilized in the study, with the exception of pefloxacin, to which UCTH *Streptococcus spp* shown sensitivity. The pattern of resistance generated was determined to be multiple antibiotic resistance (MAR), which implies that no bacteria isolated from wastewater samples from both hospitals exhibited resistance to a single antibiotic to all drugs tested.

## CONCLUSIONS

Based on the results of the study, the bacteria Streptococcus spp, Escherichia spp and Staphylococcus spp were identified in the wastewater at Ratatotok General Hospital, Southeast Minahasa. The bacteria identified showed an interpretation of being resistant to the antibiotic Amoxicillin and some bacteria is still sensitive for Gentamicin.

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