



Faculty of Resource Science and Technology

**Identification and Characterisation of Coliform from Tebalau River Water Environment
in Betong, Sarawak.**

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Bachelor of Science with Honours
(Resource Biotechnonology)
2022

**Identification and Characterisation of Coliform from Tebalau River Water Environment
in Betong, Sarawak.**

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of
Science with Honours
(Resource Biotechnology)

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Acknowledgement

First of all, I would like to thank God for His tremendous blessings and strength that allowed me to complete my final year project. Through Him who strengthens me, I can accomplish everything.

Dr. Samuel Lihan, Senior Lecturer in the Faculty of Resource Science and Technology (FRST), my supervisor, was instrumental in helping me to complete my project successfully. He provided me with constant encouragement and guidance throughout the entire project.

Apart from that, I would like to express my gratitude to my parents, Mr. Kabilan A/L Murugaiyan and Mdm. Prema Kabilan, for their unwavering support and encouragement. My family continually reminded and encouraged me to give this endeavor my all. Since the beginning, they have been my greatest source of inspiration.

I owe a deep sense of acknowledgment to the Faculty of Resource Science and Technology for giving me the resources I needed to complete my study, as well as Khairunnisa Binti Mohammad Hamdi, a postgraduate student at the Virology Laboratory, for her ongoing support in the laboratory.

Finally, I would like to express my gratitude to my fellow friends for always being there for me and providing me with the moral support I needed. Besides that, it is my privilege to thank everyone else who contributed to the project's completion. Your gifts are greatly valued and will be remembered for a long time.

Identification and Characterisation of Coliform from Tebalau River Water Environment in Betong, Sarawak.

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ABSTRACT

Enterobacteriaceae are a Gram-negative bacteria family that can cause urinary tract infections (UTIs), lung infections, soft tissue infections, osteomyelitis, and a variety of other illnesses. The rise in antibiotic resistance bacteria has been a concerning issue due to the ineffectiveness of antibiotic drugs to treat illness. The high concentration of fecal indicator in river water indicates the potential risk public is exposed to. The main purpose of this research is to determine the microbial count of total coliform and fecal coliform, isolate and identify all the 20 isolates. All the isolated coliform from Tebalau river water were characterised by antibiotics susceptibility. DNA was extracted and used as template for 16S rDNA PCR. The PCR product were run on agarose gel electrophoresis to separate the DNA molecules based on their size. The 16S rDNA sequencing was done, and the results showed that 10 isolates were from Enterobacteriaceae family, 3 isolates from Chromobacteriaceae, 3 isolates from Pseudomonadaceae, and the remaining isolates were each from Bacillaceae, Yersiniaceae and Aeromonadaceae families. Kirby –Bauer disc diffusion method was carried out to determine antibiotic susceptibility test. Out of 20 isolates, 19 isolates were found to be resistance to more than four antibiotics. In conclusion, communities residing nearby the river water should be aware of the health hazards caused by the bacteria in the water. I recommend experts to do further research in identifying and characterizing bacteria from Tebalau river water environment as there is a lack of research on water and waterborne bacteria reported from the study area.

Keywords: Enterobacteriaceae, 16S rDNA, Kirby-Bauer disc diffusion, fecal coliform, public health risk.

ABSTRAK

Enterobacteriaceae ialah keluarga bakteria Gram-negatif yang boleh menyebabkan jangkitan saluran kencing (UTI), jangkitan paru-paru, jangkitan tisu lembut, osteomyelitis, dan pelbagai penyakit lain. Peningkatan bakteria rintangan antibiotik telah menjadi isu yang membimbangkan kerana ketidakberkesanan ubat antibiotik kepada penyakit. Kepekatan penunjuk najis yang tinggi dalam air sungai menunjukkan potensi risiko orang ramai terdedah. Tujuan utama penyelidikan ini adalah untuk menentukan kiraan mikrob jumlah koliform dan koliform najis, mengasing dan mengenal pasti kesemua 20 pencilan tersebut. Kesemua koliform terpencil dari air sungai Tebalau dicirikan oleh kerentanan antibiotik. DNA telah diekstrak dan digunakan sebagai tempalats untuk 16S rDNA PCR. Produk PCR dijalankan pada elektroforesis gel agarose untuk memisahkan molekul DNA berdasarkan saiznya. Penjujukan 16S rDNA telah dilakukan, dan keputusan menunjukkan bahawa 10 pencilan adalah daripada keluarga Enterobacteriaceae, 3 pencilan daripada Chromobacteriaceae, 3 pencilan daripada Pseudomonadaceae, dan pencilan selebihnya adalah setiap satu daripada keluarga Bacillaceae, Yersiniaceae dan Aeromonadaceae. Kaedah Kirby-Bauer disc diffusion telah dijalankan untuk menentukan ujian kerentanan antibiotik. Daripada 20 isolat, 19 isolat didapati tahan terhadap lebih daripada empat antibiotik. Kesimpulannya, masyarakat yang tinggal berdekatan dengan air sungai harus sedar tentang bahaya kesihatan yang disebabkan oleh bakteria di dalam air. Saya mengesyorkan pakar untuk membuat kajian lanjut dalam mengenal pasti dan mencirikan bakteria dari persekitaran air sungai Tebalau kerana terdapat kekurangan penyelidikan mengenai air dan bakteria bawaan air yang dilaporkan dari kawasan kajian.

Kata kunci: Enterobacteriaceae, 16SrDNA, Kirby-Bauer disc diffusion, koliform najis, risiko kesihatan awam.

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List of Abbreviations

AGE	Agarose Gel Electrophoresis
AST	Antimicrobial susceptibility testing
bp	Base pair
°C	Degree Celsius
CFU	Colony forming units
DNA	Deoxyribonucleic acid
ddH ₂ O	Double-distilled water
dNTPs	Deoxyribonucleotide triphosphate
<i>E. coli</i>	<i>Escherichia coli</i>
ESBL	Extended spectrum beta-lactamase
EtBr	Ethidium bromide
g	Gram
hrs	Hours
ICU	Intensive Care Unit
<i>K. pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Kb	Kilobyte
L	Litre
LB	Lysogeny broth

m	meter
MAR	Multiple antibiotic resistance
MgCl ₂	Magnesium chloride
mg/L	Milligrams per Liter
mL	Milliliter
μl	Microliter
PCR	Polymerase chain reaction
pH	Potential of Hydrogen
rDNA	Recombinant Deoxyribonucleic acid
rpm	Rotation per minute
TAE	Tris-acetate-EDTA
TSA	Trypticase Soy Agar
UV	Ultraviolet
V	Voltage
%	Percentage

Chapter 1 : Introduction

1.1 Background

Tourists and nearby residents commonly use the river water environment for recreational activities such as fishing, swimming, water sports, and many more. Pollution in the water, mainly caused by fecal is known as fecal pollution. Improper sewage disposal and domestic pet waste can lead to fecal pollution (Boehm & Sassoubre, 2014). This river water pollution is often considered as a significant issue in upcoming countries as it brings health risks to the public. The economy of the growing country would face a downfall if rivers and recreational water environments are closed down due to pollution as it would cause the rate of tourists to drastically drop which then consequently affects the economy of the country. Out of the many health risks the public may be exposed to, diarrhea, gastrointestinal illness, and urinary tract illness as suggested by presented reports are considered to be the common threats.

Enterobacteriaceae are a type of bacteria group that belong to the bacteria family of Gram-negative bacteria. This bacteria family also consists of *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and so on (Cabral, 2010). Indicator bacteria such as fecal coliform and Enterobacteriaceae allow us to determine the level of bacteria present and whether the seawater is safe enough for the public to be utilized. The permitted level that won't be hazardous to the public is ≤ 35 colony forming units (CFU) of culturable *Enterococci* per 100 mL (Smeets *et al.*, 2010, as cited in Adeniji *et al.*, 2019). These contaminants bring an impact on the recreational water environment in many growing countries.

The characteristics of Enterobacteriaceae to thrive aerobically and anaerobically in extreme temperatures and pH, allows them to withstand the external factors to survive. Apart from *Klebsiella*, all the other genera are motile as they possess flagella that aids in their movement. The presence of cytotoxic enterotoxin causes watery diarrhea and the presence of cytotoxic enterotoxin, causes tissues to be damaged (Evans & Evans, 1996). Children are the primary victim of recreational water environment pollution as they are at a higher risk compared to adults. This is because children often swallow the river water when they swim and are unaware of the danger that might cause them. River water is considered the main source of exposure to pathogens (Arnold *et al.*, 2016). Hence, children are usually the victim as they generally have weak immune systems toward pathogens, making them more prone to diseases and will be unable to cure the disease fast.

Antibiotic-resistant bacteria are considered a threat to the efficacy of antibiotics, as these bacteria are able to withstand harsh conditions. Along with that, antibiotic-resistant bacteria can multiply and develop even during the existence of antibiotics. The primary cause of the occurrence of antibiotic-resistant bacteria is the overuse of antibiotics as described by Sir Alexander Fleming in early 1945 (Abraham, 1963). Once infected by the bacteria, it takes a long period of time to recover, and sometimes it's untreatable and causes fatality. Hence, this is why it is crucial to identify and characterize bacteria especially in recreational water environments to make sure the safety of the public is protected from antibiotic-resistant bacteria.

Tebalau river is a wonderful place in Sarawak that attracts visitors with its forests and simplicity with a hint of nature. This recreational water environment provides the public with

several activities such as fishing, swimming, and trekking. There are many waterborne diseases that cause health risks via direct exposure such as accidental ingestion of contaminated water and many more (Alipour et al., 2014). Thus, it is important to bring awareness to the public and determine the bacteria that carry risk to the health of the public.

1.2 Objectives:

1. To determine the microbial count of total coliform and fecal coliform in water samples from Tebalau river.
2. To isolate and identify the coliform from the water samples of Tebalau river.
3. To characterize antibiotic susceptibility of the isolated coliform from the Tebalau river.

Chapter 2 : Literature Review

2.1 Tebalau river, Betong

Tebalau river is located at the northwest of Sungai Mupoh and north of Sungai Selimau. The Tebalau river has 134 metres as an elevation. The latitude of the Tebalau river is 1.53333° and at the longitude of 111.6 °. Tebalau river is used by the communities nearby for drinking, bathing, swimming and many other purposes. Since the river is used for various purposes, the deterioration of water quality in the river should be monitored as it poses a great impact on the aquatic biota and the human health. The river water is contaminated with pathogenic bacteria such as *E. coli*, Enterobacteriaceae family, Pseudomonadaceae family and many more.

2.2 Total coliform and fecal coliform

The standard bacterial indicator of fecal contamination in wastewater and other waters, is fecal coliforms (Berg *et al.*, 1978). For decades, the concentration of indicator bacteria in ocean waters has been used to assess the safety of recreational water (Noble *et al.*, 2003).

Total coliforms are a type of bacterium that can be found in abundance in the environment. *E. coli* (*Escherichia coli*) is the sole member of the entire coliform bacteria family that can only be found in the intestines of mammals, including humans. In addition to other microorganisms, each human excretes 100-400 billion coliforms per day. *E. coli* in water suggests recent fecal contamination and may indicate the presence of disease-causing pathogens

such as bacteria, viruses, and parasites, among other things. Coliform bacteria are facultatively anaerobic, Gram-negative, non-spore-producing rod-shaped bacteria that grow red colonies with a metallic (golden) sheen within 24 hours on an end-type lactose medium. Contamination of human-animal wastes from seeping animal manure, inadequately treated septic and sewage discharge, storm water runoff, or domestic animals are the primary causes of total coliform. Bacteria and dangerous germs from the sources may be discharged into the river during and after precipitation. It's possible that there isn't enough area to properly dispose of human waste directly into the river. The coliform in the water have no taste, smell, or color. As a result, determining if bacteria are present is challenging (Divya & Solomon, 2016).

2.3 Enterobacteriaceae

The family of Enterobacteriaceae consists mainly of *Enterobacter*, *Klebsiella*, *Citrobacter*, *Escherichia coli*, and *Serratia* genera. Opportunistic infections are infections that cause more harm to people who have weak immune systems compared to people with healthy immune systems. This family of bacteria causes many opportunistic infections in humans such as infection in the gastrointestinal tract, diarrhea, pneumonia, and meningitis (Church, 2015). These Gram-negative bacteria grow well at 37 °C in an optimum culture medium (Abbas *et al.*, 2014). The influence of fecal indicator bacteria in high levels in surface water provides indicators for measurable quantities of unspecified agents that directly facilitate or imply waterborne hazards like pathogens, biotoxins, and chemicals (Deshmukh *et al.*, 2016).

As for the characteristics of among the many genera in Enterobacteriaceae, *Enterobacter* has a diverse and comprehensive system for controlling membrane permeability. The ability of the Enterobacteriaceae to avoid the action of a large number of antibacterial drugs appears to be one of their most important biological characteristics (David *et al.*, 2019).

As for *Klebsiella* spp., only a few of the components have been discovered such as exotoxins, capsules, adhesins, and siderophores (Gundogan, 2014). Most *Klebsiella* spp., isolates are naturally resistant to ampicillin due to a constitutively generated chromosomal b-lactamase. One of the most important pathogens capable of manufacturing extended-spectrum b-lactamase is *K. pneumoniae* (ESBL). Hence, *K. pneumoniae* is a significant pathogen in the synthesis of antibiotics.

2.4 Antibiotic-Resistant Bacteria

Antibiotic-resistant organisms have been found in water habitats, and transposons, plasmids, and integrons have been used to pass antibiotic resistance genes on to other disease-causing bacteria. Bacteria isolated from beach sand, seawater, and sediments have been found to be resistant to antimicrobials. Furthermore, the presence of many antibiotics from industrial sources flowing in water environments has the potential to modify microbial ecosystems (Akanbi *et al.*, 2017). Antibiotics generally have many benefits such as aiding in achieving medicine and surgery. Besides that, antibiotics are able to treat or prevent infections for those who go through chemotherapy and chronic diseases such as diabetes, rheumatoid, and arthritis. This consequently allows the patients to extend their lifespan as antibiotics help to treat or prevent the infection.

The primary cause of the emergence of antibiotic-resistant bacteria is the overuse of antibiotics. Along with that, there are also bacteria species that can undergo mutation spontaneously that may cause the bacteria to develop into antibiotic-resistant bacteria. This phenomenon usually occurs through natural selection. Besides that, incorrectly prescribed antibiotics can promote the growth of resistant bacteria. Antibiotic use in agriculture has an impact on the microbiome in the environment. Antibiotics given to animals are expelled in urine and feces to the extent that they are widely disseminated through fertilizer, groundwater, and surface runoff (Abraham, 1963) which can contaminate the seawater.

There are many reports that suggest Enterobacteriaceae is resistant to antibiotics. The β lactam type in particular is now known to be dominated via the mobilization of continuously expressed single genes. These single genes play a role in encoding to increase the efficacy of drug modifying enzymes (Iredell *et al.*, 2016). It's been discovered that in the healthcare system, there will be many cases where antibiotic resistance might occur to patients with lifesaving options and therefore patients, unfortunately, will not be able to opt for the available option and this will cause fatality (Friedman *et al.*, 2016). Therefore, the use of polymyxins are recommended to utilize in empirical antibiotics mainly in ICUs where the known probability of a Gram-negative bacterium being polymyxin-only susceptible is close to 50% in the presence of carbapenem-resistant bacteria (Falagas & Kasiakou, 2005).

2.5 Public Health Risk

Human or animal fecal waste pollution of recreational water is a major public health concern. Recreational activities in wastewater-polluted river waters are also thought to cause gastrointestinal illness and millions of cases of severe respiratory illness per annum (Anbiah & Thankamony, 2021). Swimming-related illness is usually caused by microbial diseases that enter the water from point sources. To assure the safety of seawater for recreational reasons, microbiological examinations of river water are required. Several dangerous bacteria found in seawater are capable of producing diseases such as diarrhea and cholera, posing a risk to human health. Because river water are increasingly being used for waste disposal, particularly sewage drainage, which can carry a high load of pathogenic bacteria, particularly enteric groups, as this has resulted in potentially dangerous changes in the characteristics of the marine environment, posing a public health risk.

2.6 Identification and characterization of coliform in Tebalau river water

2.6.1 16S rDNA Sequencing

The identification of coliform can be done via 16S rDNA as it is used to identify bacterial isolates accurately. Besides that, 16S rDNA sequences is utilized to determine the phylogenetic relationships, the finding, and classification of novel bacterial species (Woo *et al.*, 2008). One of the most critical functions of clinical microbiology laboratories is accurate bacterial isolate identification. On a patient-by-patient basis, precise identification is critical in identifying

whether the isolate is causing true infection or is simply a colonizer or contaminant, as well as the necessary antibiotic treatment and duration (Woo *et al.*, 2008).

In addition to the widespread use of PCR and DNA sequencing, 16S rDNA has aided in the precise identification of bacteria and the discovery of new bacteria. Furthermore, even for rare isolates, 16S rDNA sequencing gives unambiguous data that is repeatable in and between laboratories. Therefore, 16S rDNA sequencing is ideal for bacterial strain identification and especially effective for bacterial species that are difficult to identify using phenotypic testing.