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Biological Sciences Department

Comparative analysis of antimicrobial resistance and heavy metal resistance in bacteria isolated from various environmental samples found in Bindura

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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Biotechnology

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Approval form

The undersigned certify that they have read the dissertation titled '**Comparative analysis of antimicrobial resistance and heavy metal resistance in bacteria isolated from various environmental samples found in Bindura** ' and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

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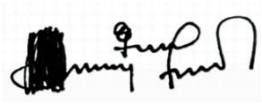
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Dedication

I dedicate this project to my parents, who have supported and believed in me through this journey at Bindura University.

Acknowledgements

I'd want to express my heartfelt gratitude to my supervisor, Dr Claudious Gufe, for his invaluable advice, patience, and support during this project. His observations substantially affected the course of this research. I'd also want to thank the staff and students of the Biological Sciences department for their support. Finally, I'd want to thank my friends and family for their ongoing support during the journey.

List of abbreviations

AMR: Antimicrobial Resistance

MIC: Minimum Inhibitory Concentration

HMR: Heavy Metal Resistance

WHO: World Health Organisation

MGE: Mobile Gene Transfer

WWTP: Wastewater Treatment Plant

HGT: Horizontal Gene Transfer

ARG: Antimicrobial Resistant Gene

PCR: Polymerase Chain Reaction

DNA: Deoxyribonucleic acid

NGS: Next Generation Sequencing

WGS: Whole Genome Sequencing

ARB: Antibiotic-Resistant Bacteria

CLSI: Clinical and Laboratory Standards Institute

AML: Amoxicillin

CIP: Ciprofloxacin

TE: Tetracycline

CN: Gentamicin

MTZ: Metronidazole

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ABSTRACT

Antimicrobial resistance (AMR) and heavy metal resistance (HMR) are becoming more serious hazards to public health, particularly in low-income nations with weak environmental regulations. Co-selection of these qualities is possible when resistance genes are carried on mobile genetic elements. The purpose of this study was to evaluate the AMR and HMR profiles of bacteria isolated from environmental samples in Bindura, Zimbabwe, and to see if there was a significant link between these resistance types. Thirty environmental samples (soil, water, and sediment) were obtained from 30 Bindura locations. Bacteria were isolated from a selective medium and identified using morphological and biochemical testing. The antimicrobial susceptibility of amoxicillin, ciprofloxacin, and tetracycline was investigated using disc diffusion. Agar dilution was used to test heavy metal resistance to lead, silver, zinc and mercury. A MIC of ≥200 μg/mL was considered resistant. *Escherichia coli* (80%) and *S. aureus* (53% of locations) were the most common isolates. Ciprofloxacin had the highest resistance rate (68% of isolates), followed by amoxicillin (61%). Heavy metals showed the highest rate of lead resistance (48%). The Pearson correlation revealed a small but statistically significant positive relationship between AMR and HMR ($r = 0.275$, $p = 0.010$). Isolates resistant to all three metals exhibited considerably greater antibiotic resistance ratings than metal-susceptible isolates ($p < 0.001$). Co-resistant bacteria thrive in Bindura's polluted settings. While there is a strong link between AMR and HMR, the modest correlation shows that other variables play an important role in resistance propagation. It is advised that antibiotics and heavy metals be monitored more closely and their sources controlled.

Keywords: Antimicrobial resistance, heavy metal resistance, co-selection, environmental samples, bacteria.

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CHAPTER 1

1.0 INTRODUCTION

This chapter describes the background information of the study, problem statement, justification, research questions and objectives of the study.

1.1 Background of the study

The increasing rise and existence of antimicrobial resistance (AMR) and heavy metal resistance pose a huge threat to public health. Antimicrobial resistance occurs when microbes like bacteria, fungi, and viruses evolve, developing resistance to antimicrobial medications, for example, antibiotics used for treating infections (WHO 2020). Bacteria are under selective pressure to acquire adaptive mutations or genes that allow them to survive the use of antibiotics. The resistance genes acquired cause unrelenting sickness in humans and animals (Anderson & Hughes, 2017). These increases spread risks, hospital stays and increase the death rate. Bacteria have developed mechanisms to resist the effects of antibiotics, such as production of drug-inactivating enzymes, target site modification, efflux pumps, and reduced membrane permeability (Davies & Davies, 2010). These adaptive strategies enable resistant bacteria to persist in hostile environments, spread resistance genes, and pose a significant threat to global health. Antimicrobial resistance is a natural process that has accelerated and been exacerbated due to human activities. Antimicrobial resistance of bacteria may be caused by various factors, such as health care facilities, agricultural practices, industrial disposal, and veterinary medicine. The dependence on antibiotics has resulted in the emergence of a resistant strain of bacteria, mainly known as the superbugs (Algammal et al 2023).

Alongside the AMR crisis is the growing problem of environmental contamination by heavy metals. Heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), and nickel (Ni) are non-biodegradable and persist in the environment for prolonged periods (Jaishankar et al., 2014). The increasing pace of metal pollution in many developing countries can be attributed to industrial activities, mining, and agriculture. In the case of Zimbabwe, mining of nickel and gold is common, especially in mining towns such as Bindura, resulting in heavy metal pollution in soils and rivers around such areas (Mtetwa et al., 2017). Prior studies conducted in the country indicated the presence of high levels of nickel (between 0.8 and 2.4 mg/L) in the Mazowe river, exceeding WHO guidelines for drinking water quality, which is 0.07 mg/L (Mtetwa et al.,

2017; ZEMA, 2019). Until now, no study has provided an extensive analysis of the type of microorganisms thriving in such contaminated environments, nor their resistance levels. Heavy metals affect microbial communities' structure and impose selective pressures. Therefore, there will be metal-tolerant or heavy metal-resistant (HMR) bacteria.

According to (Nnaji et al, 2024), “Heavy metal resistance is the ability of bacteria to thrive and survive in an environment contaminated with heavy metals, for example, lead, mercury, cadmium, arsenic and chromium, just to name a few”. Heavy metal resistance in bacteria may be caused by wastewater discharge into surface water, contaminating water bodies such as rivers and reservoirs. Heavy metals are natural components in the environment; however, their concentrations are being increased by human activities such as agriculture, industrial, mining and medical waste discharge, and they become toxic to bacteria (Dahms 2018). The release of heavy metals into the environment automatically affects the indigenous microbial population and microbial activities. Heavy metals interfere with normal cellular processes by damaging cell membranes, altering enzyme activity, affecting cellular functions and disrupting DNA structure. Consequently, microorganisms develop survival strategies, including genetic mutation to withstand the selective pressure caused by the metals (Hemme et al, 2016).

In Zimbabwe, most industries release waste into dams and rivers, and improper agricultural activities increase the levels of heavy metal toxification in the surrounding water bodies and soil. This leads to native strains of bacteria selectively surviving by mutation. An understanding of how heavy metal resistance in bacteria may become a duo with antimicrobial resistance allows manipulation and formulation of new drugs that can counteract the new strains. The Zimbabwean health sector will benefit profoundly from this study, as it gives new insights into how to tackle drug mechanisms. With the growing industrial and mining sector of Zimbabwe comes a huge problem, such as industrial disposal in water bodies and land pollution from toxic metals used in their processes. (Sharma 2020). For example, Sites in Penhalonga and Shurugwi that were tested showed microbial contamination of fecal origin above the recommended World Health Organization (WHO) and Zimbabwe effluent guidelines for domestic and drinking purposes. Heavy metals and mineral nutrients were detected at all sites, with some levels exceeding safety

specifications. Some physicochemical parameters measured in situ at some sites were very extreme and above the recommended guidelines.

Conducting a comparative analysis of antimicrobial-resistant bacteria and heavy metal-resistant bacteria is of paramount importance, as it gives an understanding of resistance dynamics. It gives insights and knowledgeon how all environmental factors have an impact on the emergence and spread of resistant bacterial stains. This project outcome can help the countries municipal in regulating and managing them use of antibiotics and heavy metals. My project aims to brin more understanding on to the topic of the correlation between heavy metal resistance and antimicrobial resistance.

According to Akhta (2013), in the Karachi, a coastal area in Pakistan, he discovered resistance in 62% of Gram-positive bacteria and 38% of Gram-negative bacterial strains. Heavy metal resistance was found in 30% of the collected population. This proves that a there ia an existing relationship between the two traits.

1.2 Problem statement

Antimicrobial resistance has shakem the medical world by reducing the effectiveness of antibiotics used to treatdieaseas (WHO, 2020). Although clinical sources are often the focus of AMR surveillance, the environment constitutes an important and often neglected reservoir of resistant bacteria and resistance genes (Berendonk et al., 2015). The ecological dimension of AMR is particularly relevant in developing countries where environmental monitoring is limited, and pollution control infrastructure is weak. There is an increase in environmental heavy metal and antimicrobial contamination due to many illegal mining activities and improper waste management in Bindura, Zimbabwe, which puts selection pressure on co-resistant bacteria, promoting their formation and dissemination. This offers a substantial public, animal, and environmental health risk because bacteria resistant to one kind of substance, such as heavy metals, may also be resistant to antibiotics, aided by horizontal gene transfer and mutation. A comparison investigation is required to determine the magnitude of this core resistance in various contexts and its contribution to the worldwide antimicrobial resistance challenge. This is problematic since the interrelated resistance mechanisms for heavy metal resistance (HMR) and antimicrobial resistance (AMR) are frequently found on mobile genetic elements (MGEs), such as plasmids and integrons. This is easily transmitted from resistant bacteria to susceptible bacteria. These MGEs may be transmitted

across bacteria, allowing for the fast spread of HMR and AMR genes across various microbial communities. Moreover, exposure to heavy metals and antimicrobials in many contexts (e.g., industrial waste, agricultural runoff, and sewage) can exert a significant selection pressure, boosting the survival and spread of co-resistant bacteria. The coexistence of heavy metals and antimicrobial resistance is a growing worry, since resistance to heavy metals might unintentionally select for antibiotic-resistant bacteria, worsening the worldwide danger of AMR and potentially leading to treatment failures for human and animal illnesses. Different environmental samples (such as soil, water, and food) are exposed to different quantities and kinds of heavy metals and antimicrobials. A comparative investigation is necessary to understand the size and processes of co-resistance in various contexts, which is critical for creating effective methods to counteract the spread of antimicrobial resistance.

1.3 JUSTIFICATION OF THE STUDY

The rise of antimicrobial resistance (AMR) is a significant public health crisis in Zimbabwe and worldwide, with resistant infections leading to increased morbidity and mortality. Understanding the prevalence and mechanisms of AMR in native bacterial populations is important for local healthcare systems to effectively address and manage resistant infections. Moreover, due to industrial activities and agricultural practices, heavy metal pollution has become a pressing issue in many regions, including Zimbabwe. Heavy metals can have toxic effects on both human health and ecosystems. Investigating heavy metal resistance (HMR) in bacteria is essential for assessing the ecological implications of pollution and the potential risks to human health through contaminated water and soil.

An increasing amount of evidence indicates that the exposure to heavy metals may promote the co-selection of antibiotic resistance. An understanding of the dynamics of this co-selection can help explain the interactions and relationships between environmental contaminants and microbial resistance, offering insights into how to mitigate these issues. While AMR and HMR are well-studied in many regions, there is a significant gap in research specific to Zimbabwe, particularly in the Bindura area. This study will provide essential local data that can inform public health policies, environmental management strategies, and future research agendas.

The study will provide fundamental data on the occurrence and diversity of bacterial isolates resistant to both antimicrobials and heavy metals in Bindura. Analyzing bacteria from various

matrices, such as water, sediments, and soils, will elucidate the extent of co-resistance and help clarify whether heavy metal exposure enhances antimicrobial resistance. Identification of resistance genes will add to the limited literature on the genetic mechanisms linking metal and antibiotic resistance in sub-Saharan Africa (Gwenzi et al., 2021).

1.4 Purpose of the study

1.4.1 AIM

To compare antimicrobial and heavy metal resistance in bacteria isolated from various environmental samples collected from Bindura.

1.4.2 Specific Objectives

- To isolate and identify bacteria from contaminated environmental samples in Bindura, Zimbabwe.
- To determine the heavy metal resistance of isolated bacteria against selected heavy metals (mercury, lead and silver and zinc)
- To determine the antibiotic resistance profile of isolated bacteria against commonly used and available antibiotics in Zimbabwe.
- To determine the prevalence of bacteria with co-resistance to heavy metals and antibiotics.

1.5 Research questions/Hypothesis

1.5.1 Research questions

1. What bacterial species are prevalent in various contaminated environmental samples collected in Bindura?
2. What are the patterns and levels of antimicrobial resistance exhibited by the isolated bacteria?
3. To what extent are these bacterial isolates tolerant to heavy metals found in the environmental samples?
4. Is there a significant relationship between antimicrobial resistance and heavy metal resistance in the studied isolates?

1.5.2 Hypothesis

H_0 (Null hypothesis): There is no statistically significant association between heavy metal resistance (to Hg, Pb, Ag, Zn) and antimicrobial resistance (to amoxicillin, ciprofloxacin, and tetracycline gentamicin and metronidazole) among bacterial isolates from Bindura environmental samples.

H_1 (Alternative hypothesis): There is a statistically significant positive association between heavy metal resistance and antimicrobial resistance among the isolated bacteria, indicating potential co-selection mechanisms.

1.5 Significance of the study

The results from this study shall be important in many ways. First of all, this will be important in increasing knowledge in regard to the mechanisms of resistance that microbes develop and their effect on the environment. Secomdly, the studyshall help form a platform where scientists interested in studying the environment microbiology and the health of individuals can work together. Bacteria that have developed resistance can be used in bioremediation process in order to clean the contaminated environment. Understanding of profiles and mechanisms of resistance could help in formulating ways to use resistant bacteria to detoxify the contaminated environments. Most scientists in today's world focus either on the study of antimicrobial resistance or heavy metal resistance; but in this study, both types of resistances shall be studied together. The main aim of this study is finding a connection between antimicrobial resistance and heavy metal resistance. Since we have knowledge about the two types of resistances independently, it will be easy to find whether there is connection between them.

CHAPTER 2 LITERATURE REVIEW

2.0 INTRODUCTION

This chapter describes antimicrobial resistance and heavy metal resistance are widely studied because of their impact on environmental quality, ecosystem balance, and human health. Literature from different parts of the world indicates that environmental pollution contributes significantly to the emergence and spread of resistant microorganisms (Martinez, 2009). Antibiotics released into the environment through hospitals, pharmaceutical industries, livestock production, and domestic waste create selective pressure that favors resistant bacterial strains. Similarly, heavy metals introduced through mining, industrial processes, and agricultural chemicals accumulate in soils and water systems, influencing microbial survival and adaptation.

Research has shown that bacteria possess several mechanisms that enable them to resist both antimicrobial agents and heavy metals. These mechanisms include enzymatic degradation, mutation of target sites, efflux pumps, and biofilm formation (Poole, 2002). In many cases, genes responsible for antimicrobial resistance and heavy metal resistance are genetically linked, allowing co-resistance to develop under environmental stress conditions (Pal et al., 2017). This relationship has raised concern among researchers because contamination by heavy metals may indirectly increase antibiotic resistance even in the absence of antibiotics.

Several studies conducted in polluted rivers, wastewater treatment plants, and mining environments have reported high prevalence of multidrug-resistant bacteria associated with heavy metal contamination. In Africa, limited research has focused specifically on environmental resistance patterns despite increasing mining and urbanization activities. Therefore, there is a knowledge gap regarding the comparative occurrence of antimicrobial resistance and heavy metal resistance in environmental samples collected from mining regions such as Bindura. This literature review examines previous studies related to AMR, heavy metal pollution, microbial resistance mechanisms, and environmental contamination in order to provide a theoretical framework for the present study.

2.1 Antimicrobial resistance (AMR)

2.1.1 Sources and Drivers of AMR

The environment serves as a significant reservoir of antimicrobial resistance (AMR) microbes and genes and is increasingly recognized as a key source of clinical AMR. Modern human activities impose an additional burden on environmental AMR due to environmental pollution. promoting its transmission to the clinical setting and posing a serious threat to human health and welfare. Therefore, a comprehensive review of AMR transmission from the environment to the clinic, along with proposed effective control strategies, is crucial. This review systematically summarized current research on the transmission of environmental AMR to clinical settings. Furthermore, the transmission pathways, horizontal gene transfer (HGT) mechanisms, as well as the influential drivers including triple planetary crisis that may facilitate AMR transfer from environmental species to clinical pathogens are highlighted. In response to the growing trend of AMR transmission, we propose insightful mitigation strategies under the One Health framework, integrating advanced surveillance and tracking technologies, interdisciplinary knowledge, multisectoral interventions, alongside multiple antimicrobial use and stewardship approaches to tacking development and spread of AMR.

Antimicrobial resistance has been documented beyond clinical settings, in soil, surface waters, sewage effluents, and agricultural systems (Berendonk et al., 2015). The main drivers include excessive antimicrobial usage in human medicine, livestock, and aquaculture, as well as the release of pharmaceutical effluents and hospital waste (Kümmerer, 2009). In many developing regions, including Zimbabwe, inadequate waste management and poor sanitation further exacerbate environmental accumulation of antibiotic residues and resistance genes (ManyiLoh et al., 2018).

2.1.1 AMR mechanisms

Antimicrobial resistance (AMR) occurs when bacteria develop the ability to withstand the effects of an antibiotic that would normally kill them or inhibit their growth. Resistance can be intrinsic(a natural, inherent trait) or acquired (developed through evolution or gene transfer).

The four main mechanisms of antimicrobial resistance are

1. **Limiting Uptake/Decreased Accumulation:**The bacteria reduce the uptake of the drug such that it does not reach a sufficient concentration to have an effect on the bacteria.

2. **Reduced Permeability:** Alterations in the permeability of the bacterial membrane result in reduced influx of the drug into the bacteria. It is usually due to alterations of the LPS of Gram negative bacteria's outer membrane.
3. **Modification of the Drug Target:** The antibiotic target protein is modified such that the drug fails to attach to it. Modification can be achieved through mutations in the gene coding for the protein, post-translational modification such as methylation of the protein, or using a different form of the targeted protein. For instance, bacteria develop resistance to penicillin drugs by modifying the PBPs proteins.
4. **Enzymatic Inactivation or Drug Modification:** Bacteria produce enzymes that alter or destroy antibiotics thereby limiting their action. Enzymes that destroy antibiotics include lactamases, which break down the beta-lactam ring structure present in antibiotics such as penicillin drugs. Other enzymes include CATs, which add acetate groups to chloramphenicol to inactivate it.
5. **Active Drug Efflux:** Bacteria use s efflux pumps to actively transport the antibiotics out of the cell. This lowers the intracellular concentration. Multidrug efflux pumpsare a common mechanism for both intrinsic and induced resistance.

Acquired resistance often involves horizontal gene transfer of genes coding for resistance determinants, such as those for drug-inactivating enzymes or efflux pumps.

2.1.2 AMR detection methodologies

Detecting AMR is critically important as it enables researchers to identify solutions at an early stage. Although conventional culture-based and molecular based techniques are still widely used to generate AMR data, newer and faster technologies have been developed. In recent years, advanced methods based on microscopy and spectroscopy have been increasingly incorporated into AMR detection (Kaprou et al, 2021).

2.1.2.1 Culture-based/ Phenotypic methods

Phenotypic resistance detection methods identify bacterial growth in the presence of antimicrobial agents. They are generally categorized into manual or automated techniques. According to Kaprou et al. (2021), manual approaches encompass disk diffusion , gradient test , agar dilution and broth microdilution. Automated commercial platforms (VITEK® 2 COMPACT, Sensititre™ ARIS™

2X, and Alfred 60AST system) use these methods as foundational principles. Broth dilution-based platforms typically use ready-made cartridges or plates, including positive controls and gradient concentrations of antibiotics. Sensititre panels belong to the category of microdilution methods. Typically, such panels are plastic multi-well micro-titer plates precision-dosed with dried antimicrobial agents. For instance, the Sensititre panel method was used for the determination of the susceptibility of carbapenem-resistant Klebsiella pneumoniae to polymyxins (Anjum et al, 2017). These methods produce qualitative and quantitative results. EUCAST and the US-based CLSI are the leading international authorities responsible for updating antimicrobial susceptibility testing standards on an annual basis (Kaprou et al, 2021).

2.1.2.2 Molecular- based methods

This type of assays address the detection of antimicrobial resistant genes (ARG), such as multiplex targeting. As stated by Kaprou et al (2021), "The methods are inclusive of PCR-based methods, isothermal amplification methods and DNA microarrays. Molecular-based methods for detecting ARGs, as well as their expression, take advantage of the developments in amplification and nucleic acid hybridization techniques. Molecular-based techniques can offer ARGs detection in a fast and sensitive manner. ARGs encode the ability of bacteria to survive and grow in the presence of antibiotics. In the past, scientists were solely targeting a small fraction of ARGs, but with the decrease in the cost of next-generation sequencing (NGS) technologies and the subsequent expansion in bacterial whole genome sequencing (WGS), the availability of ARG targets in various databases has enormously expanded."

There are non-conventional methods like genome sequencing and metagenomics, which are promising ways of identifying and detecting ARGs. The methods are inclusive of matrix-assisted laser desorption/ ionization, time of flight mass spectrometry, and Fourier transform infrared spectrometry.

2.2 Heavy metal resistance

2.2.1 Mechanism for heavy metal resistance

Along the way, most bacterial strains have evolved genetically to have systems that can cop in heavy metal infested environment. High concentrations of heavy metals can damage proteins and nucleic acids, disrupt osmotic balance, and interfere with oxidative phosphorylation. The mechanisms are as follows:

1. **Bioaccumulation**

This is the gradual buildup of chemical substances like pesticide, heavy metals and other pollutants in the tissues of living organism such as bacteria overtime. Some bacteria can accumulate heavy metals in their cells to sequester them away from critical metabolic pathways (Zhou & Ning, 2017). The process of bioaccumulation involves uptake, storage and elimination.

i. Uptake

Bioaccumulation is initiated when an environmental pollutant crosses the cellular membrane into bacterial cells. Although the process of uptake remains partially obscure, it has been established that chemicals such as heavy metals tend to move or diffuse, passively from a place of high concentration to one of low concentration. This intracellular movement is driven by chemical potential, which serves as the primary thermodynamic force encouraging the solutes entry into the organism. Furthermore, this entry process is highly modulated be the specific characteristics of the molecule (lipophilic or hydrophilic) (Chojnacka, 2010).

ii. Storage

The factors that affect uptake continue to operate inside, hindering the metal from returning to the outer environment. Some chemicals are attracted to specific sites and, by binding to proteins or dissolving in fats, are temporarily stored (Balali-Mood et al, 2021) Heavy metals like mercury and certain other water-soluble chemicals bind tightly to specific sites within the body. When binding occurs, even highly water-soluble chemicals can accumulate. This is illustrated by cobalt, which binds very tightly and specifically to sites in the liver and accumulates there despite its water solubility. Similar accumulation processes occur for mercury, copper, cadmium, and lead(Fatullayeva et al, 2024)The storage of toxic chemicals in fat reserves serves to detoxify the chemical by removing it from contact with other organs. However, when fat reserves are utilized to provide energy for an organism, the materials stored in the fat may be remobilized within the organism and may again be potentially toxic. If appreciable amounts of a toxicant are stored in fat and fat reserves are quickly used, significant toxic effects may be seen from the remobilization of the chemical (Freese et al, 2019).

2. **Biotransformation:** Specific bacteria are capable of transforming toxic metals into less harmful forms through biochemical processes., cas an example, certain strains can reduce Cr (VI) to the less toxic Cr (III) (Padhan et al., 2021).
3. **Efflux Pumps**: Many bacteria have developed efflux systems that expel heavy metals from their cells, allowing them to reduce internal concentrations (Vats et al., 2022). P-type ATPases are efflux systems that utilize the energy from ATP hydrolysis to transport metal ions across the cytoplasmic membrane.
4. **Enzymatic detoxification**: Bacteria can produce enzymes that modify heavy metals into less toxic forms (Haque et al., 2022).
5. **Extracellular barrier:** This involves preventing the metal ions from entering the cells. It can include the cell wall or capsule acting as a barrier, which may adsorb metal ions due to the presence of groups like carboxyl, amino, phosphate, and hydroxyl groups.
6. **Extracellular Sequestration:** Toxic metals are kept out of the cytoplasm by being sequestered outside the cell. This can involve forming insoluble metal complexes or retaining the ions in the periplasm. For example, some bacteria form sulfide compounds with silver ions in the periplasm.
7. **Intracellular Sequestration:** Inside, metal ions can be bound and neutralized by specific chelating agents or metal-binding proteins. Metal binding Proteins areCysteinerich polypeptides likemetallothionein effectively trap toxic heavy metals such as Cd, Hg, Pb, and Zn. Glutathione and metallochaperones are also involved.

These mechanisms not only enable bacterial survival but can also lead to increased tolerance in populations exposed to heavy metals over time. However, the efficacy of these resistance mechanisms can be compromised by high levels of metal pollution, which can overwhelm bacterial systems and lead to community collapse (Sun et al., 2016).

2.2.2 Detection Methodologies

Several methods are used to detect the presence of heavy metals in the environment and the native biota in the area. Resolutions such as biosensors have been of great importance as they allow detection of a metal just as it reaches above threshold levels. Examples of biosensors used are optical biosensors, electrochemical biosensor and piezoelectric biosensors, all of which can be

used for heavy metal detection in wastewater (Anjum et al, 2017). In addition, molecularly imprinted polymer sensors are synthetic receptors with high selectivity and stability, allowing them to be an ideal sensor for in-field diagnostics and environmental monitoring.

2.3. Sources of AMR and HMR resistance

2.3.1 Wastewater

Wastewater is water that contains physical, chemical and biological pollutants after use in homes, industries, and or institutions. The main types of wastewater are domestic wastewater, blackwater (toilet waste), and grey water (washing, bathing, and kitchen water), industrial wastewater, agricultural wastewater, and storm runoff. According to Rizzo et al (2013), wastewater treatment plants (WWTPs) are critical hotspots for the selection and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Hazra, 2022). However, conventional treatment processes remove bacterial biomass but not necessarily resistance genes, leading to their persistence in discharge waters (Li et al., 2020, Bharadava et al, 2026). In Bindura, the absence of functional WWTPs means that raw sewage is discharged directly into streams, potentially accelerating horizontal gene transfer. Wastewater contains metals such as Zinc, lead and chromium, just to name a few.

2.3.2 Soil

The soil accumulates metals from wastewater irrigation, fertilizer, and pesticides and industrial waste. Long term wastewater irrigation causes a significant increase in Cu, Zn, Ni and Pb and other metals and metal are known as persistent pollutants (Kodani 2023). The natural microbes in the soil acquire AMR and HMR genes from constant and prolonged exposure to heavy metals. Soil naturally hosts microbes, but AMR rises when manure, sewage sludge, or contaminated wastewater are applied, allowing resistant bacteria to persist and transfer genes. Similarly, soil accumulates metals from fertilizers, pesticides, industrial waste, and wastewater irrigation, with studies in Zimbabwe showing significant increases in copper, zinc, cadmium, nickel, chromium, and lead that can remain for decades. Rivers and streams receive untreated sewage, hospital waste, and agricultural runoff, spreading resistant bacteria and genes downstream. In Zimbabwe, resistance levels are consistently higher downstream of wastewater plants than upstream

(Takawire&Mbanga, 2023). Metals from mining runoff, industrial discharge, and soil erosion bind to sediments in rivers, where bacteria also develop resistance.

2.2.3. Water bodies

Water bodies such as dams, lakes, and groundwater act as reservoirs for resistance genes, while biofilms allow bacteria to survive and exchange genes over time.The majority of rural areas, such as Bindura, have water sources for drinking that are still the traditional ones, which are dams, wells, rivers, streams and ponds (Zvidzai et al, 2007). Contamination comes from landfill leachate, sewage, and mining activities, and in Zimbabwe, poor waste disposal and leachate from landfills can release toxic metals like lead, cadmium, chromium, and mercury into groundwater. In Bindura, landfills are not always properly located, designed or managed, in which case some surface water contamination may occur (Kadziya&Chikosha 2013). Mining waste is the most critical issue in Bindura, a major mining town for nickel and gold. Mining releases nickel, lead, cadmium, mercury, and chromium into the environment, and studies show higher concentrations of these metals in soils and plants around Bindura compared to non-mining areas. These metals accumulate in soil and crops, enter the food chain through bioaccumulation, and create strong selection pressure for resistant bacteria. Mining environments also promote AMR through co-selection, where metal and antibiotic resistance genes are carried on the same plasmids, and through stress-induced mutations in microbes.

2.2.4. Mining waste

In Bindura specifically, mining pollution contaminates soil, water, and crops, with elevated metal levels detected in plants consumed by local communities. At the same time, studies have reported microbial contamination of water supplies in peri-urban areas. The combination of mining pollution and poor waste management results in both heavy metal and microbial contamination, increasing the risk of AMR and HMR together. Overall, AMR and HMR are interconnected, and in places like Bindura, mining pollution combined with wastewater contamination creates conditions where bacteria evolve faster, resistance genes spread more widely, and human health risks grow through exposure via water and food.

2.2.5 Pollution

Heavy metals in the environment pose an extremely deadly threat to humans and the ecosystem. This might cause the deterioration of habitats and the decline of biodiversity. Bacteria have developed several mechanisms to mitigate the toxic effects of heavy metals in the environment. Heavy metals such as lead, silver, and cadmium are prevalent contaminants in most ecosystems, especially those in regions with intensive industrial activity and urbanization (Mapira, 2007). Mining, industrial discharges, and agricultural activities are the primary sources of these pollutants (Jaishankar et al., 2014). The relationship between pollution and heavy metal resistance in bacteria is inherently complex. Pollution from industrial activities has increased the concentration of heavy metals in various environments, leading to a series of ecological consequences. For example, in the Sakubva River in Mutare, Zimbabwe, pollution from sewage and industrial effluent has created severe ecological challenges, impacting aquatic life and human health (Mapira, 2007). In Bindura, artisanal and industrial mining of nickel and gold contribute significantly to heavy metal contamination of soil and water (Mapanda et al, 2007). These metals can disrupt microbial metabolism, inhibit growth and may lead to death. Introduction of heavy metals into the environment through industrial discharge, agricultural runoff and wastewater discharge has been linked with the reduction of biodiversity and alteration of microbial community structure. Unlike organic pollutants, heavy metals cannot be biodegraded and thus exert long-term selective pressure on microbial populations. Studies worldwide have reported significant bacterial tolerance to metals in contaminated soils and waters. In Zimbabwe, Mubayiwa et al. (2020) found high levels of metal-resistant *Pseudomonas* and *Bacillus* isolates from mining-impacted soils, indicating adaptive microbial responses. Similar reports from Nigeria and South Africa confirm that persistent metal contamination enriches for resistant microbial communities (Okoh et al., 2017; Tchounwou et al., 2012).

2.3.6 Health and socioeconomic implications

2.4 Co-selection of Antibiotic and Heavy Metal Resistance

2.4.1 Genetic and Physiological Linkages

Coselection refers to the phenomenon where both antibiotic and metal resistance genes exist on the same genetic factor, allowing the selection of one type of resistance through maintaining the other (Seiler &Berendonk, 2012). Plasmids with blaCTXM and czcA or merA resistance genes,

for example, create organisms that are simultaneously resistant to β-lactams and mercury (Zhu et al., 2013).

2.4.2 Horizontal Gene Transfer and Co-resistance

Through horizontal gene transfer, such as conjugation, transformation, and transduction, resistance can be rapidly spread in bacterial populations (Partridge et al., 2018). In addition, heavy metals have been shown to promote increased levels of bacterial conjugations and activation of integrons leading to faster acquisition of ARGs (He et al., 2017). This is why multi-drug resistant strains can often be found in mining sites, despite the lack of presence of any antibiotics

2.4.3 Environmental and Public Health Implications

The presence of co-resistant bacteria in the environment means that they can easily spread via environmental contamination to humans and animals. Human exposure to such contaminated areas would lead to infection with co-resistant bacteria and commensals that can pass their plasmids to clinically important isolates (Martinez, 2017).

2.5 Comparative Analyses of AMR and HMR in Environmental Isolates

Comparative studies conducted in different environmental compartments demonstrate that there is a disparity in resistance profiles depending on the nature and extent of contamination. For example, Guo et al. (2019) discovered that antibiotics and heavy metal-resistant genes were more abundant in downstream sediment samples near mining areas compared to upstream sediment samples. Additionally, Sampson et al. (2020) discovered a statistically significant difference in multidrug resistance indices in Escherichia coli isolated from agricultural soil samples contaminated with copper (Cu) and zinc (Zn). There are limited studies in Zimbabwe that have examined the association between antimicrobial resistance and heavy metal resistance among environmental isolates. However, studies conducted in South Africa's agricultural and industrial environments have shown strong associations between metal contamination and antibiotic resistance phenotypes (Matongo et al., 2019)

2.6 Research gap

While there is increased interest in the topics of antimicrobial resistance and environmental contamination, few studies have explored the relationship between antimicrobial resistance and

heavy metal resistance among environmental microorganisms in Bindura. Available literature within Zimbabwe on AMR has predominantly concentrated on AMR in a clinical setting and environmental pollution, especially from mining industries.

Although awareness regarding the issues of antimicrobial resistance and environmental pollution has been increasing, very few studies have been conducted to understand the possible link between these two aspects in relation to environmental microbes in Bindura. Previous literature on antimicrobial resistance and environmental pollution in Zimbabwe has concentrated mainly on antimicrobial resistance within clinical environments and the problem of pollution caused by mining activities, but there has been no effort to establish any co-resistance pattern in environmental samples. Also, there is a lack of information on resistant microbes in mining environments like Bindura, which leads to a lack of understanding about the impact of heavy metal pollution on antimicrobial resistance.

CHAPTER 3

3.0 Materials and methods

3.0 Introduction

This chapter describes the research methods and procedures used to investigate antimicrobial resistance and heavy metal resistance in environmental samples collected from Bindura. The methodology provides a structured approach for sample collection, microbial isolation, resistance

testing, and statistical analysis to ensure the reliability and validity of the findings. According to Creswell and Creswell (2018), an appropriate methodology is essential for generating accurate and reproducible scientific results.

Soil, sewage, water from the river, and sediments from designated areas that relate to mining, farming, and residential activity within Bindura were collected as the source of samples for the study. The samples were collected in accordance with the exposure of the area to pollutants and human activities. Bacterial isolates were identified and characterized using standardized microbiology procedures. Resistance determination was done through sensitivity testing against various antibiotics, such as tetracycline and amoxicillin, according to standard procedures, like the Kirby-Bauer disk diffusion test (Clinical and Laboratory Standards Institute [CLSI], 2021).

Heavy metal resistance testing involved exposing bacterial isolates to varying concentrations of selected heavy metals, including lead, mercury, zinc, and silver. Laboratory experiments were performed under controlled conditions to determine bacterial tolerance levels and resistance profiles. Data generated from antimicrobial susceptibility and heavy metal resistance tests were analyzed statistically to compare resistance patterns among isolates from different environmental sources. The methodology adopted in this study was therefore suitable for examining the relationship between antimicrobial resistance and heavy metal resistance among environmental microorganisms in Bindura.

3.1 Study area

The study was conducted in Bindura(urban,peri urban)Mashonaland Central Province, Zimbabwe. The region lies approximately 88 km northeast of Harare and is known for nickel and gold mining, agriculture, and urban development. Industrial and mining operations often discharge effluents and tailings that contaminate the Mazowe River and adjacent agricultural soils (Mtetwa et al., 2017). These features make Bindura an appropriate model area for examining the environmental co-selection of antimicrobial and heavy metal resistance.

3.2 Sample collection

A total of 90 samples were collected aseptically from various sampling sites in Bindura. 22 samples were water samples while 31 samples were soil, 9 samples were sediment and 4 samples were sewage. Some of the samples consisted of both water and soil. All the sampling tools used were sterilized in an autoclave at 121^0C for 15 minutes before sample collection. They were collected using the necessary personal protective equipment like gloves, face masks and laboratory coats. This was to ensure that both the sample and the collector are protected. The samples were all labeled with their respective data such as date, time, place of collection and kind of sample collected for proper documentation purposes. Samples were transferred to the laboratory in a cooler box to prevent direct sunlight exposure. On arrival, samples were logged in a logbook. The logbook was meant to document experiment progress during experimentation period. Samples were kept in a refrigeration unit for 2 hours after arrival in the laboratory as an acclimatization period.

3.3 Sample preparation and isolation of microorganisms.

The area to be used for experimental purposes was sterilized using a cotton swab and 95% alcohol. Following the instructions from Miller (2004), one pill of Ringer's solution was dissolved in 500ml of distilled water. 9mls of the solution was poured into test tubes. The test tubes were sealed and autoclaved for 15 min at 121 °C. Serial dilution was carried out according to Miller (2024) and Werner (2023), giving a solution of 10^{-1} to 10^{-4}.

3.3.1 Media preparation.

Different agar types were used for this method for different purposes. Nutrient agar was used for general bacterial growth. MacConkey agar was used for the selective and differential purposes of Gram-negative bacteria. Mannitol salt agar was used as a selective medium to select for salt-tolerant bacteria.

3.3.1.1 Nutrient agar

62 g of nutrient agar was dissolved using a balance according to Eckman (2021). The spirit burner was used to heat the agar to dissolve it. The agar was autoclaved for 15 min at 121°C. The agar was left to cool to 45 °C. After cooling down, the agar was poured into sterile petri dishes and left to solidify. The samples and the serial dilutions were inoculated into the petri dishes using the

spread plate method. The petri dishes were sealed, labeled appropriately and incubated for 24-48 hours at 35-37 °C.

3.3.1.2 McConkey agar

McConkey agar is used to isolate Gram-negative enteric bacteria and to differentiate between lactose fermenters and non-fermenters. The agar was prepared following the manufacturer's instructions. To prepare it, 49.53 g of medium was mixed into 1000 ml of distilled water and heated to boiling until fully dissolved. The mixture was then sterilized by autoclaving at 121°C for 15 min. After cooling to about 45°C, the agar was poured into petri dishes.

3.3.1.3 Mannitol salt agar

Mannitol salt agar is used to select for salt-tolerant bacteria. 38 grams were accurately measured using a balance and dissolved in 1000ml of distilled water. The solution was heated with a spirit burner and later autoclaved for 15 min at 121 ^{0}C. After autoclaving, the agar was left to cool to 45 ^{0}C and then poured into sterilized petri dishes. All this was done as per the manufacturer's instructions. Using the spread plate method, the samples were inoculated after the agar had settled and solidified.

3.4 Inoculation of samples

The agar plates were sealed and labeled for the identification process. The label includes the name of the sample, date and time of incubation. The samples were given pseudo names or unique identification numbers/names, which were shorter and easier to remember, for example, Chiwaridzo shops dump site was named sample A1. The first serial dilution was named A1^{1,} going on and on. The sample was inoculated using the spread plate method. An L-shaped spreader was used to spread a small volume of the sample around the agar. The glass spreader was dipped in 70% ethanol and passed over a lit Bunsen burner to sterilize is for the next sample (Sagar, 2022). The agar plate was incubated upside down to avoid moisture (if present) from falling back on the sample. Incubation was done for 24-48 hours at 35-37°C.

3.4.1 Identification of isolates

The bacterial isolates on the agar plates were initially characterized based on the colony morphology observed, such as colony shape, size, texture, colour, elevation and opacity. All the

observed characteristics were recorded in the logbook. Gram staining was done to see if the bacteria were gram-positive or gram-negative, and the shape of the bacteria, as this aided in choosing the biochemical tests to perform.An isolate was carefully placed on a microscope slide using a sterile inoculating loop. The sample was air-dried for 5 minutes. Using a dropper, 3 drops of crystal violet were put onto the slide and left for 60 seconds. Crystal violet was rinsed off using distilled water, and iodine was added to the slide using a dropper. The iodine was left there for 60seconds and later rinsed off. 95% ethanol was added to the slide for 10 seconds and was later rinsed off. Safranin was added to the slide for 30 seconds and rinsed off. The slide was air-dried for 5 minutes, and the slide was mounted on a microscope for viewing. A purple color was regarded as Gram-positive, and a red/pink color was regarded as Gram-negative.

3.4.2 Biochemical tests

3.4.2.1 Catalase test

50% hydrogen peroxide was diluted to 3% hydrogen peroxide using distilled water. This used the formula($V_1C_1=V_2C_2$). A 100ml solution of 3% hydrogen peroxide was made. A well-isolated pure bacterial isolate, which was 18-24 hours old, was placed on a microscope slide using an inoculating loop. 3 drops of hydrogen peroxide were placed on top of the isolate using a dropper. Formation of bubbles or effervescence was recorded as a positive, and no bubbles were recorded as a negative (Sagar, 2022; Reiner, 2010).

3.2.2.2 Indole test

This test demonstrates the ability of bacteria to decompose the amino acid tryptophan to indole. It is used to identify Enterobacteria (MacWilliams, 2016). 4mls of tryptophan broth were poured into a test tube, and the tube was inoculated aseptically by taking the growth from an 18 to 24-hour culture. 3 drops of Kovacs reagent were put in the test tube. The tubes were incubated at 37 °C for 24 hrs (Darkoh et al., 2015)

3.2.2.3 Oxidase test

The oxidase test was done following the protocol described by Dahal (2023). An isolated colony of the test isolate was picked using a sterile inoculating loop and smeared over a Whatman number 1 filter paper strip. Two drops of Kovacs' oxidase reagent were dropped onto the bacterial smear. The color change and time taken for the color change to happen were noted for up to 60 seconds.

A color change to purple or deep blue within 60 seconds was recorded as a positive result, while no color change within the time was recorded as a negative result (Chavan et al., 2022).

3.5 Antimicrobial susceptibility test

Firstly, 38.1g of Mueller Hinton was measured using a balance and suspended in $1dm^3$ of distilled water and allowed to stand for 15min. The solution was boiled to dissolve the agar completely, then autoclaved at 121°C at 15 psi for 15 min. The agar was poured into petri dishes and left to solidify for 15 minutes, and the width of the agar was 4 mm. The Kirby-Bauer disc diffusion method, followed by Clinical and Laboratory Standards Institute (CLSI) criteria, was used (CLSI M02-A13, 2018). A 0.5 McFarland standard suspension of each isolate was produced in sterile saline and evenly swabbed onto Mueller-Hinton agar plates. The following antibiotic discs (Oxoid, UK) were applied using sterile forceps:

- Amoxicillin (AML) 25 µg, with CLSI breakpoint (mm) of S ≥ 17, I 14-16, R < 13.
- Ciprofloxacin (CIP) 5 µg, with CLSI breakpoint (mm) of S ≥ 21, I 16-20, R ≤ 15
- Tetracycline (TE): 30 µg; with CLSI breakpoint (mm) of S≥ 19, I 15-18, R ≤ 14.
- Gentamicin (CN): 10 µg, with CLSI breakpoint (mm) of S ≥ 15, I 13-14, R ≤ 12
- Metronidazole (MTZ): 50 µg, with CLSI breakpoint (mm) of S ≥ 24, 21-23, R ≤ 20
- *Escherichia coli* ATCC 25922 was used as a quality control strain. Plates were incubated at 37 °C for 18-24 hours. Zone diameters were measured to the closest millimeter using digital calipers. Antibiotic discs are placed on the surface of the inoculated plate.

3.4 Heavy metal resistance testing

High-purity metal salts were dissolved in deionized water to make stock solutions of (1000mg/L). metal salts used were silver nitrate ($AgNO_3$), Lead nitrate ($Pb(NO_3)_2$), mercuric chloride ($HgCl_2$) and zinc sulphate ($ZnSO_4 \cdot 7H_2Oa$) using the formular. The solutions were filtered using a (0.22 µm pore size) (Pal et all, 2017). Molten Muller Hinton agar was prepared according to manufactures instruction, autoclaved to sterilize and cooled to 50 °C. Sterile test tubes were label according to the concentration of the metal. 5mL of the broth was dispensed into the tube. Concentrations of 50, 100, 200, and 400 µg/mL for each metal were prepared and put in the molten agar. A control tube containing nutrient broth without heavy metals was prepared. Overnight bacterial cultures were adjusted to 0.5 McFarland standard (~1.5×10^8 CFU/mL). Using a micropipette, 0.1 ml of

the standardized inoculum was added to each test tube. The tubes were mixed gently to ensure even distribution. All the tubes were incubated at 37 °C for 24 hours. After incubation, the tubes were examined for cloudiness or turbidity, which indicates bacterial growth. Isolates having MIC > 200 µg/mL for any metal were categorized as resistant, using breakpoints adopted from Nies' (1999) environmental isolates.

3.6 Data analysis

The data was analyzed using IBM SPSS software. Pearson's correlation coefficient (r) was used to determine the linear association between antibiotic resistance (0-3) and heavy metal resistance (0-3) per isolate. The mean antibiotic resistance scores were compared using one-way ANOVA across heavy metal resistance groups (none, single-metal, and multi-metal). Chi-square was used to test for a significant association between heavy metals, and A p-value of <0.05 was judged statistically significant. Graphs were created using Excel. For each tested antibiotic and heavy metal resistance rates were calculated.

Prevalence = (number of each bacterial species detected / total number of bacteria isolated) * 100

Resistance of isolates = (resistant isolates towards each antibiotic or heavy metal / total number of bacteria isolated per genus) * 100

CHAPTER 4

4.1 Results

4.0 Introduction

This chapter describes the empirical findings derived from the comparative analysis of antimicrobial resistance (AMR) and heavy metal resistance (HMR) within the environmental samples collected in Bindura. The primary objective of this section is to present the laboratory data, also illustrating the prevalence of the bacteria by their sources and their rate of resistance to

antibiotics and heavy metals. byevaluating the susceptibility profiles of these microorganisms against a spectrum of commonly used antibiotics and commonly used heavy metals. This study aims to identify a potential correlation between heavy metal resistance and the emergence of multidrug resistance. The data synthesized in this chapter serve as the foundation for assessing the public health and ecological implications of microbial resistance.

4.1 Prevalence of bacteria by sources

Table 4.1 shows the distribution and prevalence of bacteria isolated from the different types of environmental samples obtained from various sites in Bindura. As seen from the table, at least one type of bacterial isolate was found in all 90 sampled sites, indicating wide microbial contamination in the sampling area. Types of samples include water samples, soil samples, sediment samples, sewage samples, sludge samples, effluent samples, vegetable samples, animal waste samples, and mine tailing samples.

Of all bacterial isolates, the one that occurred frequently was *Escherichia coli* and was isolated from nearly all types of sample sources such as water sources, sewage, soils, wetlands, mine tailings, composts, dump sites, and fish ponds. The occurrence of this microorganism is an indicator of wide fecal contamination in the environment. The second most isolated bacterial organism was *Staphylococcus aureus* and occurred widely in soils, water samples, sediments, sewage, and waste materialsOther commonly isolated organisms include Shigella, Bacillus mycoides, *Pseudomonas aeruginosa*, *Klebsiella*, *Bacillus licheniformis*, *Bacillus subtilis*, *Pseudomonas*, and other types of *Staphylococcus*. Mixed bacteria were also observed in many samples such as sewage, wetlands, dumps, and wastewater sources.

Water samples collected from rivers, dams, fish ponds, streams, and wells had some pathogenic organisms like E. coli, Shigella, and S. aureus, implying potential health implications due to the use of water. High bacterial diversities were noted in sewage sludge and wastewater samples, indicating their pollution by human and domestic wastes. In addition, mine tailings and mining-associated soils hosted organisms like E. coli and various Bacillus species.

Overall, the findings demonstrate that environmental samples collected from Bindura harbor diverse bacterial communities, including potentially pathogenic and environmentally resistant organisms.

Table 4.1 Prevalence of bacteria by sources

Code	Source	Bacteria Identidied	Sample type
A1	FSE bridge	*S. aureus, E. coli*	water
A2	Twin Lodge River	*S. aureus, Shigella, E. coli.*	Water
A3	Chiravi bridge	*S. aureus, Shigella*	Water
A4	Shashi stream	*E. coli, Shigella*	Water
A5	Shashi mountain soil	*B. mycoides*	Soil
A6	Chakamanga farm soil	*E. coli*	Soil
A7	Chiwaridzo dumpsite	*E. coli*	Soil
A8	Aerodrome dump site	*E. coli*	Soil
A9	Chipadze dump site	*S. aureus, E. coli*	Soil
A10	Waste water pre-maturation	*S. aureus, E. coli*	Sewage
A11	Mature/final wastewater	*E. coli*	Sewage
A12	Chiwaridzo sewage run off	*E. coli*	Sewage
A13	Shashi's illegal mining settlement	*S. aureus*	Soil
A14	FSE farm	*S. aureus*	Soil
A15	Kangara dam water	*S. aureus, Shigella, P. aeruginosa*	Water
A16	1084 Shashi household well	*E. coli*	Water
A17	Kangara dam	*S. aureus*	Sediment
A18	Kudyauripo farm	*B. mycoides, E. coli*	Soil
A19	Chipindura dumpsite	*E. coli, S. aureus*	Soil
120	Chipindura high ground	*E. coli*	Soil
A21	Chipadze sewage runoff	*S. aureus, E. coli*	Sewage
A22	Manhenga river	*S. aureus, E. coli*	Sediment
A23	Sanitary waste dumpsite	*S. aureus, E. coli*	Soil
A24	Manhenga	*S. aureus, E. coli*	Soil
A25	Wood Brooke	*E. coli,*	Soil
A26	Wood Brooke 2	*B. mycoides, E. coli, P. Aeruginosa*	Soil
A27	Botha mine	*E. coli*	Mine tailings
A28	Freda Rebecca mine	*Bacillus spp*	Mine tailings

A29	Trojan mine	*E. coli*	Mine tailings
A30	Ran mine	*E. coli*	Mine tailings
A 31	Madziva	*Bacillus spp, B. mycoides, B. licheniformis*	Soil
A32	Chanaka	*Shigella, Pseudomonas spp, P. aeruginosa, S. aureus, E. coli*	Water
A33	Galliver	*B. licheniformis, B. mycoides, Klebsiella*	Soil
A 34	Dombotombo	*E. coli Klebsiella*	Water
A 35	Chiwarire dam	*Shigella, P. aeruginosa, B. licheniformis*	Sediment
A36	Bindura Park	*B. mycoides, B. licheniformis*	Soil
A37	Bindura Park 2	*B. mycoides, Shigella*	Soil
A38	Bindura Park 3	*B. mycoides, E. coli, Shigella*	Soil
A39	Nyakabau compost	*S. aureus, B. mycoides, P aeruginosa. B. licheniformis,*	Soil
A40	Buse Farm 1	*E. coli, S. aureus, Shigella*	Soil
A41	Buse farm 2	*P. aeruginosa, E. coli, Klebsiella, B. licheniformis, B,*	Animal waste
A42	Buse Farm 3	*B. mycoids, Bacillus spp, E. coli, B. mycoids, P. aeruginosa*	Soil
A43	Piki shop's front	*Klebsiella, E. coli, S. aureus, B. mycoides,*	Soil
A44	Piki shops dumpsite	*B. mycoids, Klebsiella*	Soil
A45	Piki shops dumpsite	*P. aeruginosa, S. aureus, B. mycoides*	Soil
A46	Shasi Hayes	*Shigella, Staphylococcus, E. coli, P. aeruginosa*	Water
A47	Chinos fish pond	*B. subtilis, P. aeruginosa*	Sediment
A48	Chinos fish pond	*S. aureus, Shigella*	Water

A49	Tendai hall	*B. mycoids, S. aureus, E. coli, B. licheniformis, P. Aeruginosa*	Soil
A50	Sheila compost	*S. aureus, Pseudomonas spp, B. mycoides,*	Soil
A51	Woodbrooke farm wi	*B. licheniformis, P. aeruginosa, E. Coli*	Soil
A52	Wood Brooke farm w2	*S. aureus, B. mycoides, B. licheniformis, Klebsiella*	Soil
A53	Pig stay	*Klebsiella, P. aeruginosa, S. aureus*	Sludge
A54	Pig stay	*Klebsiella, P. aeruginosa*	Effluent
A55	Pote river	*S. aureus, Shigella*	Sediment
A56	Pote river	*B. licheniformis, Shigella*	Water
A57	Trojan pond	*Pseudomonas spp, Bacillus spp, E. coli,*	Effluent
A58	Zegu campus	*B. mycoids, S. aureus, Shigella*	Soil
A 59	Chiwaridzo community tap	*E. coli*	Water
A60	FSE tap	*E. coli*	Water
A61	Shashi primary	*E. coli*	Water
A62	Bindura prison garden	*Shigella, P. aeruginosa, Klebsiella,*	Water
A63	Dzingai fish pond	*S. aureus, Shigella, P. aeruginosa*	Water
A64	Aerodrome tap	*E. coli*	Water
A65	Bindura railway	*P. aeruginosa, Staphylococcus, E. coli. S. aureus.*	Soil
A66	Opposite railway tap	*E. coli*	Water
A67	Main campus road	*S. aureus, B. subtilis. Shigella, P. aeruginosa,*	Sediment
A68	Marketplace 1	*B. subtilis. S. aureus, E. coli,*	Vegetables
A69	Marketplace 2	*P. aeruginosa, S. aureus*	Water

A70	Marketplace 3	*B. mycoids, S. aureus, E. coli*	Soil
A 71	Construction site tank	*E. coli, S. aureus.*	Water
A 72	Construction site	*E. coli, S. aureus, shigella, B. licheniformis*	Water
A73	Wet land	*P. aeruginosa, Shigella, S. aureus.*	Water
A74	Wetland	*P. aeruginosa, klebsiella*	Sediment
A75	Wetland	*Shigella, P. aeruginosa,*	Water
A76	Shingi fish pond	*Bacillus spp, P. aeruginosa, E. coli*	Sediment
A77	Borehole	*E. coli, Staphylococcus, S. aureus*	Water
A78	Shoki	*Shigella, bacillus*	Water
A79	10 missed calls 1	*Klebsiella, P. aeruginosa,*	Soil
A80	10 missed calls 2	*S. aureus, Shigella, E. coli. Staphylococcus spp,*	Soil
A81	Chawagona grounds	*B. mycoids, Staphylococcus, S. aureus,*	Soil
A82	Chawagona farm	*B. mycoids, S. aureus, E. coli*	Soil
A83	Bindura hospital ground	*P. aeruginosa, B. licheniformis*	Soil
A84	Shashi Mountain opposite	*B. mycoids, E. coli, S. aureus, Pseudomonas,*	Soil
A85	Shashi Mountain opposite	*B. mycoids, E. coli, Shigella, B. licheniformis*	Soil
A86	Woodbrooke oxidation pond	*Klebsiella, S. aureus, B. licheniformis, E. coli.*	Sewage sludge
A87	Woodbrooke oxidation pond	*Klebsiella, B. licheniformis, Shigella, E. coli, S. aureus.*	Sewage effluent
A88	ZAOGA gum tree	*E. coli, S. aureus, Shigella, Pseudomonas,*	Soil
A89	ZAOGA church	*E. coli, S. aureus, Shigella*	Soil
A90	Ezekiel Guti Mountain	*B. mycoides, Shigella, E. coli*	Soil

4.2 Antimicrobial resistance rates of isolated bacteria

Table 4.2 and Figure 4.1 show the percent resistance rates of bacterial isolates to five antibiotics; namely amoxicillin, ciprofloxacin, tetracycline, gentamicin, and metronidazole. From the data obtained, different resistances were noted on the various types of bacterial isolates tested.

Tetracycline generally had the highest level of resistance from the various types of bacterial isolates. The highest resistance recorded to tetracycline occurred on Pseudomonas species with 87.5%, followed by Bacillus licheniformis (77.778%), E. coli (76.470%), and S. aureus (75%). Therefore, it can be seen that tetracycline is less effective against the various bacterial isolates from the environment in Bindura. E. coli, which was the most common isolate, had high resistance to ciprofloxacin (66.67%), tetracycline (76.470%), and amoxicillin (60.784%), whereas gentamicin and metronidazole resistance levels were lower (35.294% and 37*Klebsiella* species demonstrated relatively high resistance to ciprofloxacin (62.857%) and amoxicillin (57.143%), while *P. aeruginosa* showed moderate resistance levels across all antibiotics tested. *Bacillus subtilis* recorded the lowest resistance rates overall, with equal resistance levels of 33.333% for all antibiotics.

The results further indicate that multidrug resistance was common among several bacterial isolates, particularly among Gram-negative bacteria such as *E. coli*, *Klebsiella* species, and *Pseudomonas* species. The widespread occurrence of antimicrobial resistance among environmental isolates suggests possible exposure to antibiotics or environmental selective pressures in the study area.

Figure 4.1 illustrates these resistance trends graphically, showing tetracycline resistance as the dominant resistance pattern among most isolates.

Table 4.2 shows the rates of resistance of bacteria to antibiotics in%

isolate	**Amoxicillin**	**Ciprofloxacin**	**tetracycline**	**Gentamicin**	**Metronidazole**
E. coli	60.784	66.67	76.470	35.294	37.255
S. aureus	45	57.5	75	42.5	45
Shigella	46.429	50	67.857	39.285	39.285
*Klebsiella*spp	57.143	62.857	42.857	42.857	51.428
P. aeruginosa	48.4848	45.4545	51.5151	42.4242	42.4242

*Pseudomonas*spp	50	50	87.5	50	50
B. licheniformis	61.111	55.556	77.778	44.444	50
B. mycoides	54.1667	58.333	58.333	41.667	50
B. subtilis	33.333	33.33333333	33.333	33.333	33.333
*Bacillus*spp	66.667	66.667	50	50	50
*Staphylococcus*spp	57.142	57.143	42.857	57.143	57.142

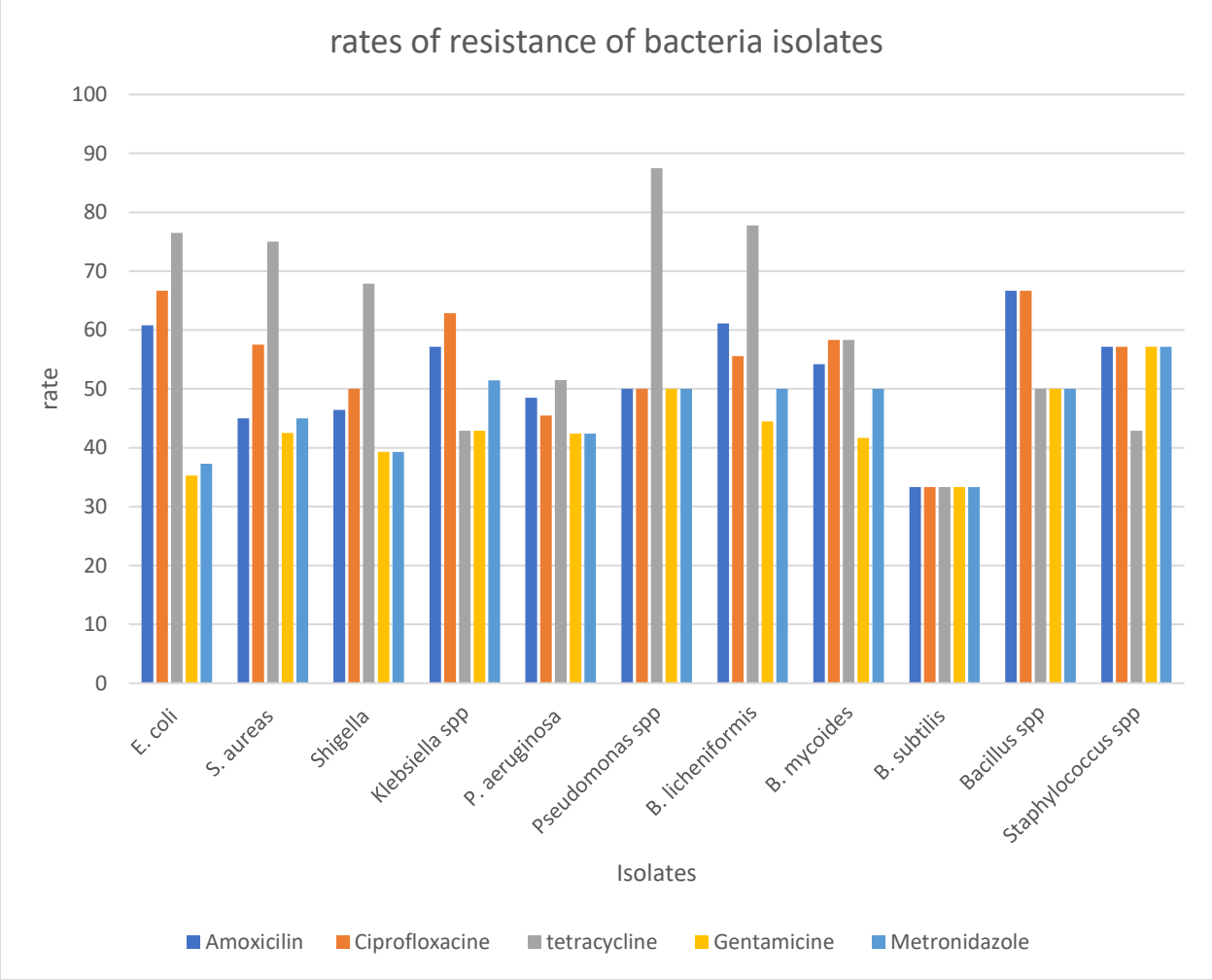


Figure 4.1 shows the rates of resistance of bacteria to antibiotics.

4.3 Resistant rates of bacteria to heavy metals

Table 4.3 and Figure 4.3 show the resistance percentages of bacterial isolates to selected heavy metals, such as Ag (silver), Pb (lead), Zn (zinc), Hg (mercury). From the data, it is evident that several bacterial isolates possessed notable resistance to heavy metals implying adaptation to toxic environment. Pb and Zn showed relatively higher resistance levels to most bacterial isolates. Lead resistance was highest in the Bacillus group (83.333%) followed by B. licheniformis (77.778%) and *Klebsiella* spp. (77.143%). The same pattern was recorded in the case of zinc resistance in *Klebsiella spp*. (71.429%), *Staphylococcus s*pp. (71.429*%), E. coli* (70.588%), and *S. aureus (70%).*

E. coli showed a relatively high resistance level to lead (74.51%) and zinc (70.588%). This shows the ability of *E. coli* to survive in heavy metal-laden environments. Similarly, *S. aureus* had notable resistance levels to mercury (65%) and lead (70%). However, aluminium had lower levels of resistance to most isolates, with *E. coli* having the least resistance level of 37.255%.

The *Pseudomona*s species and *P. aeruginosa* were moderately resistant to heavy metals, whereas *B. subtilis* was less resistant to the heavy metals. The presence of heavy metals-resistant bacteria is an indication of constant exposure to polluted environment due to activities such as mining, sewage dumping, and industrial effluent dumping in Bindura.

Figure 4.2 illustrates the resistance of bacteria isolates to heavy metals, whereby Pb and Zn are the most commonly resistant metals.

Table 4.3 shows the resistance rates of bacteria to heavy metals in %

isolate	**Silver (Ag)**	**Lead (Pb)**	**Zinc (Zn)**	**Mercury (Hg)**
E. coli	54.902	74.51	70.588	58.824
S. aureus	52.5	70	70	65
Shigella	42.857	64.286	64.286	42.857
*Klebsiella*spp	51.429	77.143	71.429	57.143
P. aeruginosa	42.424	66.667	60.606	48.485
*Pseudomonas*spp	37.5	62.5	62.5	62.5
B. licheniformis	50	77.778	66.667	61.111
B. mycoides	45.833	66.667	66.667	54.167

B. subtilis	33.333	66.667	66.667	33.333
*Bacillus*spp	50	83.333	66.667	50
*Staphylococcus*spp	57.143	71.429	71.429	57.143

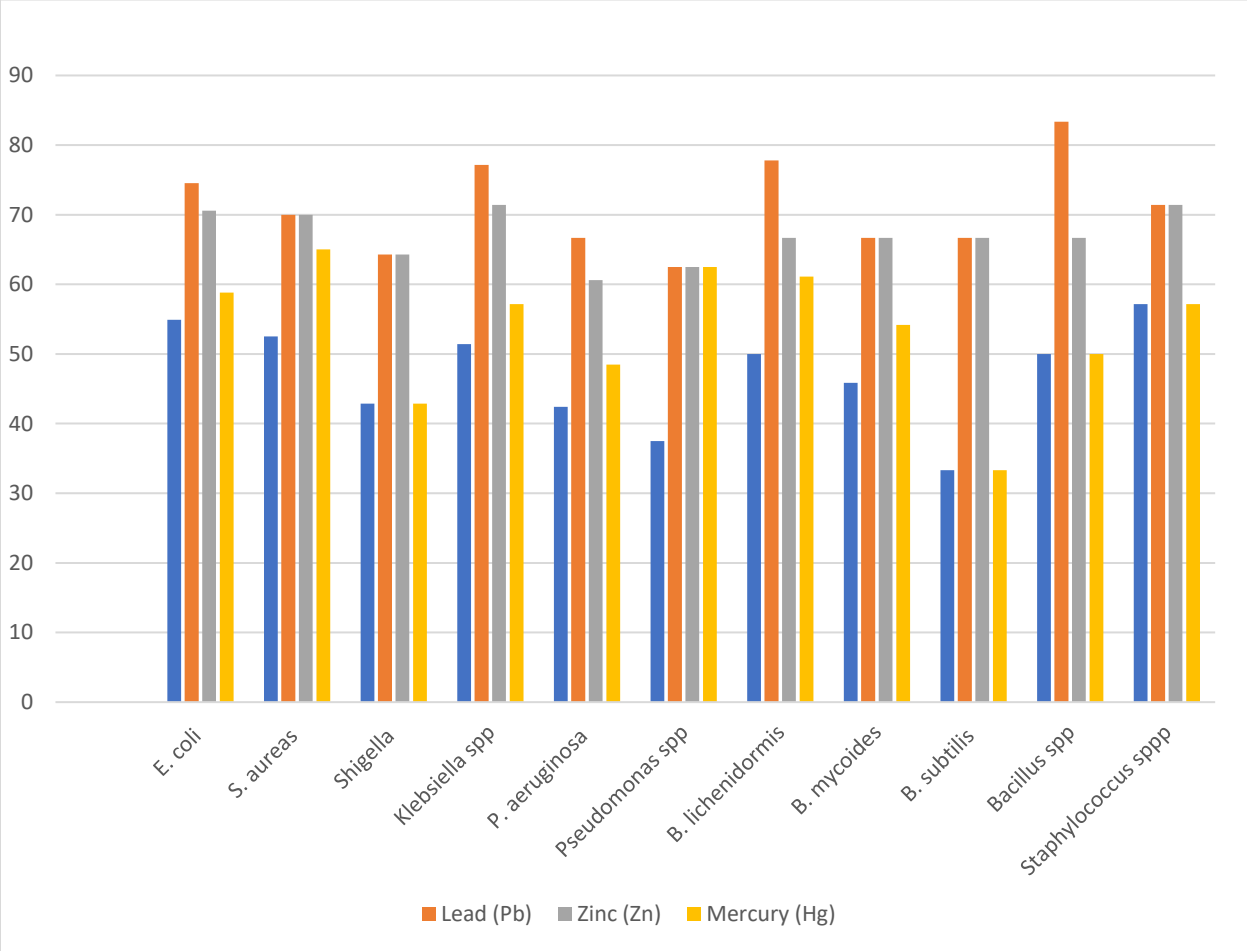


Figure 4.2 shows the rate of resistance of bacteria to heavy metals.

4.4 Statistical evaluation of co-resistance

Pearson's correlation analysis found a moderate positive correlation between the number of antibiotic resistances and heavy metal resistances per isolate ($r = 0.405$, 95% CI: 0.112 to 0.425, $p = 0.001$). The link is statistically significant, but only explains 7.6% of the variation ($r^2 = 0.076$).

Antibiotic resistance ratings varied significantly among heavy metal resistance categories, as seen by one-way ANOVA ($F(3, 156) = 7.21$, $p < 0.001$). Triple-metal resistant isolates exhibited substantially higher antibiotic resistance scores (mean = 2.7 ± 0.4) compared to metal-susceptible isolates (mean = 1.2 ± 0.6, $p < 0.001$).

Pearson's correlation analysis revealed several significant relationships between heavy metal resistance and antibiotic resistance among bacterial isolates. A moderate positive correlation was observed between Zinc and tetracycline ($r = 0.404$, $p = <0.001$), which indicated that isolates resistant to zinc were more likely to be resistant to tetracycline. Mercury-resistant isolates also showed a positive association with tetracycline ($r = 0.206$, $p = 0.007$). In addition, aluminium resistance was positively correlated to amoxicillin resistance ($r = 0.132$, $p = 0.037$) and gentamicin resistance ($r = 0.167$, $p = 0.008$). Chi-square analysis was used to determine whether there was a significant association between heavy metal resistance and antibiotic resistance among the bacterial isolates. The results show that some heavy metals are significantly associated with resistance to specific antibiotics. This suggests the occurrence of a co-resistance mechanism. A strong association was observed between resistance to zinc and resistance to tetracycline ($X^2 = 27.927$, $p< 0.001$). Isolates resistant to zinc showed a much higher proportion of tetracycline resistance (76.7%) compared to zinc-susceptible isolates (34.0%). This indicates that resistance genes for zinc may be linked or selected together in bacterial populations. Mercury resistance was also significantly associated with tetracycline resistance ($X^2 = 7.201$, $p = 0.007$). About 72.3% of mercury-resistant isolates were resistant to tetracycline compared to 52.2% of mercury-susceptible isolates. Similarly, silver resistance and lead resistance showed significant relationships with tetracycline resistance ($p = 0.016$ & $p = 0.33$, respectively). There was a significant correlation between aluminium and amoxicillin ($X^2 = 4.362$, $p = 0.037$) and gentamicin resistance ($X^2 = 6.956$, $p = 0.008$). One-way ANOVA shows a statistically significant difference.

CHAPTER5

5.0 Discussion

The existence of *S. aureus* is a concerning matter, especially given that the bacteria in question are opportunistic pathogens. As indicated by Gufe eta al (2022), the high prevalence of *S. aureus* indicates unsanitary conditions, which can cause minor diseases such as skin disease and life-threatening conditions such as septicemia. The isolation of the bacteria in dumpsites is evidence that human health hazard behavior can cause pathogenic bacteria to inhabit the environment. *Bacillus mycoides* was identified mostly in soil samples. This limited distribution is expected, as Bacillus species are primarily soil inhabitants The study sought to carry out a comparative study on antimicrobial resistance (AMR) and heavy metals resistance (HMR) among environmental bacterial isolates from various environmental samples collected within Bindura town of Zimbabwe. The results that are reported in chapter four shed light on issues concerning the prevalence, patterns, and possible co-selection of resistance traits in environmental bacterial isolates. In this regard, the present section discusses these results in relation to the existing body of knowledge, research questions, hypotheses, and their possible implications on public health and environmental management in Zimbabwe. The most frequent bacteria were the *E. coli* (20.32%) and *S. aureus*(15.94%), *Klebsiella* (13.94%) and *P. aeruginosa*(12.35%) and *Shigella* (11.15%) and *B. mycoids*(9.56%) species. However, *B. subtilis*, *Bacillus spp*, *Staphylococcus* and *Pseudomonas* were found to have the least frequency with the prevalence rate of (1.95%), (2.39%), (2.78%), and (3.18%). Previous studies have indicated that some of the mentioned bacteria are commonly used as indicators of fecal and environmental pollution (Odonkor and Ampofo 2013).Escherichia coli were isolated from 51 locations, where it occurred either as a sole isolate or together with other organisms. The extensive occurrence of E. coli from various matrices such as river, stream, dumping site, sewage effluent, and mine is an indication of possible fecal pollution of Bindura. These findings are similar to those reported by Nyarota (2025), who indicated that

there is microbial pollution of fecal origin in various water bodies in Zimbabwe beyond World Health Organization recommendations. E. coli isolations from mining-affected areas including Botha Mine (A27), Freda Rebecca Mine (A28), Trojan Mine (A29), and Rine Mine (A30) show that heavy metal pollution does not affect the viability of this microbe, and hence, may have developed adaptive resistance mechanism and are less commonly associated with fecal contamination (DeVos et al, 2009). Shigella species were detected in samples from Twin Lodge River (A2), Chiravi Bridge (A3), and Shashi Stream (A4), just to name a few. The presence of *Shigella*, a known enteric pathogen responsible for shigellosis (dysentery), in surface water bodies raises significant public health concerns, particularly for communities that rely on untreated water for domestic purposes. This poses a great concern, as indicated by Lubeck et al (2025), as studies showed that *Shigella* was responsible for causing diarrhea in children across 14 countries. The diversity of bacterial species observed across different sampling sites reflects the complex microbial ecology of Bindura's contaminated environments. As noted, sites with higher anthropogenic activity, such as dumpsites, sewage outfalls, and mining areas, exhibited greater bacterial diversity compared to less disturbed sites. This observation supports the concept that human activities create ecological niches that select for specific bacterial communities (Berendonk et al., 2015).

The antimicrobial susceptibility testing revealed alarmingly high levels of resistance among environmental isolates (Table 2). Across all isolates tested, resistance to ciprofloxacin (a fluoroquinolone) was the most prevalent (60% of isolates). Similarly, Ruiz-Lievano (2024) discovered a high rate of fluoroquinolone resistance in UPEC in India (>60%). Amoxicillin (61% of isolates) did not come as a surprise, as the antibiotic is frequently used.Recent data in Vietnam have shown that the amoxicillin resistance rate of H. pylori differs across regions and it seems to have increased over time, from 1.1% in 2015 to 10.4% in 2016 and 15% in 2018 (Tran et al, 2022). Tetracycline resistance was comparatively lower. These findings are concerning, given that ciprofloxacin and amoxicillin are among the most commonly prescribed antibiotics in Zimbabwe's healthcare system (Gwenzi et al., 2021). The high resistance to ciprofloxacin is particularly worrisome because fluoroquinolones are considered critically important antimicrobials for human medicine by the World Health Organization (WHO, 2019). The widespread environmental resistance to this drug class suggests selective pressure, likely originating from both clinical and

agricultural sources. In Zimbabwe, ciprofloxacin is frequently used to treat respiratory and urinary tract infections, and its residues may enter the environment through patient excreta, improper disposal of unused medications, and pharmaceutical manufacturing effluents (ManyiLoh et al., 2018). Amoxicillin resistance at 61% is consistent with the extensive use of this β-lactam antibiotic in both human and veterinary medicine. The relative ease with which bacteria acquire β-lactamase enzymes, which hydrolyse the β-lactam ring explain the high prevalence of resistance to this drug class (Davies & Davies, 2010). The lower tetracycline resistance (11%) may reflect reduced usage of tetracyclines in the Bindura area compared to other antibiotic classes. However, this cannot be confirmed without local antibiotic consumption data.

Lead resistance was the most predominant among the resistance to heavy metals (48% of the isolates had only lead resistance. While 33% showed resistance to both lead and zinc together, 30% had multiple resistances to the three metals. Zinc resistance occurred in 35% of the isolates (zinc alone) and was often coupled with resistance to other heavy metals. The silver resistance was seen in 33% of isolates (silver alone) and occurred in multiple heavy metal resistance. The high rate of lead resistance occurs amid Bindura's mining background. Lead is one of the environmental contaminants of gold and nickel mining, and continued pollution leads to selection of the resistant strains in the environment (Mapanda et al., 2007). The strategies used by bacteria to be resistant to lead include efflux pump (P-type ATPase), sequestration by metal-binding proteins like metallothioneins and bioaccumulation (Naika& Dubey, 2013). Zinc resistance (35%) arises from the fact that it is one of the essential trace elements in many industrial uses. Although zinc is needed in small quantities by bacteria in enzyme catalysis, excess zinc becomes toxic, hence the development of resistance by bacteria (efflux and sequestration).

As far as the null hypothesis is concerned, which claims the lack of any significant relationship between HMR and AMR, it should be rejected based on Pearson's correlation and ANOVA results. The alternative hypothesis is therefore accepted; however, the low correlation coefficient ($r = 0.275$) shows that only a small amount of variability associated with antibiotic resistance is accounted for by heavy metal resistance. As far as the existence of co-selection is concerned, it cannot be ruled out in the present case; however, due to its low significance, one may assume that there are other factors (such as patterns of antibiotic usage, presence of runoffs from agricultural lands, and horizontal gene transfer rates) which account for higher levels of AMR. As a result, the findings presented in Chapter 4 offer convincing evidence of a positive relationship between the

two parameters. There are several different ways in which the co-existence between heavy metals and antibiotics could be genetically justified. As stated in Chapter 2, when genes associated with antibiotic resistance and metal resistance are located on the same MGE, both traits are preserved when any of the selective agents is applied to the bacterial population.

Study limitations are as follows:

1. Identification methodology: Biochemical tests have limited discriminatory power; misidentification rates of 10-15% are possible for Enterobacteriaceae.
2. Sample size and scope: ninety sampling points provide a snapshot but cannot capture temporal (seasonal) variation in resistance profiles.
3. Metal resistance definition: The 200 μg/mL MIC breakpoint is adapted from literature; no Zimbabwe-specific standards exist.
4. Confounding variables: Water temperature, pH, organic matter content, and other physicochemical parameters were not measured but may influence resistance expression.
5. Genetic characterization: Resistance genes were not identified using PCRtherefore mechanisms remain speculative.

CHAPTER 6

6.0 Conclusion and recommendations

6.1 Conclusions

There was successful identification of the existence of bacteria that exhibit resistance to heavy metals and antibiotics in environmental samples from Bindura. Different types of bacteria isolated from the environment, including *E. coli, S. aureus, P. aeruginosa,* Shigella *spp*., *Klebsiella spp*., and *Bacillus spp*., were resistant to various extents of heavy metals and antibiotics. It was discovered that there was high resistance to heavy metals such as lead, mercury. Furthermore, there was also resistance to various common antibiotics used today, namely tetracycline, amoxicillin and ciprofloxacin. Some associations observed statistically between some heavy metals and antibiotics indicate the possibility of the presence of co-selection and co-resistance of different types of bacteria from the environment. From the results obtained, environmental pollution with heavy metals might play an important role in the development and dissemination of bacterial resistance. This could be a possible danger in the environment, public health and disease management within the Bindura community and its surrounds. There exists a relationship between resistance to heavy metals and resistance to antibiotics, particularly in regions that are heavily polluted with heavy metals. The co-presence of heavy metals and antibiotics in the same environmental setup causes the presence of both HMRGs and ARGs. The connection between the two genes is multifaceted and complex. It is associated with such processes as co-selection, which does not only provide conditions for horizontal gene transfer among microorganisms including pathogens but also makes microorganisms more adaptable to different environments. In clinics, the presence of this connection promotes the appearance of multiple drug resistant bacteria such as P. aeruginosa and S. aureus that are posing a significant public health problem due to the limited treatment options available for these bacteria.

6.2 Recommendations

The following recommendations are suggested for the public health Authorities Bindura and future.

1. The government and local authorities should enforce stricter environmental protection regulations to control pollution from mining and industrial activities.
2. Public health authorities should promote rational use of antibiotics in both human and veterinary medicine to reduce the emergence and spread of resistant bacteria.
3. Community awareness campaigns should be implemented to educate residents about the dangers of environmental pollution and antimicrobial resistance.
4. Further molecular studies should be conducted to identify the specific genes responsible for heavy metal resistance and antimicrobial resistance among the bacterial isolates.
5. Conduct 16S rRNA sequencing for definitive species identification
6. Perform PCR screening for specific resistance genes (e.g., *bla*TEM, *merA, czc*)
7. Include seasonal sampling (wet vs. dry season)
8. Measure heavy metal concentrations in water/soil samples, not just bacterial resistance.
9. Future research should investigate the transmission pathways of resistant bacteria from the environment to humans and animals within mining communities.

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APPENDICES

Appendix A

Appendix 1Table A1: isolate morphologies

Isolate	Color	Margin	Surface texture	Humidity	Growth Pattern	Opacity	Elevation	Colony shape
A1a	Creamish /light tan	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A1b	Yellow	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A2a	Pale	Entire	Smooth	Moist	Not spread	Transparent	Flat	Circular
A2b	Off white	Wavy	Dull	Dry	Spread	Opaque	Doom shaped	Round
A2c	Yellow	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A3a	Cream	Entire	Rough	Dry	Spread	Opaque	Doom shaped	Circular
A3b	Pale	Entire	Smooth	Moist	Not spread	Transparent	Low convex	Round
A3c	Yellow	Entire	Smooth	Moist	spread	Opaque	Flat	Circular
A4a	Colorless	Entire	Smooth	Moist	Not Spread	Transparent	Flat	Circular
A4b	Brown	Entire	Smooth	Dry	Spread	Opaque	Doom shaped	Irregular
A4c	White	Wavy	Dull	Dry	Spread	Opaque	Flat	Circular
A4d	Golden yellow	Entire	Smooth	Moist	Spread	Opaque	Flat	Circular

A5a	Browm	Filamentous	Smooth	Moist	Spread	Opaque	Raised	Irregular
A5b	Cream	Entire	Smooth	Dry	Not spread	Opaque	Flat	Circular
A5c	White	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A6a	White	Entire	Smooth	Moist	Spread	Transluce nt	Flat	Circular
A6b	Cream	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A6c	Yellow	Wavy	Smooth	Dry	Spread	Opaque	Flat	Irregular
A6d	Brown	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A7a	Golden yellow	Entire	Smooth	Moist	Spread	Opaque	Flat	Circular
A8a	Yellow	Wavy	Smooth	Moist	Not spread	Opaque	Doom shaped	Irregular
A8b	Pale white	Entire	Smooth	Moist	Spread	Opaque	Flat	Irregular
A8c	White	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A8d	White	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A8e	White	Wavy	Smooth	Dry	Spread	Opaque	Doom shaped	Irregular
A9a	Creamish	Entire	Dull	Moist	Spread	Opaque	Flat	Circular
A9b	Yellow	Wavy	Smooth	Moist	Spread	Opaque	Flat	Irregular

A9c	White	Wavy	Dull	Moist	Not spread	Opaque	Flat	Irregular
A10a	White	Entier	Dull	Moist	Not spread	Opaque	Flat	Circular
A10b	Yellow	Wavy	Moist	Dry	Spread	Opaque	Flat	Irregular
A10c	Cream	Entire	Smooth	Moist	Spread	Opaque	Flat	Circular
A11a	Cream	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A11b	Cream	Entire	Sooth	f\dry	Spread	Opaque	Flat	Circular
A12a	Off white	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A12b	White	Entire	Smooth	Dry	Not spread	Opaque	Flat	Circular
A13a	White	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A13b	Yellow	Entire	Dull	Moist	Spread	Opaque	Flat	Circular
A13c	White	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A14a	Yellow	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A15a	Wjite	Entire	Smooth	Dry	Not spread	Opaque	Flat	Circular
A15b	Off white	Wavy	Dull	Dry	Spread	Opaque	Doom shaped	Round
A15c	Brown	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A16a	Cream	Entire	Rough	Dry	Spread	Opaque	Doom shaped	Circular

A16b	Off white	Wavy	Dull	Moist	Spread	Opaque	Doom shaped	Round
A16c	Yellow	Entire	Smooth	Moist	spread	Opaque	Flat	Circular
A16d	Cream	Wavy	Dull	Dry	Spread	Opaque	Flat	Irregular
A17a	Brown	Entire	Smooth	Dry	Spread	Opaque	Doom shaped	Irregular
A17b	White	Wavy	Dull	Dry	Spread	Opaque	Flat	Circular
A17c	Golden yellow	Entire	Smooth	Moist	Spread	Opaque	Flat	Circular
A18a	Browm	Filamentous	Smooth	Moist	Spread	Opaque	Raised	Irregular
A18b	Cream	Entire	Smooth	Dry	Not spread	Opaque	Flat	Circular
A18c	White	Entire	Dull	Dry	Spread	Opaque	Flat	Circular
A19a	Cream	Entire	Dull	Dry	Spread	Opaque	Flat	Circular
A19a	White	Entire	Dull	Dry	Spread	Opaque	Flat	Circular
A20a	White	Entire	Dull	Dry	Spread	Opaque	Flat	Circular
A20b	White	Entire	Dull	Dry	Spread	Opaque	Flat	Circular
A20c	Brown	Entire	Dull	Dry	Spread	Opaque	Flat	Circular
A21a	Off white	Entire	Smooth	Dry	Spread	Opaque	Flat	Circular
A21b	yellow	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A22a	Brown	Entire	Smooth	Dry	Not spread	Opaque	Flat	Circular
A22b	Off white	Wavy	Dull	Dry	Spread	Opaque	Doom shaped	Round

A22c	Yellow	Entire	Smooth	Moist	Not spread	Opaque	Flat	Circular
A23a	Cream	Entire	Rough	Dry	Spread	Opaque	Doom shaped	Circular
A23b	Off white	Wavy	Dull	Moist	Spread	Opaque	Doom shaped	Round
A23c	Yellow	Entire	Smooth	Moist	spread	Opaque	Flat	Circular
A23d	White	Entire	Smooth	Moist	Spread	Opaque	Flat	Circular
A24a	Cream	Wavy	Dull	Dry	Spread	Opaque	Flat	Irregular
A24b	yellow	Entire	Smooth	Dry	Spread	Opaque	Doom shaped	Irregular
A25a	White	Wavy	Dull	Dry	Spread	Opaque	Flat	Circular
A25b	Golden yellow	Entire	Smooth	Moist	Spread	Opaque	Flat	Circular
A26a	White	Wavy	Dull	Dry	Not spread	Opaque	Flat	Irregular
A26b	Browm	Filamentous	Smooth	Moist	Spread	Opaque	Raised	Irregular
A26c	Cream	Entire	Smooth	Dry	Not spread	Opaque	Flat	Circular
A27a	White	Entire	Smooth	Moist	Spread	Translucent	Raised	Irregular
A27b	Brown	Wavy	Smooth	Moist	Spread	Opaque	Doom shaped	Irregular
A28								
A29								
A30								

Table A2: Biochemical tests

Isolate	Biochemical tests				Selective agar	
	Catalase	Oxidase	Indole	Gramm test	Mannitol salt agar	McConkey agar
A1a	Positive	Negative	Negative	Negative	Positive	Negative
A1b	Weak Positive	Negative	Negative	Positive	Positive	Negative
A2a	Positive	Negative	Negative	Negative	Positive	Positive
A2b	Positive	Negative	Positive	Positive	Positive	Positive
A2c	Weak Positive	Negative	Positive	Positive	Positive	Negative
A3a	Positive	Negative	Negative	Positive	Positive	Negative
A3b	Positive	Negative	Negative	Negative	Positive	Positive
A3c	Positive	Negative	Negative	Positive	Positive	Negative
A4a	Positive	Negative	Negative	Negative	Positive	Positive
A4b	Positive	Negative	Negative	Negative	Positive	Negative
A4c	Positive	Negative	Negative	Negative	Positive	Negative
A4d	Weak Positive	Negative	Positive	Positive	Positive	Negative
A5a	Positive	Negative	Negative	Positive	Positive	Negative
A5b	Positive	Negative	Negative	Negative	Positive	Negative
A5c	Positive	Negative	Negative	Positive	Positive	Negative
A6a	Positive	Negative	Negative	Positive	Positive	Negative
A6b	Positive	Negative	Negative	Positive	Positive	Negative
A6c	Weak Positive	Negative	Positive	Negative	Positive	Negative
A6d	Positive	Negative	Negative	Negative	Positive	Negative
A7a	Weak Positive	Negative	Positive		Positive	Negative
A8a	Weak Positive	Negative	Positive	Negative	Positive	Negative

A8b	Positive	Negative	Negative	Positive	Positive	Negative
A8c	Positive	Negative	Negative	Positive	Positive	Negative
A8d	Negative	Negative	Negative	Positive	Positive	Negative
A8e	Positive	Negative	Negative	Positive	Positive	Negative
A9a	Positive	Negative	Negative	Positive	Positive	Negative
A9b	Positive	Negative	Negative	Positive	Positive	Negative
A9c	Positive	Negative	Negative	Positive	Positive	Negative
A10a	Positive	Negative	Negative		Positive	Negative
A10b	Weak Positive	Negative	Positive	Positive	Positive	Negative
A10c	Positive	Negative	Negative	Negative	Positive	Negative
A11a	Positive	Negative	Negative	Negative	Positive	Negative
A11b	Negative	Negative	Negative	Negative	Positive	Negative
A12a	Positive	Negative	Negative	Positive	Positive	Negative
A12b	Positive	Negative	Negative	Negative	Positive	Negative
A13a	Positive	Negative	Negative	Negative	Positive	Negative
A13b	Weak Positive	Negative	Positive	Negative	Positive	Negative
A13c	Positive	Negative	Negative	Negative	Positive	Negative
A14a	Positive	Negative	Negative	Negative	Positive	Negative
A15a	Weak positive	Negative	Positive	Negative	Positive	Negative
A15b	Positive	Negative	Negative	Negative	Positive	Negative
A15c	Positive	Negative	Negative	Negative	Positive	Negative
A16a	Positive	Negative	Negative	Negative	Positive	Negative
A16b	Positive	Negative	Negative	Negative	Positive	Negative
A16c	Negative	Negative	Negative	Negative	Positive	Negative
A16d	Positive	Negative	Negative	Negative	Positive	Negative
A17a	Positive	Negative	Negative	Negative	Positive	Negative
A17b	Positive	Negative	Negative	Negative	Positive	Negative
A17c	Negative	Negative	Negative	Negative	Positive	Negative

A18a	Positive	Negative	Negative	Negative	Positive	Negative
A18b	Positive	Negative	Negative	Negative	Positive	Negative
A18c	Positive	Negative	Negative	Negative	Positive	Negative
A19a	Positive	Negative	Negative	Negative	Positive	Negative
A19a	Positive	Negative	Negative	Negative	Positive	Negative
A20a	Positive	Negative	Negative	Negative	Positive	Negative
A20b	Positive	Negative	Positive	Negative	Positive	Negative
A20c	Positive	Negative	Negative	Negative	Positive	Negative
A21a	Positive	Negative	Negative	Negative	Positive	Negative
A21b	Positive	Negative	Negative	Negative	Positive	Negative
A22a	Positive	Negative	Positive	Negative	Positive	Negative
A22b	Positive	Negative	Negative	Negative	Positive	Negative
A22c	Weak Positive	Negative	Positive	Negative	Positive	Negative
A23a	Weak positive	Negative	Positive	Negative	Positive	Negative
A23b	Positive	Negative	Negative	Posivive	Positive	Negative
A23c	Weak Positive	Negative	Positive	Negative	Positive	Negative
A23d	Positive	Negative	Negative	Positive	Positive	Negative
A24a	Positive	Negative	Negative	Negative	Positive	Negative
A24b	Weak Positive	Negative	Positive	Negative	Positive	Negative
A25a	Positive	Negative	Negative	Positive	Positive	Negative
A25b	Negative	Negative	Negative	Positive	Positive	Negative
A26a	Positive	Negative	Negative	Negative	Positive	Negative
A26b	Positive	Negative	Negative	Negative	Positive	Negative
A26c	Positive	Negative	Negative	Positive	Positive	Negative
A27a	Positive	Negative	Negative	Negative	Positive	Negative
A27b	Positive	Negative	Negative	Negative	Positive	Negative

A28

A29

A30

*Appendix B*Appendix2

Table B1: Resistance and Susceptibility table

		ANTIBIOTICS									
	BACTERIA FOUND	A g	P b	Z n	H g	Al	AM L	CI P	T E	CN	MTZ
A1a	*S. aureus*	S	R	R	R	R	S	R	S	R	R
A1b	*E. coli*	S	R	S	S	R	S	R	S	R	S
A2a	*S. aureus*	S	R	S	R	R	R	R	S	R	S
A2b	*E. coli*	S	R	S	S	S	R	R	R	S	S
A2c	*Shigella*	S	R	S	S	S	R	S	S	S	R
A3a	*Klebsiellaspp*	S	R	S	R	S	R	R	S	S	R
A3b	*S. aureus*	S	R	S	S	R	R	R	S	R	R
A3c	*Shigella*	S	R	S	R	R	R	R	S	S	S
A4a	*P. aeruginosa*	R	R	S	R	R	R	S	S	R	R
A4b	*E. coli*	S	R	S	S	S	R	R	S	R	R
A4c	*Shigella*	S	R	S	S	R	R	R	S	R	R
A4d	*Klebsiellaspp*	S	R	S	R	R	R	R	R	S	S
A5a	*B. mycoids*	R	R	R	R	S	R	R	S	S	R
A5b	*P. aeruginosa*	R	R	R	S	R	R	R	S	R	R
A5c	*Klebsiellaspp*	R	R	R	S	R	R	R	S	R	R
A6a	*E. coli*	R	R	R	R	R	R	R	S	S	S
A6b	*S. aureus*	R	R	R	R	R	S	R	S	S	R
A6c	*Klebsiellaspp*	R	R	R	R	R	S	R	S	R	S
A6d	*Shigella*	R	R	R	S	R	S	S	S	R	S

A7a	*E. coli*	R	R	R	S	S	S	R	R	R	R
A8a	*S. aureus*	S	R	R	S	R	R	S	R	R	S
A8b	*E. coli*	R	R	R	R	R	S	S	S	R	S
A8c	*P. aeruginosa*	R	R	R	R	R	R	S	S	S	R
A8d	*Klebsiellaspp*	R	R	R	S	S	R	R	R	S	S
A8e	*E. coli*	R	R	R	S	R	R	R	S	S	R
A9a	*S. aureus*	R	R	R	R	R	R	R	S	S	R
A9b	*Klebsiellaspp*	R	R	R	S	R	R	R	S	R	R
A9c	*E. coli*	R	R	R	R	S	R	R	S	S	S
A10a	*S. aureus*	R	R	R	S	R	R	R	S	R	R
A10 b	*B. licheniformis*	R	R	R	S	R	R	R	S	S	R
A10c	*E. coli*	R	R	R	S	R	R	R	S	S	R
A11a	*B. licheniformis*	R	R	R	R	S	R	R	S	R	R
A11 b	*E. coli*	R	R	R	R	R	R	R	S	R	R
A12a	*Klebsiellaspp*	R	R	R	S	R	R	R	S	S	R
A12 b	*S. aureus*	R	R	R	S	R	R	R	S	R	R
A13a	*P. aeruginosa*	R	S	S	S	R	R	R	S	S	R
A13 b	*B. licheniformis*	S	S	S	S	R	R	S	S	R	R
A13c	*S. aureus*	R	S	S	S	S	S	R	S	S	R
A14a	*E. coli*	S	R	S	S	R	R	R	S	S	R
A15a	*S. aureus*	S	R	S	R	R	R	R	R	S	R
A15 b	*Shigella*	R	S	S	S	R	R	R	S	S	R
A15c	*P. aeruginosa*	S	S	S	R	S	R	R	S	R	R
A16a	*E. coli*	S	S	S	S	R	R	R	S	S	R

A16b	*S. aureus*	S	S	S	R	R	R	R	S	S	R
A16c	*Klebsiella*	R	S	S	R	R	S	S	S	R	R
A16d	*Shigella*	S	S	S	R	R	R	R	S	S	R
A17a	*B. mycoids*	S	S	S	S	S	R	R	S	S	R
A17b	*E. coli*	S	S	R	R	R	S	S	S	S	R
A17c	*P. aeruginosa*	S	R	S	S	R	R	R	S	S	R
A18a	*E. coli*	R	S	S	R	R	R	R	S	S	R
A18b	*S. aureus*	S	S	S	R	R	R	R	S	R	R
A18c	*E. coli*	S	S	R	S	R	R	R	R	S	R
A19a	*S. aureus*	R	R	R	R	S	S	S	S	R	R
A19a	*B. mycoids*	R	S	S	R	R	R	R	S	S	R
A20a	*E. coli*	S	S	S	R	R	R	R	S	S	R
A20b	*S. aureus*	R	S	S	S	S	R	R	S	S	S
A20c	*E. coli*	R	S	R	S	R	R	R	S	R	S
A21a	*S. aureus*	R	S	S	S	S	R	R	S	R	R
A21b	*P. aeruginosa*	S	S	S	S	S	R	R	S	R	R
A22a	*E. coli*	S	S	R	R	R	R	R	R	R	R
A22b	*S. aureus*	S	S	S	S	R	S	R	S	S	R
A22c	*Klebsiella*	S	S	R	S	R	R	R	S	S	R
A23a	*B. mycoids*	S	S	S	R	R	S	R	S	R	R
A23b	*E. coli*	S	S	S	S	R	R	R	S	R	R
A23c	*S. aureus*	S	S	S	S	R	R	S	R	S	R

A23d	*E. coli*	S	S	S	S	S	S	R	S	R	R
A24a	*B. mycoids*	R	R	S	S	R	S	S	S	S	R
A24b	*E. coli*	R	R	R	R	R	R	R	S	R	R
A25a	*B. mycoids*	R	R	R	R	R	R	R	S	S	R
A25b	*P. aeruginosa*	R	R	R	S	R	S	R	S	R	R
A27a	*E. coli*	R	R	R	R	R	R	S	S	R	S
A27b	*Pseudomonas*	R	R	S	R	R	R	R	S	R	S
A28	*E. coli*	S	R	R	R	R	R	R	R	R	R
A29a	*E. coli*	R	S	S	R	S	R	R	R	R	R
A30b	*E. coli*	S	S	R	R	R	R	R	R	R	R
A31a	*Bacillus spp.*	S	S	R	R	R	R	R	R	R	S
A31b	*B. mycoids*	S	S	R	R	R	R	R	R	R	S
A 31c	*B. licheniformis*	S	R	R	R	R	R	R	R	S	S
A32a	*Shigella*	R	R	R	S	R	R	R	R	S	S
Ab	*Pseudomonas spp*	R	R	R	R	S	R	R	S	S	R
Ac	*P. aeruginosa*	R	R	R	R	R	R	R	R	S	R
Ad	*S. aureus*	S	R	R	R	R	R	R	R	S	R
A32e	*E. coli*	R	R	R	S	R	R	R	R	S	R
A33a	*B. licheniformis*	R	R	S	S	R	R	R	R	S	R
A33b	*B. mycoids*	S	R	S	S	R	R	R	R	S	R
A33c	*Klebsiellaspp*	R	R	S	S	R	R	R	S	S	R
A34a	*E. coli*	R	S	S	R	S	R	R	S	R	R
A34b	*Klebsiellaspp*	R	R	S	S	R	R	S	S	R	R

A35a	*Shigella*	S	S	R	R	R	R	S	S	R	R
A35b	*P. aeruginosa*	R	S	R	R	R	R	S	R	R	R
A35c	*B. licheniformis*	R	S	R	R	S	R	R	R	R	S
A36a	*B. mycoids*	R	R	R	R	R	R	R	R	R	S
A36b	*P. aeruginosa*	R	R	R	R	R	R	R	R	R	R
A37a	*B. mycoids*	R	R	R	R	R	R	R	R	R	S
A37b	*Shigella*	R	R	R	R	S	S	R	R	R	R
A38a	*B. mycoids*	R	R	R	R	R	S	R	R	R	R
A38b	*E. coli*	R	R	R	R	S	S	R	S	R	R
A38c	*Shigella*	R	R	R	R	R	S	R	S	R	S
A40a	*S. aureus*	R	R	S	R	S	R	R	R	S	S
A40b	*B. mycoids*	R	S	S	R	S	S	R	R	R	S
A40c	*P. aeruginosa*	R	R	S	R	S	S	S	R	S	S
A40d	*B. licheniformis*	R	R	R	R	R	R	S	R	S	S
A40a	*E. coli*	R	R	R	R	S	R	S	R	S	S
A40b	*S. aureus*	R	S	R	R	R	R	R	S	S	S
A40c	*Shigella*	R	R	R	S	R	R	R	R	S	S
A41a	*P. aeruginosa*	R	R	R	R	R	R	R	S	S	S
A41b	*E. coli*	S	R	R	R	R	R	R	R	S	R
A41c	*Klebsiellaspp*	R	S	R	R	S	R	R	R	S	R
A41d	*B. licheniformis*	R	R	R	R	R	R	R	R	R	R
A42a	*B. mycoids*	R	R	R	S	R	R	R	R	S	R
A42b	*Bacillus spp.*	S	R	R	S	R	R	S	R	R	R
A42c	*S. aureus*	R	S	R	S	S	R	S	S	S	R
A42d	*P. aeruginosa*	S	R	R	R	R	R	S	S	S	S
A42e	*E. coli*	R	R	R	S	R	R	S	S	R	R
A43a	*Klebsiellaspp*	R	R	R	S	R	R	S	S	R	S
A43b	*E. coli*	R	R	S	S	R	R	S	S	R	S

A43c	*S. aureus*	R	R	S	R	R	R	R	S	R	S
A44a	*B. mycoids*	S	R	S	S	R	R	R	S	R	S
A44b	*Klebsiellaspp*	R	R	S	S	S	R	R	S	S	R
A45a	*P. aeruginosa*	R	R	R	S	R	R	R	S	S	S
A45b	*S. aureus*	R	R	S	R	R	R	R	S	S	R
A45c	*E. coli*	S	R	R	R	R	R	R	S	R	R
A46a	*Shigella*	R	R	R	R	R	R	R	S	S	R
A46b	*Staphylococcus spp*	R	R	R	R	R	R	R	S	S	S
A46c	*P. aeruginosa*	S	R	R	S	S	R	R	S	S	R
A46d	*E. coli*	R	R	R	R	R	R	R	S	S	R
A47a	*B. subtilis*	R	R	R	R	S	R	R	S	S	R
A47b	*P. aeruginosa*	R	R	R	S	R	R	R	S	R	R
A48a	*S. aureus*	S	S	R	S	S	S	R	S	S	R
A48b	*Shigella*	R	R	R	S	R	R	R	S	S	R
A49a	*B. mycoids*	S	R	R	R	S	R	R	S	S	S
A49b	*P. aeruginosa*	S	S	R	R	S	R	R	R	S	S
A49c	*S. aureus*	R	R	R	R	R	R	R	R	R	R
A49d	*E. coli*	R	R	R	R	R	R	R	R	S	R
A49e	*B. licheniformis*	R	S	R	R	R	R	R	R	S	S
A50a	*S. aureus*	R	R	R	S	R	R	R	R	R	S
A50b	*Pseudomonas spp*	S	S	R	R	R	R	R	R	S	S
A50c	*S. aureus*	R	S	R	R	R	R	R	R	S	S
A50d	*B. mycoids*	S	S	R	R	R	R	R	R	R	S
A51a	*B. licheniformis*	R	R	R	S	R	R	R	S	S	S
A51b	*P. aeruginosa*	R	S	R	R	S	R	R	R	S	S
A51c	*E. coli*	R	S	R	R	R	S	R	R	S	R
A52a	*S. aureus*	R	R	R	R	S	S	R	R	R	R

A52b	*B. mycoids*	S	R	R	R	S	R	R	R	S	R
A52c	*B. licheniformis*	R	R	R	R	S	R	R	R	S	R
A52d	*Klebsiellaspp*	R	R	R	R	S	S	R	R	R	R
A53a	*Klebsiellaspp*	R	R	R	R	R	S	R	R	R	R
A53b	*P. aeruginosa*	S	R	R	R	R	S	R	R	S	R
A53c	*S. aureus*	R	R	R	R	R	S	R	R	R	R
A54a	*Klebsiellaspp*	R	R	R	R	R	S	R	R	R	R
A54b	*P. aeruginosa*	R	R	R	R	R	S	S	R	R	R
A55a	*S. aureus*	R	R	R	R	R	S	S	S	R	R
A55b	*Shigella*	R	R	R	R	R	R	R	R	R	S
A56a	*B. licheniformis*	S	R	R	R	R	R	R	R	R	S
A56b	*Shigella*	S	R	R	R	R	R	R	R	R	S
A57a	*Pseudomonas*	R	R	R	R	R	R	R	R	R	R
A57b	*Bacillus spp.*	R	R	R	R	R	R	R	R	R	R
A57c	*E. coli*	S	R	R	R	R	R	R	R	R	S
A58a	*B. mycoids*	R	R	R	R	R	R	R	R	S	R
A58b	*S. aureus*	R	R	R	R	S	R	R	R	S	R
A58c	*Shigella*	R	R	R	R	S	R	R	R	S	S
A59	*E. coli*	R	R	R	S	S	R	R	R	S	S
A60	*E. coli*	S	S	S	S	S	S	S	S	S	S
A61	*E. coli*	R	R	S	R	R	R	R	R	R	S
A62a	*Shigella*	R	S	R	R	S	R	R	R	S	R
A62b	*P. aeruginosa*	R	S	S	R	S	R	R	R	S	S
A62c	*Klebsiellaspp*	R	R	R	R	R	R	R	R	R	R
A63	*E. coli*	S	S	S	S	S	S	S	S	S	S
A64a	*S. aureus*	R	R	R	S	S	S	R	R	R	S
A64b	*Shigella*	R	R	R	S	S	S	R	R	R	R
A64c	*P. aeruginosa*	R	R	R	S	S	S	R	R	R	S
A65a	*P. aeruginosa*	R	R	R	S	S	S	R	S	R	R

A65b	*Staphylococcus spp*	S	R	R	R	S	R	S	S	S	R
A65c	*S. aureus*	R	R	R	S	R	S	S	S	S	S
A65d	*E. coli*	R	R	R	R	R	S	R	S	R	R
A66a	*E. coli*	R	R	R	R	R	S	R	S	S	S
A67a	*S. aureus*	S	R	R	S	R	R	R	S	S	S
A67b	*B. subtilis*	R	R	R	S	R	R	R	S	R	R
A67c	*Shigella*	R	R	R	R	S	S	R	S	R	R
A67d	*P. aeruginosa*	R	R	R	S	R	S	R	S	S	R
A68a	*B. subtilis*	S	R	R	S	R	R	R	S	R	R
A68b	*S. aureus*	R	R	R	S	S	R	R	R	R	S
A68c	*E. coli*	R	R	R	S	R	S	R	R	R	S
A69a	*P. aeruginosa*	R	R	R	S	R	R	R	R	R	R
A69b	*S. aureus*	S	R	R	R	R	S	R	R	R	R
A70a	*B. mycoids*	R	R	R	R	R	R	R	S	S	S
A70b	*S. aureus*	R	R	R	R	S	R	R	R	S	S
A70a	*E. coli*	R	R	R	R	R	R	R	R	R	S
A70b	*S. aureus*	R	R	S	S	R	S	R	R	R	S
A71a	*E. coli*	R	R	S	S	R	S	R	R	S	S
A71b	*S. aureus*	R	R	R	R	R	R	R	R	R	S
A71c	*Shigella*	S	R	R	S	S	S	R	R	R	S
A71d	*B. licheniformis*	R	R	R	R	R	S	R	R	R	S
A72a	*P. aeruginosa*	R	R	R	R	S	S	S	R	S	R
A72b	*Shigella*	R	R	R	S	R	R	R	R	S	S
A72c	*S. aureus*	R	S	R	R	R	S	R	R	S	S
A73a	*P. aeruginosa*	R	S	R	R	R	S	R	R	S	S
A73b	*Klebsiellaspp*	S	S	R	S	R	S	R	R	R	R
A74a	*Shigella*	R	S	S	R	S	S	R	R	S	R
A74b	*P. aeruginosa*	R	R	R	R	R	R	R	S	R	R
A74c	*Shigella*	R	R	R	R	S	R	R	S	S	S

A75a	*Bacillus spp*	R	R	R	S	R	R	R	S	R	R
A75b	*P. aeruginosa*	R	R	R	R	S	R	R	S	S	R
A75b	*E. coli*	R	R	R	R	R	R	R	S	R	R
A76a	*E. coli*	R	R	R	R	S	R	R	S	S	R
A76b	*Staphylococcus spp*	S	R	R	R	R	R	R	S	R	R
A76c	*S. aureus*	R	R	R	R	S	R	R	R	R	R
A77a	*Shigella*	R	R	R	R	R	R	R	S	R	R
A77b	*Bacillus spp*	R	R	R	R	R	R	R	S	S	R
A78a	*Klebsiellaspp*	R	R	R	R	R	R	R	S	S	R
A78b	*P. aeruginosa*	S	R	R	R	S	R	S	S	S	S
A79a	*S. aureus*	R	S	R	R	S	S	S	S	S	R
A79b	*Shigella*	R	R	R	S	R	S	R	S	R	S
A79c	*E. coli*	R	S	R	R	R	S	R	S	R	R
A79d	*Staphylococcus spp*	R	R	R	R	S	S	R	S	R	R
A80a	*B. mycoids*	R	R	R	R	R	S	R	S	R	R
A80b	*Staphylococcus spp*	R	R	R	S	R	R	R	S	S	R
A80c	*S. aureus*	R	S	R	R	R	R	R	R	S	R
A81a	*B. mycoids*	R	R	R	S	R	R	R	R	S	R
A81b	*S. aureus*	R	R	R	S	R	R	R	R	R	S
A81c	*E. coli*	R	R	R	S	R	R	R	R	S	R
A82a	*P. aeruginosa*	R	R	S	S	R	S	R	R	S	R
A82b	*B. licheniformis*	R	R	R	S	S	R	R	R	R	R
A83a	*B. mycoids*	R	R	R	S	R	R	R	R	R	S
A83b	*E. coli*	R	R	R	S	R	S	R	R	R	R
A83c	*S. aureus*	R	R	R	R	R	R	R	R	R	S
A83d	*Pseudomonas spp*	R	R	R	R	R	R	R	S	R	S

A84a	*B. mycoids*	S	R	R	R	S	R	R	S	R	R
A84b	*E. coli*	R	R	R	R	R	R	R	S	R	S
A84c	*Shigella*	R	R	R	R	S	S	R	S	S	R
A84d	*B. licheniformis*	R	R	R	R	S	S	R	S	S	R
A85a	*Klebsiella*	S	R	R	R	S	R	R	S	S	R
A85b	*S. aureus*	R	R	R	R	R	R	R	S	R	R
A85c	*B. licheniformis*	R	R	R	S	S	R	R	S	S	S
A86d	*E. coli*	R	S	R	R	R	S	R	S	R	R
A87a	*Klebsiella*	S	R	R	R	R	S	R	S	S	R
A87b	*B. licheniformis*	R	S	R	R	R	S	S	S	S	R
A87c	*Shigella*	R	S	R	S	R	R	S	R	R	R
A87d	*E. coli*	R	R	S	R	R	R	R	R	S	R
A87e	*S. aureus*	S	R	R	R	R	R	R	S	S	R
A88a	*E. coli*	R	R	R	R	R	R	R	S	R	R
A88b	*S. aureus*	R	R	R	R	R	R	R	R	R	R
A88c	*Pseudomonas spp*	R	R	R	S	R	R	R	R	S	R
A89a	*E. coli*	R	R	R	R	R	R	R	R	R	R
A89b	*S. aureus*	S	R	R	R	R	R	R	S	R	R
A89c	*Shigella*	R	R	R	R	R	R	R	S	R	R
A90a	*B. mycoids*	R	R	R	S	R	R	R	R	R	R
A90b	*Shigella*	R	R	R	R	S	S	R	R	S	R
A90c	*E. coli*	R	S	R	R	R	R	R	S	R	S

Table B2: One-way ANOVA for Al

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
AML AST	Between Groups	.858	1	.858	4.404	.037
	Within Groups	48.114	247	.195		
	Total	48.972	248			
CIP AST	Between Groups	.007	1	.007	.055	.815
	Within Groups	30.788	247	.125		
	Total	30.795	248			
TE AST	Between Groups	.241	1	.241	1.043	.309
	Within Groups	38.870	168	.231		
	Total	39.112	169			
CN AST	Between Groups	1.739	1	1.739	7.099	.008
	Within Groups	60.502	247	.245		
	Total	62.241	248			
MTZ AST	Between Groups	.000	1	.000	.002	.969
	Within Groups	56.297	247	.228		
	Total	56.297	248			

Table B3:One-way ANOVA for Hg

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AML AST	Between Groups	.005	1	.005	.027	.869
	Within Groups	48.967	247	.198		
	Total	48.972	248			
CIP AST	Between Groups	.087	1	.087	.702	.403
	Within Groups	30.708	247	.124		
	Total	30.795	248			
TE AST	Between Groups	1.657	1	1.657	7.431	.007

	Within Groups	37.455	168	.223		
	Total	39.112	169			
CN AST	Between Groups	.020	1	.020	.078	.781
	Within Groups	62.221	247	.252		
	Total	62.241	248			
MTZ AST	Between Groups	.011	1	.011	.049	.825
	Within Groups	56.286	247	.228		
	Total	56.297	248			

Table B4: one-way ANOVA for Silver

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AML AST	Between Groups	.564	1	.564	2.879	.091
	Within Groups	48.408	247	.196		
	Total	48.972	248			
CIP AST	Between Groups	.049	1	.049	.397	.529
	Within Groups	30.746	247	.124		
	Total	30.795	248			
TE AST	Between Groups	1.337	1	1.337	5.944	.016
	Within Groups	37.775	168	.225		
	Total	39.112	169			
CN AST	Between Groups	.116	1	.116	.460	.498
	Within Groups	62.125	247	.252		
	Total	62.241	248			
MTZ AST	Between Groups	.068	1	.068	.299	.585
	Within Groups	56.229	247	.228		
	Total	56.297	248			

Table B5: one-way ANOVA for zinc

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AML AST	Between Groups	.043	1	.043	.219	.640
	Within Groups	48.928	247	.198		
	Total	48.972	248			
CIP AST	Between Groups	.103	1	.103	.831	.363
	Within Groups	30.692	247	.124		
	Total	30.795	248			
TE AST	Between Groups	6.425	1	6.425	33.023	.000
	Within Groups	32.687	168	.195		
	Total	39.112	169			
CN AST	Between Groups	.748	1	.748	3.002	.084
	Within Groups	61.493	247	.249		
	Total	62.241	248			
MTZ AST	Between Groups	.093	1	.093	.408	.523
	Within Groups	56.204	247	.228		
	Total	56.297	248			

Table B6: One-way ANOVA for Pb

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AML AST	Between Groups	.115	1	.115	.583	.446
	Within Groups	48.857	247	.198		
	Total	48.972	248			

CIP AST	Between Groups	.198	1	.198	1.598	.207
	Within Groups	30.597	247	.124		
	Total	30.795	248			
TE AST	Between Groups	1.040	1	1.040	4.590	.034
	Within Groups	38.072	168	.227		
	Total	39.112	169			
CN AST	Between Groups	.620	1	.620	2.486	.116
	Within Groups	61.621	247	.249		
	Total	62.241	248			
MTZ AST	Between Groups	.125	1	.125	.550	.459
	Within Groups	56.172	247	.227		
	Total	56.297	248			

Table B7: correlation

		Ag	Pb	Zn	Al	Hg
CIP AST	Pearson Correlation	.053	1	.137*	.107	.005
	Sig. (2-tailed)	.403		.031	.163	.938
	N	249	249	249	170	249
AML AST	Pearson Correlation	-.010	.137*	1	-.032	-.129*
	Sig. (2-tailed)	.869	.031		.680	.043
	N	249	249	249	170	249
TE AST	Pearson Correlation	.206**	.107	-.032	1	.039
	Sig. (2-tailed)	.007	.163	.680		.616
	N	170	170	170	170	170
CN AST	Pearson Correlation	.018	.005	-.129*	.039	1

	Sig. (2-tailed)	.781	.938	.043	.616	
	N	249	249	249	170	249
MTZ AST	Pearson Correlation	-.014	.038	-.041	-.288**	.009
	Sig. (2-tailed)	.825	.555	.522	.000	.891
	N	249	249	249	170	249

Table B8: Chi-square

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.869[a]	1	.090		
Continuity Correction[b]	2.360	1	.125		
Likelihood Ratio	2.993	1	.084		
Fisher's Exact Test				.115	.060
Linear-by-Linear Association	2.857	1	.091		
N of Valid Cases	249				