Ref no: _____

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Biomedical Sciences

Phenotypic and Molecular Antimicrobial Resistance Profiles of Bacteria from Wastewaters and Inanimate Surfaces of Tertiary Hospitals in Addis Ababa, Ethiopia

Msc Thesis

By

Misganu Yadesa Tesema

June, 2026

Bishoftu, Ethiopia

Phenotypic and Molecular Antimicrobial Resistance Profiles of Bacteria from Wastewaters and Inanimate Surfaces of Tertiary Hospitals in Addis Ababa, Ethiopia

A Thesis Submitted to College of Veterinary Medicine and Agriculture, Addis Ababa University, in Partial Fulfillments of the Requirement for the Degree of Masters in Health Biotechnology

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Approved for submittal to thesis assessment committee

We verify that student **Misganu Yadesa's** MSc thesis entitled **"Phenotypic and Molecular Antimicrobial Resistance Profiles of Bacteria from Wastewaters and Inanimate Surfaces of Tertiary Hospitals in Addis Ababa, Ethiopia"** has been carried out under our direct supervision and recommend the thesis to be accepted as a fulfilling the requirements for a degree of Master of Science in Heath Biotechnology.

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Addis Ababa University

College of Veterinary Medicine and Agriculture

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This is to certify that the thesis prepared by **Misganu Yadesa** entitled **"Phenotypic and Molecular Antimicrobial Resistance Profiles of Bacteria from Wastewaters and Inanimate Surfaces of Tertiary Hospitals in Addis Ababa, Ethiopia"** and submitted in partial fulfillment of the requirements for the degree of Master of Science in Health Biotechnology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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ACKNOWLEDGEMENTS

First and at most I am grateful to Almighty God to bring me here in all ups and down of my life journey.

I would like to express my deepest gratitude to my major advisor, Dr. Tesfaye Admassu (PhD), for the invaluable guidance, patience, and encouragement throughout this process.

I would like to express my sincere and heartfelt gratitude to my co-advisor Professor Tesfaye Sisay for constructive advice, technical support and encouragement throughout the course of the study and for providing the resources necessary to conduct this study.

I am profoundly grateful for the mentorship of my co-advisor Dr. Bayable Atnafu (PhD) for the positive communication, guidance and support for this work.

I am deeply indebted to Dambi Dollo University for their institutional support and financial sponsorship, which were instrumental in the commencement and pursuit of my MSc degree.

I am grateful to the Office of the Vice President for Research and Technology Transfer, Addis Ababa University, for the financial assistance provided through the thematic research program, which allowed me to focus entirely on my studies.

I am deeply thankful to the staffs of the Biotechnology Research Center, Addis Ababa University, for their invaluable academic guidance and for fostering an environment of excellence during my graduate studies.

I am grateful to Department of Biomedical Sciences, College of Veterinary Medicine and Agriculture, Addis Ababa University, for their support for the accomplishment of this work.

I would like to express my sincere gratitude to Tikur Anbessa Specialized Hospital and Zewditu Memorial Hospitals for permitting me access to their facilities for the sample collection process. My research would not have been possible without the cooperation of the administration, the IPC department and all staffs who assisted me during the data collection process.

I am grateful to my lab colleagues for their partnership and for the many helpful discussions that contributed to the development of this project.

Lastly, I thank my friends and family for their unwavering encouragement.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
ARB	Antimicrobial Resistant Bacteria
AST	Antimicrobial Susceptibility Testing
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
CRE	Carbapenem-Resistant Enterobacterales
ESBL	Extended-Spectrum B-Lactamase
GNB	Gram Negative Bacteria
GPB	Gram Positive Bacteria
GRAM	Global Research on Antimicrobial Resistance
HAI	Healthcare-Associated Infections
HGT	Horizontal Gene Transfer
IPC	Infection Prevention and Control
IS	Inanimate Surfaces
LMIC	Low- And Middle-Income Countries
MDR	Multidrug-Resistance
MGE	Mobile Genetic Elements
PDR	Pandrug-Resistant Bacteria
TASH	Tikur Anbessa Specialize Hospital
WHO	World Health Organization
WW	Wastewater
XDR	Extensively Drug-Resistant
ZMH	Zewuditu Memorial Hospital

ABSTRACT

Antimicrobial resistance (AMR) is a major global public health threat driven by inappropriate antimicrobial use and the emergence of resistant organisms. In Ethiopia, AMR-associated deaths are estimated to reach 89,200 by 2030. Therefore, to assess the presence of resistant bacteria and their associated genes in hospital environments, a cross-sectional study was conducted between October 2023 and October 2025 in Addis Ababa, Ethiopia. A total of 38 samples were collected from wastewaters (WW) and inanimate surfaces (IS) of Tikur Anbessa Specialized Hospital (TASH) and Zewuditu Memorial Hospital (ZMH). Samples were processed using culture-based methods, biochemical testing, and MALDI-TOF MS (EXS3000) for bacterial identification. Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method, while antimicrobial resistance genes (ARGs) detected by polymerase chain reaction (PCR). A total of 92 bacterial isolates were identified, comprising 63 (68.5%) from IS and 29 (31.5%) from WW samples. Among Gram-negative bacteria, 100% of *K. pneumoniae* isolates exhibited a multidrug-resistant (MDR) phenotype, while 89% of *S. aureus* were MDR from Gram-positive. The PCR revealed high frequencies of both chromosomal and plasmid-mediated ARGs. The most frequent chromosomal genes detected were *blaTEM* (94%), *sul1* (53%), *blaCITM* (38.5%) and *blaCTXM* (37.5%), while high frequent plasmid-mediated ARGs were *blaTEM* (92%), *tetA* (65.6%), *sul1* (56%), *blaCTXM* (50%), *blaNDM* (43.8%) and less frequent genes included *blaOXA-48*, *blaKPC*, *blaFOXM*, *blaEBCM*, *blaMOXM*, *blaDHAM* and *ermB*. Data analysis (SPSS v.26) indicated significant association ($p < 0.05$) between phenotypic resistance and the presence of specific carbapenamase genes. The findings indicate a high burden of AMR in hospital environments and highlight the importance of implementing on-site hospital WW treatment and strengthening surface disinfection practices to reduce the spread of AMR.

Keywords: antimicrobial resistance, hospital wastewater, antimicrobial resistance genes, hospital inanimate surface, Ethiopia

1. INTRODUCTION

1.1. Background of the Study

Antimicrobials are natural products of microorganisms or chemically synthesized compounds that can prevent other microorganisms from proliferating and thriving (Kumar et al., 2019). However, due to misuse, abuse and overuse, antimicrobials might lose their activity. Then, microorganisms evolve adaptive responses that make them resistant to treatments, rendering them ineffective and making the treatment of infections more challenging or impossible. This phenomenon is known as antimicrobial resistance (AMR) (Tesema, 2025; WHO, 2015). Despite numerous local and global antimicrobial consumption regulations, antimicrobial consumption was projected to increase by 200% by 2030. Among factors increasing AMR, overconsumption of antimicrobials is one (Amangelsin et al., 2023).

Once antimicrobials are consumed by humans and animals, only some portions are assimilated. The majorities of them (up to 90%) are excreted as the parent or partially metabolized substrate in the urine and faeces. These residues can end up unabsorbed and may eventually be released into the environment (Sabri et al., 2020). The emergence and survival of resistant bacteria in the environment is mainly resulted from selection pressures imposed by the presence of unmetabolized antimicrobials or their residues released into the environment. Furthermore, what worsens the phenomena is that the selection pressures is not only against target bacteria but also against non-target bacteria (Jabeen et al., 2023). A major threat to the welfare of humans could arise from the formation of extremely virulent super-bacteria resistant to the majority of antimicrobial medications when antimicrobial resistance genes (ARGs) in bacteria increasingly accumulate (Tao et al., 2022).

Antimicrobial resistance is one of the most significant threats to global public health in the 21st century. In 2016, AMR was forecasted to cause 10 million deaths annually by 2050 (O'NEILL, 2016). Again in 2024, empowering the 2016 estimation based on data spanning 30 years (1990-2021), the worrisome rate predicted that by 2050 AMR will directly cause approximately 39 million deaths and be associated with an additional 169 million deaths. This equates to three deaths every minute (Naghavi et al., 2024).

AMR is projected to cause 89,200 deaths and substantially increase mortality in Ethiopia by 2030 if current trends continue (GRAM, 2023). Paying attention to this global issue, the World Health Organization (WHO) identified strategic and operational priorities to address antimicrobial resistant bacteria (ARB) infections from 2025 to 2035 (WHO, 2023).

Antimicrobial resistance causes a multifaceted crisis. It causes a major financial impact on both domestic and global economies. It lengthens hospital stays; because ARBs necessitate the use of last-line antimicrobials, which are significantly more costly than first- and second-line antimicrobial therapy, it necessitates more costly medications and intense care (Zhen et al., 2021). According to the World Bank, if AMR is not controlled, it will result in yearly gross domestic product losses ranging from $1 trillion to $3.4 trillion by 2030. Particularly, AMR threatens and impacts low- and middle-income countries (World Bank, 2017) as the previous study estimates suggested that the most risk attributable to, and associated with, AMR are in Sub-Saharan Africa and South Asia (Ranjbar and Alam, 2024). Nevertheless, to divert this scenario, it is necessary to have full data of the characterized existing ARGs whether they are unstable (plasmid) or stable (chromosomal) (Naghavi et al., 2024), that might encouradge the development of new antimicrobials targeting Gram negative bacteria (GNB) estimated to avert 11.08 million AMR-related deaths by 2050 globally (Assefa et al., 2025).

Ethiopia has acknowledged the rising threat of AMR and launched the 4th National Action Plan on Antimicrobial Resistance for 2026-2030 on December 01, 2025 (WHO, 2025).

Tertiary hospitals are high-pressure environments characterized by heavy antimicrobial selection pressure (Sebre et al., 2020). Healthcare facilities act as major reservoirs for antimicrobial-resistant organisms, with wastewater (WW) systems facilitating their dissemination into the environment. It provides ideal conditions for horizontal gene transfer (HGT), allowing resistance genes to spread between different bacterial species via plasmids and be discharged into municipal sewer systems. Compared to municipal WW, hospital WW can have up to 10 times more ARGs and resistant bacteria (Hassoun et al., 2020).

Consequently, inanimate surfaces (IS) such as bed rails, medical equipment, and door handles act as reservoirs where bacteria might persist for weeks and more, facilitating healthcare-associated infections (HAIs) (Laizer et al., 2025). When ARBs and ARGs are released from hospitals into WW with no adequate treatment, aquatic habitats, animals and people are in danger apart from ecological damage and ARB evolution (Yao et al., 2021).

In different environmental settings, the opportunity for vertical and horizontal gene transmission (HGT) is highlighted by the identification of the particular chromosomal DNA and plasmids DNA that carry those responsible resistance genes, respectively. This allows for their selective potential and quick spread across human, animal, and environmental reservoirs

in addition to healthcare facilities. This genomic information is essential for directing antimicrobial stewardship initiatives, leading targeted infection control, and speeding up the creation of innovative diagnostic and treatment strategies to address Ethiopia's worsening public health emergency (Assefa et al., 2025). The risk of highly spreading ARBs and ARGs is frequently increased in low- and middle-income countries (LMICs) due to problems with WW infrastructure and uneven environmental disinfection practices (Naghavi et al., 2024).

In Ethiopia, particularly in densely populated urban centres like Addis Ababa, tertiary hospitals such as Tikur Anbessa Specialised and Zewditu Memorial Hospitals handle a high volume of complex cases. However, data integrating both phenotypic and molecular profiles of AMR from these environmental sources remain scarce (Sebre et al., 2020; Yosef et al., 2025). However, antimicrobial stewardship, infection control strategies, and the prioritization of medication and vaccine development can all be guided by documenting the changing nature of AMR, particularly changes in resistance to important antimicrobial classes like the carbapenems (Naghavi et al., 2024). The most prevalent GNB extensively drug-resistant (XDR) in Ethiopia, such as *Klebsiella* species, *Escherichia coli* (*E. coli*), *Acinetobacter* species, and *Pseudomonas aeruginosa* (*P. aeruginosa*), speed up the transmission of ARGs from hospital settings to patients, to the environments and to the large communities. This can seriously threaten the public health with the rise of multidrug-resistant (MDR), XDR, and pandrug-resistant (PDR) bacteria, which can lead to higher rates of morbidity and mortality, longer hospital stays, expensive medical bills, and difficult treatment choices (Assefa et al., 2025).

The majority of existing investigations focus exclusively on clinical isolates from patients, leaving a huge knowledge gap regarding the environmental resistance that surrounds them. Therefore, this study aims to address the gap by analyzing the phenotypic and molecular AMR profiles of bacterial isolates from WW and IS at these two major hospitals. As a part of One Health, it provides imperative data to improve national infection control policies and sanitation strategies in Ethiopia by identifying resistance patterns as well as specific genetic determinants.

1.2. Statement of the Problem

One of the concerning issues contributing to AMR is the discharge of ARB from healthcare facilities into a variety of ecosystems, including aquatic and terrestrial settings. Hospital contamination causes the bacteria to mobilize more ARGs and/or boost environmental ARBs

(Murray et al., 2021). Hospital WW flowing into the surroundings and water sources pose a risk of AMR and the development of ARB that may affect humans, animals, and environments (Xue et al., 2022).

Hospital WW and IS are two underestimated reserviors of AMR. However, they are hubs for the emergence and transmission of AMR. Antimicrobial resistant bacteria have the potential to contaminate the hospital environments, as they are present on the surfaces of inanimate materials, making it easier to contaminate patients, medical personnels, and guests to spread the infection. They are also common in hospital WW due to inadequate sanitation and hygiene standards, overpopulation in healthcare facilities, and increased patient loads. Hospital WW is a powerful mixture of harmful bacteria and antimicrobials residues that are discharged from patient urine, faeces, and other human fluids. Superbugs are produced as a result of these ideal conditions for the selection and horizontal transfer of resistance genes. This WW frequently finds its way into municipal sewage systems or the environment with no or little treatment, where it may contaminate water supplies and spread AMR throughout the neighbourhood. Antimicrobial resistant bacteria are common in WW possibly at an extremely high rate, and one of the primary causes of their environmental spread is the discharge of hospital effluents into the sewage system without sufficient treatment. One of the main causes of healthcare-associated infections (HAIs) is this environmental pollution.

In Ethiopia, high levels of resistance to commonly used antimicrobials are already evident in surveillance data, which is mostly derived from clinical samples (blood, urine, and wound swabs). Nevertheless, comprehensive information on the environmental AMR reservoirs in healthcare institutions is severely lacking in the country. There are serious repercussions from this information gap. Infection prevention and control (IPC) regulations could not be fully data-driven and could not be enough to break the link of transmission if the genetic profile of environmental AMR and the particular contamination hotspots are unknown. Public health in Addis Ababa and downstream communities are seriously at risk from the direct discharge of untreated, resistance-laden WW from these large hospitals into the environment. By identifying the most urgent resistance concerns circulating within the hospital, an understanding of the environmental resistance can help to advise and enhance empirical antimicrobial therapy guidelines. Effective resource allocation and impact measurement are challenging when public health interventions are adopted without a baseline understanding of the issue.

Therefore, this study, by scaffolding the phenotypic AMR profiling with molecular detection, including plasmid-mediated ARGs detection, will narrow the gaps.

1.3. Objectives

1.3.1. General Objective

The general objective of this study is to evaluate the phenotypic and genotypic antimicrobial resistance patterns of hospital WW and IS bacterial isolates through susceptibility testing and PCR based molecular screening.

1.3.2. Specific Objectives

The specific objectives of the present study are:

- To determine the prevalence of bacterial isolates from hospital wastewater and inanimate surface samples.
- To evaluate the phenotypic antimicrobial susceptibility profiles of the bacterial isolates.
- To characterize chromosomally-mediated antimicrobial resistance genes (ARGs) using PCR.
- To assess the potential for horizontal gene transfer by identifying plasmid-mediated ARGs using PCR.

1.4. Significance of the Study

The present study of AMR characterization from TASH and ZMH WW and IS has much significance in clinical safety, public health, and environmental integrity. It helps the hospital epidemiologists understand how superbugs persist in the clinical environment, leading to better-targeted disinfection protocols. The hospitals obtain the evidence from phenotypical and molecular characterization of the AMR to provide life-saving IPC measures specifically tailored to the most at-risk patients and also to the environments.

Additionally, this study serves to show where we are and what to do, reflecting the real magnitude of AMR within the two tertiary hospitals and provides evidence to show that Ethiopia needs a comprehensive One Health approach and nationwide AMR surveillance. Detecting the plasmid-mediated ARGs from the hospitals WW and IS samples give significant data based alert to understand how urban agriculture and downstream water users are exposed to high-risk pathogens. Due to the discharge of TASH and ZMH WW into local rivers (such as the Akaki River) or municipal sewage systems, the ARB and ARGs were able

to enter into the wider ecosystem. These findings demonstrate the critical necessity for specialized on-site treatment facilities, such as WW treatment plants (WWTPs), to eliminate these risks before they depart the hospital settings. The prevalence of ARBs and detection of ARGs on the cleaned IS is used as a performance indicator for hospital cleaning and waste management procedures.

Furthermore, this study made a strong case for more stringent antimicrobial stewardship programmes (ASP) in Addis Ababa by highlighting the presence of MDR bacteria and criticizing the illogical use of broad-spectrum medication. Once ARBs reach this environment as a habitat, they use it to exchange resistance characteristics through horizontal gene transfer (HGT). The finding identified and filled gaps in current ARGs dissemination from hospital environments to communities via plasmid-mediated to infectious pathogens and also to commensal bacteria.

Generally, this investigation has significance in direct clinical activity to reduce morbidity and mortality by identifying surface and water-borne pathogens or non-pathogenic origins that carry ARGs and also rare emerging bacteria in the country. It can be used as the evidence underpinning for policy-driven decisions for the antimicrobial resistance prevention and containment strategic plan of the national action plan on AMR in Ethiopia. These findings also can be used by national and international scientific communities from multidisciplinary areas for understanding the African region and the global AMR profile.

1.5. Scope of the Study

This study was conducted in two public hospitals, TASH, the largest referral and teaching hospital and ZMH in Addis Ababa, Ethiopia. It focused on two primary reservoirs, IS and WW, where bacteria inhabit outside the human body. IS are high-touch areas, including medical equipment (ventilators, IV poles, and tables...), critical wards (intensive care units, neonates intensive care units, and operation theaters…) which include fomites (bed rails, linens, and doorknobs). WW is the flow collected from various parts of the hospital drainage system, including influent (untreated raw sewage from wards and labs) and effluent (a composite WW leaving hospitals towards the municipal system). The study performed on the prevalence of Gram positive and Gram negative bacterial HAIs, opportunistic bacteria and rare emerging bacteria. Bacterial identification was preceded by biochemical tests, the golden standard for bacterial identification, and matrix-assisted laser desorption ionization time of

flight mass spectrometry (MALDI-TOF MS), the rapid and accurate technique, for the identification of bacteria.

The investigation employed phenotypic AMR profiling to determine the antibiogram (the pattern of resistance) using the Kirby-Bauer disk diffusion method against common antimicrobials, and multiplex polymerase chain reaction (mPCR), a molecular profiling. The presence of plasmid-mediated ARGs on IS suggests that a harmless environmental bacterium might pick up resistance genes from a pathogen and transport into the WW. These mobile genes may eventually make their way to the Akaki River, where they might be transferred to bacteria in the soil or in the water used for nearby agriculture.

2. LITERATURE REVIEW

2.1. Global Crisis of Antimicrobial Resistance

2.1.1. Antimicrobial Resistance Threats and Its Drivers

Bacteria, viruses, fungi, and parasites can evolve to the extent where they become resistant to treatments. This phenomenon is known as antimicrobial resistance (AMR) (Tesema, 2025). Antimicrobial resistance affects people of all ages and all nations, making it one of the top ten global public health problems confronting human beings. Prolonged disease not only causes mortality and incapacity but also lengthens hospital stays, necessitates more costly medications, and puts those afflicted in a difficult financial situation (Naghavi et al., 2024). Antimicrobial resistance is increasingly acknowledged as one of the most serious health risks to humans, animals and the environment. The main drivers of AMR, the global public health threat and a silent pandemic, are the misuse and overuse of antimicrobials, poor sanitation, inadequate awareness, poor regulation and insufficient IPC in hospitals and on farms (James, 2025).

This encourages ARGs to develop, proliferate, and accumulate in a variety of environments (Wang et al., 2020). Additionally, the hospital acquired sub-lethal dosages of antimicrobials released into the environment, which may apply selective pressure to the environment's vulnerable microorganisms (Teshome et al., 2020). Furthermore, bacteria that carry clinically significant resistance genes in natural ecosystems, in wild animals, and even in isolated communities, which have not been exposed to antimicrobials, may disseminate ARGs due to HGT and enhanced mutagenesis (Silva and Khare, 2024).

Antimicrobial resistant microorganisms afflict 8.2 million individuals each year, and more than 35,000 people lost their lives in 2018 in the US. Resistant antimicrobial diseases caused high economic costs to China, 77 billion dollars(Ratna et al., 2025; Zhen et al., 2021).

Previous studies in Ethiopia consistently report high antimicrobial resistance rates; however, most focus on clinical isolates, with limited attention to environmental reservoirs such as hospital WW and IS. For instance, MDR prevalence was reported in Arba Minch (70.4%) (Regasa et al., 2021), Addis Ababa (64.77%) (Tilahun et al., 2025), Bule Hora (63.9%) (Dhengesu et al., 2022), Shashemene (75.3%) (Bonso et al., 2023), Bahir Dar (49.3%) (Sitotaw et al., 2024), Gondar (52.6%) (Geta and Kibret, 2022), Wolaita Sodo (50.5%) (Adugna and Sivalingam, 2022), Hawassa (29.5%) (Mekengo et al., 2021), Mekelle (79.1%)

(Asfaw, 2018), Dire Dawa (85%) (Teshome et al., 2020) and Debre Tabor (85%) (Yenew et al., 2022).

2.1.2. Global and National Frameworks for Antimicrobial Resistance Surveillance

In order to improve understanding and create strategies against AMR, the WHO launched the global antimicrobial resistance and surveillance system (GLASS) in 2015. Ethiopia has also recognized the alarming threat of AMR and launched the 4th National Action Plan on Antimicrobial Resistance for 2026-2030 on December 01, 2025 (WHO, 2025).

Therefore, a systematic method for gathering, evaluating, and disseminating information about AMR at the international and local levels is facilitated by the GLASS. This includes information about AMR in the environment, food chain, and humans (WHO, 2021). One important element in managing and avoiding this pathogenic disease is prompt and precise identification. The next one is phenotypic and genotypic techniques to detect ARGs from the bacteria (Caliskan and Alocilja, 2023).

2.2. Epidemiology of AMR in Healthcare Settings

2.2.1. Healthcare-Associated Infections (HAIs) and Environmental Reservoirs

Healthcare- associated infections, often known as nosocomial infections, affect people receiving medical treatment. About 10% of infections in LMICs and 7% in industrialized counties are attributable to HAIs (Khan et al., 2017) and every year, the rate of HAIs rises by 0.06% (Raoofi et al., 2023). HAIs typically affect one out of ten patients; however, in LMICs and among high-risk patients, such as those in intensive care units (ICUs), the frequency can be significantly greater. According to the WHO report, 136 million cases of AMR disease linked to healthcare occur annually worldwide (WHO, 2024).

These infections result in extended hospital stays, incapacity, and financial hardship since they happen during hospital stays. Central line-associated bloodstream infections, urinary tract infections linked to catheter use, surgical site infections, and ventilator-associated pneumonia are among the illnesses that are commonly encountered (Khan et al., 2017).

The problem of HAIs is critical, particularly in intensive care units (ICUs) that house high-risk patients with immunocompromised conditions, due to the rise of highly pathogenic and high-risk bacterial strains like "ESKAPE" pathogens: *Enterococcus faecium* (*E. faecium*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter*

baumannii (*A. baumannii*), *P. aeruginosa*, and *Enterobacter* species) (Abban et al., 2023; Elbehiry et al., 2025). In LMICs, up to 25% of those admitted to hospitals may be at risk for HAIs (Bardossy et al., 2016). West Africa, Southern Africa, East Africa, and Central Africa were shown to have a combined current prevalence of HAI of 15.5%, 6.5%, 19.7%, and 10.3% of patient populations, respectively (Melariri et al., 2024), showing the problem is relatively higher in East Africa. In Ethiopia HAI was reported as 11.9% (Atalay et al., 2024). Therefore, in order to contain antimicrobial resistance and prevent HAIs, intervention-based advancements in IPC at the national and facility levels are essential (WHO, 2022).

2.2.2. Hospital Environmental Reservoirs of AMR

One of the main sources of AMR contaminants in the environment is WW from medical institutions. It raises the dissemination of ARGs in microorganisms and increases AMR in the environmental microorganisms (Murray et al., 2021). It carries 10 times more ARGs than WW from public sources. It contaminates municipal WW. The contaminated urban WW can accumulate and spread AMR across communities and in the environment as a result of inappropriate waste management, and lack of regulation policy implementation on WWTP systems (Hassoun et al., 2020).

In Ethiopia, there are reports of higher incidence (90.89%) of the total bacterial pathogens in hospital WW, indicating severe environmental impact (Tilahun et al., 2025). Additionally, different hotspot areas had different bacterial resistance profiles, but medical WW contains the highest concentration of resistant bacteria (Geta and Kibret, 2022).

The presence of ARBs in hospital WW demonstrated that they propagated in WW and disseminated into the environment (Endalamaw et al., 2024). The possibility of distribution of AMR from hospital WW can be reduced by proper WW management, infection control, and antimicrobial stewardship (Tilahun et al., 2025).

Furthermore, MDR bacteria are more common in hospital WW due to poor cleaning and hygiene standards, overcrowding in healthcare facilities, and increased patient and worker transmission (Gashaw, et al., 2024).

Hospital IS are highly touched areas which are thought to be responsible for over 25% of hospital acquired infection (HAI) cases, increasing the potential for spreading nosocomial diseases within hospital settings and increasing mortality (Cobrado et al., 2017).

In Bahir Dar, Ethiopia, the overall incidence of bacterial contamination of inanimate hospital IS was 60.1% (Ayalew et al., 2019). Additionally, a study indicated that the rates of

contamination on IS of neonatal intensive care units with MDR and carbapenemase-producing (CP) critical GNB were 80.8% and 70.6%, respectively (Kindu et al., 2023). Furthermore, in Harar, Ethiopia, the study revealed that the prevalence of bacteria in hospital IS was 94.2% (Bodena et al., 2019).

2.2.3. Healthcare Waste Management and Infection Control

The United Nations Environment Programme stated that hospital waste constitutes one of the most dangerous and the most critical environmental challenges for the global population. This is due to the fact that 1/3 of healthcare facilities worldwide do not follow proper biomedical waste management procedures (Egbenyah et al., 2021; UNEP, 2022).

Contrary to the fact that proper management of healthcare waste is essential to achieving sustainable sanitation management for all, as envisioned in sustainable development goal 6 by 2030, it needs much work in the Ethiopian context (Tadesse and Dolamo, 2022). An evaluation of healthcare waste management practices in private clinics in Addis Ababa, Ethiopia, revealed that 61.2% of the clinics had poor practices. Of these, 56.8% had poor waste segregation practices, 55.0% had poor waste collection practices, 85.6% had poor waste transportation practices, 63.3% had poor waste storage practices, 61.9% had poor waste treatment, and 57.9% had poor disposal systems. This showed that Addis Ababa's private clinics may not have complied with WHO guidelines for the handling of medical waste (Wassie et al., 2022). On the other hand, according to a study conducted at government hospitals, the average percentage of infectious hospital waste produced in Addis Ababa was 33%. This is far higher than the 15-20% worldwide criteria established by the World Bank (Abebe, 2017; World Bank, 2017). Adequate infectious waste management was significantly hampered by inadequate storage spaces, trash containers, and waste segregation facilities.

The risk of exposure and contamination is increased when there are inadequate facilities to separate infectious wastes from ordinary waste (Berhe et al., 2025). It was suggested to take into account the connection between AMR, sanitary and nosocomial diseases (Tadesse and Dolamo, 2022).

2.3. Molecular Mechanisms of Antimicrobial Resistance

As the primary source of diseases brought on by ARB in clinical settings, it is useful to talk about how AMR arises in a bacterial population in order to comprehend the issue of AMR. A community of bacteria that was initially sensitive to antimicrobials can develop acquired resistance through horizontal gene transfer (HGT) or genetic changes that result in the

acquisition of resistance genes. Additionally, due to bacterial multiplication (vertical evolution), resistant subpopulations take over when the mutation appears and transmit the gene on to their offspring (Holmes et al., 2016).

Among the three mechanisms for the spread of genetic materials, such as conjugation, transduction, and transformation, conjugation is the most effective and frequent method of genetic information exchange. Transposons or integrons connected to mobile genetic elements (MGE) can also acquire resistance genes. Transposons are customized DNA segments that are unable to replicate on their own yet carry many resistance genes. Their ability to travel throughout the genome makes it unchallenging for the ARGs to migrate from the chromosome to the plasmid (Holmes et al., 2016; Tesema, 2025). The most common mechanisms through which bacteria might develop antimicrobial resistance are: (1) limiting the antimicrobial's access by altering the entry pathways or reducing their quantity; (2) activating efflux pumps in their cell walls to eliminate the antimicrobials that enter the cell; (3) modifying or eliminating the antimicrobial through the use of enzymes; and (4) changing the antimicrobial's targets by altering intracellular enzymes so that they are unable to latch onto it (Tesema and Birhanu, 2024).

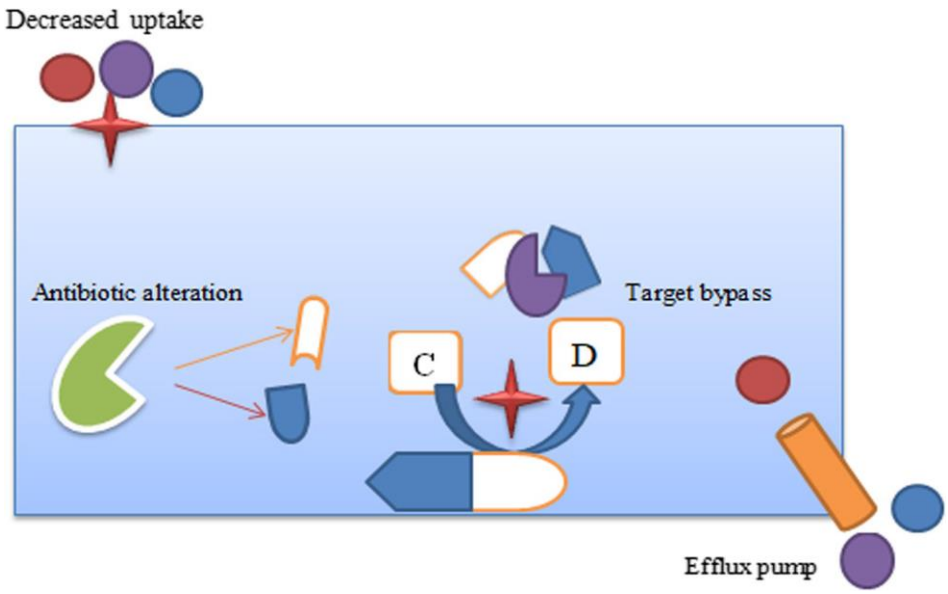

Figure 1. Molecular mechanism of antimicrobial resistance.

The diagram shows that enzymatic inactivation of antimicrobials (alteration), alteration of a drug target (target bypass), decreased outer membrane permeability (decreased uptake), and active drug efflux (efflux pump) are the four basic mechanisms of AMR. The figure adopted from Tesema and Birhanu (2024).

2.3.1. Broad-Spectrum β-Lactams and Extended-Spectrum β-Lactamases

Extended-spectrum β-lactamases (ESBL) are enzymes that hydrolyze penicillins, third-generation cephalosporins, and monobactams. AmpC β-lactamases can hydrolyze most penicillin, a wide array of cephalosporins, and most β-lactam combinations. By hydrolyzing second-, and third-generation cephalosporins and monobactams, ESBLs provide resistance to penicillins. Because ESBLs are encoded on transferable conjugative plasmids, they can spread widely not just among bacteria of the same species, but also among species that are quite distinct from one another. Additionally, the plasmid-mediated ARGs to fluoroquinolones and aminoglycosides were also reported (Diriba et al., 2020). Among the multiple ESBLs reported in a range of diseases, CTXM, TEM, and SHV types proved to be the most versatile and spread across distinct epidemiological environments (Ur et al., 2018). Despite extensive evidence on ESBL-producing clinical isolates, data on their prevalence in hospital environmental reservoirs remain limited in Ethiopia.

2.3.2. Emergence of Carbapenem Resistance and Carbapenemases

Carbapenemases can hydrolyze carbapenems and a wide variety of other β-lactams. As a last resort, carbapenems have been employed to combat anaerobic, Gram-positive, and Gram-negative bacteria (Başaran and Öksüz, 2025). In hospital-acquired infections, carbapenems such as imipenem, meropenem, doripenem, and biapenem have been utilized extensively.

Usually, these carbapenems are only used in patients infected with MDR bacteria, such as those that produce AmpC or ESBL (Smith et al., 2025).

A serious health risk is HAIs brought on by carbapenemase-producing bacteria. In carbapenemase-producing bacterial colonized patient surroundings, carbapenemase-encoding genes were commonly found, particularly on floors (97% of detection frequency), door frames (52%), and no frequently touched surfaces (42%). Additionally, these genes were found in corridors next to carbapenemase-producing colonized patient rooms (92%), control rooms (100%), and non carbapenemase-producing bacteria colonized patient rooms (78%). The frequency of these genes decreased as the distance from carbapenemase-producing bacteria colonized rooms increased (Richer et al., 2025).

The development of carbapenemase genes, primarily KPC, NDM, VIM, IMP, and OXA-48-like, is the most prevalent mode of carbapenem resistance globally. These genes are those that code for carbapenemase in β-lactamase (bla) (Smith et al., 2025).

Since the majority of them are plasmid-encoded, HGT can readily spread these carbapenem resistance genes. Patients who do not utilize the carbapenem but contact environments and hosts colonized with CP bacteria can develop host colonization and infection cases due to the quick spread (Caliskan and Alocilja, 2023).

The threatening part of carbapenem-resistant enterobacterales (CRE) is that many CRE-colonized individuals spread the bacteria without being infected by them. Additional threatening is that the transfer of genetic elements can occur in the food chain and the environment (CDC, 2019). Therefore, a major hazard to public health is the advent of CP bacteria that produce NDM with a growing number of variations (Caliskan and Alocilja, 2023).

2.4. Methodological Approaches for AMR Detection

2.4.1. Phenotypic and Culture-Based AMR Detection

To direct endeavours on AMR investigation and surveillance, the WHO global action plan for AMR was created (WHO, 2021): (i) rapid detection using established methods for high-income countries and LMICs, (ii) measurement of differences in occurrence and prevalence between high-income countries and LMICs, and (iii) the capacity to compare food and medical samples in addition to environmental monitoring.

To evaluate the antimicrobial resistance profiles of target bacteria, clinical and public health laboratories employ antimicrobial susceptibility testing (AST) frequently. Disk diffusion, E-tests, and broth and agar dilution tests are the common culture-based AST techniques. The food and drug administration (FDA) approved these tests, which entail isolating pure cultures of the possible pathogens (Khan et al., 2019). The gold standard for AST is specifically the Kirby Bauer disk diffusion method, in which bacteria are seeded on Mueller Hinton agar (MHA) plates using an antimicrobial disk and the resistance profile is then determined by measuring the zone of inhibition (Al-Zahrani, 2018). Breakpoints and the majority of commonly used standard interpretations of AST are suggested by the european committee for antimicrobial susceptibility testing (EUCAST) and the clinical and laboratory standards institute (CLSI) in order to ascertain the susceptible, intermediate, and resistant profiles of tested bacteria (Cusack et al., 2019).

One significant disadvantage of culture-based methods is that uncultured non-pathogenic environmental bacteria may serve as significant repositories of resistance, making identification more difficult (Pruden et al., 2018).

2.4.2. Genotypic and Molecular-Based AMR Detection

Bacteria can have innate, acquired, or adaptive AMR (Lee, 2019). Due to innate resistance, the impermeability of the outer membrane found in the Gram-negative bacterial cell envelope causes the bacteria to demonstrate glycopeptide resistance (Christaki et al., 2020). When a previously susceptible bacteria develops a resistance mechanism through a mutation or the acquisition of new genetic material from an outside source, this is known as acquired resistance (Holmes et al., 2016). The resistance to one or more antimicrobials brought on by a particular environmental cue (such as stress, growth status, pH, ion concentrations, nutritional circumstances, or sub-inhibitory antimicrobial levels) is known as adaptive resistance. Adaptive resistance is temporary, as opposed to intrinsic and acquired resistance.

Once the inciting signal is eliminated, adaptive resistance, which enables bacteria to react to antimicrobial challenges more quickly, usually returns to its initial state (Lee, 2019). Molecular AMR detection approaches are efficient ways to quickly identify ARGs from bacterial culture and environmental samples (Tesema, 2025).

Molecular epidemiology of colistin resistance, carbapenemases, β-lactamases, and other resistance genes in Ethiopia showed a worrisome landscape of fast changing AMR, seriously impairing the efficacy of last-resort antimicrobials (Legese et al., 2022). PCR-based techniques, which use particular primers that anneal to single-stranded DNA after denaturing the target DNA at a high temperature, are among the most effective and popular rapid molecular tools for quantifying and profiling genes containing resistance at the species and genus levels (Hussain et al., 2025). Multiplex PCR (mPCR), one of PCR advancements, provides a more robust, quick and multiple ARGs detection in a single PCR reaction tube (Reynoso et al., 2021). Key benefits of molecular approaches, including increased sensitivity and specificity, quickly increase their application in the world (Tesema, 2025).

In order to fully understand the ARGs dissemination, it is better to detect chromosomal and plasmid-mediated ARGs. The term chromosomal-mediated AMR describes resistance mechanisms, such as mutation and intrinsic resistance, that are encoded by genes found on the bacterial chromosome. The fact that chromosomal AMR is vertically inherited is an important feature. During cell division, daughter cells inherit it (Belay et al., 2024). The detection of specific ARGs within the genomic DNA, coupled with their absence in the plasmid DNA might suggest chromosomal-mediated ARGs. This indicates that the resistance determinant has been stably integrated into the host genome, potentially leading to the long-

term persistence of these resistant lineages within the hospital environment even in the absence of direct antimicrobial selection pressure (Toribio et al., 2024).

Plasmid-mediated ARGs dissemination can be carried out via transformation, transduction, or conjugation (Arnold et al., 2022). The mobile genetic elements (MGEs) facilitate the accumulation and spread of ARGs in both Gram positive and Gram negative bacteria by sharing genetic elements of resistance with other non-resistant bacterial species through horizontal gene transfer (HGT). The MGEs facilitate intracellular DNA mobility (from the chromosome to a plasmid or between plasmids) and intercellular DNA mobility (Zheng et al., 2021). The availability of free ARGs, which commensal environmental bacteria can acquire, is assessed using plasmid DNA detection (Pruden et al., 2018).

3. MATERIALS AND METHODS

3.1. Description of Study Area

This study was conducted in Addis Ababa, the capital of Ethiopia and the diplomatic hub of Africa, which hosts the African Union headquarters and numerous international organizations. The Tikur Anbessa Specialized Hospital (TASH) and Zewditu Memorial Hospital (ZMH) were selected due to their large number of patients and wide range of medical services. TASH is the country's largest teaching and referral hospital, opened in 1972 and incorporated into Addis Ababa University in 1998. It serves with over 700 beds and offers more than 26 different medical services. A hospital having more than 19 different medical services, ZMH was established in 1933. It is one of the biggest hospitals in central Addis Ababa and provides all round health care services (Kebede et al., 2022) (Figure 3).

3.2. Study Design

This study employed a facility-based cross-sectional design to assess antimicrobial resistance in hospital environments between October 2023 and October 2025 in Addis Ababa, Ethiopia. The research focused on the investigation of bacterial prevalence, AMR profiling and ARGs detection within WW and IS samples collected from TASH and ZMH (Figure 2).

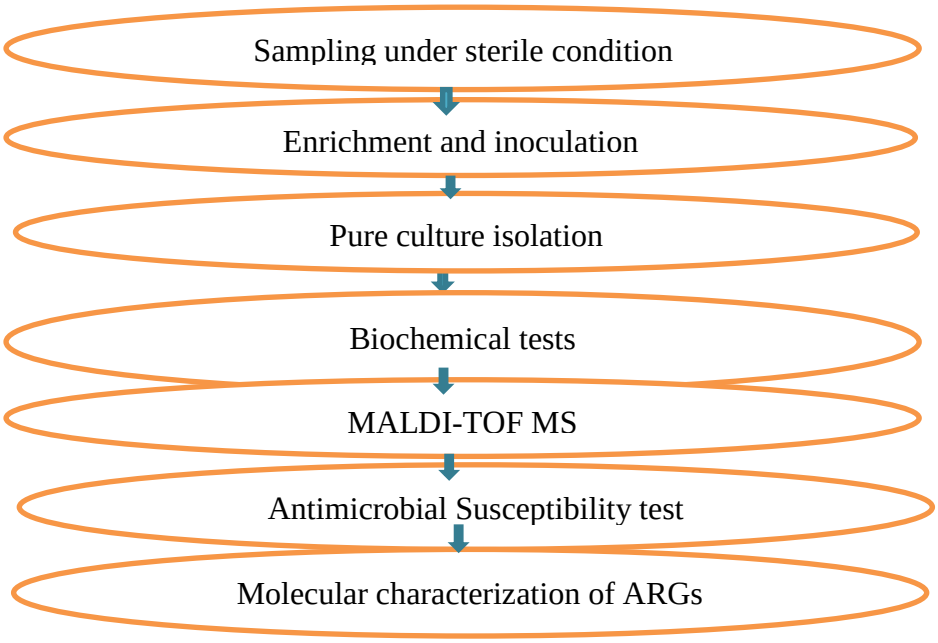

Figure 2. Schematic workflow of the experimental design and bacterial identification process.

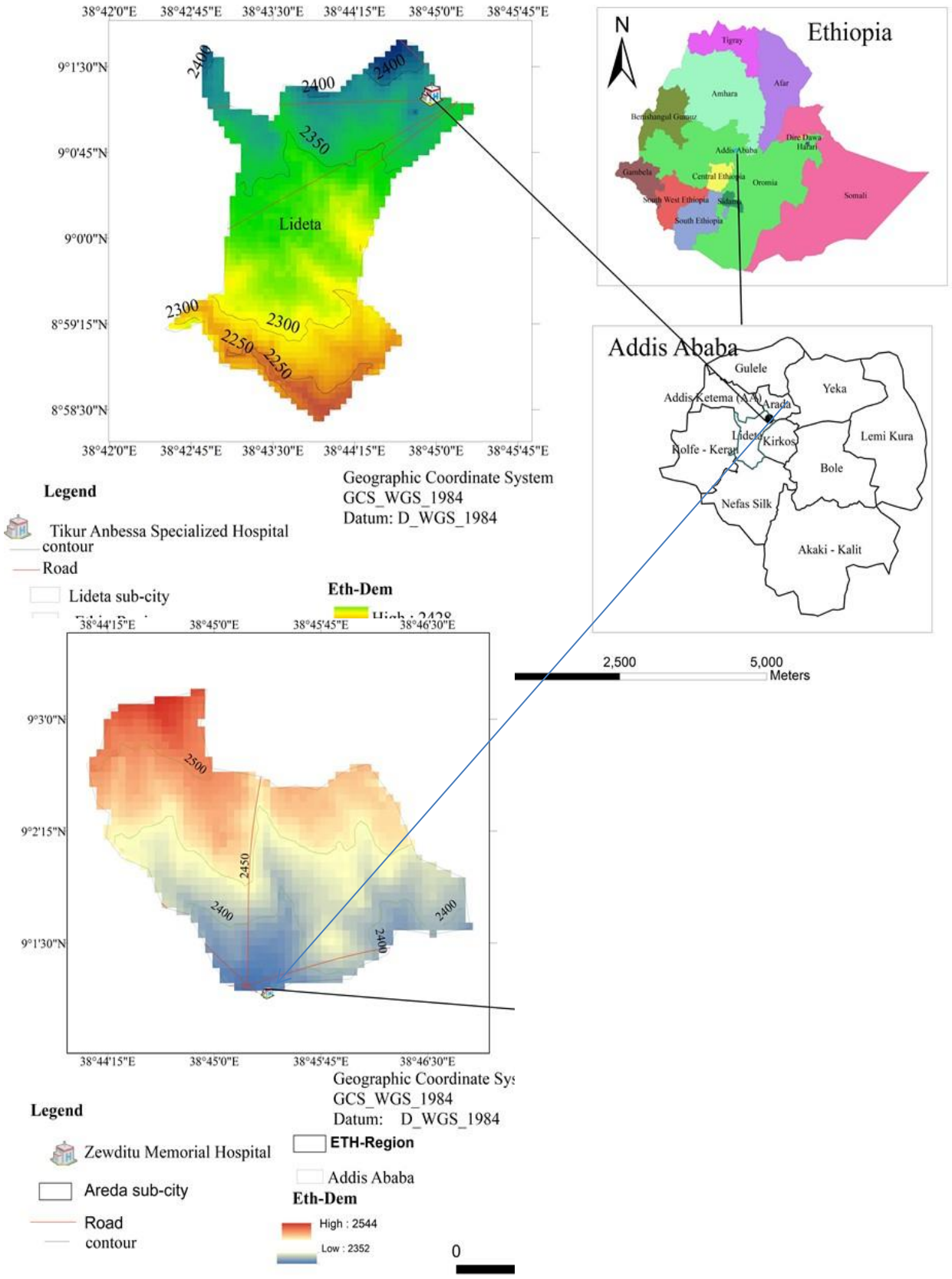

Figure 3. Map of the study area.
The map was designed by ArcGis 10.8 version.

IS and WW sources at the Tikur Anbessa Specialized Hospital, the University hospital (under Addis Ababa University) and which is managed under the Federal Ministry of Health (FMOH) and ZMH, which is managed by Addis Ababa City Health Bureau (AACHB), were chosen for sample collection (Figure 4).

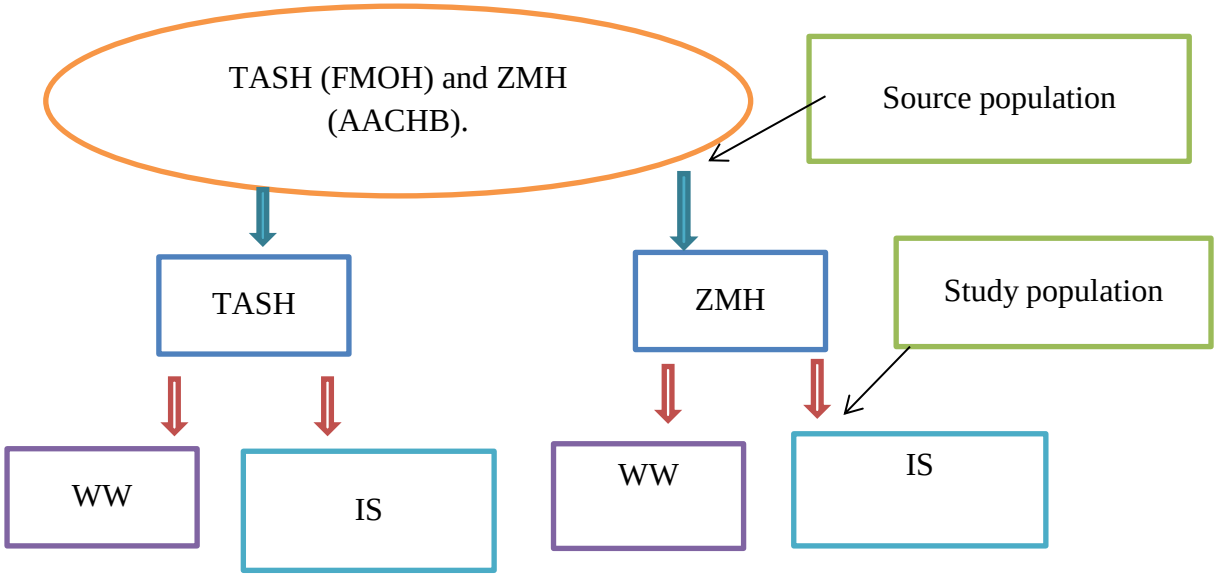

Figure 4. Schematic representation of the study workflow.

The figure illustrating the target population and specific sample matrices.

Key: AACHB, Addis Ababa City Health Bureau; FMOH, Federal Ministry of Health; IS, inanimate surface; TASH, Tikur Anbessa Specialized Hospital; WW, wastewater; ZMH, Zewditu Memorial Hospital

3.3. Sampling and Sample Type

WW samples were collected using the grab sampling approach following the environment protection authority (EPA) and American Public Health Association (APHA) instructions for WW sampling procedures (Bill et al., 2017). Grab samples were from a single moment in time; they were highly influenced by daily fluctuations in WW flow and composition. IS sampling was employed according to the guideline of Chansareewittaya (2021). Samples were collected using purposive sampling from high-risk hospital areas, including ICUs, operating rooms, and WW discharge points. The pre-developed data collection format was used to enter the sample ID (source name, sampling unit).

A total of thirty eight (38) samples (due to logistical constraints or exploratory design) were collected from both TASH and ZMH. Hospital WW samples were collected from TASH and

ZMH specified sampling locations in the WW sewage system. To obtain a flow representative sample, the actual samples were obtained by grab sampling collected in the morning hours at 8:00-11:00 am. Five untreated WW samples were collected from different outlets within the different sections of the TASH included the intensive care unit, the surgery or operation room, pediatric ward, hospital laboratory, delivery room, and from the TASH composite. Four untreated WW samples were also collected from ZMH. The sample collection sites were medical wards and pediatric ward, delivery room, operation and intensive care unit and ZMH composite.

At each site, a WW sample was collected in a 200 mL sterile bottle. To avoid personal contamination, a fresh set of powder free disposable gloves, appropriate clothing, safety goggles and heavy duty gloves were also used during cleaning and removal of the manhole covers in each sample site. After collection, samples were transported and stored in a light proof cooler with ice packs and shipped within 2 hours for analysis to the health biotechnology laboratory of the biotechnology research centre, Addis Ababa University.

IS samples were collected from various objects of TASH and ZMH departments. To obtain a representative sample, the samples were obtained right after sterilization of the specific surfaces in the morning hours at 8:00-11:00 am and afternoon 2:00-5:00 pm. Seventeen (17) IS swab samples were collected from TASH. The samples were collected from selected wards included the neonates intensive care unit room 1, neonates intensive care unit room 2, neonates intensive care unit room 3, operation room 1, operation room 2, operation room 3, operation room 4, general intensive care unit room 1, general intensive care unit room 2, general intensive care unit room 3, general intensive care unit room 4, pediatrics room 1, pediatrics room 2, delivery room 1, delivery room 2, main lab room 1, and main lab room 2.

Similarly, twelve (12) surface swab samples were collected from ZMH included the intensive care unit room 1, intensive care unit room 2, intensive care unit room 3, neonates intensive care unit room 1, neonates intensive care unit room 2, delivery room 1, operation room 1, operation room 2, pediatrics unit room 1, pediatrics unit room 2, medical ward room 1, and medical ward room 2.

Samples from IS (bed railing, bed sheet, cardiac monitor knobs, surgical tables, ventilator knobs, door knob, nursing table, patient side table, inpatient floors, and chairs) were collected and processed for isolation of bacteria. Sterile cotton swabs pre-moistened in a sterile normal saline solution (0.85% NaCl) were used for sampling surfaces.

At each site, the area was swabbed in two directions at right angles to each other in a close zigzag pattern, rotating the swab to ensure that the entire surface of the swab was used according to the guideline of Chansareewittaya (2021). After sample collection, the swabs were placed in a pre-labeled, sterile 15 mL screw-top falcon tube sealed with a cap, and the swabs were taken to the laboratory for analysis within 2 hours of collection (Dawodu and Akanbi, 2021).

3.4. Sample Processing and Isolation of Bacteria

One milliliter sample from WW was thoroughly mixed in 9 mL of buffered peptone water to distribute the bacteria uniformly, and vortexed to homogenize the samples and settle the large debris. The serial ten-fold dilutions were prepared up 10^{-7} by sterile physiological saline (0.85% NaCl) from homogenized sample in a sterilized test tube. Blood agar base (HiMedia, India) was prepared using distilled water according to the manufacturer's instructions. The medium was heated with frequent agitation and boiled to be melted completely and sterilized by autoclaving at 121°C for 15 min. Then, the agar medium was cooled to 40-50°C and 5% sheep blood was added and mixed gently. The 5% sheep blood agar media poured into sterile glass petri dish on a flat surface to a uniform depth of 4 mm, and allowed to solidify. The plates were dried until excess surface moisture evaporated to make the media moist but free of water droplets on the surface. The agar capability and quality to provide the conducive environment for the cultivation of bacteria was checked by streaking control bacterial strains on the agar medium.

Then, 1 mL aliquots of the relevant dilutions (10^{-2}-10^{-6}) were inoculated on 5% sheep blood agar plates and incubated at 37°C for 24/48 hours. Then, plates with 25-250 pure colonies were selected for subsequent procedures. The colonies were prepared from each unique colony and subcultured on to 5% sheep blood agar for further processing. The colonies were isolated on the basis of their hemolysis, shape, pigment, color, size and other parameters (Dawodu and Akanbi, 2021).

To maintain and store the isolated pure colonies, tryptic soy broth (TSB) medium (HiMedia, India) was prepared; the isolated pure colony was inoculated into 5 mL of TSB (HiMedia, India) and incubated at 37°C for 24 hours. The TSB without test organism was included as negative control. After the end of the incubation period, the well grown test organisms were preserved with 25% glycerol and stored at -20 °C (Obayiuwana et al., 2018).

3.5. Bacterial Identification Methods

The bacteria were identified on the basis of their colony morphology, growth on their respective selective media, and other bacteriological tests. The bacterial isolates identification was performed based on Bergey's manual determinative bacteriology and online advanced bacterial identification software (ABIS online: https://www.tgw1916.net/) (Robert et al., 1957; Sorescu and Stoica, 2021). Briefly, based on their morphological distinction such as size, pigment, color, shape and etc, pure colonies were isolated. The systematic identification workflow was performed as presented elsewhere (Figure 5 and Figure 6).

Gram staining and 3% KOH

After pure culture was obtained, bacterial isolates were classified as Gram positive and Gram negative using 3% KOH and Gram staining procedures. To test Gram reaction, the pure isolates were taken and put on to sterilized glass slides. After making a smear, the slides were left in a safe place to air dry the smear. Then, samples were heat fixed onto a clean, and grease free slide. A crystal violet solution was added in drops to the sample and then allowed to stand for 1 minute. Then, the stain was washed under running tap water. Two drops Iodine solution (Gram's iodine) was added and allowed to stand for 1 minute, and then washed with tap water. The sample was rinsed with 2 mL of ethanol for 30 seconds and then gently washed in running water. Two milliliter of safranin was added to the slide, allowed to stand for 1 minute, and washed. The slide was allowed to air dry again and mounted on the microscope stage to be viewed under high power microscope objective lens. The dark purple color was recorded as GPB and pale to dark red was recorded as GNB (Dawodu and Akanbi, 2021).

Gram negative bacteria were inoculated on MacConkey (MAC) agar and Eosin methylene blue (EMB) agar, a selective and differential media, to test for lactose fermentation. The cultures on MAC agar differentiated as lactose fermenter (pink/red) and lactose non fermenter (colorless), and the culture on EMB agar differentiated as lactose fermenter (metallic green sheen, pink, purple, or mucoid colonies) and lactose non-fermenter (colorless).

The oxidative and fermentative properties of the isolates were assessed using the oxidation and fermentation (OF) test. Following this, oxidase and catalase tests were performed to identify the genera and species of the isolates. The fermentative bacteria (Enterobacterales) were subcultured onto EMB to observe the dark blue-black colonies with a green metallic

sheen characteristic of *E. coli* and brown, dark-centered and mucoid colonies for *K. pneumoniae.* Among these, *P. aeruginosa* from the oxidative group was subcultured onto Pseudomonas selective agar to observe blue to greenish colonies.

Gram positive bacteria were subcultured on the same blood agar plate and further identified via biochemical tests, such as oxidation and fermentation (OF), oxidase, catalase, coagulase and salt tolerance test. Catalase producer GPB cocci were subcultured onto mannitol salt agar (MSA) (Accumix, Spein) and incubated at 37°C for 24/48 hours and differentiated as mannitol fermenter (yellow) and nonfermenter (pink/red) followed by the coagulase test to differentiate between coagulase-positive and coagulase-negative *Staphylococcus* species. Catalase-negative GPB cocci were grown on the same blood agar and identified using hemolytic patterns (i.e., alpha, beta, and gamma hemolysis), followed by biochemical tests for the identification of *Streptococci* and *Enterococci* spp.

3.5.1. Phenotypic and Biochemical Based Identification

Biochemical tests were performed according to procedures of Dawodu and Akanbi (2021) with some modifications. The biochemical tests included catalase, oxidase, oxidation-fermentation, coagulase, indole, sulfur production, motility, triple sugar iron agar, methyl red (MR) test, citrate, urease, Voges Proskauer (VP) test, sugar fermentation tests (Figure 5 and Figure 6).

Catalase test

This test was carried out to demonstrate the presence of catalase, an enzyme characterized with the release of oxygen from hydrogen peroxide. A drop of 3% hydrogen peroxide solution was added to the sterile slide containing a loop full of the isolate, and when active bubbling /foaming formed immediately, it was recorded as catalase positive result. The positive control, *S. aureus* (ATCC 25923), and negative control, *E. coli* (ATCC 25922) were used (Hafezi and Khamar, 2024).

Oxidase test

The oxidase reagent strips were moistened with a drop of sterile water. The colonies of the test organisms were taken using sterilized stick or glass rod (not an oxidized wire loop) and rubbed on the strip. The *P. aeruginosa* (ATCC 27853) was used as positive control and *E. coli* (ATCC 25922) used as negative control. The formation of red purple color within 20 seconds was recorded as positive (Dawodu and Akanbi, 2021).

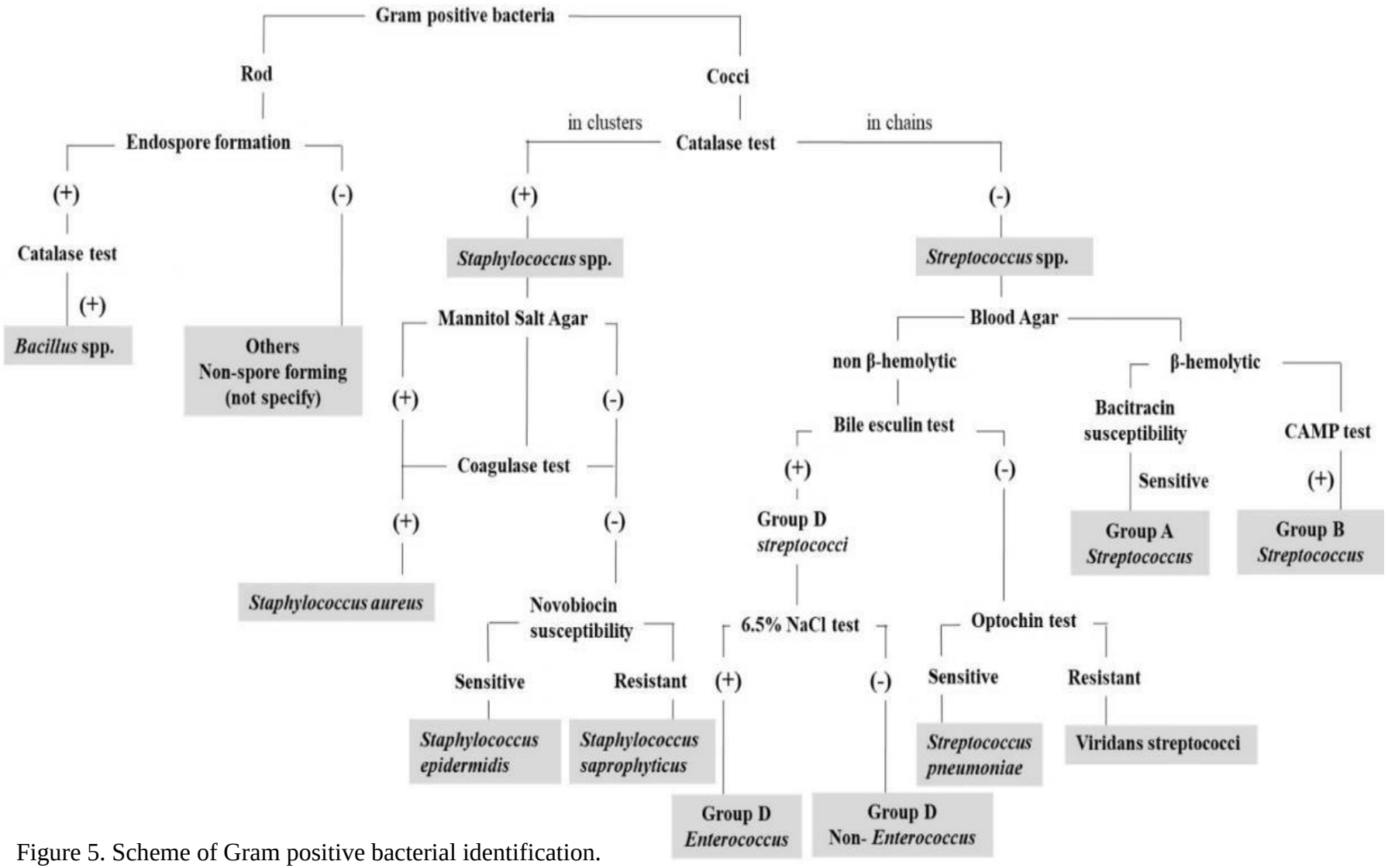

Figure 5. Scheme of Gram positive bacterial identification.
Adopted from Chansareewittaya (2021).

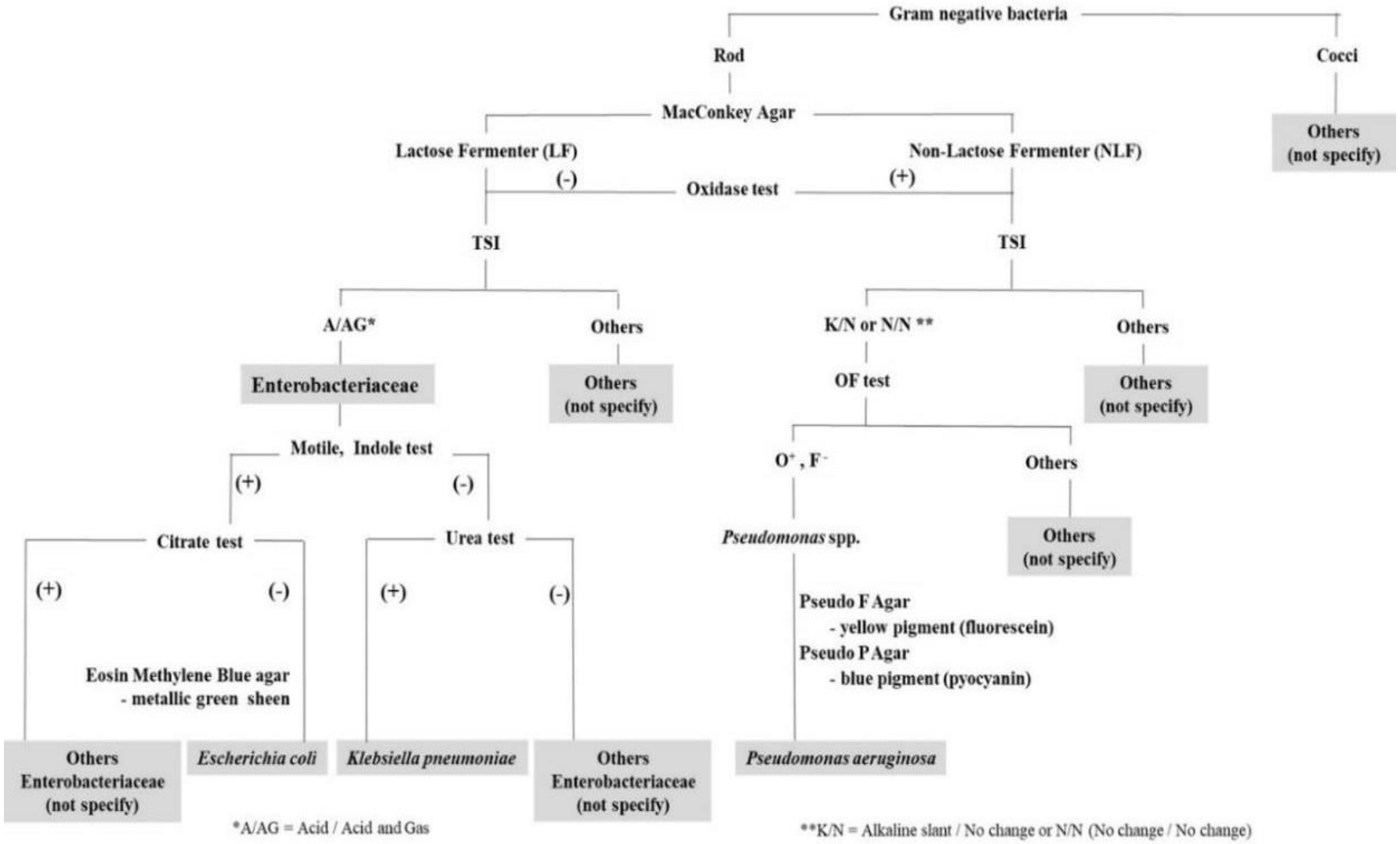

Figure 6. Scheme of Gram negative bacterial identification.
Adopted from Chansareewittaya (2021).

Oxidation fermentation (OF)

The pure isolated colonies from an 18-24 hours fresh culture were prepared. The OF basal medium was allowed to equilibrate to room temperature. The agar was inoculated with test organisms in duplicate by stabbing to nearly 1/4 inch from the bottom. The sterile paraffin was applied to one of the tube. The cap of the overlaid tube was tightened and the caps of the non-overlaid tube loosen. Then, tubes were incubated aerobically at 37ºC for 24/48 hours. A control tube of the OF basal medium should be inoculated and incubated in parallel with the OF tests. A positive carbohydrate utilization test was indicated by the development of a yellow color in the medium and a negative carbohydrate utilization test was indicated by the absence of a yellow color (media remains green or turns blue) (Zhang et al., 2022). The *P. aeruginosa* (ATCC 27853) was used as oxidative control and *E. coli* (ATCC 25922) used as fermentative control (Hafezi and Khamar, 2024).

Voges Proskauer test

To test bacteria that produce butanediol from glucose fermentation, 2.5 mL of culture was transferred into a new sterile culture tube, and 0.6 mL (or 12 drops) of Barritt's reagent A was added. Then, 0.2 mL (or 4 drops) of Barritt's reagent B was added. The tube was carefully shaken for 30 seconds to 1 minute to expose the medium to atmospheric oxygen. The tube was allowed to stand for 30 minutes. Voges Proskauer (VP) positive tubes exhibited red color on at the top; whereas, negative tests showed yellow. *K. pneumoniae* (ATCCC 700603), as positive and *E. coli* (ATCC 25922), as negative control were included (Nagaraja et al., 2025).

Indole Test

The indole test was conducted in inoculated sterilized screw capped test tube (5 mL) containing tryptophan broth (Himedia, India) incubated for 24 hours. After incubation, 0.5 mL of Kovac's reagent was added. The positive test was recorded by the presence of red ring within 10 minutes in the surface layer, and test tubes in which there was no formation of red ring recorded as negative. *E. coli* (ATCC 25922), as positive and *K. pneumoniae* (ATCCC 700603), as negative control were included (Hafezi and Khamar, 2024).

Citrate utilization test

To test citrate utilization by bacteria, Simmon's citrate agar medium (Himedia, India) was prepared in screw capped test tube and allowed to set in a slanting position. A sterile wire loop was used to inoculate the test organism on to the slant medium and incubated at 37˚C for

24/48 hours. The formation of bright blue color in the medium was considered as a positive citrate test. *K. pneumoniae* (ATCCC 700603), as positive and *E. coli* (ATCC 25922), as negative control were included (Dawodu and Akanbi, 2021).

Urease test

Urease test was conducted to identify bacteria that produce urease. A sterilized slant test tube with urea broth agar was inoculated with test organism and incubated at 37°C for at least 6 hours. For test bacteria producing urease enzyme, the color of the broth changed from light orange to magenta and for negative remained a yellowish color. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as positive and negative control, respectively (Zhang et al., 2022).

Triple Sugar Iron Test

Triple sugar iron test was used to determine the ability of the bacteria to ferment sugar and produce hydrogen sulfide. Straight inoculating needle was used to pick up an isolated colony and inoculate the TSI slant by first stabbing the butt down to the bottom then surface of the slant was streaked and was withdrawn. The inoculated tubes with test organisms were incubated at 37 °C for 24 hours (Mekengo et al., 2021). *E. coli* (ATCC 25922) as fermenter control and *P. aeruginosa* (ATCC 27853) non fermenter control were used. Three kinds of results may be obtained from the reactions (Dawodu and Akanbi, 2021).

Hemolytic Test

A hemolysis test was conducted using sterilized blood agar base mixed with 5% sheep blood, inoculated with test organism and incubated at 37^{o}C for 24/48 hours for hemolysis. Bacteria that can completely lyse the blood cells, causing a clearing around the colony, were considered as β hemolytic; bacteria that can partially break down the blood cells, causing a green or brown discoloration of the agar around the colony, were considered as α hemolytic and bacteria that cannot lyse the cells, causing no change in the agar, were considered as γ hemolytic. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as positive and negative control organisms, respectively (Zhang et al., 2022).

Methyl Red (MR) test

To perform the MR test, sterilized screw capped test tubes with MRVP broth (cooled at room temperature) were inoculated with test bacteria for 24/48 hours at 37°C. Two and a half millilitres of culture was transferred into a new sterile culture tube into which 5 drops of the

MR reagent was added. The MR-positive showed a red color as a result of high acid production and due to the decrease in the pH of the culture medium to 4.4. The MR negative test tubes showed yellow color indicating lower acidity in the medium. *E. coli* (ATCC 25922), as positive and *K. pneumoniae* (ATCCC 700603), as negative control were included (Zhang et al., 2022).

a) Sugar fermentations: Acid butt, alkaline slant (yellow butt, red slant, A/K): glucose has been fermented but not sucrose or lactose. Acid butt, acid slant (yellow butt, yellow slant, A/A): lactose and/or sucrose has been fermented. Alkaline butt, alkaline slant (red butt, red slant, K/K): glucose, lactose, and sucrose has not been fermented.
b) Gas production: Indicated by bubbles in the butt. With large amounts of gas, the agar may be broken or pushed upward.
c) Hydrogen sulfide production: was indicated by a blackening of the butt as a result of the reaction of H_2S with the ferrous ammonium sulfate to form black ferrous sulfide. The black precipitate indicates that the bacteria were able to produce hydrogen sulfide (H_2S) from sodium thiosulfate. The results would be recorded as acid over acid (A/A), H_2S positive.

3.5.2. Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Based Species Identification

MALDI-TOF MS EXS3000 assay (Zybio Inc., a Chinese company) was executed for the identification of bacterial species according to Cruz et al. (2024) with some modifications. Briefly, the pure isolates were cultivated on blood agar media. A thin smear of 24 hours old pure cultures were put on a target plate with a sterilized wooden toothpick in duplicate. An α-hydroxy-4-hydroxycinnamic acid (HCA), the primary reagent of matrix, involved ionization reaction with the sample and serves to create a co-crystal state to prevent the sample from being totally burned by the laser. Formic acid (70%; 0.5 µL) and acetonitrile (0.5 µL) were added and allowed to dry at room temperature. The matrix plate spots were overlaid with 1 µL of matrix solution (α-cyano-4-hydroxycynnamic acid (CHCA) in 50% acetonitrile and 2.5% trifluoro acetic acid) and let dry before the insertion of the plate into the instrument. For bacterial isolates, the CHCA matrix and the on plate extraction standard procedure were pretreated. Pulsed lasers (337 nm/355 nm) were used by the EXS3000 to supply energy for the ionization process. A single charge was assigned to the majority of the ions in the sample. The target plate was loaded into the EXS3000 MALDI-TOF MS instrument. The bacterial database was selected. The data was compared to a database to identify the bacterial species.

As directed by the manufacturer, the instrument was calibrated. The manufacturer claimed that a scores ≤ 1.69 were considered to be unreliable, scores 1.70–1.99 were considered to be low confidence identifications, and scores ≥ 2.00 were considered to be high confidence identifications. When MALDI-TOF MS identifications between duplicate tests matched exactly (identical genus and/or species), MALDI-TOF MS identification was considered final. A bacterial sample was subjected to a protein extraction method and then analyzed by mass spectrometry. Pattern matching analysis software of mass spectra allows identifying bacterial species and subspecies unambiguously. Then, ID and respective scores obtained were recorded (Figure 7).

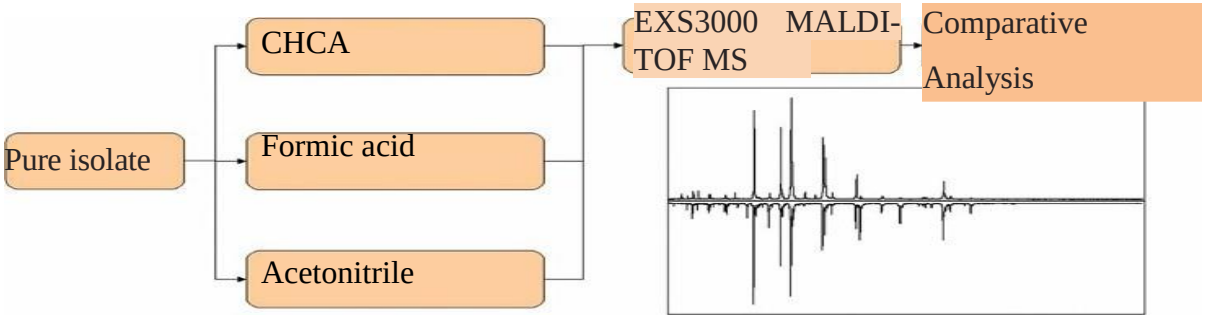

Figure 7. A general overview of MALDI-TOF MS procedure.

3.6. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was executed utilizing Mueller Hinton agar (MHA) by the Kirby Bauer disk diffusion method following standard procedures and interpreted as per the CLSI (2024) guidelines. Briefly, the 0.5 McFarland turbidity standards were prepared by mixing a 1% $BaCl_2$ and 1% H_2SO_4 as follows: A 99 mL of distilled water was added to 1 mL of concentrated H_2SO_4 in a beaker and mixed well. Then, a 1% (v/v) solution of H_2SO_4 was prepared. A 0.5 g of dihydrate barium chloride salt ($BaCl_2.2H_2O$) was dissolved in 50 mL of distilled water. Then, 1% (w/v) of $BaCl_2$ was prepared, and then both solutions (the 1% $BaCl_2$ and the 1% H_2SO_4) were combined and completely mixed to form a turbid suspension. A 0.6 mL of $BaCl_2$ solution was added to 99.4 mL of H_2SO_4 solution to make up to 100 mL and mixed well. The prepared stock solution of 0.5 McFarland turbidity standards was transferred into about 2-3 mL screw capped tubes and stored at room temperature in a well-sealed container in the dark until use. MHA was prepared using distilled water according to the manufacturer's instructions. The medium was heated with

frequent agitation and boiled to be dissolved completely and sterilized by autoclaving at 121 °C for 15 min. The agar medium was cooled to 40-50 °C and poured into sterile glass petri dish on a flat surface to a uniform depth of 4 mm. Prior to use, plates were dried until surface moisture completely evaporated. Media must be moist but free of water droplets on the surface. Before use, the ability of the agar to support the growth was checked by streaking control bacterial strains cultures on the agar medium. To check whether the media was supportive for the growth or not, each batch of representative member of the species was inoculated and tested.

From less than 24 hours fresh bacterial isolate, a few colonies were taken with a wire loop and added to 3 mL 0.85% saline containing test tubes. The turbidity of the test bacterial suspension with that of 0.5 MacFarland (vigorously shaken before use) were compared against a white background with contrasting black line under adequate light. The turbidity was increased by adding colonies and reduced by adding sterile saline. A sterile cotton swab was dipped into the standardized bacterial suspension. The excess inoculum was removed by lightly pressing the swab against the tube wall at a level above that of the liquid. The MHA was inoculated by streaking with the swab containing the inoculum and rotated by 360° until the inoculum evenly distributed. The surface of the medium was allowed to stand for less than 15 minutes to allow for absorption of excess moisture.

Antimicrobial disks were placed on the surface of the inoculated and dried plate using sterile forceps and immediately pressed down lightly to ensure complete contact between the disk and the agar surface. The disks were set at the distance of 24 mm center to center and 10-15 mm from the edge of the plate. Five antimicrobial disks were placed on a 90 mm plate and 10 antimicrobial disks on a 150 mm plate. One plate was inoculated with a control strains and incubated together with test strains. Each plate was incubated upside-down at 37 °C. We used a panel of twenty two (22) commonly prescribed antimicrobials that were classified into 8 antimicrobials classes and 12 subclasses against groups of bacteria proposed to indicate AMR level (Thermo Scientific™ Oxoid™, UK) (Table 1). These antimicrobials were chosen based on clinical efficacy, prevalence of resistance, minimizing emergence of resistance, FDA clinical indications for use, current consensus recommendations for first choice and alternative drugs and cost (Thapa et al., 2020). Seventy five (75) bacterial isolates were subjected to antimicrobial susceptibility testing. Gram positive bacteria were tested against gentamicin (GEN, 10 μg), cefoxitin (CTX, 30 μg), ampicillin (AMP, 10 μg), clindamycin (CD, 2 μg), vancomycin (VAN, 30 μg), nitrofurantoin (NIT, 30 μg), ciprofloxacin (CIP, 5

μg), sulfamethoxazoletrimethoprim (SXT, 1.25/23.75 μg), erythromycin (ERY, 15 μg), oxacillin (10 μg), norfloxacin (NOR, 10 μg), tetracycline (TE, 30 μg), doxycycline (DOX, 30 μg) and based on the availability of interpretive criteria for their inhibition zone diameters in CLSI (2024).

Gram negative bacteria were subjected to the following antimicrobials: ampicillin (AMP, 10 μg), piperacillin (PI, 100 μg), gentamicin (GEN, 10 μg), ceftazidime (CAZ, 30 μg), aztreonam (AT, 30 μg), meropenem (MRP, 10μg), ciprofloxacin (CIP, 5 μg), tetracycline (TE, 30 μg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg), nitrofurantoin (NIT, 30 μg), amoxicillin/clavulanic acid (AMC, 10 μg), cefuroxime (CXM, 30 μg), cefoxitin (CTX, 30 μg), amikacin (AK, 30 μg), tobramycin (TO, 10 μg), and norfloxacin (NO, 10 μg).

The zone of inhibition was observed after 16 to 18 hours considering slow growing organisms that might require longer incubation period. The zones of inhibition were measured using a caliper ruler and the result was interpreted and reported based on the CLSI (2024) guidelines as resistant (R), intermediate (I) or susceptible (S).

Table 1. Antimicrobial panels used for antimicrobial susceptibility testing (AST)

Class	**Sub class**	**Antimicrobial agents for AST**
β-lactams	2nd generation cephalosporins	cefuroxime (CXM, 30 μg) cefoxitin (CTX, 30 μg)
	3rd generation cephalosporins	cefotaxime (FOX, 30 μg) ceftazidime (CAZ, 30 μg)
	Carbapenems	meropenem (MRP, 10μg)
	Monobactam	aztreonam (AT, 30 μg)
	Penicillins	ampicillin (AMP, 10 μg) piperacillin (PI, 100 μg) oxacillin (OX, 10 μg) amoxicillin/clavulanic acid (AMC, 10 μg)
Aminoglycoside	Aminoglycosides	gentamicin (GEN, 10 μg) amikacin (AK, 30 μg) erythromycin (ERY, 10 μg) tobramycin (TO, 10 μg)
Sulfonamide	Sulfomethoxazole	trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg)
Quinolones-Fluoroquinolone	Quinolones-Fluoroquinolones	ciprofloxacin (CIP, 5 μg) norfloxacin (NO, 10 μg)
Tetracyclines	Tetracyclines	tetracycline (TE, 30 μg) doxycycline (DOX, 30 μg)
Macrolides	Macrolides	clindamycin (CD, 10 μg)
Glycopeptides	Glycopeptides	vancomycin (VAN, 10 μg)
Nitrofurans	Nitrofurans	nitrofurantoin (NIT, 30 μg)

3.6.1. Double Disk Synergy Test for ESBL Confirmation

Phenotypically, ESBL production was tested by the double disk synergy test (DDST) using ceftazidime and AMC. The synergy created a characteristic keyhole or champagne cork shape (CLSI, 2024; Naseer, 2017).

3.6.2. Multidrug Resistance Patterns and Multiple Antimicrobial Resistance Index (MARI)

The MARI of the bacterial species was derived as described by Bhaurao et al. (2022) following the mathematical equation: MAR index = y/x; where, 'y' indicates the total number of antimicrobials against which the bacterial species displayed resistance, while, 'x' indicates the total number of antimicrobials used against the bacterial species. When the MARI was higher in value than 0.2, it indicated that antimicrobials were being used intensively in that area and implies an environment with a high-risk of AMR's proliferation.

3.7. Molecular Characterization of Antimicrobial Resistance Genes

3.7.1. Extraction and Purity Assessment of Chromosomal and Plasmid DNA

DNA extraction were performed following standard protocols with some modification: the chromosomal DNA (Kiledal & A Maresca, 2023), plasmid DNA (Das & Dash, 2015) (Appendix 8) and PCR amplification (Appendix 9).

3.7.2. Quantitative and Qualitative Assessment of DNA

The quantity and quality of the DNA obtained was assessed spectrophotometrically at 260 and 280 nm, and the A260/A280 ratio was used to assess whether there was contamination with proteins or not. This spectrophotometric evaluation was performed in triplicate on the samples of extracted DNA, in a NanoDrop™ 2000/2000c Spectrophotometers (Thermo Fisher Scientific Inc, Massachusetts, USA) according to the manufacturer's guidelines. The NanoDrop lower pedestal was washed with 2 µL of ddH_2O and wiped with tissue paper and blanked with 1X TE buffer. The optical density (OD) was measured by clicking on the measure option on the software and the concentration and the OD260/OD280 ratio were recorded. Between each sample measurement, the pedestal was wiped with tissue paper and blanked. When reading was finished, the data generated were exported as excel format, then the pedestal was washed with ddH_2O and wiped with tissue paper, and the program was closed. The result was analyzed based on the criteria that ratios lower than 1.8 indicates the presence of proteins and/or other UV interacting agents and a ratio above 2.0 implies that the

samples may be contaminated with chloroform or phenol. A ratio 1.8-2.0 confirms that the absorption was due to the presence of the nucleic acids.

In order to verify the integrity of chromosomal and plasmid DNA, 3 µL DNA were subjected to gel electrophoresis on 1.8% (w/v) agarose gel, supported by loading dye, stained with ethidium bromide (0.5 µg mL^{-1}). The DNA migration was performed in 1X TAE running buffer at voltage of 120 V for 120 minutes. A 100 bp molecular weight standard (Solis BioDyne, Estonia) was used. The agarose gel was visualized under UV transilluminator of DW04S-3E gel documentation system. The visualization of single, very thick, and bright band at the very top suggests the chromosomal DNA, while the plasmid DNA appeared furthest down the gel, the fastest migrating band (Chongqing, China) (Weerakkody and Witharana, 2024).

3.7.3. Polymerase Chain Reaction (PCR) Amplification and Gel Electrophoresis of Antimicrobial Resistance Genes

To detect the presence of specific ARGs, both multiplex PCR (mPCR) and singleplex PCR (sPCR) were executed for all 92 bacterial isolates. For mPCR amplification was performed using group-specific primers and for sPCR target specific primers designed to the target resistance genes listed in Table 2 (Macrogen Europe, Nairobi). All mPCR and sPCR reactions were carried out in a Long Gene® Fast Gradient Thermal Cycler A300 (Hangzhou LongGene Scientific Instruments Co., Ltd., Zhejiang, China) according to protocols described (Awad et al., 2015; Bogaerts et al., 2013; Chen et al., 2007; Guarddon et al., 2014; Pérez-Pérez & Hanson, 2002; Poirel et al., 2012; Trung et al., 2015). The PCR protocols and conditions were detailed in Appendix 9 and Appendix 10. The multiplex technique was optimized in order to ensure simultaneous and accurate detection of multiple gene targets within a single reaction tube to increase diagnostic throughput.

Table 2. List of primers used for antimicrobial resistance gene (ARG) screening

Target gene	**Primer sequence (5′ to 3′)**	**Bp**	**References**
blaCTXM	F:TGTGCAGYACCAGTAARGTKATGGC R:GGTRAARTARGTSACCAGAAYCAGCGG	590	(Trung et al., 2015)
blaTEM	F:TCGCCGCATACACTATTCTCAGAATGAC R:CAGCAATAAACCAGCCAGCCGGAAG	422	
blaSHV	F: TGTATTATCTC (C/T)CTGTTAGCC (A/G)CCCTG R:GCTCTGCTTTGTTATTCGGGCCAAGC	739	

For degenerate primers: D = A, G or T; R = A or G; Y = C or T; K = G or T; W = A or T, S = G or C			
blaPER	F: AGTGTGGGG GCCTGACGAT R: GCAACCTGCGCAATRATAGCTT	725	(Bogaerts et al., 2013)
blaGES	F: CTGGCAGGGATCGCTCACTC R: TTCCGATCAGCCACCTCTCA	600	
blaVEB	F: CGACTTCCATTTCCCGATGC R: TGTTGGGGTTGCCCAATTTT	376	
blaMOXM	F: GCTGCTCAAGGAGCACAGGAT R: CACATTGACATAGGTGTGGTGC	520	(Pérez-Pérez and Hanson, 2002)
blaDHAM	F: AACTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC	405	
blaACCM	F: AACAGCCTCAGCAGCCGGTTA R: TTCGCCGCAATCATCCCTAGC	346	
blaCITM	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462	
blaEBCM	F: TCGGTAAAGCCGATGTTGCGG R: CTTCCACTGCGGCTGCCAGTT	302	
blaFOXM	F: AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCGGATTGG	190	
blaVIM	F: TGTCCGTGATGGTGATGAGT R: ATTCAGCCAGATCGGCATC	437	(Bogaerts et al., 2013)
blaNDM	F: ACTTGGCCTTGCTGTCCTT R: CATTAGCCGCTGCATTGAT	603	
blaIMP	F: ACAYGGYTTRGTDGTKCTTG R: GGTTTAAYAAARCAACCACC	387	
blaKPC	F: TCGCCGTCTAGTTCTGCTGTCTTG R: ACAGCTCCGCCACCGTCAT	353	
blaOXA-48	F: ATGCGTGTATTAGCCTTATCG R: CATCCTTAACCACGCCCAAATC	265	
blaOXA-58 group	F: GGGGCTTGTGCTGAGCATAGT R: CCACTTGCCCATCTGCCTTT	688	(Bogaerts et al., 2013)
blaOXA-23 group	F: CCCCGAGTCAGATTGTTCAAGG R: TACGTCGCGCAAGTTCCTGA	330	
blaOXA-24/143 group	F: GCAGAAAGAAGTAAARCGGGT R: CCAACCWGTCAACCAACCTA	271	
blaGIM	F: GTTCGGCCACCTCGAATTG R: TCGACACACCTTGGTCTGAA	477	(Poirel et al., 2012)
blaAIM	F: CTGAAGGTGTACGGAAACAC R: GTTCGGCCACCTCGAATTG	322	
blaSIM	F: TACAAGGGATTCGGCATCG R: TAATGGCCTGTTCCCATGTG	570	
blaDIM	F: GCTTGTCTTCGCTTGCTAACG R: CGTTCGGCTGGATTGATTTG	699	
ermB	F: GATACCGTTTACGAAATTGG R: GAATCGAGACTTGAGTGTGC	364	(Chen et al., 2007)
sul1	F: CGCACCGGAAACATCGCTGCAC R: TGAAGTTCCGCCGCAAGGCTCG	163	(Awad et al., 2015)

tetA	F: TTGGCATTCTGCATTCACTC R: GAAGGCAAGCAGGATGTAGC	125	Guarddon et al., 2014)

Agarose gel electrophoresis was done according to Weerakkody and Witharana (2024) with minor modifications. The choice of gel matrices and gel concentration depends on the size of amplicon, as the concentration of the agarose determine the pores size and 1X TAE (pH 8.0) buffer was prepared following manufacturer's guidelines. An agarose concentration of 1.5-2.5% (Himedia, India) was prepared based on the amplicon size of PCR products analyzed using 1X TAE buffer. The agarose was heated until completely dissolved and uniformly in black and decker microwave energy (SL1 3YD, England). Ethidium bromide of 0.5 µg mL^{-1} final concentration was added to the melted agarose. The gel casting tray was adjusted and the comb was placed, then the melted agarose was poured into gel cast half way up the comb. After proper solidification, the comb carefully removed, and the gel was transferred carefully into the agarose gel electrophoresis chamber. A written records of which sample to load in each well of the gel was prepared prior to loading samples. A DNA ladder of 100 bp (Solis BioDyne, Estonia) was used to estimate the molecular weight of the amplified products. A 5 µL of the DNA sample with 1 µL of 6X loading dye was loaded to each well of the gel with care to avoid puncture of the bottom of the wells during loading the samples. The wells checked to be closest to the negative (black) electrode and the electrophoresis tank was filled with 1X TAE buffer until it completely cover the agarose gel. Then, the lid was placed on the agarose chamber and connected to the electrode that connects to the power supply. The power supply was adjusted to 120 V and run for 1:30 hours. Then, the gel was removed from the electrophoresis chamber and the amplified products were visualized using a UV-transilluminator DW04S-3E Gel Documentation System (Chongqing, China) according to the manufacturer's protocol.

3.8. Quality Control

All standard operating procedures (SOPs), including labelling and data recording, culture media preparation, biochemical tests, antimicrobial susceptibility tests, DNA isolation and PCR were strictly followed. The sterility of the prepared culture media was checked by incubating 5% of the batch at 37 °C overnight and evaluated for possible bacterial growth. Quality control of all prepared media was also checked by inoculating standard strains such as *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853), and *K. pneumoniae* (ATCCC 700603), which were acquired from the Ethiopian Public Health Institute (EPHI). The potency of the tested antimicrobials was evaluated using the control

strains. McFarland standard (0.5 McFarland) was used to standardize the inoculum density of the bacterial suspension. The concentration and quality of DNA were checked prior to PCR.

3.9. Ethical Approval and Consideration

The study protocol was approved by the College of Natural and Computational Sciences Institutional Review Board Committee (CNS-IRB), Addis Ababa University (Ref. no. CNCSDO/162/15/2023). A formal letter of support was subsequently obtained from Addis Ababa University, Institute of Biotechnology (AAU-IOB) to communicate with the Tikur Anbessa Specialized Hospital and Zewditu Memorial Hospital. The purpose of the study was explained to both hospitals emphasizing that their permission was voluntary, and that all data would be handled anonymously although the results derived from this study would be published. Prior to sample collection, informed verbal and written consent was obtained from both hospitals before the commencement of sample collections.

3.10. Data Management and Statistical Analysis

The data were coded, entered into a Microsoft Excel spreadsheet. Data were analyzed using SPSS version 26 (IBM-SPSS Inc., Chicago, IL, US). Descriptive statistics and chi-square tests were used to assess associations, with statistical significance set at $p < 0.05$.

4. RESULTS

4.1. Isolation and Identification of Bacteria

A total of 38 samples, comprising hospital WW and IS were processed for the isolation and identification of bacteria. All samples (100%) yielded bacterial growth, indicating a high frequency of environmental contamination in both WW and IS matrices (Table 3) from all sample sites (Appendix 1).

Table 3. Frequency of positive recovery across distinct hospital environmental sample types

Hospitals	Sample type	Positive sample, N (%)
TASH (n = 22)	WW (n = 5)	5 (100)
	IS (n = 17)	17 (100)
ZMH (n = 16)	WW (n = 4)	4 (100)
	IS (n = 12)	12 (100)
Total (n = 38)		38 (100)

Samples were processed, cultured, and subcultured until pure isolates were obtained (Figure 8).

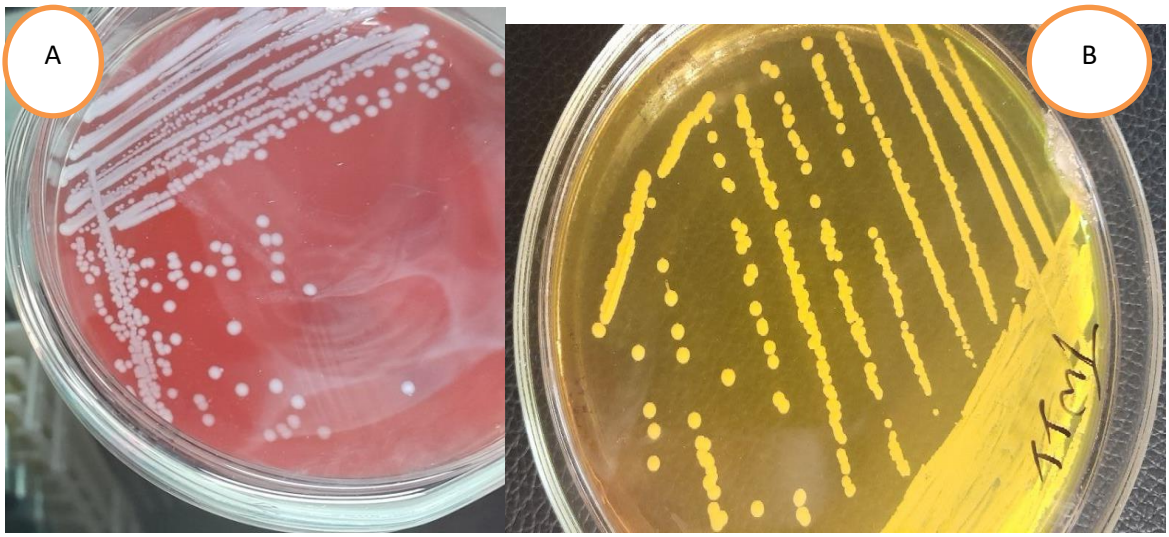

Figure 8. Phenotypic presentation of bacterial isolates on distinct culture media.

'A' depicts the non hemolytic (γ-hemolysis) E. coli culture on 5% blood agar plate.

'B' exhibits positive mannitol fermentation by S. aureus on mannitol salt agar (MSA), selective and differential media.

Once pure isolates were obtained, biochemical tests were performed to identify the bacterial species. The biochemical tests, such as gram staining, catalase test, oxidation-fermentation (OF) test, coagulase test, methyl red (MR) test, voges-proskauer (VP) test, indole test, oxidase test, hydrogen sulfide production test, motility test, citrate utilization test, and triple sugar iron agar test, were performed. Biochemical identification results are summarized in Table 4; detailed profiles are provided in Appendix 2.

All presumptive isolates obtained through culture and biochemical tests were analyzed by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS). Protein spectra generated by the Bruker Microflex LT system showed clear and reproducible peaks within the 2000 to 20,000 Da range. Identification scores were interpreted using Bruker Biotyper criteria, with values < 1.7 unreliable; 1.7-2.0 probable genus; ≥ 2.0 probable species. MALDI-TOF MS successfully identified all isolates at the species level in accordance with Bruker Biotyper score criteria. Overall identification showed high concordance with conventional biochemical test methods.

In addition, MALDI-TOF MS also detected several nonpathogenic or low risk bacterial species such as *Bacillus flexus* (*B. flexus*), emerging opportunistic pathogen such as *Leclercia adecarboxylata* (*L. adecarboxylata*), rare opportunistic pathogen linked merely to heathcare like *Ureibacillus massiliensis* (*U. massiliensis*), opportunistic bacterial species, *Acinetobacter lwoffii* (*A. lwoffii*) and *Raoultella ornithinolytica* (*R. ornithinolytica*) *(*which can exist as part of the normal human gut flora without causing harm) and pathogenic bacteria belonging to the ESKAPE pathogens. These organisms are commonly associated with hospital environments. Because the aim of this work was to characterize the total AMR patterns, these non-pathogenic, emerging rare opportunistic isolates were documented and included in all downstream analyses, including prevalence reporting, AST and PCR genotyping. In total, all 92 isolates (100%) undergo species-level identification. Identification scores by species are summarized in Appendix 3. The spectral variability observed across bacterial isolates also provides qualitative support for subsequent clustering analyses (Figure 9).

Table 4. Summary of biochemical test results for the identification of bacterial isolates

S/N	Isolate ID	Bacteria	S/N	Isolate ID	Bacteria	S/N	Isolate ID	Bacteria
1.	TS101	*E. coli*	2.	ZS41	*P. aeruginosa*	3.	TS64	*Acinetobacter* spp
4.	TS14	*E. coli*	5.	ZW33	*P. aeruginosa*	6.	TS22	*S. aureus*
7.	TS23	*E. coli*	8.	TW13	Klebsiella spp.	9.	TS63	*S. aureus*
10.	TS32	*E. coli*	11.	TS121	*Klebsiella* spp	12.	TW11	*S. aureus*
13.	TS53	*E. coli*	14.	ZW11	*Klebsiella* spp	15.	TW24	*S. aureus*
16.	TS72	*E. coli*	17.	TW31	*Klebsiella* spp	18.	ZS111	*S. aureus*
19.	TS81	*E. coli*	20.	TS12	*Enterobacter* spp.	21.	ZS31	*S. aureus*
22.	TW12	*E. coli*	23.	TS131	*Enterobacter spp.*	24.	ZS52	*S. aureus*
25.	TW21	*E. coli*	26.	TS31	*Enterobacter spp.*	27.	Zs82	*S. aureus*
28.	TW32	*E. coli*	29.	TS42	*Enterobacter spp.*	30.	Zw42	*S. aureus*
31.	Tw42	*E. coli*	32.	TS51	*Enterobacter spp.*	33.	TS102	*CoNS* spp
34.	TW51	*E. coli*	35.	TS71	*Enterobacter spp.*	36.	TS111	*CoNS* spp
37.	ZS112	*E. coli*	38.	TW43	*Enterobacter spp.*	39.	TS141	*CoNS* spp
40.	ZS121	*E. coli*	41.	ZS122	*Enterobacter spp.*	42.	TW14	*CoNS* spp
43.	ZS13	*E. coli*	44.	ZS32	*Enterobacter spp.*	45.	ZS11	*CoNS* spp
46.	ZS21	*E. coli*	47.	ZS42	*Enterobacter spp.*	48.	ZS81	*CoNS* spp
49.	ZS44	*E. coli*	50.	ZS51	*Enterobacter spp.*	51.	TS161	*CoNS* spp
52.	ZS61	*E. coli*	53.	ZS83	*Enterobacter spp.*	54.	TS62	*CoNS* spp
55.	ZW14	*E. coli*	56.	ZW13	*Enterobacter spp.*	57.	ZS23	*Enterococcus* spp
58.	ZW22	*E. coli*	59.	Zw41	*Enterobacter spp.*	60.	ZS34	*Enterococcus* spp
61.	ZW32	*E. coli*	62.	ZS71	*Enterobacter spp.*	63.	ZW23	*Enterococcus* spp
64.	ZW43	*E. coli*	65.	TS171	*Enterobacter spp.*	66.	ZS35	*Enterococcus* spp
67.	ZS101	*Klebsiella* spp.	68.	ZW21	*Citrobacter* spp.	69.	ZS84	*Enterococcus* spp
70.	ZS91	*Klebsiella* spp.	71.	TS11	*Raoultella* spp	72.	TS13	*Bacillus* spp
73.	TW41	*Klebsiella* spp.	74.	TS21	*Acinetobacter* spp	75.	TW22	*Bacillus* spp
76.	ZS102	*Bacillus* spp	77.	TS41	*Acinetobacter* spp	78.	TW45	*Bacillus* spp
79.	TS61	*Bacillus* spp	80.	TS33	*Bacillus* spp	81.	ZS22	*Bacillus* spp

82.	TS151	*Bacillus* spp	83.	TS52	*Bacillus* spp	84.	ZS33	*Bacillus* spp
85.	TW23	*Bacillus* spp	86.	TW44	*Bacillus* spp	87.	ZS43	*Bacillus* spp
88.	TS33	*Bacillus* spp	89.	ZS113	*Bacillus* spp	90.	ZW12	*Bacillus* spp
91.	ZW31	*Bacillus* spp	92.	ZS12	*Bacillus* spp			

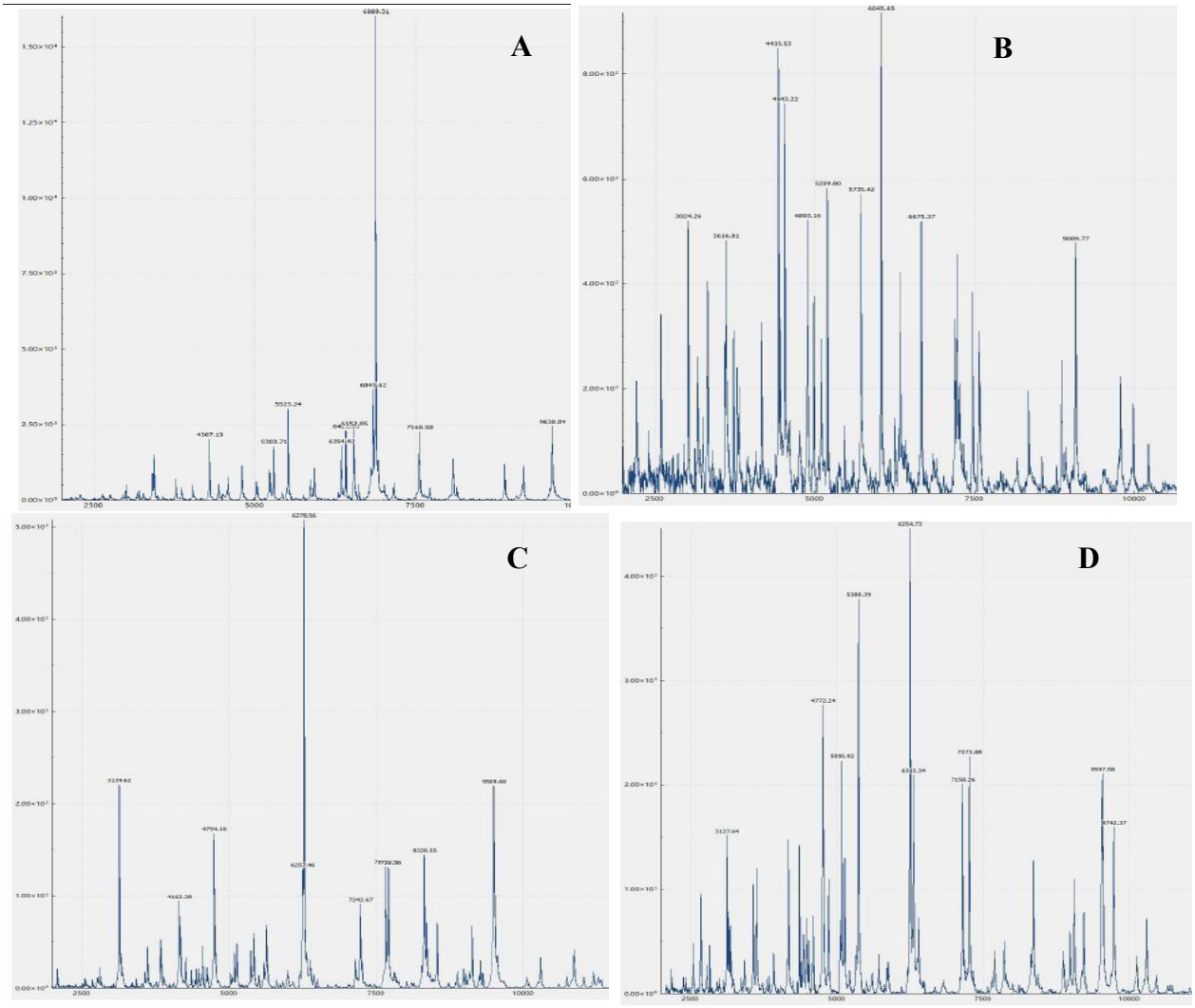

Figure 9. Representative MALDI-TOF MS spectra of identified bacterial isolates from wastewater and inanimate surface samples of TASH and ZMH.
Panel A presents S. aureus, which displayed dominant peaks between 5000 and 7000 Da, consistent with ribosomal protein signatures. Panel B shows P. aeruginosa, with high intensity peaks detected between 3500 and 7000 Da. Panel C depicts E. coli, which showed distinct spectral clusters between 4500 and 7500 Da. Panel D illustrates E. cloacae, which showed distinct spectral clusters between 4000 and 7000 Da.

4.2. Prevalence and Spatial Distribution of Bacterial Isolates

In this study, a total of 92 bacterial strains were successfully isolated and analyzed. Among these, 52.2% (48/92) isolates were recovered from TASH, while 47.8% (44/92) were recovered from ZMH. This indicates that TASH was observed to be slightly larger source of the bacterial isolates within the study scope (Figure 10). Among the sample types, WW accounted for 31.5% (29/92); whereas, IS accounted for 68.5% (63/92).

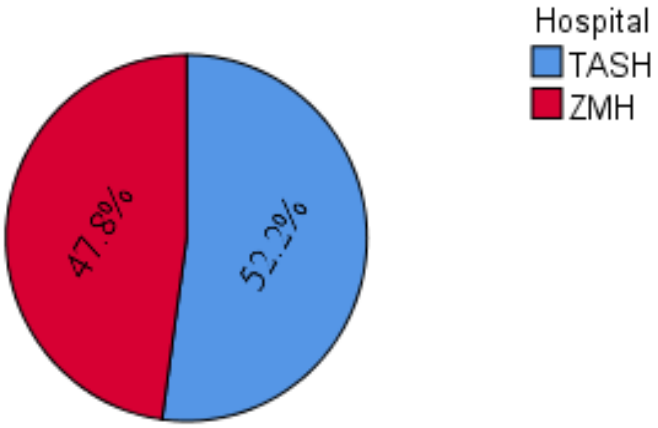

Figure 10. Prevalence of bacterial isolates across TASH and ZMH settings.
Abbreviations: TASH, Tikur Anbessa Specialized Hospital; ZMH, Zewuditu Memorial Hospital

4.2.1. Prevalence of Bacterial Isolates in TASH Wastewaters and Inanimate Surface Sampes

Out of 48 bacterial isolates recovered from TASH, 33.3% (16/48) were from WW and 66.7% (32/48) were from IS. This indicates that IS were the predominant sources of the bacterial isolates. Among the WW isolates, 56.3% (9/16) were GNB, while 43.8% (7/16) were GPB. Similarly, among the IS isolates, 62.5% (20/32) were GNB; whereas, only 37.5% (12/32) were GPB. This exhibits that GNB were dominant in both WW and IS samples. Overall, GNB were predominant (60.4%; 29/48), followed by GPB (39.6%; 19/48) (Table 5).

Table 5. Distribution of Gram-positive and Gram-negative bacterial isolates across TASH WW and IS samples

Sample Source	Isolates		Total, n (%)
	Gram Positive, n (%)	Gram Negative, n (%)	
TASH-WW (n = 16)	7 (43.8)	9 (56.2)	16 (33.3)
TASH-IS (n = 32)	12 (37.5)	20 (62.5)	32 (66.7)
Total	19 (39.6)	29 (60.4)	48 (100)

Among the TASH WW isolates, *E. coli* was the most prevalent GNB at 55.6% (5/9), followed by *Klebsiella oxytoca* (*K. oxytoca*) at 22.2% (2/9), while *K. pneumoniae* and *Enterobacter cloacae* (*E. cloacae)* each accounted for 11.1% (1/9). Among GPB, *S. aureus, Bacillus cereus* (*B. cereus*) and *B. flexus* were each isolated at 28.6% (2/7); whereas,

Staphylococcus epidermidis (*S. epidermidis*was relatively the least prevalent at 14.3% (1/7). Overall, GNB (56.3%; 9/16) were more prevalent than GPB (43.8%; 7/16) (Figure 11).

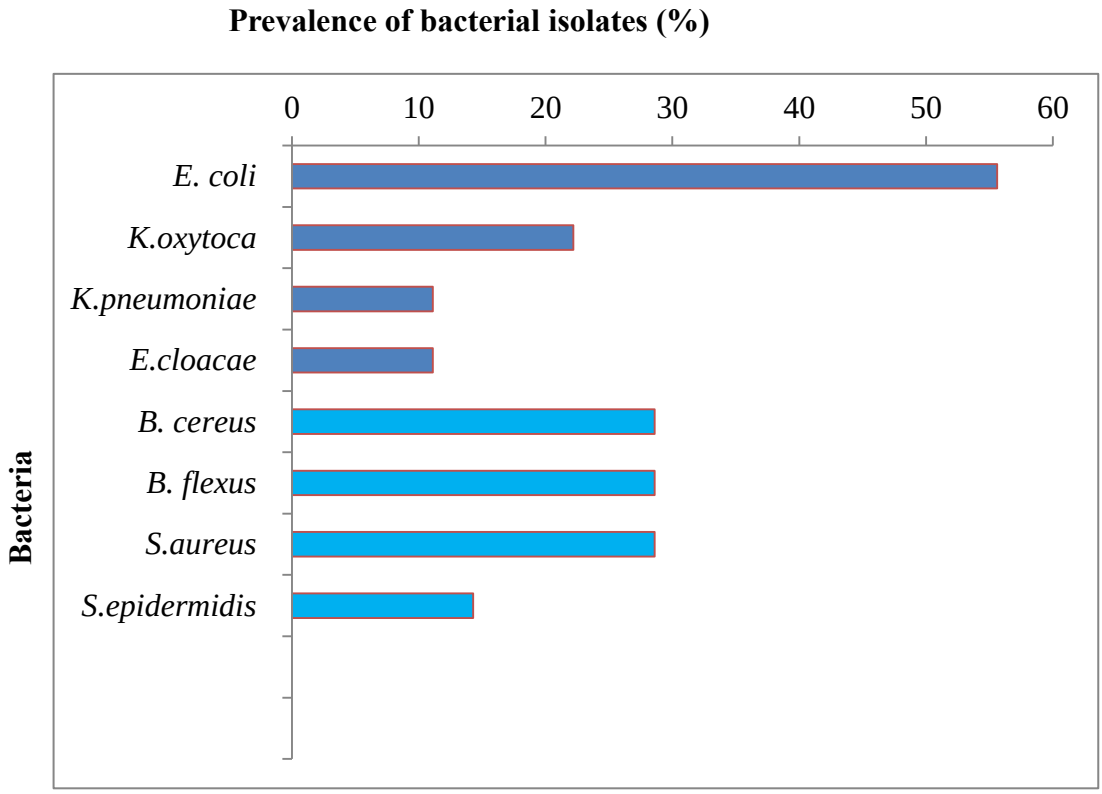

Figure 11. Prevalence of bacterial isolates from TASH wastewater samples.

This figure depicts the prevalence of bacterial isolates from TASH wastewater samples. The blue colr indicates GNB, while the light blue color indicates the GPB.

When compared by specific sampling sites within the TASH drainage WW samples, 31.3% (5/16) of bacterial isolates were recovered from the ICU ward, suggesting more contamination in the area, followed by emergency wards and operation wards (each accounted for 25% (4/16). Conversely, pediatrics wards (12.5%; 2/16) and the composite sample site (6.3%; 1/16) were less contaminated area (Figure 12).

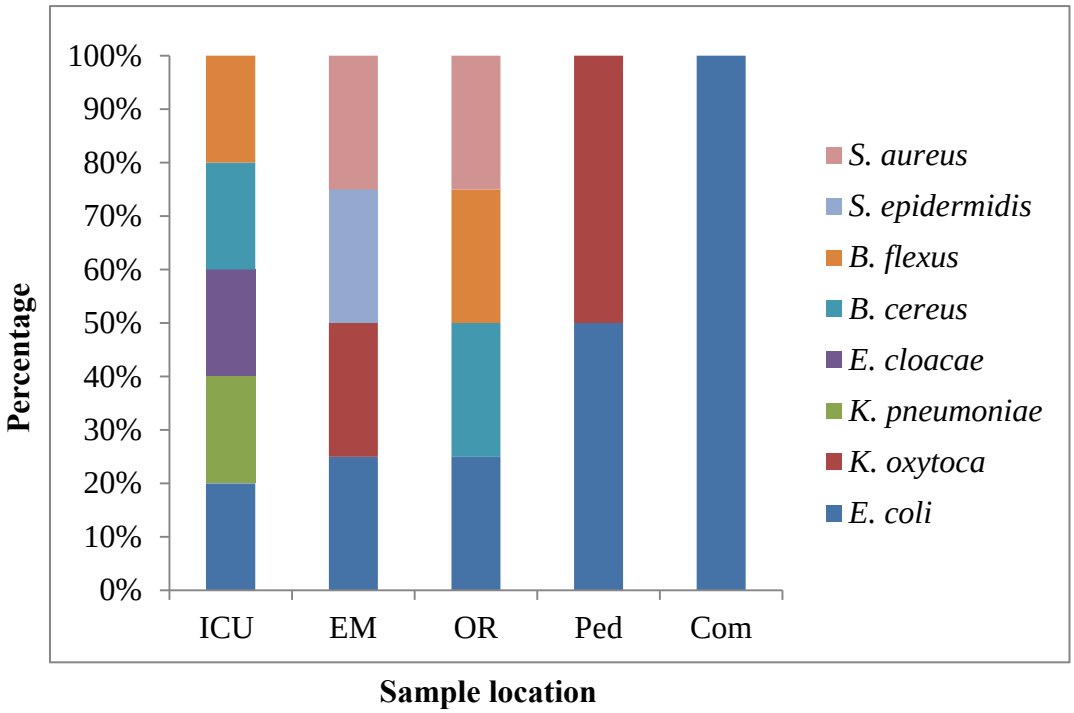

Figure 12. Percentage contamination of TASH wards by bacterial isolates from wastewater samples.

The stacked column graph illustrating the spatial distribution of bacterial isolates across TASH wastewater wards. Em, emergency wards; OR, operation wards; Ped, pediatrics wards and ICU, intensive care unit wards.

From the TASH IS samples, *E. coli* (35%; 7/20) was the most prevalent GNB, followed by *E. cloacae* (30%; 6/20) and *A. baumannii* (10%; 2/20), the WHO priority-1 pathogen was isolated. The *R. ornithinolytica* and *L. adecarboxylata* were each isolated in less frequency (5%; 1/20). From GPB, *S.epidermidis* and *B. cereus* were the most frequently isolated both constituting 25% (3/12). These were followed by *S.aureus* and *Staphylococcus caprae* (*S. caprae*), which each accounted for 16.7% (2/12). Whereas, *U. massiliensis*, and *B. flexus* were rare bacteria, each identified at lower frequentcy of 8.3% (1/12) (Figure 13**)**. Overall, among IS isolates of TASH, GNB were more prevalent (62.5%; 20/32) than GPB (37.5%; 12/32).

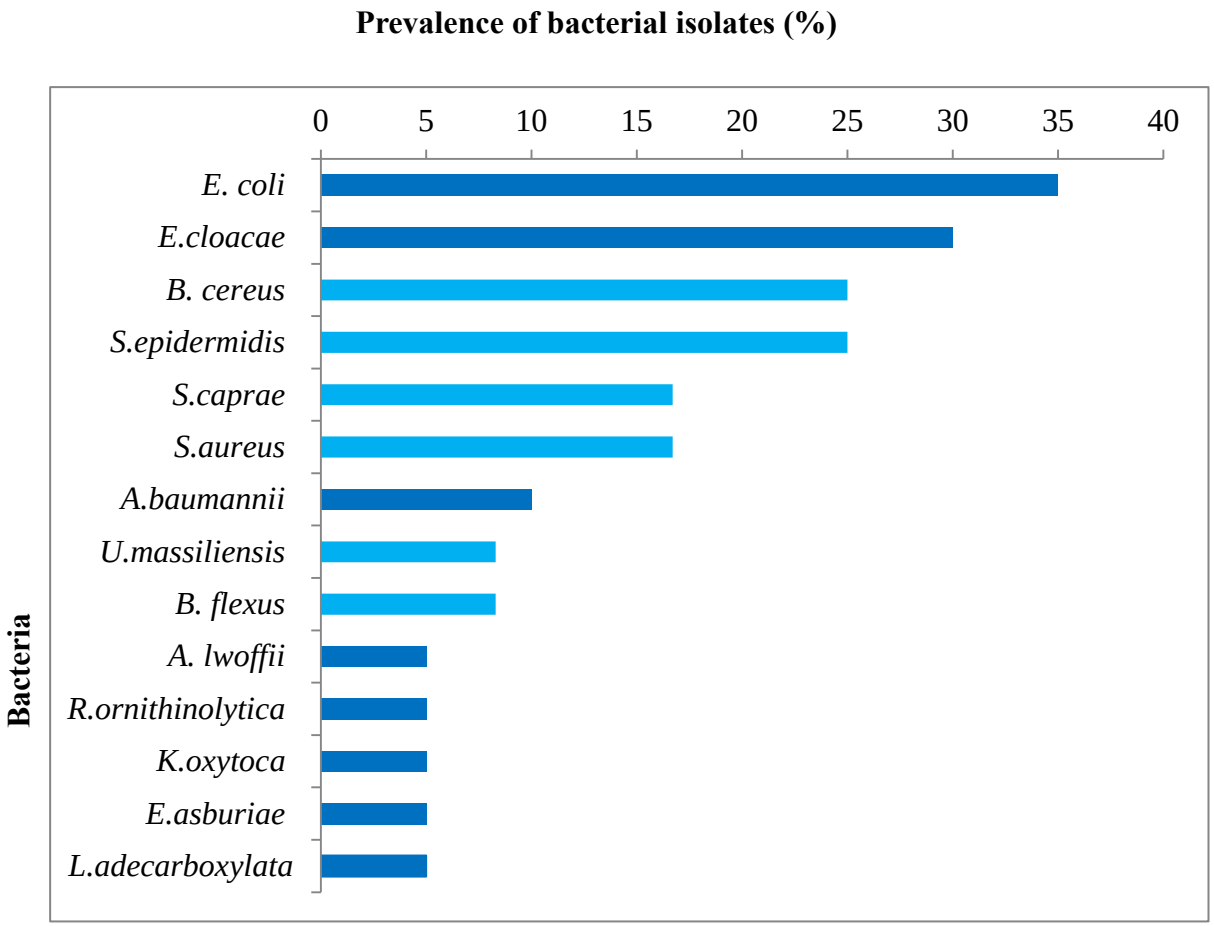

Figure 13. Prevalence of bacterial isolates from TASH inanimate surface samples.

This figure shows the frequency isolation of both GNB and GPB isolates from TASH inanimate surface samples. The blue color indicates GNB, while the light blue color indicates the GPB.

Among TASH inanimate wards, both neonatal intensive care unit (room 1) and operation room (room 3) were found to be the most contaminated wards accounting for 13% (4/32) of the isolate (Figure 14). The density of diverse isolates in the neonatal intensive care unit often suggest a significant bacterial load on a high-touch medical equipment (incubators, ventilators) likely exacerbated by frequent contact by healthcare workers and caregivers. Similarly, the operation room outcomes may reflect a high selective pressure of surgical-grade disinfectants, potentially leading to the persistence of specific resilient organisms.

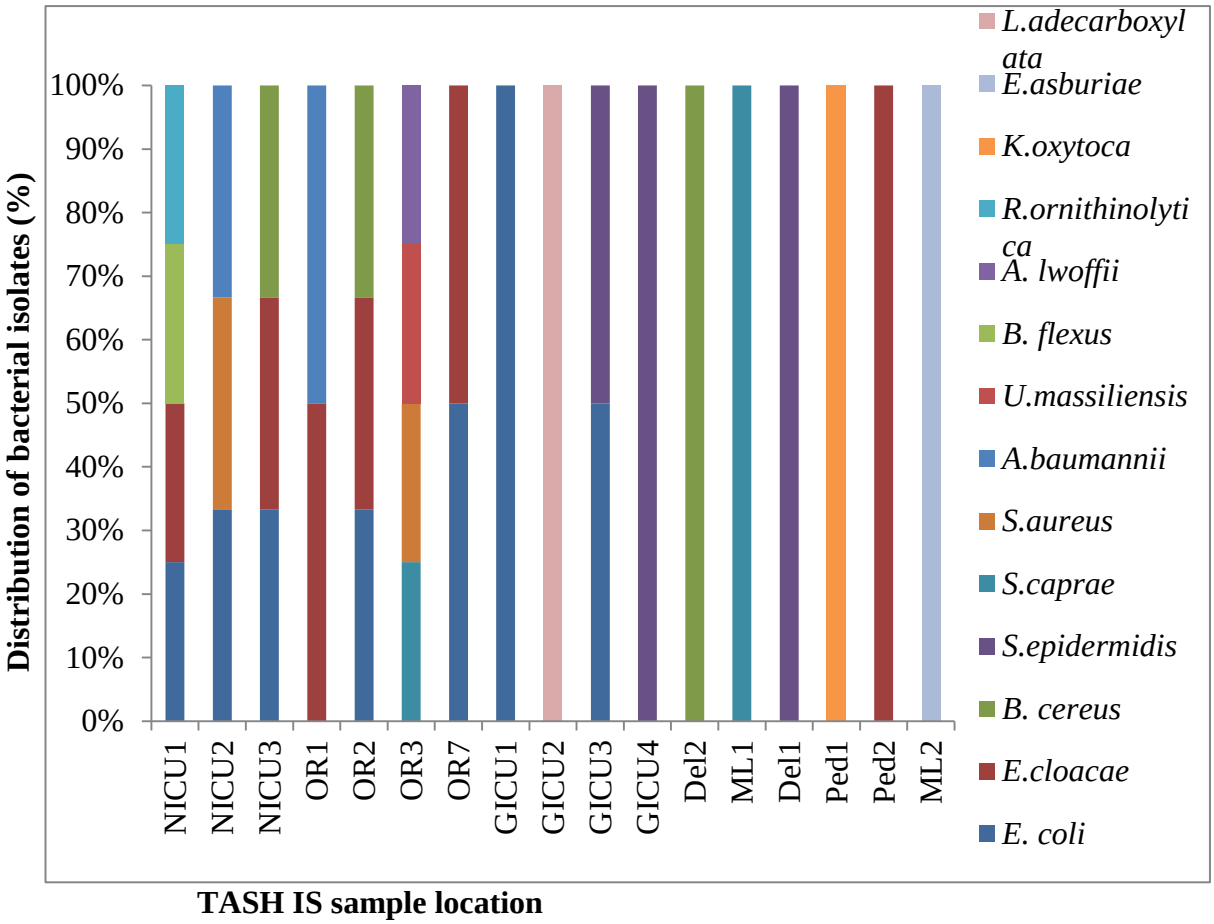

Figure 14. Percentage contamination of TASH wards by bacterial isolates from inanimate surface samples.

Key: NICU1, neonatal intensive care unit room 1; NICU2, neonatal intensive care unit room 2; NICU3, neonatal intensive care unit room 3; OR1, operation room 1; OR2, operation room 2; OR3, operation room 3; OR7, operation room 7; GICU1, general intensive care unit room 1; GICU2, general intensive care unit room 2; GICU3, general intensive care unit room 3; GICU4, general intensive care unit room 4; Ped1, pediatrics room 1; Ped2, pediatrics room 2; Del1, delivery room 1; Del2, delivery room 1; ML1, main laboratory unit room 1; ML2, main laboratory unit room 2.

The microbiological profiling from TASH WW and IS samples identified 15 distinct bacterial species demonstrating varying distribution. Among the GNB, the prevalence in WW vs. Is was as follows: *E. coli* (55.6% vs. 35%), *E. cloacae* (11.1% vs. 30%) and *K. oxytoca* (22.2% vs. 5%); whereas, from GPB, *B. flexus* (28.6% vs. 8.3%), *B. cereus* (28.6% vs. 25%) and *S. aureus* (28.6% vs. 16.7%) as depicted in Figure 11 and Figure 13. This indicates that surface to patient transmission, surface shedding into the hospital drainage system, drainge system to

biofilm crawal up to the surface and bacterial population links between WW and IS are concurrent risks.

4.2.2. Prevalence of Bacterial Isolates across ZMH Wastewaters and Inanimate Surfaces

A total of 44 bacterial isolates were obtained from ZMH. Out of these 29.5% (13/44) were from WW and 70.5% (31/44) were from IS. In ZMH WW, GNB were the most prevalent (69.2%; 9/13) compared to GPB (30.8%; 4/13). Conversely, IS exhibited higher frequency of GPB (54.8%; 17/31) than GNB (45.2%; 14/31). Similar to the findings at TASH, IS from ZMH were the predominant sources of bacterial contamination (70.5%; 31/44) (Table 6).

Table 6. Distribution of Gram-positive and Gram-negative bacterial isolates across ZMH WW and IS samples

Sample Source	Isolates		Total, n (%)
	Gram Positive, n (%)	Gram Negative, n (%)	
ZMH-WW (n = 13)	4 (30.8)	9 (69.2)	13 (29.5)
ZMH-IS (n = 31)	17 (54.8)	14 (45.2)	31 (70.5)
Total (n = 31)	21 (47.7)	23 (52.3)	44 (100)

Among ZMH WW isolates, *E.coli* was the most prevalent GNB isolate at 44.4% (4/9), followed by *E. cloacae* (22.2%; 2/9). *P. aeruginosa* was recovered at a frequency of 11.1% (1/9), further indicating the high-risk nature of the ZMH drainage system. The *Citrobacter freundii* (*C. freundii*), the rare opportunistic bacteria, was also isolated at 11.1% (1/9). Regarding the GPB isolates, *B. cereus* was the most dominant species accounting for 50% (2/4). Both *Enterococcus faecalis* (*E. faecalis)* and *S. aureus* were isolated each at 25% (1/4). (Figure 15).

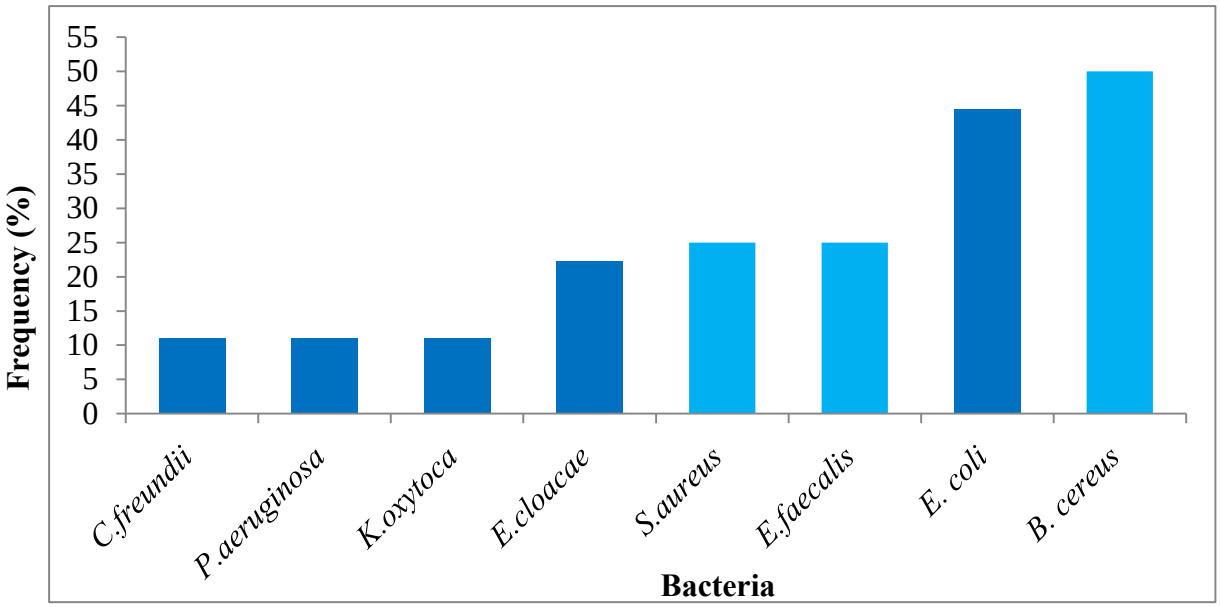

Figure 15. Prevalence of bacterial isolates from ZMH wastewater samples.

The bar chart depicts the prevalence of bacterial isolates in ZMH wastewater. The blue color indicates GNB, while the light blue indicates the GPB.

Among the ward drainage systems sampled at ZMH, the medical ward showed the highest bacterial recovery (30.8%; 4/13), followed by the pediatrics, the ICU and the composite sample site, each contributing 23% (3/13) of the recovered bacterial isolates. This findings show that the medical ward was the primary source of bacterial contaminants at ZMH drainage system (Figure 16).

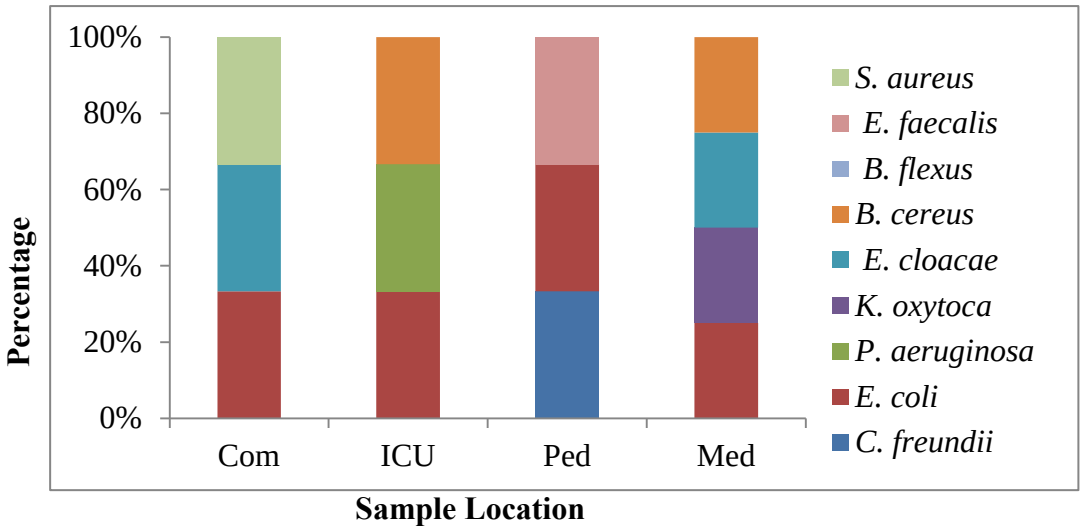

Figure 16. Percentage contamination of ZMH wards by bacterial isolates from wastewater samples.

Key: Med, medical ward; Ped, pediatrics ward; ICU, intensive care unit ward; Com, composite sample site

Among the IS of ZMH isolates, *E. coli* and *E. cloacae* were the most frequently recovered GNB at 43% (6/14) and 36% (5/14), respectively. Conversely, clinically significant pathogens such as *K. pneumoniae* and *P. aeruginosa* were isolated at lower frequencies of 14% (2/14) and 7% (1/14), respectively. Among the GPB isolates, *B. cereus* and *S. aureus* were exhibited the highest prevalence at 29% (5/17) and 24% (4/17), respectively. The enterococci, specifically *E. faecalis* and *E. faecium* were each isolated at the frequency of 12% (2/17); whereas, both species were absent at TASH samples (Figure 17).

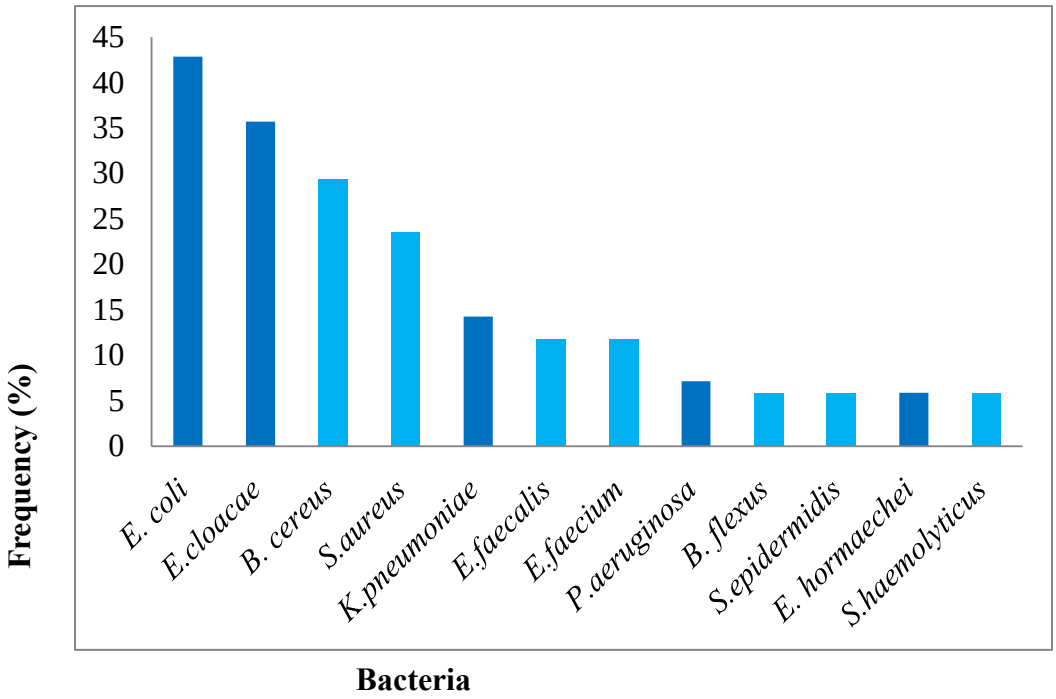

Figure 17. Prevalence of bacterial isolates from ZMH inanimate surface samples.

The bacterial isolates from IS was demonstrated by bar chart showing the prevalence range 6%-43%. The blue colr indicates GNB, while the light blue color indicates the GPB.

Among the IS sample sites, intensive care unit (room 3) was the most contaminated area (16%; 5/31), followed by neonatal intensive care unit (room 1) and pediatrics unit (room 2) both constituted (13%; 4/31) of different bacterial isolates (Figure 18).

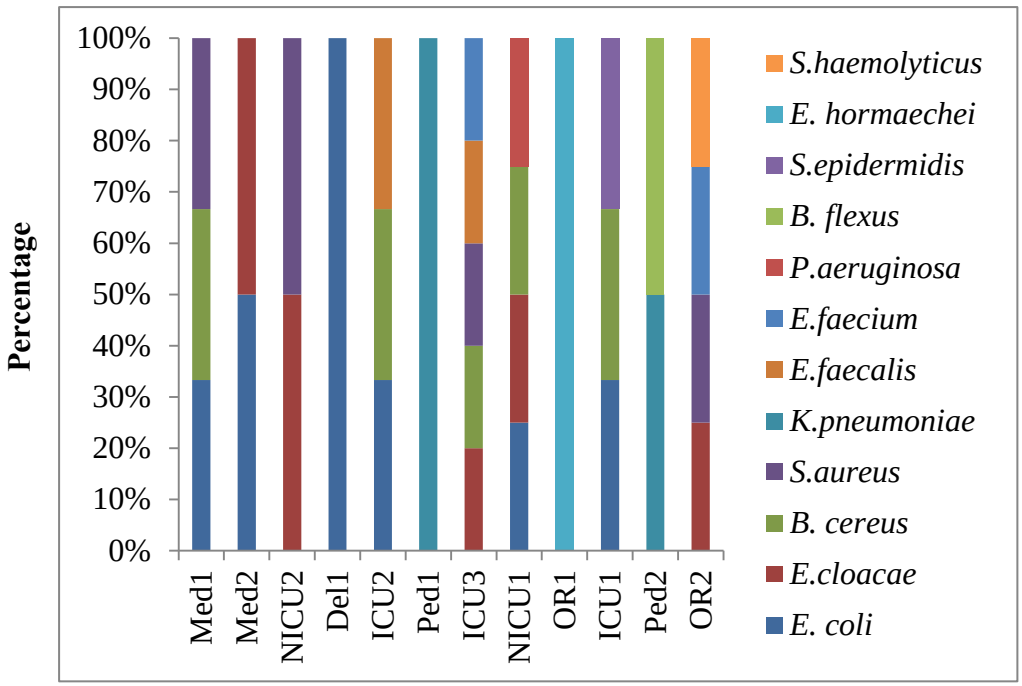

Figure 18. Percentage contamination of ZMH wards by bacterial isolates from inanimate surface samples.

Key: ICU1, intensive care unit room 1; ICU2, intensive care unit room 2; ICU3, intensive care unit room 3; NICU1, neonatal intensive care unit room 1; NICU2, neonatal intensive care unit room 2; Del1, delivery room 1; OR1, operation Room 1; OR2, operation room 2; Ped1, pediatrics room 1; Ped2, pediatrics room 2; Med1, medical ward room 1; Med1, medical ward room 1.

Similar to TASH, the frequency of bacterial isolates prevalence varied between WW and IS of ZMH. Prevalence of GNB (WW vs. IS) included: *E. coli* (44.4% vs. 43.5%), *E. cloacae* (22.2% vs. 36%) and *P. aeruginosa* (11.1% vs. 7%), while that of GPB including, *B. cereus* (50% vs. 29%), *S. aureus* (25% vs. 24%) and *E. faecalis* (25% vs. 12%) (Figure 15 and Figure 17).

4.3. Antimicrobial Susceptibility Patterns and Spatial Distribution of Resistance Profiles

Bacterial isolates from TASH and ZMH WW and IS which are considered as hotspots for contamination were subjected to phenotypic test, AST, to determine the AMR profile of the bacterial isolates (Figure 19). A total of 75 bacterial isolates were subjected to AST against

respective antimicrobial disks and demonstrated different patterns of susceptibility to antimicrobial disks (Table 7).

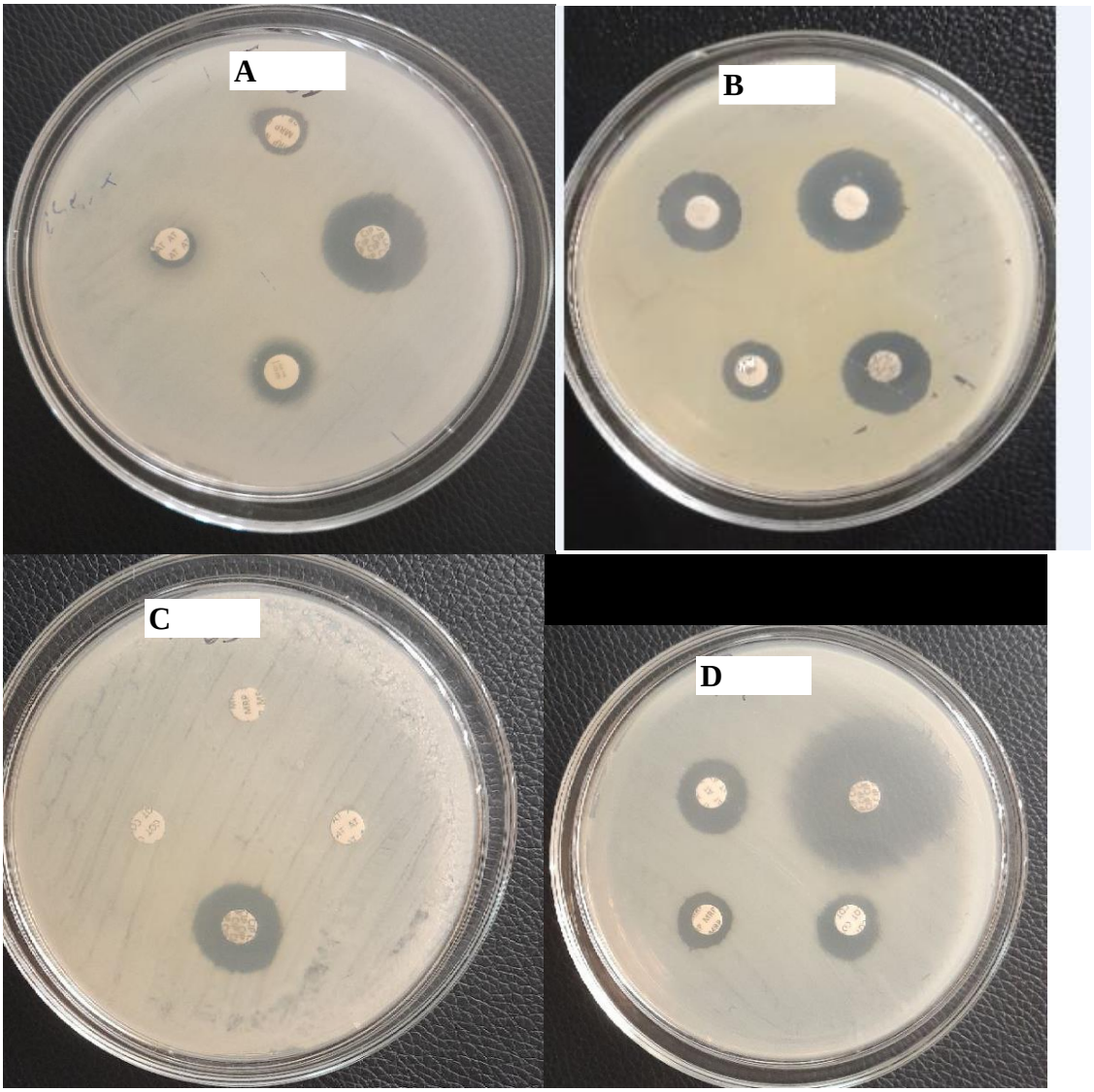

Figure 19. Phenotypic AST profiles via the Kirby-Bauer disk diffusion method.
The figures illustrate the patterns of the zones of inhibition against distnict panels of antimicrobials which is measured and categorized as 'S', 'I', and 'R'.
The letter 'A' depicts the AST pattern of E. coli against aztreonam, ampicillin, meropenem and ciprofloxacin, while 'B' shows S. aureus against vancomycin, clindamycin, oxacillin and ciprofloxacin.
The letters 'C' and 'D' demonstrate AST pattern of K. pneumoniae against meropenem, ciprofloxacin, ceftazidime and aztreonam.

Table 7. Antimicrobial susceptibility status of GNB isolates obtained from WW and IS of TASH and ZMH

Gram negative bacteria	SP	Antimicrobial panels and AST status (%)															
		AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	SXT	NIT	AMC	CXM	CTX	AK	TM	NO
C. freundii (n = 1)	S	NA	100	NA	100	100	-	100	NA	100	NA	NA	-	-	-	-	-
	I	NA	-	NA	-	-	100	-	NA	-	NA	NA	100	-	-	100	100
	R	NA	-	NA	-	-	-	-	NA	-	NA	NA	-	100	100	-	-
K. pneumoniae (n = 3)	S	NA	-	66.7	-	-	-	-	-	-	-	-	-	-	NA	NA	NA
	I	NA	33.3	-	33.3	-	-	-	33.3	100	66.7	66.7	66.7	66.7	NA	NA	NA
	R	NA	66.7	33.3	66.7	100	100	100	66.7	-	33.3	33.3	33.3	33.3	NA	NA	NA
K. oxytoca (n = 4)	S	NA	75	75	75	25	-	75	25	25	25	-	-	-	NA	NA	NA
	I	NA	25	25	25	75	50	25	75	75	75	66.7	100	50	NA	NA	NA
	R	NA	-	-	-	-	50	-	-	-	-	-	-	50	NA	NA	NA
E. coli (n = 22)	S	31.8	40.9	72.7	31.8	45.5	4.5	59.1	50	68.2	27.3	9.1	27.3	NA	NA	NA	NA
	I	18.2	40.9	18.2	36.4	22.7	45.5	27.3	40.9	22.7	40.9	18.2	72.7	NA	NA	NA	NA
	R	50	18.2	9.1	31.8	31.8	50	13.6	9.1	9.1	31.8	-	-	NA	NA	NA	NA
L. adecarboxylata (n = 1)	S	100	100	100	100	100	-	100	100	100	100	NA	-	NA	NA	NA	NA
	I	-	-	-	-	-	100	-	-	-	-	NA	100	NA	NA	NA	NA
	R	-	-	-	-	-	-	-	-	-	-	NA	-	NA	NA	NA	NA
E. asburiae (n = 1)	S	NA	-	100	-	-	-	-	100	100	-	NA	-	NA	NA	NA	NA
	I	NA	100	-	100	100	100	100	-	-	100	NA	100	NA	NA	NA	NA
	R	NA	-	-	-	-	-	-	-	-	-	NA	-	NA	NA	NA	NA
E. hormaechei (n = 1)	S	NA	-	100	100	100	-	100	-	-	100	NA	-	NA	NA	NA	NA
	I	NA	100	-	-	-	100	-	-	100	-	NA	100	NA	NA	NA	NA
	R	NA	-	-	-	-	-	-	100	-	-	NA	-	NA	NA	NA	NA
R. ornithinolytica (n = 1)	S	NA	-	-	-	-	-	-	-	-	-	-	-	NA	NA	NA	NA
	I	NA	100	-	-	-	-	100	100	-	-	100	100	NA	NA	NA	NA
	R	NA	0	100	100	100	100	-	-	100	100	-	-	NA	NA	NA	NA
E. cloacae (n = 14)	S	NA	35.7	64.3	21.4	35.7	-	35.7	28.6	42.9	35.7	NA	28.6	NA	NA	NA	NA
	I	NA	28.6	14.3	42.9	14.3	42.9	28.6	35.7	50	42.9	NA	57.1	NA	NA	NA	NA
	R	NA	35.7	21.4	35.7	50	57.1	35.7	35.7	7.1	21.4	NA	14.3	NA	NA	NA	NA

Gram negative bacteria	SP	Antimicrobial panels and AST status (%)															
		AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	SXT	NIT	AMC	CXM	CTX	AK	TM	NO
P. aeruginosa (n = 2)	S	NA	50	100	100	-	-	100	NA	NA	NA	NA	NA	-	50	-	NA
	I	NA	-	-	-	100	50	-	NA	NA	NA	NA	NA	50	50	100	NA
	R	NA	50	-	-	-	50	-	NA	NA	NA	NA	NA	50	-	-	NA
A. baumannii (n = 2)	S	NA	-	100	-	NA	-	100	50	-	NA	NA	NA	-	-	50	NA
	I	NA	100	-	50	NA	50	-	50	100	NA	NA	NA	50	100	50	NA
	R	NA	-	-	50	NA	50	-	-	-	NA	NA	NA	50	-	-	NA
A. lwoffii (n = 1)	S	NA	-	100	100	NA	-	100	-	-	NA	NA	NA	-	-	-	NA
	I	NA	100	-	-	NA	-	-	100	100	NA	NA	NA	100	-	100	NA
	R	NA	-	-	-	NA	100	-	-	-	NA	NA	NA	-	100	-	NA

Abbreviations: SP, susceptibility pattern; S, susceptibile; I, intermediate; R, resistanct; NA, not applicable; -, 0.

Table 8. Antimicrobial susceptibility status of GPB isolates obtained from WW and IS of TASH and ZMH

Gram positive bacteria	SP	AMP	GEN	CIP	TE	SXT	NIT	OX	CD	VAN	FOX	NO	ERY	DOX	DOX
S. aureus (n = 9)	S	66.7	66.7	33.3	-	77.8	11.1	11.1	88.9	33.3	22.2	33.3	-	66.7	66.7
	I	22.2	22.2	66.7	66.7	11.1	22.2	11.1	11.1	66.7	66.7	55.6	66.7	22.2	22.2
	R	11.1	11.1	-	33.3	11.1	66.7	77.8	-	-	11.1	11.1	33.3	11.1	11.1
S. caprae (n = 2)	S	-	100	50	-	50	-	-	-	-	-	50	-	-	-
	I	100	-	-	50	-	100	-	-	100	50	50	100	100	100
	R	-	-	50	50	50	-	100	100	-	50	-	-	-	-
S. epidermidis (n = 5)	S	20	100	100	-	100	-	-	60	-	20	40	-	20	20
	I	80	-	-	60	-	100	-	20	100	20	40	60	80	80
	R	-	-	-	40	-	-	100	20	-	60	20	40	-	-
S. haemolyticus (n = 1)	S	100	100	-	-	100	-	-	-	100	100	-	-	100	100
	I	-	-	100	100	-	100	-	100	-	-	100	100	-	-
	R	-	-	-	-	-	-	100	-	-	-	-	-	-	-

E. faecium (n = 2)	S	50	NA	100	-	NA	-	NA	NA	50	NA	-	-	50	50
	I	50	NA	-	50	NA	50	NA	NA	50	NA	50	100	50	50
	R	-	NA	-	50	NA	50	NA	NA	-	NA	50	-	-	-
E. faecalis (n = 3)	S	-	NA	100	-	NA	-	NA	NA	66.7	NA	100	66.7	-	-
	I	66.7	NA	-	66.7	NA	66.7	NA	NA	33.3	NA	-	-	66.7	66.7
	R	33.3	NA	-	33.3	NA	33.3	NA	NA	-	NA	-	33.3	33.3	33.3

4.3.1. Antimicrobial Resistance Profiles and MARI Scores of Bacterial Isolates from TASH Wastewaters and Inanimate Surfaces

Among GNB isolates from TASH WW samples, *E. coli* isolates, (60%; 3/5) exhibited resistance to ampicillin and meropenem. Notably, 100% (1/1) of *K. pneumoniae* demonstrated resistance to piperacillin, ceftazidime, aztreonam, meropenem, ciprofloxacin, tetracycline and cefuroxime. Similarly, 100% (1/1) of *E. cloacae* showed resistance to gentamicin, ceftazidime, aztreonam, ciprofloxacin, tetracycline and nitrofurantoin. Half (50%; 1/2) of *K. oxytoca* showed resistance to meropenem and cefotaxime. Among antimicrobials tested against GNB, meropenem showed a highest level of resistance (55.5%; 5/9).

Among GPB isolates, 100% (1/1) of *S. epidermidis* displayed resistance to tetracycline, oxacillin, clindamycin, and vancomycin; whereas, 50% (1/2) of *S. aureus* showed resistance to multiple agents, including tetracycline, nitrofurantoin, oxacillin, erythromycin, doxycycline, and vancomycin. Among the antimicrobials tested against GPB isolates, tetracycline, oxacillin and vancomycin each exhibited the highest rate of resistance (66.7%; 2/3). The frequently observed AMR underscore the dissemination of AMR *staphylococci* via the hospital WW. The detailed AMR profiles of TASH WW bacterial isolates are presented in Table 9.

Table 9. Antimicrobial resistance profiles of bacterial isolates from TASH WW samples

Bacteria	Antimicrobial panels and AMR status (%)											
Gram negative	AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	NIT	CXM	CTX	MARI
K. pneumoniae (n = 1)	NA	100	-	100	100	100	100	100	-	100	-	0.7
K.oxytoca (n = 2)	NA	-	-	-	-	50	-	-	-	-	100	0.2
E.coli (n = 5)	60	20	20	20	20	60	20	-	20	-	NA	0.24
E.cloacae (n = 1)	NA	100	100	100	100	-	100	100	100	-	NA	0.8
Total (n = 9)	33.3	33.3	22.2	33.3	33.3	55.5	33.3	22.2	22.2	11.1	11.1	
Gram positive	CIP	TE	SXT	NIT	OX	CTX	ERY	NOR	DOX	CD	VAN	
S.aureus (n = 2)	-	50	-	50	50	-	50	-	50	-	50	0.3
S. epidermidis (n = 1)	-	100	-	-	100	-	-	-	-	100	100	0.2
Total (n = 3)	-	66.7	-	33.3	66.7	-	33.3	-	33.3	33.3	66.7	

Key: NA, not applicable; -, 0; AMP, ampicillin; PI, piperacillin; GEN, gentamicin; AT, azitronam; MRP, meropenem; CIP, ciprofloxacin; TE, tetracycline; FOX, cefoxitin; SXT, trimethoprim/sulfamethoxazole; ERY, erythromycin; CAZ, ceftazidime; MRP, meropenem; NIT, nitrofurantoin; AK, amikacin; FOX, cefotaxime; CXM, cefuroxime; OX, oxacillin; DOX, doxycycline; CD, clindamycin; VAN, vancomycin.

All TASH ward drainage systems isolates demonstrated MAR index ≥ 0.2, with the sole exception of those from the pediatrics ward. This indicates that these sample sites represent critical high-risk environments with intense antimicrobial selective pressure. Notably, isolates from ICU ward drainage exhibited the highest resistance profile with MAR index of 0.5, indicating the most frequent contamination with ARB, closely followed by the operation ward drainage site, which showed MAR index of 0.4 as depicted in Figure 20 (see Appendix 4).

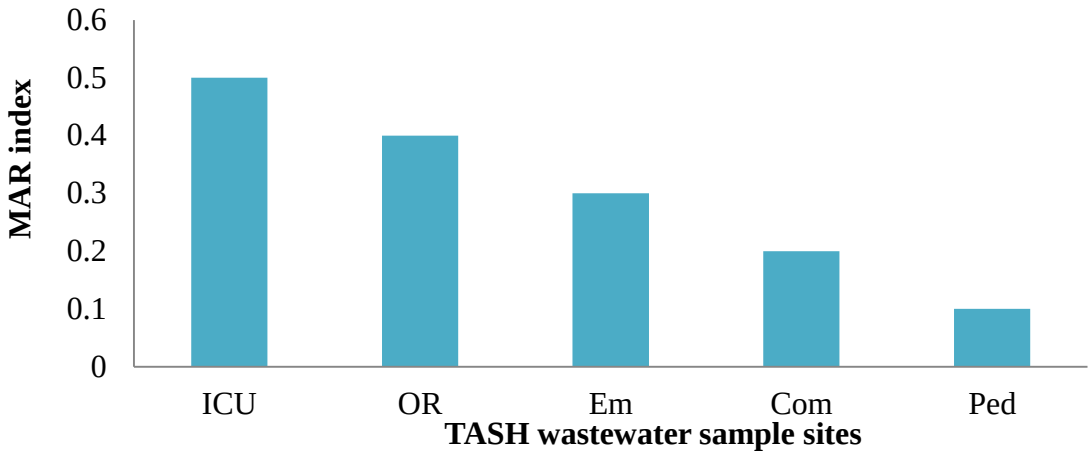

Figure 20. Spatial contamination of TASH wards by antimicrobials resistance bacterial isolates from wastewater samples.

The figure depicts the antimicrobial resistance percentage of the TASH wastewater drainages. ICU, intensive care unit; OR, operation ward; Em, emergency ward; Com, composite sample site; Ped, pediatrics ward

The AMR patterns among GNB isolated from TASH IS varied widely. For instance, 100% (1/1) of *A. Iwoffii* demonstrated resistance to meropenem. Similarly, all (100%; 1/1) of *R. ornithinolytica* showed resistance to gentamicin, ceftazidime, aztreonam, meropenem, trimethoprim/sulfamethoxazole, and nitrofurantoin. Meanwhile, (50%; 1/2) of *A. baumannii* exhibited resistance to both ceftazidime and meropenem. Among GPB isolates, all of *S. aureus* (100%; 2/2), *S. caprae* (100%; 2/2) and *S. epidermidis* (100%; 3/3) showed resistance to both oxacillin and vancomycin. All of *S. caprae* and *S. epidermidis* showed resistance to cefoxitin, and also all of *S. caprae* demonstrated resistance to clindamycin. Both oxacillin and vancomycin showed the highest level of resistance (100%; 7/7) among the tested antimicrobials (Table 10).

Table 10. AMR profiles of bacterial isolates from TASH IS samples

Bacteria	Antimicrobial panels and AMR status (%)												
Gram negative	AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	SXT	NIT	AK	FOX	MARI
E. coli (n = 7)	28.6	14.3	14.3	28.6	28.6	42.9	14.3	-	14.3	14.3	NA	NA	0.2
R. ornithinolytica (n = 1)	NA	-	100	100	100	100	-	-	100	100	NA	NA	0.7
K.oxytoca (n = 1)	NA	-	-	-	-	-	-	-	-	-	-	-	0
E. cloacae (n = 6)	NA	50	16.7	33.3	33.3	33.3	33.3	33.3	-	-	NA	NA	0.3
L.adecarboxylata (n = 1)	-	-	-	-	-	-	-	-	-	-	NA	NA	0
A. baumannii (n = 2)	NA	-	-	50	NA	50	-	-	-	NA	-	50	0.2
A. Iwoffii (n = 1)	NA	-	-	-	NA	100	-	-	-	NA	100	-	0.2
E.asburiae (n = 1)	NA	-	-	-	-	-	-	-	-	-	NA	NA	0
Total (n = 20)	10	20	15	30	25	40	15	10	10	10	5	5	
Gram positive	GEN	CIP	TE	SXT	NIT	OX	FOX	ERY	NOR	CD		VAN	MARI
S. aureus (n = 2)	-	-	-	-	50	100	50	-	-	-		100	0.3
S. caprae (n = 2)	-	50	50	50	-	100	100	-	-	100		100	0.5
S. epidermidis (n = 3)	-	-	33.3	-	-	100	100	66.7	33.3	-		100	0.6
Total (n = 7)	-	14.3	28.6	14.3	14.3	100	85.7	28.6	14.3	28.6		100	

Among TASH IS isolates, those from the neonates intensive care unit (room 1) exhibited the highest level of MDR, demonstrating MAR index of 0.7, followed by isolates from operation room (room 2) and pediatrics room (room 2), which each accounted for MAR index of 0.6 as showed in Figure 21 (see Appendix 5).

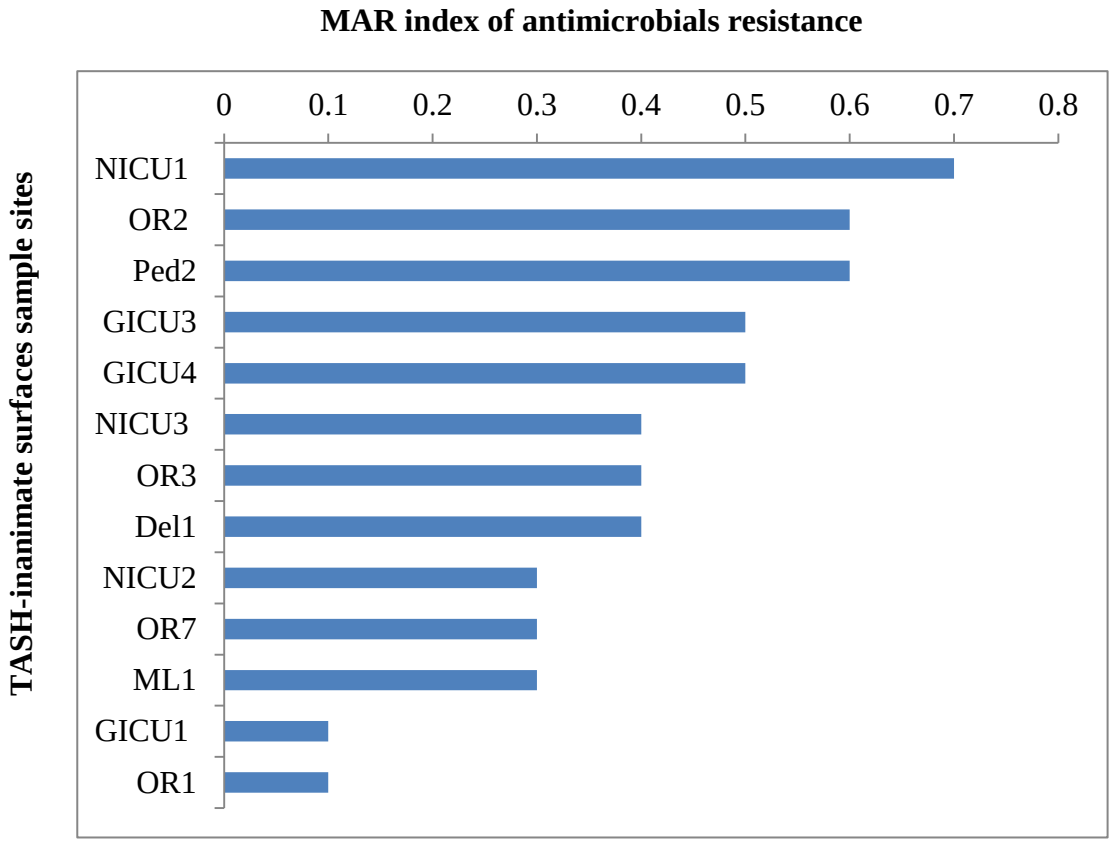

Figure 21. Contamination of TASH wards by antimicrobial resistance bacterial isolates from inanimate surface samples.

Key: NICU1, neonatal intensive care unit room 1; NICU2, neonatal intensive care unit room 2; NICU3, neonatal intensive care unit room 3; OR1, operation room 1; OR2, operation room 2; OR3, operation room 3; OR7, operation room 7; GICU1, general intensive care unit room 1; GICU2, general intensive care unit room 2; GICU3, general intensive care unit room 3; GICU4, general intensive care unit room 4; Ped1, pediatrics room 1; Ped2, pediatrics room 2; Del1, delivery room 1; Del2, delivery room 1; ML1, main laboratory unit room 1; ML2, main laboratory unit room 2.

4.3.2. Antimicrobial Resistance Profiles and MARI Scores of Bacterial Isolates from ZMH Wastewaters and Inanimate Surfaces

Among the ZMH WW GNB isolates, 100% (1/1) of *C. freundii* showed resistance to amikacin and cefotaxime. Similarly, 100% (1/1) of *K. oxytoca* and 100% (2/2) of *E. cloacae* exhibited resistance to meropenem, while 50% (2/4) of *E. coli* demonstrated resistance to meropenem. Among GPB isolates, 100% (1/1) of *S. aureus* showed resistance to tetracycline, nitrofurantoin, and oxacillin marking the most predominant profile, and 33.3% (1/3) of *E. faecalis* demonstrated resistance to both gentamicin and doxycycline as detailed in Table 11.

Among the isolates from the ZMH WW drainage system, those from the medical ward drainage showed the highest frequency of MDR with MAR index of 0.6, followed by the composite drainage site, which exhibited MAR index of 0.3 as depicted in Figure 22 (see Appendix **6**).

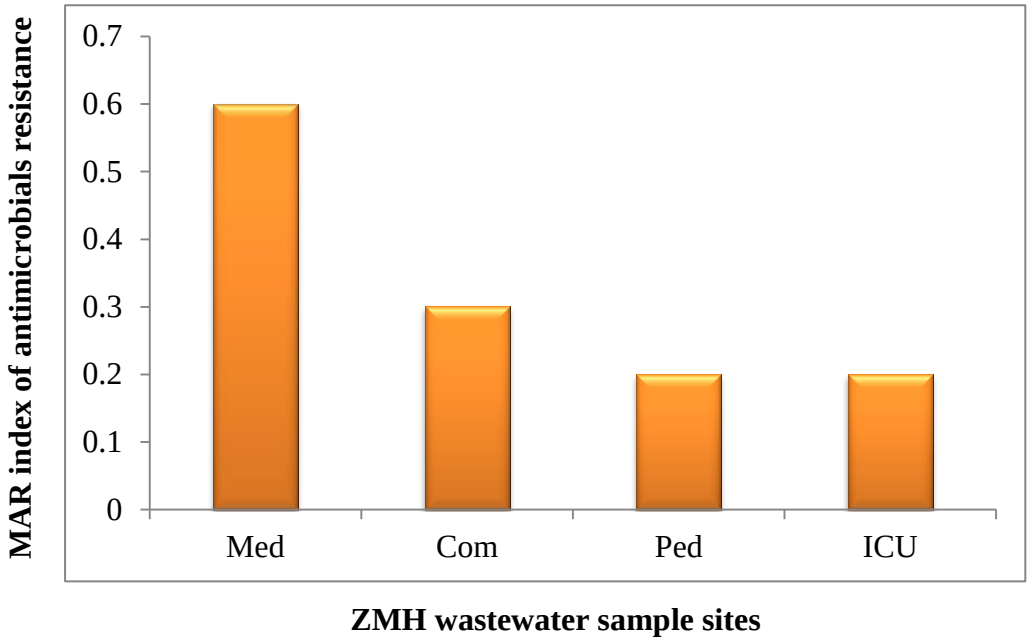

Figure 22. Percentage contamination of ZMH wards by antimicrobial resistance bacterial isolates from wastewater samples.

Key: Med, medical ward; Ped, pediatrics ward; ICU, intensive care unit ward; Com, composite

Table 11. Antimicrobial resistance profiles of bacteria from ZMH WW samples

Bacteria	Antimicrobial panels and AMR status (%)													MARI
Gram negative	AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	SXT	NIT	AK	CXM	FOX	
C. freundii (n = 1)	NA	-	NA	-	-	-	-	NA	-	NA	100	-	100	0.2
K. oxytoca (n = 1)	NA	-	-	-	-	100	-	-	-	-	NA	-	-	0.1
E. coli (n = 4)	50	25	-	25	25	50	-	-	-	50	NA	-	NA	0.2
E. cloacae (n = 2)	NA	-	-	50	50	100	-	50	-	50	NA	-	NA	0.3
P. aeruginosa (n = 1)	NA	100	-	-	-	-	-	NA	NA	NA	-	NA	100	0.3
Total (n = 9)	22.2	11.1	-	33.3	33.3	55.6	-	11.1	-	33.3	11.1	-	22.2	
Gram positive	GEN	CIP	TE	SXT	NIT		OX		ERY	NOR	DOX	VAN		
S. aureus (n = 1)	-	-	100	-	100		100		-	-	-	100		0.4
E. faecalis (n = 3)	33.3	-	-	-	-		-		-	-	33.3	-		0.1
Total (n = 4)	25	-	15	-	15		25		-	-	25	25		

Among GNB isolates from IS of ZMH, 100% (1/1) of *P. aeruginosa* and *E. hormaechei* showed resistance to meropenem and tetracycline, respectively; whereas, half (50%; 1/2) of *K. pneumoniae* exhibited resistance to piperacillin, gentamicin, aztreonam, meropenem, ciprofloxacin, tetracycline, nitrofurantoin and cefotaxime. The 66.7% (4/6) of *E. coli* demonstrated resistance to ampicillin; whereas, 50% (3/6) exhibited resistance to ceftazidime, aztreonam, meropenem and nitrofurantoin. The 80% (4/5) of *E. cloacae* showed resistance to meropenem, while 60% (3/5) of them exhibited resistance to aztreonam. Among GPB isolates, 75% (3/4) of *S. aureus* showed resistance to nitrofurantoin and oxacillin; all 100% (1/1) of *S. epidermidis* demonstrated resistance to oxacillin and vancomycin. All 100% (1/1) of *S. haemolyticus* showed resistance to oxacillin. All 100% (2/2) of *E. faecalis* exhibited resistance to ampicillin, 50% (1/2) to tetracycline, nitrofurantoin, erythromycin, vancomycin). Half (50%; 1/2) of *E. faecium* showed resistance to ampicillin, tetracycline, nitrofurantoin, norfloxacin and vancomycin (Table 12).

In the study conducted at ZMH, the most prevalent sources of IS contamination by ARB were the pediatrics room 2 with MAR index of 0.7, followed by delivery room 1 with MAR index of 0.6 as demonstrated in Figure 23 (see Appendix 7).

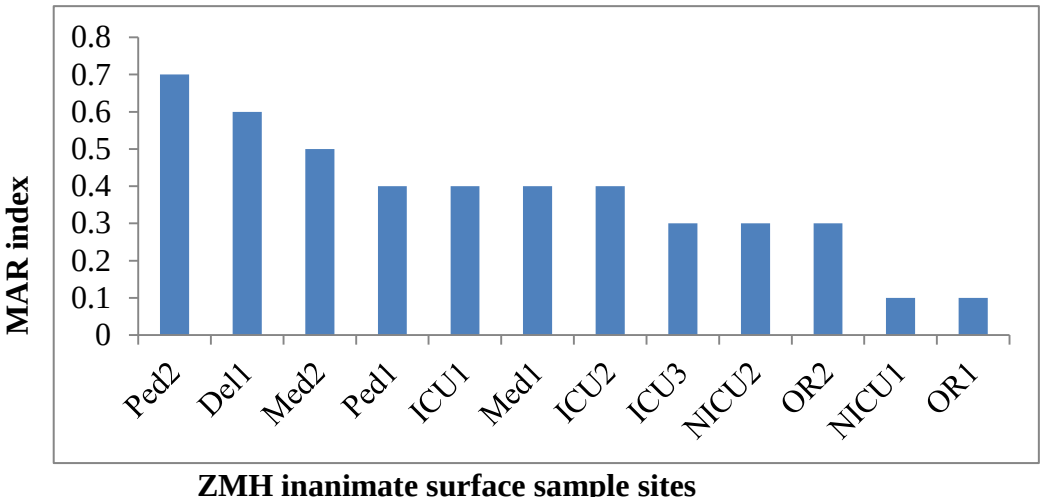

Figure 23. Percentage contamination of ZMH wards by antimicrobial resistance bacterial isolates from inanimate surface samples.
Key: ICU1, intensive care unit room 1; ICU2, intensive care unit room 2; ICU3, intensive care unit room 3; NICU1, neonatal intensive care unit room 1; NICU2, neonatal intensive care unit room 2; Del1, delivery room 1; OR1, operation room 1; OR2, operation room 2; Ped1, pediatrics room 1; Ped2, pediatrics room 2; Med1, medical ward room 1

Table 12. Antimicrobial resistance profiles of bacterial isolates from ZMH IS samples

Bacteria	Antimicrobial panels and AMR status (%)												
Gram negative bacteria	AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	SXT	NIT	CXM	FOX	MARI
K. pneumoniae (n = 2)	NA	50	50	100	100	100	100	50	-	50	-	50	0.6
E. coli (n = 6)	66.7	16.7	-	50	50	50	16.7	33.3	16.7	50	-	NA	
E. hormaechei (n = 1)	NA	-	-	-	-	-	-	100	-	-	-	NA	0.1
E. cloacae (n = 5)	NA	20	20	20	60	80	40	20	20	20	40	NA	0.3
P. aeruginosa (n = 1)	NA	-	-	-	-	100	-	NA	NA	NA	NA	-	0.1
Total (n= 15)	26.7	20	13.3	40	53.3	66.7	33.3	33.3	13.3	33.3	13.3	6.7	
Gram positive	AMP	GEN	CIP	TE	SXT	NIT	OX	ERY	NOR	VAN			
S. aureus (n = 4)	NA	25	-	25	25	75	75	50	25	50			0.4
S. epidermidis (n = 1)	NA	-	-	-	-	-	100	-	-	100			0.2
S. haemolyticus (n = 1)	NA	-	-	-	-	-	100	-	-	-			0.1
E. faecium (n = 2)	50	NA	-	50	NA	50	NA	-	50	50			0.4
E. faecalis (n = 2)	50	NA	-	50	NA	50	NA	50	-	50			0.4
Total (n= 10)	20	10	-	30	10	50	50	30	20	50			

4.3.3. Spatial Distribution and Comparative Analysis of Environmental MDR Hotspots

The bacteria are said to be MDR when resistant to 3 or more than 3 antimicrobial classes. For instance, *K. pneumoniae* showed resistance to 8 antimicrobial classes: carbapenem (meropenem), the third generation cephalosporin (ceftazidime), second-generation cephalosporin (cefuroxime), monobactam (aztreonam), quinolones-fluoroquinolones (CIP), penicillin (PI) and tetracycline (TE). Among GNB, 24.5% (13/53) showed a susceptible profile, 20.8% (11/53) exhibited resistance to one antimicrobial class, 19% (10/53) demonstrated resistance to two antimicrobial classes, and 35.8% (19/53) were MDR. Among GNB, 100% of *K. pneumoniae* (3/3) and *R. ornithinolytica* (1/1) demonstrated MDR profile; whereas, 36.4% (8/22) of *E. coli* and 50% (7/14) of *E. cloacae* were MDR. Among GPB, none showed a fully susceptible profile. A 13.6% (3/22) of the isolates exhibited resistance to a single and dual antimicrobial class, each, while 72.7% (16/22) demonstrated MDR profile. All, 100% (2/2) of *S. caprae* and 89% (8/9) of *S. aureus* showed MDR profile (Table 13).

As demonstrated in Table 14, TASH demonstrated a higher prevalence of MDR (25.3%; 19/75) than ZMH (22.7%; 17/75).

Table 13. Multidrug resistance profiles of bacterial isolates at TASH and ZMH.

Bacteria	AMR status (%)			
Gram negative	R0	R1	R2	MDR
C. freundii (n = 1)	-	-	100	-
K. pneumoniae (n = 3)	-	-	-	100
K. oxytoca (n = 4)	25	50	25	-
E. coli (n = 22)	27.3	27.3	9.1	36.4
L. adecarboxylata (n = 1)	100	-	-	-
E. asburiae (n = 1)	100	-	-	-
E. hormaechei (n = 1)	-	100	-	-
R. ornithinolytica (n = 1)	-	-	-	100
E. cloacae (n = 14)	28.6	-	21.4	50
P. aeruginosa (n = 2)	-	50	50	-
A. baumannii (n = 2)	-	50	50	-
A. lwoffii (n = 1)	-	-	100	-
Total (n= 53)	24.5	20.8	19	35.8
Gram positive	R0	R1	R2	MDR
S. aureus (n = 9)	-	11.1	-	89
S. caprae (n = 2)	-	-	-	100
S. epidermidis (n = 5)	-	-	20	80
S. haemolyticus (n = 1)	-	100	-	-

E. faecium (n = 2)	-	-	50	50
E. faecalis (n = 3)	-	33.3	33.3	33.3
Total (n= 22)	-	13.6	13.6	72.7

Abbreviations: R0, susceptible to all tested antimicrobials; R1, resistant to 1 antimicrobial class; R2, resistant to 2 antimicrobials class; MDR, multidrug resistance.

Table 14. Spatial distribution of multidrug resistance bacterial isolates across TASH and ZMH

Bacterial isolates	Non MDR	MDR	
		TASH	ZMH
C. freundii (n = 1)	+	-	-
K. pneumoniae (n = 3)	-	+	66.7
K. oxytoca (n = 4)	100	-	-
E. coli (n = 22)	63.6	18.2	18.2
L. adecarboxylata (n = 1)	+	-	-
E. asburiae (n = 1)	+	-	-
E. hormaechei (n = 1)	+	-	-
R. ornithinolytica (n = 1)	-	+	-
E. cloacae (n = 14)	43	28.5	28.5
P. aeruginosa (n = 2)	100	-	-
A. baumannii (n = 2)	100	-	-
A. lwoffii (n = 1)	+	-	-
S. aureus (n = 9)	+	33.3	55.7
S. caprae (n = 2)	-	100	-
S. epidermidis (n = 5)	+	80	-
S. haemolyticus (n = 1)	+	-	-
E. faecium (n = 2)	+	-	+
E. faecalis (N = 3)	66.7	-	+
Total (n =75)	39 (52)	19 (25.3)	17 (22.7)

Among the TASH sample types, IS showed the most frequency of GNB MDR isolates (24.1%; 7/29) than the WW (13.8%; 4/29). Similarly, comparing sample types for GPB, IS showed higher MDR frequency (70%; 7/10) than the WW (20%; 2/10) as depicted in Table 15.

Table 15. Multidrug resistance profiles of bacterial isolates from WW and IS at TASH

Bacteria	MDR status (%)		Non-MDR
Gram negative	WW	IS	
K. pneumoniae (n = 1)	100	-	-
K.oxytoca (n = 3)	-	-	100

L. adecarboxylata (n = 1)	-	-	100
E.asburiae (n = 1)	-	-	100
A.baumannii (n = 2)	-	-	100
A. lwoffii (n = 1)	-	-	100
E. coli (n = 12)	16.7	25	58.3
R. ornithinolytica (n= 1)	-	100	-
E. cloacae (n = 7)	14.3	42.9	42.9
Total (n = 29)	13.8	24.1	62
Gram positive			
S. aureus (n = 4)	25	50	25
S. caprae (n = 2)	-	100	-
S. epidermidis (n = 4)	25	75	-
Total (n = 10)	20	70	10

Abbreviation: WW, wastewater; IS, inanimate surface.

Among GNB isolates of ZMH IS, 100% (2/2) of *K. pneumoniae*, 42.9% (3/7) of *E. cloacae* showed MDR profile. Similarly, among GPB isolates, 80% (4/5) of *S. aureus* and 50% (1/2) of *E. faecium* demonstrated MDR profile (Table 16).

Table 16. Multidrug resistance profiles of bacterial isolates from WW and IS at ZMH

Bacteria	MDR status (%)		
Gram negative	WW	IS	Non-MDR
C. freundii (n = 1)	-	-	100
K. pneumoniae (n = 2)	-	100	-
K. oxytoca (n = 1)	-	-	100
E. coli (n = 10)	10	30	60
E. cloacae (n = 7)	14.3	42.9	42.8
E. hormaechei (n = 1)	-	-	100
P. aeruginosa (n = 2)	-	-	100
Total (n = 24)	8.3	33.3	58.4
Gram positive			
S. aureus (n = 5)	20	80	-
S. epidermidis (n = 1)	-	-	100
S. haemolyticus (n = 1)	-	-	100
E. faecium (n = 2)	-	50	50
E. faecalis (n = 3)	-	33.3	66.7
Total (n = 29)	3.4	20.7	17.2

4.3.4. Double Disk Synergy Test (DDST) for ESBL Confirmation

Extended-spectrum β-lactamases production was tested by the DDST using ceftazidime and amoxicillin/clavulanic acid. The synergy created a characteristic keyhole or champagne cork shape as depicted in Figure 24.

Among the tested bacteria for ESBL production, all of *K. pneumoniae* (100%; 3/3), *R. ornithinolytica* (100%; 1/1) and *C. freundii* (100%; 1/1), half of *P. aeruginosa* (50%; 1/2), *A. baumannii* (50%; 1/2), *K. oxytoca* (50%; 2/4), and *E. cloacae* (35.7%; 5/14) and *E. coli* (31.8%; 7/22) showed positive result (Figure 25).

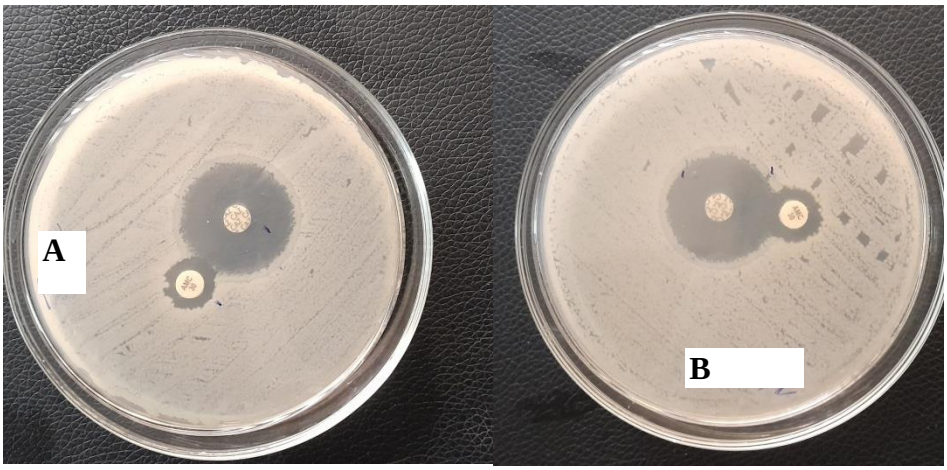

Figure 24. Double disc synergy test.

The figure illustrates the synergy between amoxicillin/clavulanic acid and ceftazidime confirming the presence of extended-spectrum beta-lactamases.

The letter 'A' depicts E. cloacae and 'B' shows E. coli.

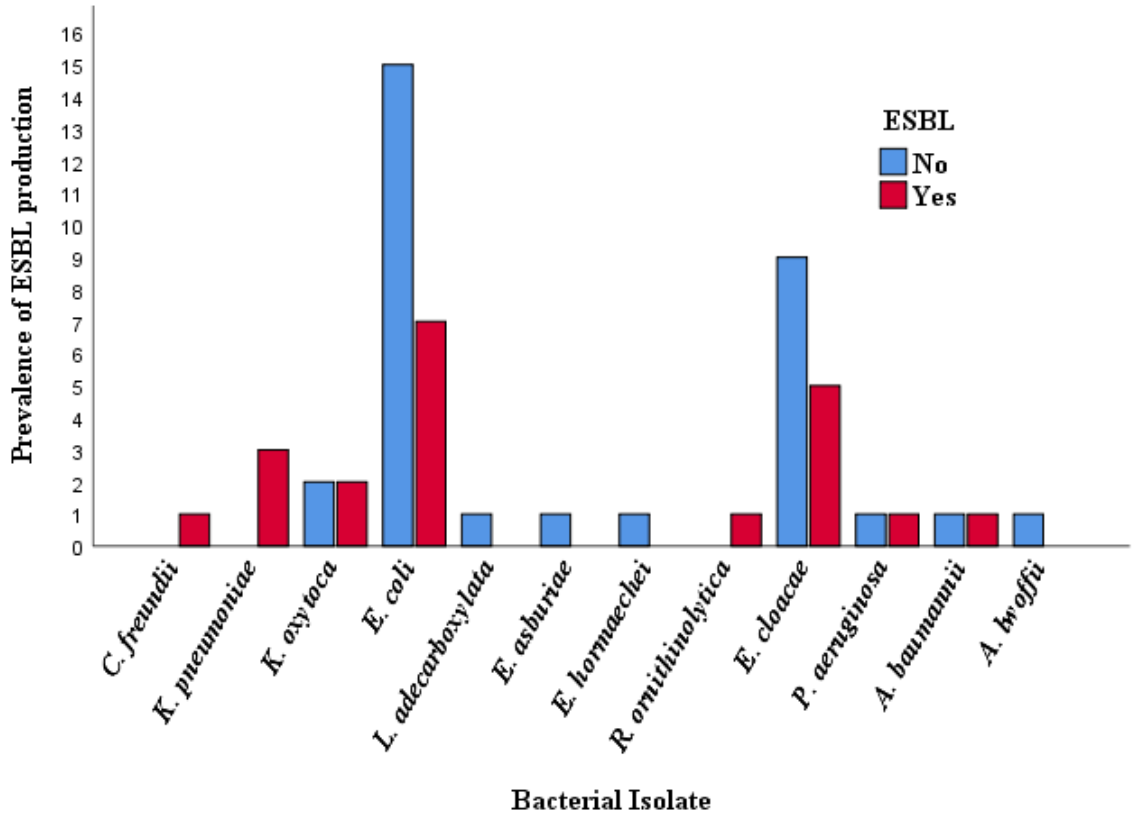

Figure 25. Phenotypic test for ESBL production among GNB isolates.

The graph demonstrates the distribution of ESBL-producing (red) and non-producing (blue) isolates.

Bacterial isolates such as *L. adecarboxylata*, *E. asburiae*, and *E. hormaechei* showed negative phenotypic ESBL production. The overall prevalence of ESBL-positive bacterial isolates was 37.7% (20/53) (Table 17).

Table 17. Prevalence of ESBL-producing isolates based on double-disk synergy test results

	Phenotypic ESBL production (%)	
Bacterial Isolate	No	Yes
C. freundii (n = 1)	100	-
K. pneumoniae (n = 3)	-	100
K. oxytoca (n = 4)	50	50
E. coli (n = 22)	68.2	31.8
L. adecarboxylata (n = 1)	100	-
E. asburiae (n = 1)	100	-
E. hormaechei (n = 1)	100	-
R. ornithinolytica (n = 1)	-	100
E. cloacae (n = 14)	64.3	35.7

P. aeruginosa (n = 2)	50	50
A. baumannii (n = 2)	50	50
A. lwoffii (n = 1)	100	-
Total (n = 53)	62.3	37.7

4.4. Molecular Detection of Antimicrobial Resistance Genes

A total of 92 bacterial isolates were subjected to ARGs detection. Multiplex PCR and sPCR result revealed the presence of *blaCTXM*, *blaTEM*, *blaCITM*, *blaEBCM*, *blaFOXM*, *blaMOXM*, *blaDHAM*, *blaKPC*, *blaNDM*, *blaOXA-24/143* group, *blaOXA-48*, *ermB*, *tetA* and *sul1*. The *blaSHV*, *blaPER*, *blaGES*, *blaVEB*, *blaACCM*, *blaVIM*, *blaIMP*, *blaOXA-58* group, *blaOXA-23* group, *blaGIM*, *blaAIM*, *blaSIM*, *blaDIM* were not detected. The PCR products are depicted in Figure 26.

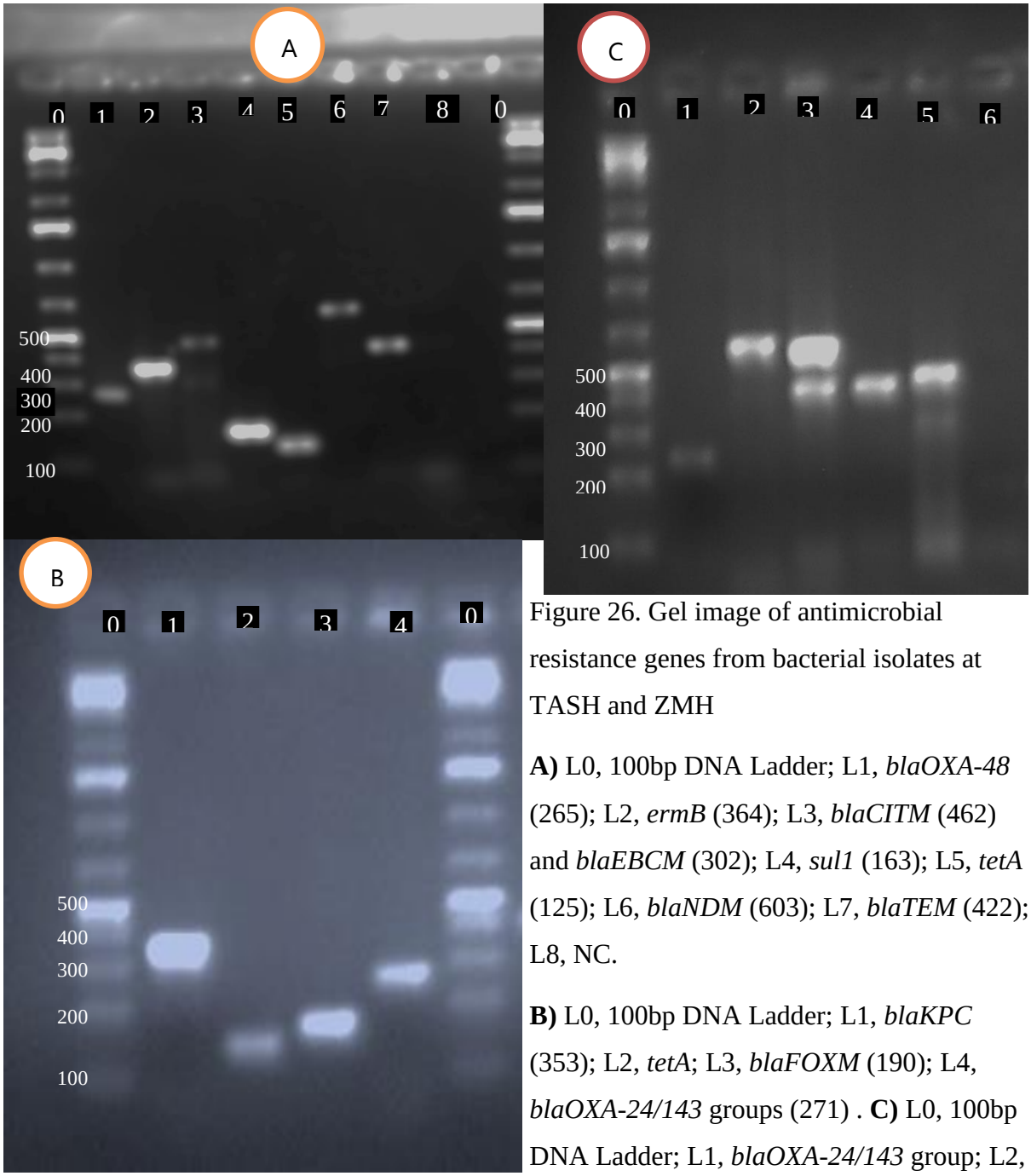

Figure 26. Gel image of antimicrobial resistance genes from bacterial isolates at TASH and ZMH

A) L0, 100bp DNA Ladder; L1, *blaOXA-48* (265); L2, *ermB* (364); L3, *blaCITM* (462) and *blaEBCM* (302); L4, *sul1* (163); L5, *tetA* (125); L6, *blaNDM* (603); L7, *blaTEM* (422); L8, NC.

B) L0, 100bp DNA Ladder; L1, *blaKPC* (353); L2, *tetA*; L3, *blaFOXM* (190); L4, *blaOXA-24/143* groups (271) . **C)** L0, 100bp DNA Ladder; L1, *blaOXA-24/143* group; L2, *blaCTXM* (590); L3, *blaMOXM* (520) and *blaDHAM* (405); L4, *blaTEM*; L5, *blaCITM* and *blaEBCM*; L6, NC.

Notes: L, lane; NC, negative control.

4.4.1. Chromosomal-Mediated AMR from TASH Wastewaters and Inanimate Surfaces

Among TASH WW isolates, *blaTEM* was the most predominant ESBL gene (81.3%; 13/16), followed by *blaCTXM* (37.5%; 6/16). This *blaTEM* gene was detected in all of *K. pneumoniae* (100%; 1/1), *K. oxytoca* (100%; 2/2), *E. coli* (100%; 5/5), *S. aureus* (100%; 2/2) and *B. flexus* (100%; 1/1) isolates. Additionally, *blaCTXM* was detected in *K. pneumoniae* (100%; 1/1), *K. oxytoca* (100%; 2/2) and *S. epidermidis* (100%; 1/1) species.

Regarding AmpC genes, both *blaCITM* and *blaFOXM* were detected in 25% (4/16) and 18.8% (3/16) of isolates, respectively. Among the CP genes, only *blaOXA-48* was isolated in 6.3% (1/16). The sulfonamide resistance gene, *sul1* was highly frequent, identified in 37.5% (6/16) of the isolates; whereas, the tetracycline resistance gene, *tetA*, and the macrolide resistance gene, *ermB*, were each detected at a frequency of 18.8% (3/16) within the total hospital WW isolate (Table 18).

Table 18. Prevalence of chromosomal-mediated ARGs in WW samples of TASH

Bacteria	Antimicrobial resistance genes, n (%)							
	blaCTXM	*blaTEM*	*blaCITM*	*blaFOXM*	*blaOXA-48*	*ermB*	*tetA*	*sul1*
K.pneumoniae (n = 1)	+	+	-	-	+	-	-	-
K. oxytoca (n = 2)	2 (100)	2 (100)	-	-	-	-	-	-
E. coli (n = 5)	2 (40)	5 (100)	3 (60)	3 (60)	-	3 (60)	3 (60)	3 (60)
E. cloacae (n = 1)	-	+	+	-	-	-	-	+
S. aureus (n = 2)	-	2 (100)	-	-	-	-	-	+
S. epidermidis (n = 1)	+	+	-	-	-	-	-	+
B. flexus (n = *2)*	-	+	-	-	-	-	-	-
B. cereus (n = 2)	-	-	-	-	-	-	-	-
Total (n = 16)	6 (37.5)	13 (81.3)	4 (25)	3(18.8)	1 (6.3)	3 (18.8)	3 (18.8)	6 (37.5)

Key: -, shows 0; +, represents 1.

Regarding the spatial distribution of chromosomal-mediated ARGs within the TASH WW drainage, emergency ward sample sites was the primary source, accounting for 28.2% (11/39), followed by ICU sample site, accounting for 25.6% (10/39) of the detected ARGs as showed in Figure 27 (see Appendix 11).

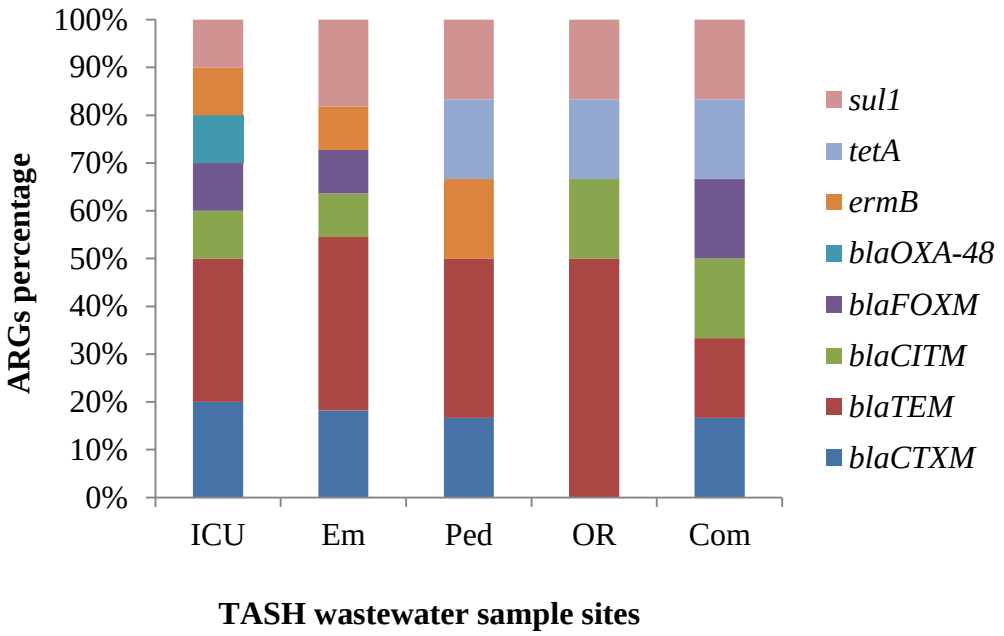

Figure 27. Chromosomal mediated ARGs abundance and distribution across TASH wastewater samples.

The figure depicts the chromosomal mediated ARGs counts per of the TASH wastewater sample locations.

ICU, intensive care unit; OR, operation ward; Em, emergency ward; Com, composite

Analysis of 32 bacterial isolates recovered from IS at TASH revealed the most prevalence of ESBL gene *blaTEM* (94%; 30/32), followed by *blaCTXM* (12.5%; 4/32). Additionally, *blaCITM* (25%; 8/32) and *blaFOXM* (6.3%; 2/32) were identified indicating that hospital IS serve as significant reservoirs for both ESBL and AmpC-producing bacteria. Notably, the CP genes were higher on IS with the detection of *blaOXA-48* (15.6%; 5/32) and *blaNDM* (12.5%; 4/32) than WW isolates where only *blaOXA-48* (15.6%; 5/32) was detected. The CP genes, *blaNDM* and *blaOXA-48* were detected in *E. cloacae*, constituting (66.7%; 4/6) and (83.3%; 5/6), respectively. The rare emerging bacteria, *L. adecarboxylata* and *R. ornithinolytica* were positive for *blaTEM* (100%; 1/1) and *sul1* (100%; 1/1).

Additionally, all *E. asburiae* harbored *blaTEM* and *tetA* genes. The *A. baumannii* was confirmed to host *blaTEM* (100%; 2/2) and *sul1* (50%; 1/2). All of *A. Iwoffii* also detected for *blaTEM* and *sul1* resistance genes. The *blaTEM* gene was detected in all of *B. flexus* (100%; 1/1) and *U. massiliensis* (100%; 1/1). Additionally, bacterial isolates showed high carriage rates for *sul1* (53%; 17/32), *tetA* (37.5%; 12/32), and *ermB* (6.3%; 2/32). When ranking all detected ARGs, *sul1* was identified as the second most dominant, surpassing only by *blaTEM*. Among the isolates, *E. coli* was identified as the primary reservoir for ARGs on IS at TASH, harboring *blaTEM* (100%; 7/7), *blaCTXM* (14.3%; 1/7), *blaCITM* (71%; 5/7), *blaFOXM* (28.6%; 2/7), *ermB* (28.6%; 2/7), *tetA* (71%; 5/7) and *sul1* (57%; 4/7). The *E. cloacae* was also identified as a common reservoir for ARGs on IS at TASH, harboring *blaTEM* (100%; 6/6), *blaCITM* (50%; 3/6), *blaNDM* (66.7%; 4/6), *blaOXA-48* (83.3%; 5/6), *tetA* (83.3%; 5/6), and *sul1* (50%; 3/6) (Table 19).

Table 19. Prevalence of chromosomal-mediated antimicrobial resistance genes (ARGs) in IS samples from TASH

Bacterial isolates	Antimicrobial resistance genes, n (%)								
	blaCTXM	*blaTEM*	*blaCITM*	*blaFOXM*	*blaNDM*	*blaOXA-48*	*ermB*	*tetA*	*sul1*
K. oxytoca (n = 1)	+	+	-	-	-	-	-	+	-
E. coli (n = 7)	+	7 (100)	5 (71)	2 (28.6)	-	-	2 (28.6)	5 (71)	4 (57)
L. adecarboxylata (n = 1)	-	+	-	-	-	-	-	-	+
E. asburiae (n = 1)	-	+	-	-	-	-	-	+	-
R. ornithinolytica (n = 1)	-	+	-	-	-	-	-	-	+
E. cloacae (n = 6)	-	6 (100)	3 (50)	-	4 (66.7)	5 (83.3)	-	5 (83.3)	3 (50)
A. baumannii (n = 2)	-	2 (100)	-	-	-	-	-	-	+
A. Iwoffii (n = 1)	-	+	-	-	-	-	-	-	+
S. aureus (n = 2)	-	2 (100)	-	-	-	-	-	-	+
S. caprae (n = 2)	-	2 (100)	-	-	-	-	-	-	2 (100)
S. epidermidis (n = 3)	2 (66.7)	3 (100)	-	-	-	-	-	-	3 (100)
B. cereus (n = *3*)	-	+	-	-	-	-	-	-	-
B. flexus (n = 1)	-	+	-	-	-	-	-	-	-
U. massiliensis (n = 1)	-	+	-	-	-	-	-	-	-
Total (n = 32)	4 (12.5)	30 (94)	8 (25)	2 (6.3)	4 (12.5)	5 (15.6)	2 (6.3)	12 (37.5)	17 (53)

The main laboratory unit (room 1) emerged as the primary environmental hotspots for ARGs at TASH IS contributing 14.3% (12/84), followed by delivery room 1 accounting for 13.1% (11/84) of the detected ARGs as depicted in Figure 28. The concentration of ARGs in these specific wards suggests a high risk for HAIs in vulnerable and immunocompromised patient populations (see Appendix 12).

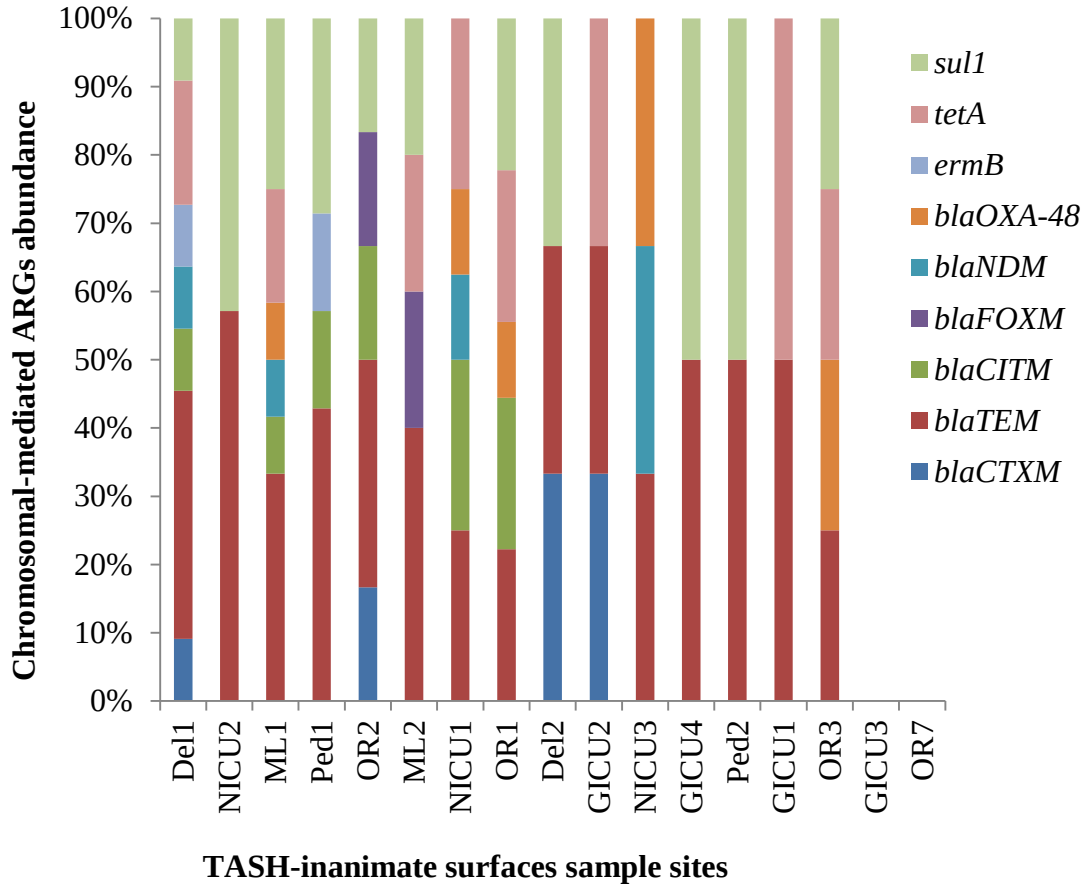

Figure 28. Spatial chromosomal-mediated antimicrobial resistance genes (ARGs) profile across TASH IS samples.

Key: NICU1, neonatal intensive care unit room 1; NICU2, neonatal intensive care unit room 2; NICU3, neonatal intensive care unit room 3; OR1, operation room 1; OR2, operation room 2; OR3, operation room 3; OR7, operation room 7; GICU1, general intensive care unit room 1; GICU2, general intensive care unit room 2; GICU3, general intensive care unit room 3; GICU4, general intensive care unit room 4; Ped1, pediatrics room 1; Ped2, pediatrics room 2; Del1, delivery room 1; Del2, delivery room 1; ML1, main laboratory unit room 1; ML2, main laboratory unit room 2.

Among 48 bacterial isolates from WW and IS of TASH, the ESBL marker *blaTEM* was identified at 94% rates in IS and at 81.3% in WW samples, while *blaCTXM* was more prevalent in WW (37.5%) than on surfaces (12.5%). Carbapenemase genes followed a similar trend toward surface accumulation, with *blaNDM* detected in 12.5% of IS isolates and lacked in WW; whereas, *blaOXA-48* was 15.6% and 6.3% (IS vs. WW). Prevalence of the *sul1* gene was higher in isolates from IS (53%) than in those from WW (37.5%). Overall, the study confirms that the hospital surface environment serves as a primary reservoir for resistance genes than its effluent.

4.4.2. Chromosomal-Mediated AMR Analysis from ZMH Wastewaters and Inanimate Surfaces

In ZMH WW isolates, *blaTEM* reached a near-universal prevalence of (92.3%; 12/13), followed by *blaCTXM* (15.4%; 2/13). The effluent also serves as a reservoir for multiple AmpC genes, most notably *blaCITM* (38.5%; 5/13) and *blaFOXM* (15.4%; 2/13), indicating a high potential for the environmental dissemination of extended-spectrum resistance. The AmpC profile also included *blaEBCM*, *blaMOXM* and *blaDHAM*, each of which was present in 7.7% (1/13) of the bacterial isolates. The carbapenemase group was dominated by *blaNDM* (23.1%; 3/13), followed by *blaOXA-48* (7.7%; 1/13). These two genes represent the major drivers of carbapenem resistance being discharged into the municipal sewage system from the facility. The tetracycline resistance gene, *tetA* (38.5%; 5/13) and sulphonamide resistance gene, *sul1* (30.8%; 4/13) were highly prevalent, suggesting that the hospital effluent is a significant source of MDR genes. These results highlight a significant environmental discharge of genes conferring resistance to ESBL genes, AmpC genes, CP genes, *tetA* gene and *sul1* genes. All of *P. aeruginosa* (100%; 1/1), *C. freundii* (100%; 1/1), *K. oxytoca* (100%; 1/1), *E. coli* (100%; 4/4), *E. cloacae* (100%; 2/2), *S. aureus* (100%; 1/1) and *E. faecalis* (100%; 1/1) isolates were found to harbor *blaTEM*, the ESBL producing gene. Additionally, (50%; 1/2) of *B. cereus* was *blaTEM* positive. All of *E. cloacae* and *C. freundii* were demonstrated the detection of *blaNDM*, carpanemase genes. *E. coli* was found to harbor *tetA* (75%; 3/4) and *sul1* (50%; 2/4) genes (Table 20).

Table 20. Prevalence of chromosomal-mediated ARGs in WW samples from ZMH

Bacterial isolate	Antimicrobial resistance genes, n (%)										
	blaCTX M	*blaTEM*	*blaCIT M*	*BlaEBC M*	*blaFOXM*	*blaMOX M*	*blaDHA M*	*blaNDM*	*blaOXA-48*	*tetA*	*sul1*
C. freundii (n = 1)	+	+	+	+	-	+	+	+	-	-	-
K. oxytoca (n = 1)	-	+	-	-	-	-	-	-	-	+	-
E. coli (n = 4)	+	4 (100)	2 (50)	-	2 (50)	-	-	-	-	3 (75)	2 (50)
E. cloacae (n = 2)	-	2 (100)	2 (100)	-	-	-	-	2 (100)	+	+	+
P. aeruginosa (n = 1)	-	+	-	-	-	-	-	-	-	-	-
S. aureus (n = 1)	-	+	-	-	-	-	-	-	-	-	+
E. faecalis (n = 1)	-	+	-	-	-	-	-	-	-	-	-
B. cereus (n = 2)	-	+	-	-	-	-	-	-	-	-	-
Total (n = 13)	2 (15.4)	12 (92.3)	5 (38.5)	1 (7.7)	2 (15.4)	1 (7.7)	1 (7.7)	3 (23.1)	1 (7.7)	5 (38.5)	4 (30.8)

The analysis of the ZMH WW drainage identified the medical ward as the primary source of ARGs, accounting for 33.3% (12/36); whereas, the pediatric ward WW sample represented the second largest reservoir constituting 30.6% (11/36) of the total detected ARGs as demonstrated in Figure 29 (see Appendix 13).

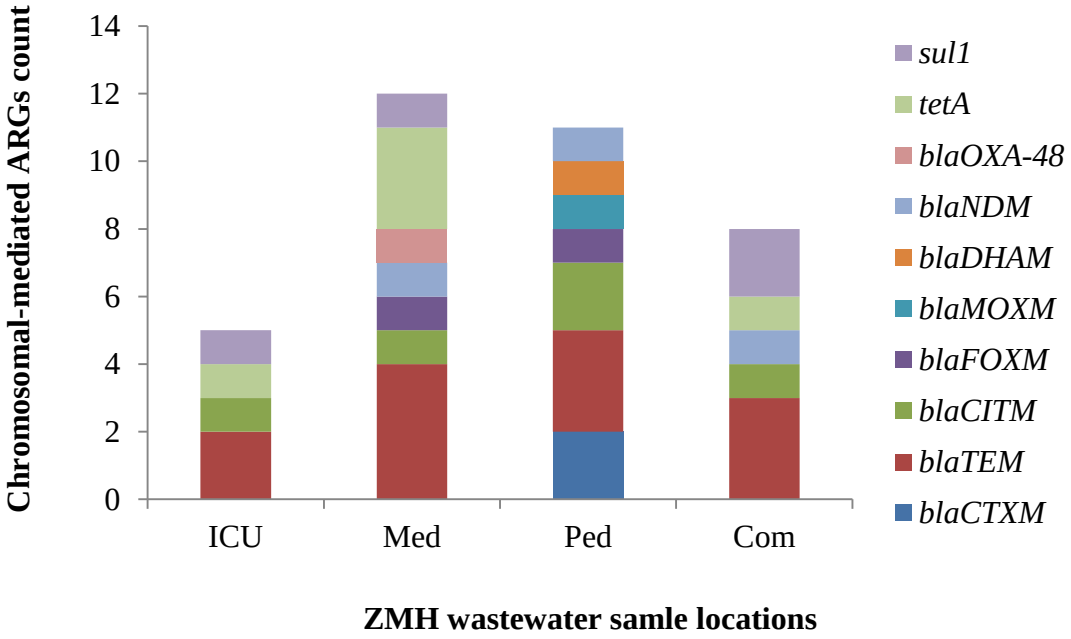

Figure 29. Spatial distribution of chromosomal-mediated ARGs across ZMH WW samples.

An evaluation of 31 bacterial isolates from ZMH IS revealed a high prevalence of *blaTEM* gene (84%; 26/31), followed by *blaCTXM* (13%; 4/31). The surfaces also harbored notable levels of the AmpC genes, *blaCITM* (22.6%; 7/31) and *blaFOXM* (13%; 4/31) of the isolates, respectively. The most frequently detected carbapenemase gene was *blaOXA-48* (9.7%; 3/31), followed by *blaNDM* (3%; 1/31). The ARGs such as *sul1* (32.3%; 10/31), *tetA* (16%; 5/31) and *ermB* (6.5%; 2/31) were identified from isolates. From ESKAPE pathogens, all of *K. pneumoniae* (100%; 2/2), *E. coli* (100%; 6/6), *P. aeruginosa* (100%; 1/1) and *S. aureus* (100%; 4/4) were detected for *blaTEM;* as well as all of *E. hormaechei* (100%; 1/1) was detected. The CoNS group, *S. epidermidis* (100%; 1/1) and *S. Haemolyticus* (100%; 1/1) were positive to *blaTEM* and *blaCTXM*. The *E. coli*, the most frequently isolated bacteria showed substantial resistance against most class of antimicrobials showing the range of resistance genes from 33.3%-100% to ESBL genes (*blaCTXM* and *blaTEM*), AmpC genes (*blaCITM* and *blaFOXM*), *ermB*, *tetA* and *sul1* (Table 21).

Table 21. Prevalence of chromosomal-mediated antimicrobial resistance genes (ARGs) in IS samples from ZMH

Bacterial isolate	Antimicrobial resistance genes, n (%)								
	blaCTXM	*blaTEM*	*BlaCITM*	*blaFOXM*	*blaNDM*	*blaOXA-48*	*ermB*	*TetA*	*sul1*
K. pneumoniae (n = 2)	-	2 (100)	-	-	-	-	-	-	-
E. coli (n = 6)	2 (33.3)	6 (100)	4 (66.7)	4 (66.7)	-	-	2 (33.3)	3 (50)	5 (83.3)
E. hormaechei (n = 1)	-	+	-	-	-	-	-	-	-
E. cloacae (n = 5)	-	5 (100)	3 (60)	-	+	3 (60)	-	2 (40)	3 (60)
P. aeruginosa (n = 1)	-	+	-	-	-	-	-	-	-
S. aureus (n = 4)	-	4 (100)	-	-	-	-	-	-	+
S. epidermidis (n = 1)	+	+	-	-	-	-	-	-	-
S. haemolyticus (n = 1)	+	+	-	-	-	-	-	-	+
E. faecium (n = 2)	-	2 (100)	-	-	-	-	-	-	-
E. faecalis (n = 2)	-	2 (100)	-	-	-	-	-	-	-
B. flexus (n = 1)	-	-	-	-	-	-	-	-	-
B. cereus (n = 5)	-	+	-	-	-	-	-	-	-
Total (n = 31)	4 (13)	26 (84)	7 (22.6)	4 (13)	1 (3)	3 (9.7)	2 (6.5)	5 (16)	10 (32.3)

The spatial evaluation of chromosomal-mediated ARG distribution across ZMH IS identified the intensive care unit (ICU) ward (room 2 and room 3), neonatal intensive care unit (room 2), and medical ward (room 1) as primary hotspots, with each site accounting for 11.5% (7/61) of the detected genes, highlighting these critical areas as significant environmental reservoirs as showed in Figure 30 (see Appendix 14).

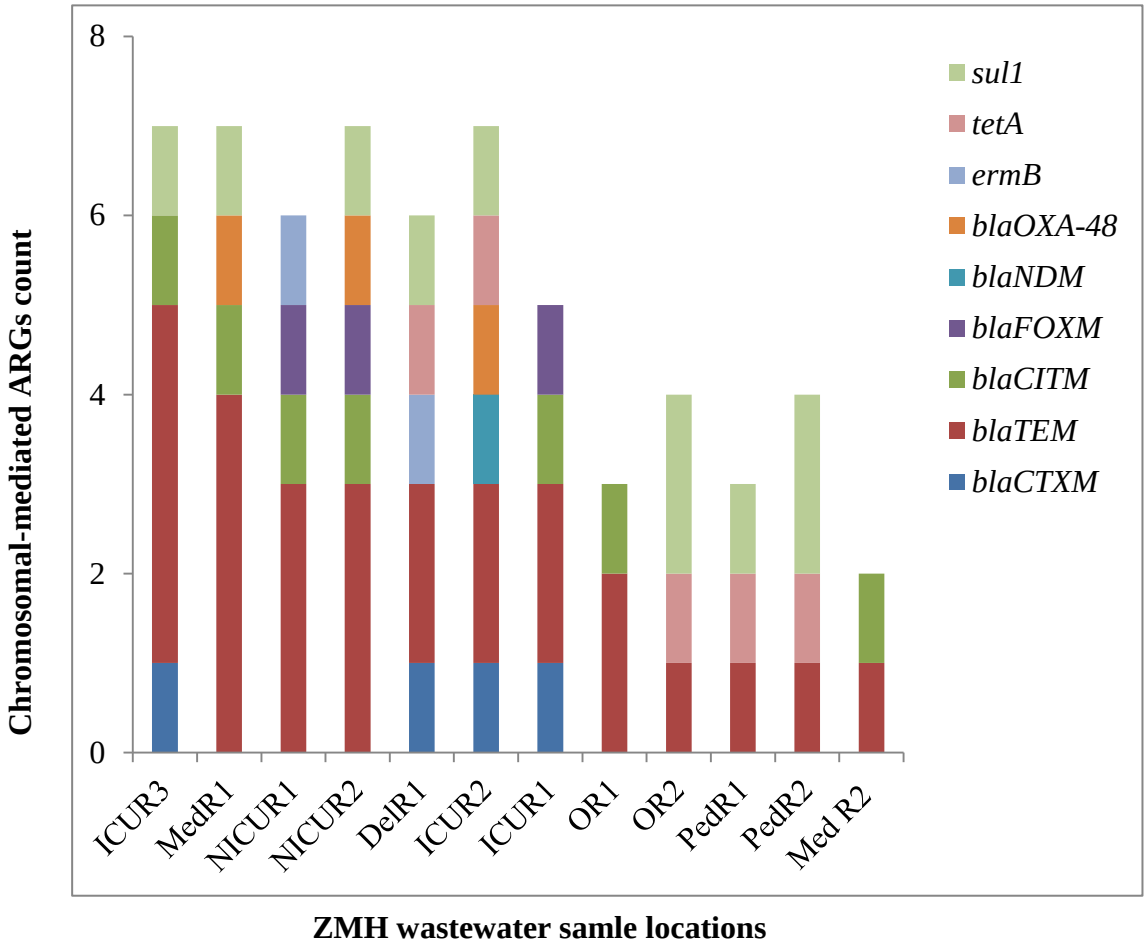

Figure 30. Spatial distribution of chromosomal-mediated ARGs across ZMH IS samples.

Key: ICU1, intensive care unit room 1; ICU2, intensive care unit room 2; ICU3, intensive care unit room 3; NICU1, neonatal intensive care unit room 1; NICU2, neonatal intensive care unit room 2; Del1, delivery room 1; OR1, operation Room 1; OR2, operation room 2; Ped1, pediatrics room 1; Ped2, pediatrics room 2; Med1, medical ward room 1; Med1, medical ward room 1.

Screening of 44 bacterial isolates from ZMH demonstrated varying prevalence of ARGs across WW and IS. Specifically, *blaCITM* was detected in *E. coli* (50% vs. 66.7%) in WW vs. IS, respectively, while *blaOXA-48* was detected in *E. cloacae* at 50% (WW) and 60% (IS). Notably, all (100%) of *S. aureus* isolates (100%) from both WW and IS harbored the *blaTEM* gene. Additionally, ARGs were detected in different frequencies across WW vs. IS: *blaCTXM* (15.4% vs. 13%), *blaTEM* (92.3% vs. 84%), *blaNDM* (23.1% vs. 3%) and *sul1* genes (30.8% vs. 32.3%). This highlights that eventhough their ARGs prevalence differed, both environmental matrices were significant habitat for ARBs.

4.4.3. Plasmid-Mediated Antimicrobial Resistance from TASH Hotspots

The plasmid-mediated resistance of TASH WW bacterial isolates was characterized by a high frequency of *blaTEM* (75%; 12/16) and *blaCTXM* (50%; 8/16) among ESBL genes. The presence of *blaCITM* (25%; 4/16) and low-frequency AmpC markers, both *blaFOXM* (6.25%; 1/16) and *blaMOXM* (6.25%; 1/16), underscores the complexity of β-lactamase diversity being discharged into the hospital WW.

The carbapenemase resistance in TASH WW was dominated by *blaNDM* (31.25%; 5/16), followed by *blaOXA-48* (18.75%; 3/16). The simultaneous detection of *blaOXA-24/143* group (6.25%; 1/16) and *blaKPC* (6.25%; 1/16) suggests that the hospital WW discharge contains a highly varied population of carbapenem-resistant bacteria. All of *K. pneumoniae* isolates (100%; 1/1) were detected for *blaNDM*, *blaOXA-48* and *blaOXA-24/143* group.

The plasmid-mediated genes of *tetA* (56.3%; 9/16), *sul1* (43.75%; 7/16) and *ermB* (18.75%; 3/16) were identified indicating the prevalence of MDR bacteria. The high prevalence of tetracycline and sulfonamide resistance genes in *E. coli* (100%; 5/5 and 60%; 3/5, repectively), and in *S. aureus* (100%; 2/2) underscores the presence of well-established MDR prevalent strains within the hospital effluent (Table 22).

Table 22. Prevalence of plasmid-mediated ARGs in WW samples from TASH

Bacteria	Antimicrobial resistance genes, n (%)											
	blaCTX M	*BlaTE M*	*blaFOX M*	*blaMOX M*	*BlaDHA M*	*blaNDM*	*blaOXA-48*	*blaOXA-24/143*	*blaKPC*	*ermB*	*tetA*	*sul1*
K.pneumoniae (n = 1)	+	+	-	-	-	+	+	+	-	-	-	-
K. oxytoca (n = 2)	2 (100)	2 (100)	+	+	+	2 (100)	+	-	+	-	-	-
E. coli (n = 5)	2 (40)	4 (80)	3 (60)	-	-	+	-	-	-	2 (40)	5 (100)	3 (60)
E. cloacae (n = 1)	+	+	-	-	-	+	+	-	-	-	+	+
S. aureus (n = 2)	2 (100)	2 (100)	-	-	-	-	-	-	-	-	2 (100)	2 (100)
S. epidermidis (n = 1)	-	+	-	-	-	-	-	-	-	-	+	+
B. cereus (n = *2)*	-	-	-	-	-	-	-	-	-	+	-	-
B. flexus (n =*2*)	-	+	-	-	-	-	-	-	-	-	-	-
Total (n = 16)	8 (50)	12 (75)	4 (25)	1 (6.3)	1 (6.3)	5 (31.3)	3 (18.8)	1 (6.3)	1 (6.25)	3 (18.8)	9 (56.3)	7 (43.8)

In the assessment of plamid-mediated ARGs of TASH WW drainage systems, the intensive care unit and emergency ward emerged as the primary reservoirs, each accounting for 29.6% (16/54) of the total identified ARGs. These findings highlight that high-acuity areas and critical environments harbor the highest concentration of resistance genes on IS as showed in Figure 31 (see Appendix 15).

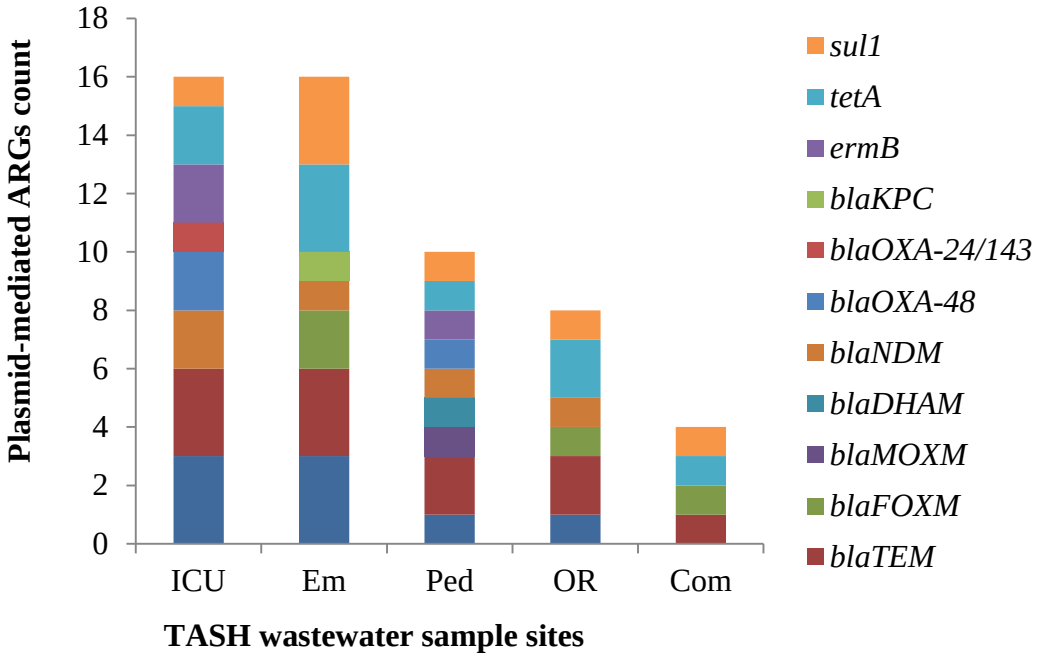

Figure 31. Spatial distribution of plasmid-mediated ARGs across TASH WW samples.

The figure depicts the plasmid-mediated resistance percentage of the TASH-WW wards.

ICU, intensive care unit; OR, operation ward; Em, emergency ward; Com, composite site; Ped, pediatrics ward

Among 32 bacterial isolates from TASH IS, the most frequently detected plasmid-mediated ESBL gene was found to be *blaTEM* (81%; 26/32), followed by *blaCTXM* (28%; 9/32). The AmpC gene, *blaFOXM* (6%; 2/32), was detected in less frequent of isolates. The most frequent detected plasmid-mediated carbapenemase gene was *blaNDM* (43.8%; 14/32), followed by *blaOXA-48* (12.5%; 4/32); whereas, *blaKPC* was less frequent (3%; 1/32). The plasmid-mediated ARGs such as *tetA* (65.6%; 21/32), *sul1* (56%; 18/32) and *ermB* (21.9%; 7/32) were detected in isolates. The *E. coli* isolates exhibited a high prevalence of MDR genotypes. It harboured *tetA* (100%; 7/7), *blaTEM* (85.7%; 6/7), *ermB* (85.7%; 6/7), *blaNDM* (71%; 5/7), *sul1* (57%; 4/7), *blaCTXM* (43%; 3/7) and *blaFOXM* (14.3%; 1/7), indicating that

it is highly MDR. The carbapenemase gene, *blaNDM* was harboured in 71% of isolates, co-occurring with *sul1* (57%) and ESBL markers such as *blaCTXM* (43%). These findings underscore a presence of high-priority resistance genes within the studied environment. All (100%; 1/1) of the *K. oxytoca* isolates exhibited an XDR genotype, harboring a combination of ESBL (*blaCTXM*, *blaTEM*), AmpC (*blaFOXM*), and carbapenemase genes (*blaNDM* and *blaKPC*). The *A. baumannii* isolates demonstrated highly resistance profile, included 100% (2/2) frequency of the carbapenemase gene *blaNDM* and the β-lactamase *blaTEM*. This highlights that carbapenems and cephalosporins totally loss their functions within this species group. Additionally, the detection of *sul1* in (50%; 1/2) of isolates suggests a diversifying resistome, HGT driven within the hospital setting. The less frequent isolates, *L. adecarboxylata*, *E. asburiae* and *R. ornithinolytica* each harbored both *blaTEM* and *tetA* (100%; 1/1) (Table 23).

Table 23. Prevalence of plasmid-mediated ARGs in IS samples from TASH

Bacteria	Antimicrobial resistance genes, n (%)								
	blaCTXM	*blaTEM*	*blaFOXM*	*blaNDM*	*blaOXA-48*	*blaKPC*	*ermB*	*tetA*	*sul1*
K. oxytoca (n = 1)	+	+	+	+	-	+	-	-	-
E. coli (n = 7)	3 (43)	6 (85.7)	+	5 (71)	-	-	6 (85.7)	7 (100)	4 (57)
L. adecarboxylata (n = 1)	-	+	-	-	-	-	-	+	+
E. asburiae (n = 1)	-	+	-	-	-	-	-	+	-
R. ornithinolytica (n = 1)	+	+	-	-	-	-	-	+	+
E. cloacae (n = 6)	3 (50)	4 (66.7)	-	6 (100)	4 (66.7)	-	-	5 (83)	4 (66.7)
A. baumannii (n = 2)	-	2 (100)	-	2 (100)	-	-	-	-	+
A. Iwoffii (n = 1)	-	+	-	-	-	-	-	-	+
S. aureus (n = 2)	+	2 (100)	-	-	-	-	-	+	+
S. caprae (n = 2)	-	2 (100)	-	-	-	-	-	2 (100)	2 (100
S. epidermidis (n = 3)	-	3 (100)	-	-	-	-	-	3 (100)	2 (66.7)
B. cereus (n = *3)*	-	+	-	-	-	-	+	-	+
B. flexus (n = 1)	-	-	-	-	-	-	-	-	-
U. massiliensis (n = 1)	-	+	-	-	-	-	-	-	-
Total (n = 32)	9 (28)	26 (81)	2 (6)	14 (43.8)	4 (12.5)	1 (3)	7 (21.9)	21 (65.6)	18 (56)

Regarding IS at TASH, the neonatal intensive care unit (room 1) was the most contaminated area (14.6%; 15/103), followed by operation room (room 2) (12.6%; 13/103) for plasmid mediated ARGs contamination as depicted in Figure 32. The high prevalence in these specific wards underscores a significant risk of hospital environmental transmission to vulnerable patients, including neonates and surgical patients (see Appendix 16).

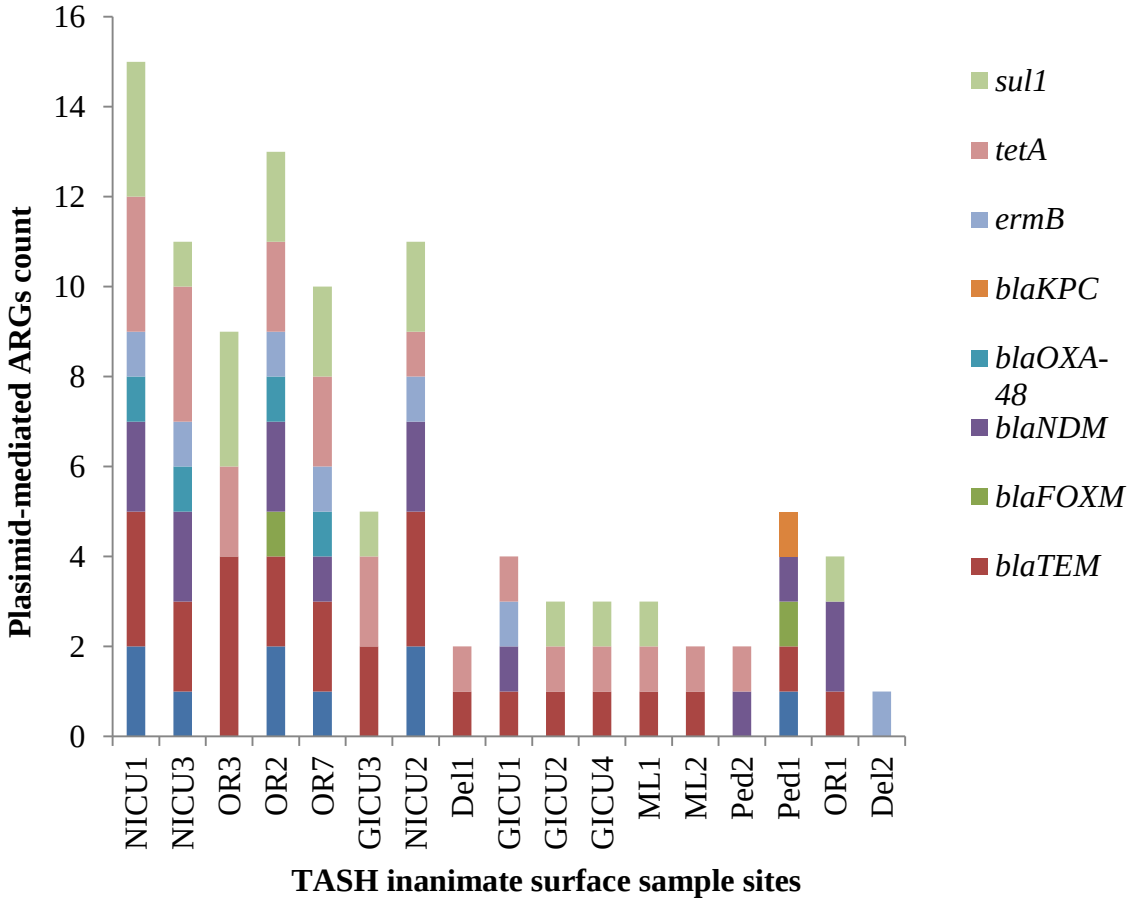

Figure 32. Abundance of plasmid-mediated ARGs in TASH IS samples.

4.4.4. Plasmid-Mediated AMR Characterization from ZMH Wastewaters and Inanimate Surfaces

Among the ZMH WW isolates, the most frequently detected plasmid-mediated ESBL gene was found to be *blaTEM* (92%; 12/13), followed by *blaCTXM* (38.5%; 5/13). The plasmid-mediated AmpC β-lactamase genes, *blaFOXM* (23.1%; 3/13) and *blaEBCM* (7.7%; 1/13) were detected from WW bacterial isolates. The most frequent plasmid-mediated

carbapenemase genes detected from ZMH WW bacterial isolates was *blaNDM* (30.8%; 4/13), followed by *blaOXA-48* (23.1%; 3/13). In addition to ESBL, AmpC and CP genes, the tetracycline, erythromycin and sulphonamide resistance genes were detected as *tetA* (46.2%; 6/13), *sul1* (38.5%; 5/13), and *ermB* (23.1%; 3/13). Among the plasmid-mediated ARGs detected in ZMH WW bacterial isolates, *tetA* was the second most frequently detected gene surpassed by *blaTEM*. All of *E. cloacae* (100%; 2/2) harbored both *blaNDM* and *blaOXA-48* genes. Additionally, *blaTEM* was detected in all of *E. coli* (100%; 4/4), *E. cloacae* (100%; 2/2), *P. aeruginosa* (100%; 1/1), *S. aureus* (100%; 1/1), *E. faecalis* (100%; 1/1) and *B. cereus* (100%; 2/2) (Table 24).

The medical ward sample site represented the major reservoir for plasmid-mediated ARGs in ZMH WW drainage system, conributing 38.1% (16/42) of the detected genes, followed by the WW composite sample site (23.8%; 10/42). The high prevalence of plasmid-mediated ARGs in these sites suggests the critical resistance pattern of the hospital WW (see Appendix 17).

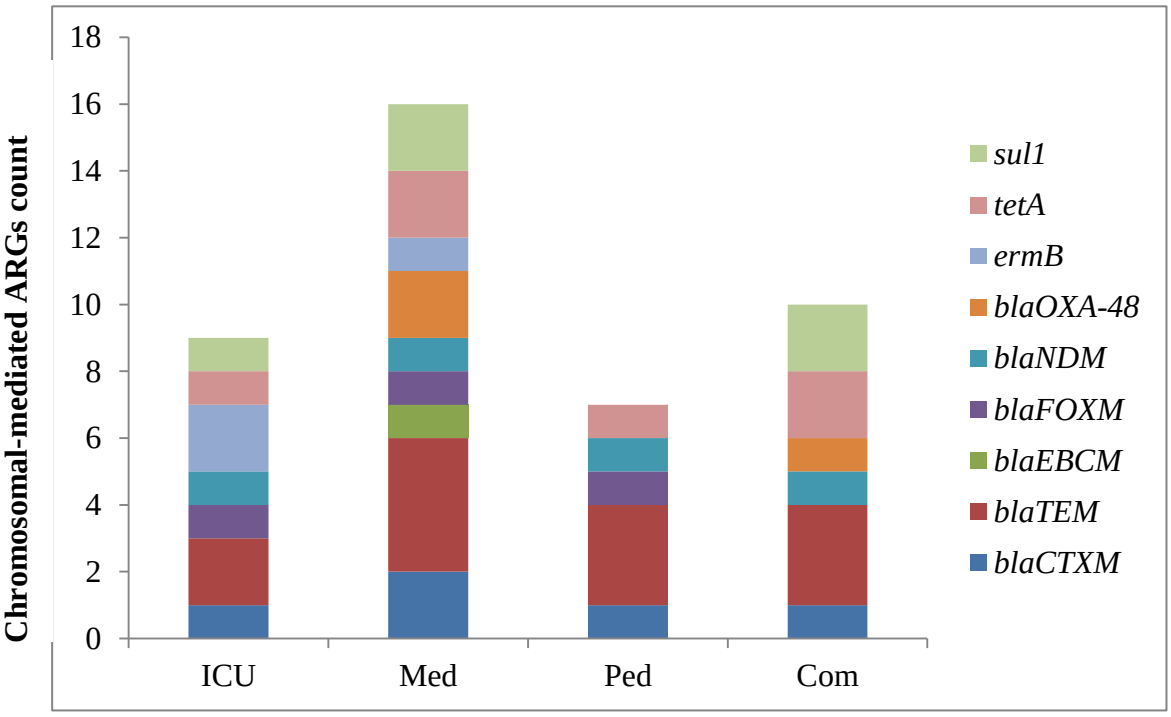

Figure 33. Spatial distribution of plasmid-mediated ARGs across ZMH WW samples.

Table 24. Prevalence of plasmid-mediated ARGs in ZMH WW samples

Bacteria	Antimicrobial resistance genes, n (%)								
	blaCTXM	*blaTEM*	*blaEBCM*	*blaFOXM*	*blaNDM*	*blaOXA-48*	*ermB*	*tetA*	*sul1*
C. freundii (n = 1)	+	+	-	-	+	-	-	-	-
K. oxytoca (n = 1)	-	+	+	+	-	+	-	-	-
E. coli (n = *4)*	2 (50)	4 (100)	-	2 (50)	+	-	2 (50)	4 (100)	2 (50)
E. cloacae (n = *2)*	2 (100)	2 (100)	-	-	2 (100)	2 (100)	-	2 (100)	+
P. aeruginosa (n = 1)	-	+	-	-	-	-	-	-	-
S. aureus (n = 1)	-	+	-	-	-	-	-	-	+
E. faecalis (n = 1)	-	+	-	-	-	-	-	-	-
B. cereus (n = *2)*	-	+	-	-	-	-	+	-	+
Total (n = 13)	5 (38.5)	12 (92)	1 (7.7)	3 (23.1)	4 (30.8)	3 (23.1)	3 (23.1)	6 (46.2)	5 (38.5)

The IS of ZMH was dominated by the plasmid-mediated *blaTEM* (83.9%; 26/31). Nearly half of the ESBL producing isolates harbored *blaCTXM* producing gene (45.2%; 14/31); whereas, *blaFOXM* (12.9%; 4/31) was detected from AmpC producing groups of isolates. The CP genes, *blaNDM* (22.6%; 7/31), *blaOXA-48* (12.9%; 4/31) and *blaKPC* (6.5%; 2/31) were detected in isolates. The plasmid-mediated genes such as *sul1* (48.4%; 15/31), *tetA* (45.2%; 14/31) and *ermB* (19.4%; 3/31) were identified in ZMH IS isolates. These findings demonstrate that a substantial portion of the hospital IS is contaminated with bacteria harboring resistance to sulfonamides, tetracyclines, and macrolides. Among the plasmid-mediated ARGs detected in ZMH IS bacterial isolates, *sul1* was the second most detected gene, constituting 48.4%, following *blaTEM* (83.9%) (Table 25).

At ZMH, the intensive care unit (room 3), medical unit (room 1) and operation room 2 were the top contributors to IS plasmid-mediated ARGs contamination, each accounting for 13.2% (12/91) of the total detected genes as demonstrated in Figure 34. The high density of resistance genes in these specific units highlights a severe risk of environmental transmission to patients undergoing critical care or invasive surgical procedures (see Appendix 18).

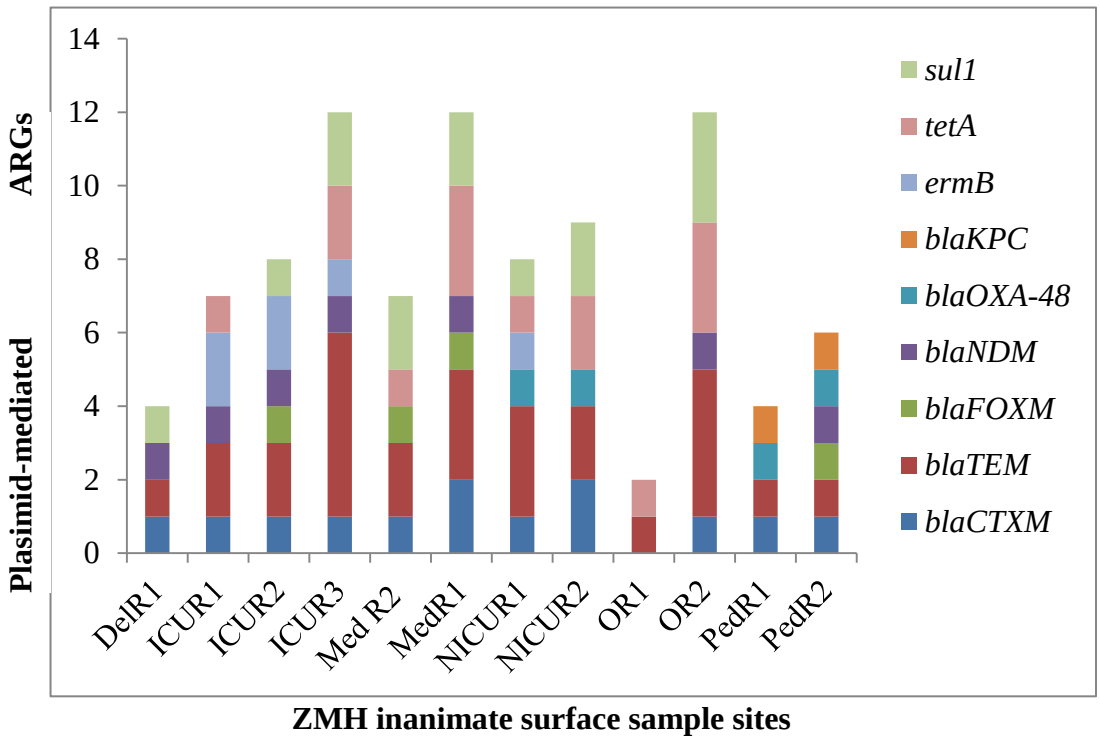

Figure 34. Spatial distribution of plasmid-mediated ARGs across ZMH IS samples.

Table 25. Prevalence of plasmid-mediated ARGs in ZMH IS samples

Bacteria	Antimicrobial resistance genes, n (%)								
	blaCTXM	*blaTEM*	*BlaFOXM*	*blaNDM*	*blaOXA-48*	*blaKPC*	*ermB*	*tetA*	*sul1*
K. pneumoniae (n = 2)	2 (100)	2 (100)	+	+	2 (100)	2 (100)	-	-	-
E. coli (n = 6)	5 (83.3)	6 (100)	3 (50)	4 (66.7)	-	-	2 (33.3)	2 (33.3)	5 (83.3)
E. hormaechei (n = 1)	-	+	-	-	-	-	-	+	-
E. cloacae (n = 5)	4 (80)	5 (100)	-	2 (40)	2 (40)	-	-	4 (80)	4 (80)
P. aeruginosa (n = 1)	-	+	-	-	-	-	-	-	-
S. aureus (n = 4)	3 (75)	4 (100)	-	-	-	-	-	4 (100)	+
S. epidermidis (n = 1)	-	+	-	-	-	-	-	+	+
S. haemolyticus (n = 1)	-	+	-	-	-	-	-	+	+
E. faecium (n = 2)	-	2 (100)	-	-	-	-	-	+	2 (100)
E. faecalis (n = 2)	-	2 (100)	-	-	-	-	+	-	-
B. flexus	-	-	-	-	-	-	-	-	-
B. cereus (n = 5)	-	+	-	-	-	-	3 (60)	-	+
Total (n = 31)	14 (45.2)	26 (83.9)	4 (12.9)	7 (22.6)	4 (12.9)	2 (6.5)	6 (19.4)	14 (45.2)	15 (48.4)

4.5. Association of Phenotypic and Molecular Antimicrobial Resistance

The level of concordance and discordance between phenotypic resistance and the presence of corresponding resistance genes was analysed. When isolates exhibiting phenotypic resistance and AMR genes were predicted, or isolates exhibited phenotypic susceptibility and no AMR genes were predicted, concordance was positive. When results from genotyping and phenotyping differed, discordance was observed. For ESBL production, the evaluated isolates (n = 53, GNB alone) showed an overall concordance of 37.7% (20/53) between the molecular data and AST results for ESBL production. However, a significant discrepancy was observed between phenotypic and genotypic profiles, as 62.3% (33/53) of isolates that were phenotypically negative for the ESBL-production were found to harbor ESBL-related ARGs upon PCR analysis. Among the Enterobacteriaceae, *E. coli* showed a concordance of 50% (11/22), *E. cloacae* (35.7%; 5/14), while *K. pneumoniae* (3/3) and *R. ornithinolytica* (1/1) both demonstrated 100% concordance though sample sizes were small (Table 26).

Table 26. Association between phenotypic β-lactam resistance and detected ESBL genes

Bacteria	ESBL gene, n (%)	Concordance, n (%)
C. freundii (n = 1)	+	-
K. pneumoniae (n = 3)	-	3 (100)
K. oxytoca (n = 4)	2 (50)	2 (50)
E. coli (n = 22)	15 (68.2)	7 (31.8)
L. adecarboxylata (n = 1)	+	-
E. asburiae (n = 1)	+	-
E. hormaechei (n = 1)	+	-
R. ornithinolytica (n = 1)	-	+
E. cloacae (n = 14)	9 (64.3)	5 (35.7)
P. aeruginosa (n = 2)	+	+
A. baumannii (n = 2)	+	+
A. lwoffii (n = 1)	+	-
Total (n = 53)	33 (62.3)	20 (37.7)

A notable discordance was observed between phenotypic meropenem susceptibility and genotypic carbapenemase profiles among the GNB isolates (n = 53). Specifically, 15.1% (8/53) of isolates exhibited phenotypic resistance to meropenem despite absence of the targeted carbapenemase genes. Conversely, 26.4% (14/53) of isolates were positive in carbapenemase PCR assay but remained phenotypically susceptible to meropenem, indicating the presence of cryptic resistance markers or the ARGs might be present but not switched on.

Among the isolates, 58.5% (31/53) demonstrated high concordance level of phenotypic resistance to meropenem with detection of correspondence carbapenemase ARGs by PCR. As presented in Table 27, the concordance level showed statistical significance (p = 0.001). This provides robust evidence that the carbapenemase genes found on hospital IS and in WW might be responsible for the carbapenem-resistant phenotypes observed.

Table 27. Association and concordance between phenotypic carbapenem resistance and detected carbapenemase genes

Bacteria	Phenotypic carbapenem resistance, n (%)	Carbapenemase gene, n (%)	Concordance, n (%)
C. freundii (n = 1)	-	+	-
K. pneumoniae (n = 3)	-	-	3 (100)
K. oxytoca (n = 4)	+	2 (50)	+
E. coli (n = 22)	4 (18.2)	4 (18.2)	14 (63.6)
L. adecarboxylata (n = 1)	-	-	+
E. asburiae (n = 1)	-	-	+
E. hormaechei (n = 1)	-	-	+
R. ornithinolytica (n = 1)	+	-	-
E. cloacae (n = 14)	-	6 (42.9)	8 (57.1)
P. aeruginosa (n = 2)	+	-	+
A. baumannii (n = 2)	-	+	+
A. lwoffii (n = 1)	+	-	-
Total (n = 53)	8 (15.1)	14 (26.4)	31 (58.5)

For tetracycline, the tested isolates (n = 75, including both GNB and GPB) showed 57.3% (43/75) discordance and 42.7% (32/75) concordance. The 49.3% (37/75) of isolates carried the *tetA* gene but remained susceptible to tetracyline. The concordance level showed statistical significance (p = 0.033) (Table 28).

Table 28. Concordance and association between phenotypic and genotypic tetracycline resistance profiles

Bacteria	Phenotypic tetracycline resistance n, (%)	*tetA* gene, n (%)	Concordance, n (%)
C. freundii (n = 1)	-	-	+
K. pneumoniae (n = 3)	2 (66.7)	-	+
K. oxytoca (n = 4)	-	2 (50)	2 (50)
E. coli (n = 22)	1 (4.5)	15 (68.2)	6 (27.3)
L. adecarboxylata (n = 1)	-	+	-
E. asburiae (n = 1)	-	+	-

E. hormaechei (n = 1)	-	-	+
R. ornithinolytica (n = 1)	-	+	-
E. cloacae (n = 14)	-	7 (50)	7 (50)
P. aeruginosa (n = 2)	-	-	2 (100)
A. baumannii (n = 2)	-	-	2 (100)
A. lwoffii (n = 1)	-	-	+
S. aureus (n = 9)	+	5 (55.6)	3 (33.3)
S. caprae (n = 2)	-	+	+
S. epidermidis (n= 5)	-	3 (60)	2 (40)
S. haemolyticus (n = 1)	-	+	-
E. faecium (n = 2)	+	-	+
E. faecalis (n = 3)	+	-	2 (66.7)
Total (n = 75)	6 (8)	37 (49.3)	32 (42.7)

Erythromycin testing showed 73.3% concordance, with a highly significant association between genotype and phenotype (p = 0.003). Although 20% of isolates carried a cryptic *ermB* genes and 6.7% showed non-genotypic resistance, the *ermB* gene stands as a common resistance gene at TASH and ZMH (Table 29).

Table 29. Association and concordance between phenotypic and genotypic erythromycin resistance profiles

Bacteria	Concordance, n (%)	Phenotypic erythromycin resistance, n (%)	*ermB* gene, n (%)
C. freundii (n= 1)	+	-	-
K. pneumoniae (n= 3)	3 (100)	-	-
K. oxytoca (n= 4)	4 (100)	-	-
E. coli (n= 22)	8 (36.4)	-	14 (63.6)
L. adecarboxylata (n= 1)	+	-	-
E. asburiae (n= 1)	+	-	-
E. hormaechei (n= 1)	+	-	-
R. ornithinolytica (n= 1)	+	-	-
E. cloacae (n= 14)	14 (100)	-	-
P. aeruginosa (n= 2)	2 (100)	-	-
A. baumannii (n= 2)	2 (100)	-	-
A. lwoffii (n= 1)	+	-	-
S. aureus (n = 9)	6 (66.7)	3 (33.3)	-
S. caprae (n = 2)	2 (100)	-	-
S. epidermidis (n = 5)	3 (60)	2 (40)	-
S. haemolyticus (n = 1)	+	-	-
E. faecium (n = 2)	2 (100)	-	-
E. faecalis (n = 3)	2 (66.7)	-	+
Total (n = 75)	55 (73.3)	5 (6.7)	15 (20)

In the evaluation of sulfonamide resistance, 53.3% of isolates demonstrated concordance between their phenotypic pattern and genotypic markers. However, a significant proportion was found to harbor the *sul1* gene (46.7%) despite testing as susceptible to trimethoprim/sulfamethoxazole. Statistical analysis confirmed a significant association between the presence of the *sul1* gene and phenotypic resistance to trimethoprim/sulfamethoxazole ($p = 0.044$). This suggests that while a large proportion of the genetic burden remains unexpressed, *sul1* is a foundational component of the sulfonamide resistance profile in these hospitals (Table 30).

Table 30. Association and concordance between phenotypic and genotypic sulfonamide resistance profiles

Bacteria	*sul1* gene, n (%)	Concordance, n (%)
C. freundii (n = 1)	-	+
K. pneumoniae (n = *3)*	-	3 (100)
K. oxytoca (n = *4)*	-	4 (100)
E. coli (n = *22)*	12 (54.5)	10 (45.5)
L. adecarboxylata (n = 1)	+	-
E. asburiae (n = 1)	-	+
E. hormaechei (n = 1)	-	+
R. ornithinolytica (n = 1)	-	+
E. cloacae (n = *14)*	9 (64.3)	5 (35.7)
P. aeruginosa (n = *2)*	-	2 (100)
A. baumannii (n = *2)*	+	+
A. lwoffii (n = 1)	+	-
S. aureus (n = *9)*	4 (44.4)	5 (55.6)
S. caprae (n = *2)*	+	+
S. epidermidis (n = *5)*	5 (100)	-
S. haemolyticus (n = 1)	+	-
E. faecium (n = *2)*	-	2 (100)
E. faecalis (n = *3)*	-	3 (100)
Total (n = 75)	35 (46.7)	40 (53.3)

5. DISCUSSION

Prevalence of bacterial isolates

The present study was carried out on a total of 38 samples that were collected from WW and IS of TASH and ZMH in Addis Ababa, Ethiopia. Bacteria were recovered from all (100%) of the samples collected from WW and IS. The 100% recovery rate from surface swabs is consistent with Mbanga et al. (2018) who reported similarly high rate in Zimbabwe, suggesting that hospital IS (such as bed rails and medical equipment) are near-universal vehicles for bacterial colonization regardless of the socioeconomic setting. However, the bacteria recovery in this study significantly exceeds the findings by Yosef et al. (2025) in Addis Ababa, Ethiopia, who reported a prevalence of only 77.5%. However, the higher prevalence observed in this study may reflect inadequate infection prevention practices and antibiotic stewardship in the studied hospitals.

The isolation of 92 bacterial strains from just two healthcare facilities highlights a significant level of bacterial contamination within the hospital environment, specifically within WW drainage and on IS. The higher frequency of isolates at TASH (52.2%) compared to ZMH (47.8%) may be attributed to the larger size of TASH, its status as a tertiary referral center, higher patient turnover, and the complexity of medical procedures performed there, all of which increase the bacterial load and diversity. The findings of the present study are consistent with those of Tilahun et al. (2025) which declare that 57.5% of bacterial strains in Ethiopia originated from hospital environments, making hospitals hotspots for environmental bacterial contamination.

The prevalence of bacterial isolates from IS of both TASH (66.7%) and ZMH (70.5%) was higher than the study conducted in Sudan, El-Mak Nimer University Hospital (43.3%), indicating that the hospitals IS are major causes for bacterial contamination despite routine cleaning efforts (Dafaalla et al., 2023).

In both TASH and ZMH WW samples, the most prevalent bacteria were GNB (56.2% and 69.2%) compared to GPB (43.8% and 30.8%), respectively. Similar reports were documented in Zimbabwe (66.18% vs. 33.82%) (Mbanga et al., 2018) and in Uganda (60% vs. 40%) (Patience et al., 2025) GNB and GPB, respectively, as the predominant environmental isolates. However, contrary to our findings, studies conducted in Addis Ababa (56.3% vs. 43.7%) (Sebre et al., 2020) and Northwest Ethiopia (53.8% vs. 46.3%) (Amsalu et al., 2024)

reported the prevalence of GPB over GNB. These differences may be due to the variation of sampling times, sampling techniques, culture methodologies, sampling sites and the presence of already colonized and/or infected patients. The increase in GNB prevalence from 43.7% as reported by Sebre et al. (2020) to 60.4% in the current study indicates a significant difference in the hospital's bacterial ecology over the last five years. Several factors may justify this trend including selection pressure from antimicrobial use, biofilm formation in aging infrastructure, and changes in patient demography (Brepoels et al., 2025). The high prevalence of *E. coli* (55.6%) in TASH WW aligns with recent findings at a tertiary hospital in Ethiopia, that reported *E. coli* as the leading isolate in WW (52.1%) (Gashaw, et al., 2024).

In ZMH WW samples, *E. coli* accounts for 44.4% of bacterial isolates, which is a comparable figure with the previous study in Arba Minch, Southern Ethiopia (33.3%) (Regasa et al., 2021); in Accra, Ghana (30.6%) (Addae et al., 2022); and in Northern Tanzania (30.9%) (Laizer et al., 2025). ZMH IS were more contaminated than TASH IS, as more bacteria were isolated from IS of ZMH (70.5%) than of TASH (66.7%). Referring to a former study which reported 77.45% isolates from IS of ZMH (Yosef et al., 2025), lower proportion of bacterial isolates were recovered in the current study. This could be due to temporal and seasonal fluctuation; as the previous study was conducted from June to November, the current study was from October to January, the change in environmental moisture could affect the desiccation tolerance and viability of specific bacterial species (Esbelin et al., 2018). Additionally, patient density and the severity of infections in the studied wards have a significant impact on the bacterial shedding rate. A lower environmental bioburden could be the consequence of a change in the sorts of clinical cases or a lower patient occupancy rate during the current sampling period (Kaur et al., 2026).

While the ICU remains a significant reservoir for pathogens (31.25%) at TASH, this prevalence is lower than the previous report (50.3%) at the same study site (Sebre et al., 2020). This might be due to the nature of the samples, dynamic of sampling time and enhancement in IPC protocol. An increased cleaning and stricter hand hygiene in high-risk areas such as ICUs has direct relationship with a reduction in the environmental shedding of bacteria (Sathitakorn et al., 2023).

The isolation of rare and emerging bacteria like *L. adecarboxylata* and *R. ornithinolytica* in less than 5% frequency was also consistent with a report from clinical specimens in Ethiopia

(Legese et al., 2022). This suggests a continuous cycle of contamination between patients and their surroundings.

Antimicrobial susceptibility profiles of bacterial isolates

During AST test, the detection of *Bacillus* spps were excluded due to the lack of standard regarding this genus in the CLSI; whereas, 75 bacterial isolates were subjected to AST. The *K. pneumoniae* from WW and surface swabs demonstrated 100% MDR profile. These findings suggest the potential for environmental transmission, although direct transmission pathways were not assessed in this study. It mirrors a longitudinal study in Addis Ababa which investigate AST profiles in 5 year exhibited that *K. pneumoniae* resistance (reaching up to 90-100%) to ciprofloxacin and carbapenems between 2017 and 2021 (Kitaba et al., 2024). Reaching a total resistance to ceftazidime (a 3rd-generation cephalosporin) and aztreonam (a monobactam) signifies the presence of potent ESBLs or metallo- β-lactamases (MBLs), which render the first- and second-line treatments clinically useless (Szymański et al., 2024). This finding was higher than the previous study in Ethiopia which reported *K. pneumoniae* resistance to ceftazidime at 86.7% (Awoke et al., 2021).

From TASH WW, *S. epidermidis* displayed 100% resistance to tetracycline, oxacillin, clindamycin, and vancomycin. The finding of vancomycin-resistant coagulase-negative *Staphylococci* (VRCoNS) in hospital WW indicates a critical shift in the local environmental resistome, while *S. epidermidis,* a common skin commensal, able to accumulate resistance genes and potentially shifted them to more virulent pathogens like *S. aureus.* In the previous study, Tilahun et al. (2025) noted that while clinical vancomycin resistance remains low (5-10%), the environmental prevalence is rising significantly. The 100% resistance to oxacillin characterizes these isolates as methicillin-resistant *S. epidermidis* (MRSE). MRSE is difficult to eliminate from hospital WW and from IS, since it forms robust biofilms on medical inanimate objects and within plumbing systems. The 100% resistance to other antimicrobials such as clindamycin and tetracycline confirms a MDR pattern that is becoming common in Sub-Saharan African tertiary hospitals (Asante et al., 2021). Finding 100% vancomycin-resistant *S. epidermidis* strains in both WW and IS of TASH suggests a direct environmental link to potential treatment failures in the hospital.

S. aureus showed 50% resistance to multiple agents, including tetracycline, nitrofurantoin, oxacillin, erythromycin, and doxycycline in TASH WW and 100% resistant to vancomycin

and oxacillin in TASH IS. The 100% resistance to vancomycin on surfaces at TASH is clinically alarming. While vancomycin-resistant *S. aureus* (VRSA) is rare in Addis Ababa, Ethiopia (Yosef et al., 2025), its presence on hospital IS suggests a specific environmental cloning or horizontal gene transfer. The clinical investigations in Hawassa, Ethiopia, reported vancomycin susceptible *S. aureus* isolated from patient wounds (Sahle and Merid, 2024). However, our finding of 100% resistance on surfaces indicates that the environment is leading the clinical samples in terms of resistance profile possibly due to the selective pressure of drugs residues and disinfectants. The resistance characteristics of *U. massiliensis* which was previously known for its promising applications in industrial production and environmental remediation as peported by He et al. (2025) might be a threat and warning for investigation of safety before confirming it as a candidate.

From TASH IS, *R. ornithinolytica* showed a total (100%) resistance to gentamicin, ceftazidime, aztreonam, meropenem, trimethoprim/sulfamethoxazole, and nitrofurantoin. This MDR profile finding aligns with a 2024 study in Tanzania which reported *R. ornithinolytica* as the most spreader of resistance genes in African hospitals due to its presence in both water systems and on IS (Silago et al., 2022). In the present study, *A. baumannii* from IS exhibited 50% resistance to both ceftazidime and meropenem, while the previous study indicated 53.2% carbapenemase producer (Beshah et al., 2023).

Antimicrobial resistance profile for *E. coli* showed that 60% were concurrently resistant to ampicillin and meropenem. This result aligns with Adama Hospital Medical College, where resistance to ampicillin was reported to be 56% (Tufa and Birhanu, 2025). The 60% resistance to meropenem is exceeding the national base line where national surveillance data recently reported *E. coli* meropenem resistance at roughly 5–14% (Kitaba et al., 2024).

As *C. freundii* is an opportunistic pathogen that intrinsically resistant to penicillins, the current finding of total resistance to cefotaxime and amikacin suggests the presence of acquired, high-level resistance genes. This indicates a high risk of rising resistance in WW, where selective pressure is high (Fonton et al., 2025). In ZMH WW, *E. faecalis* demonstrated 33.3% resistance to both gentamicin and doxycycline. This finding is higher than the 23.5% resistance reported in clinical samples from Jimma, Ethiopia (Abamecha et al., 2015), suggesting that WW might be a selective environment for more resistant strains. A 100% meropenem resistance of *P. aeruginosa* is significantly higher than the clinical prevalence in Ethiopia, where meropenem resistance of 15% - 28% was reported (Gobezie et al., 2024),

indicating intensive use of carbapenems, biofilm forming capacity of the bacteria that enhance the spread of carbapenem resistance. Globally, the WHO has mentioned carbapenem-resistant *P. aeruginosa* as a priority-1 critical pathogen (WHO, 2024).

The prevalence of bacterial isolates in the ICU ward drainage system exhibited the highest resistance with MAR index of 0.5, making it the primary reservoir of ARB in TASH WW drainage. It demonstrates a critical insight of the hospital's resistance hotspots. The previous study suggested that the room often experience higher patient turnover and less consistent disinfection (Abosse et al., 2024), showing a more consistent disinfection is required at the TASH. From ZMH WW drainage, the medical ward drainage showed the highest frequency of MDR with MAR index of 0.6, indicating higher ARB contamination at the areas. This suggests that the medical sample site is more contaminated and provide selective pressure for the ARB entering the downstream municipal drainage. A report on the Akaki river noted that hospital WW are the primary drivers of ARB, directly affecting downstream residents (Abosse et al., 2025). This prevalence underscores that the medical sample site is primary reservoir of resistance and higher than the 11.1-33.3% average reported in WW of Felege Hiwot Comprehensive Specialized Hospital and Tibebe Ghion Specialized Hospital, in Bahir Dar, Ethiopia (Endalamaw et al., 2024).

Among TASH IS, bacterial isolates from the neonates intensive care unit (room 1) exhibited the highest level of ARB contamination with MAR index of 0.7. Neonates are immature and very susceptible to environmental contamination. The study at Jimma medical center, Ethiopia, reported the high mortality rate of neonates were due to ARB contamination (Geleta et al., 2024). The lack of sufficient disinfectant and IPC practice in critical wards like operation room is a significant warning for the hospitals.

In evaluating the distribution of MDR through different environmental sources at TASH, a significant difference was observed between IS and WW. IS demonstrated a significant high MDR prevalence of 70%; whereas, WW bacterial isolates exhibited a substantially low frequency of 20%, indicating that biofilms on IS of healthcare are becoming more stable and threatening reservoirs for MDR. Inanimate object-borne bacterial isolates at TASH were 3.5 times more likely to be MDR than those obtained from the WW. IS have more exposure being frequently touched by ARB colonized patients, healthcare workers, sub-lethal concentrations of disinfectants, which might give the survival of MDR strains (Prasek et al.,

2025). This result was nearly consistent with Hailemichael et al. (2023) at Jimma University Medical Center, Ethiopia, where MDR frequency was 60% on ISs.

Molecular characterization of antimicrobial resistance

Molecular characterization of WW bacterial isolates showed a high distribution of ESBL-encoding genes at both TASH and ZMH. At both ZMH and TASH, *blaTEM* was the most frequent chromosomal-mediated gene detected in 92.3% and 81.3%, respectively; while *blaCTXM* was the second most detected gene at both TASH and ZMH in 37.5% and 15.4%, respectively. These higher ESBL genes in both hospitals demonstrate the high circulation of ESBL genes in the referral hospitals and also possibly indicate the intake of β-lactams from various areas across Ethiopia who carry different resistant strains and referred to further treatment at these tertiary hospitals. The high prevalence of these genes is the indicator for threat of AMR in downstream communities. Recent investigation of WW from Akaki Kality, Ethiopia, reported 85% of *blaTEM*, aligns with the TASH WW (Mogessie et al., 2025). Additionally, our finding is consistent with Mou et al. (2023) from surface water in Bangladesh, with *blaTEM* detection rate of 70.6%. Another report from the sample of domestic ducks in Bangladesh detected *blaCTXM* with a frequency of 20%, which is relatively less than *blaCTXM* detected in TASH, (37.5%), and a little bit greater than the *blaCTXM* identified in ZMH (15.4%) (Ahmed et al., 2023), indicating the difference in distribution of *blaCTXM* gene in the study area.

While *blaTEM* is traditionally linked to Gram-negative Enterobacteriaceae, its presence in *S. aureus* is uncommon. However, Östholm Balkhed (2014) reported cross-species gene transfer and chromosomal integration of β-lactamases were emerging in Gram-positive pathogens. Consequently, our findings of blaTEM in 100% of *S. aureus* isolates was more than the finding of Bose et al. (2025) of *blaTEM* (85.71%). Similarly, the detection of *blaTEM* in *B. flexus* at TASH WW evidenced for the presence of putative MarR family transcriptional regulators, which is conserved multiple ARGs regulator as reported by Zhang et al. (2014). This suggests that it acts as a molecular melting pot where even non-pathogenic environmental bacteria acquire resistance genes (Yu et al., 2023) and our finding was higher than that of Ghazaei (2021) *blaTEM* (84.2%).

In TASH WW, the chromosomal-mediated molecular profile AmpC was predominated by the *blaCITM* (25%) and *blaFOXM* (18.8%) groups. In contrast, ZMH WW bacterial isolates

demonstrated a more variety but lower distribution of AmpC genes, such as *blaEBCM*, *blaMOXM*, and *blaDHAM*, each present in 7.7% of the bacterial community. The distribution of *blaCITM* at TASH is two times that of a single AmpC gene at ZMH, indicating that the CIT-type is the primary AmpC driver in the tertiary hospital. The large diversity of distribution of genes including *blaEBCM*, *blaMOXM*, and *blaDHAM* at ZMH, despite their lower frequencies, suggest resistance from many sources, possibly exhibiting various antimicrobial stewardship practices or it might indicate a different patient demography. The study from hospital environments in South Africa showed the consistent frequency of *blaCITM* (26%), *blaFOXM* (16%) and *blaDHAM* (11%) with our findings (Matloko et al., 2021).

The chromosomal-mediated molecuar analysis of carbapenem resistance at TASH and ZMH WW exhibited distinct genotypic patterns. The *blaOXA-48* gene was detected in 6.3% and 7.7% of the WW isolates at TASH and ZMH, respectively. The *blaNDM* was more frequent and predominated (23.1%) at ZMH. The presence of *blaOXA-48* indicates that the hospitals WW serve as a primary reservoir for metallo-β-lactamases (MBLs), which hinder the effective treatment. The molecular epidemiology of the current study revealed greater detection of *blaOXA-48* (0.8%) from environmental samples (Kindu et al., 2025). This is consistent with *blaNDM* (28%) from WW samples from Akaki, Ethiopia (Mogessie et al., 2025).

At TASH, the sulfonamide resistance gene (*sul1*) was highly frequent (37.5%) of WW bacterial isolates. In contrast, *tetA* and *ermB* gene were detected at a lower prevalence of 18.8% each. Additionally, at ZMH WW, the resistance profile of *tetA* with 38.5% was the most prevalent gene, followed by *sul1* (30.8%). These findings are consistent with *tetA* (45%) and *sul1* (21.7%) reported from the clinical samples in Malaysia (Chin et al., 2024).

The chromosomal-mediated molecular epidemiology of isolates from IS demonstrated predominance of the *blaTEM* gene at both study sites with detection rate in 94% of bacterial isolates at TASH and 92.3% at ZMH. This was followed by the *blaCTXM* gene, identified in 12.5% and 13% of isolates at TASH and ZMH, respectively. Nearly a consistent finding of ESBL genes were reported from Mwanza Hospital surface in Tanzania (Gamuya et al., 2025). The most frequent isolated bacteria, *E. coli*, showed substantial resistance against most classes of antimicrobials showing the range 33.3-100% to ESBL genes (*blaCTXM* and *blaTEM*), AmpC genes (*blaCITM* and *blaFOXM*) and *ermB*, *tetA* and *sul1*. Additionally, in

60% of *E. cloacae* bacterial isolates from ZMH IS, *blaCITM* were detected. The high detection rates on surfaces like bed frames, medical equipment, and door handles at ZMH suggest that these inanimate objects are not just passive furniture, but active reservoirs for superbugs (Kiros et al., 2021). In all major isolates including *E. coli, E. cloacae, K. oxytoca,* and *S. aureus*, the prevalence of resistance genes was consistently higher on surfaces than in WW, revealing that surface-dwelling bacteria exhibit higher resistance compared to those from the drainage system, highlighting strong persistence on ward touch points. This pattern suggests that hospital IS serve as a more concentrated reservoir for ARGs than WW, although both environments harbor a common resistome (Yosef et al., 2025).

Molecular analysis of WW bacterial isolates showed a high prevalence of plasmid-mediated ESBL genes. Both at ZMH and TASH, *blaTEM* was the most predominant gene detected in 92% and 75% of isolates, respectively; and *blaCTXM* in 50% and in 38.5% at TASH and ZMH, respectively. The high frequency of *blaTEM* at both hospitals reveals that it is a stable gene in the study area. The relatively larger distribution of *blaCTXM* (50% at TASH) is significant, as this ARG is linked with MGE that leads the rapid dissemination of resistance to third-generation cephalosporins. The high distribution of plasmid-mediated ESBL genes at TASH and ZMH indicates that the WW from hospital is not only a waste but also AMR reservoir. Specially, the 92% frequency of *blaTEM* at ZMH is particularly alarming signal of environmental ARGs saturation. These findings underscore the urgent attention for WW treatment to prevent these ARGs from entering the municipal water system and distributing the acquired AMR to community and downstream societies. A comparable figure was reported on *blaCTXM* (39.1%) (Song et al., 2020) from South Korea, on *blaTEM* (63.4%) and *blaCTXM* (36.6%) in Nepal (Chaudhary et al., 2023). The study on WW isolates at Parirenyatwa Hospital in Harare, demonstrated a 87.5% frequency of *blaCTXM* and 62.5% of *blaTEM* (Marembo and Chirenda, 2025). This high prevalence of *blaCTXM*, followed by *blaTEM* in Harare is reverse of our finding, possibly suggesting different regional clonal expansions of ARB in different Sub-Saharan Africa countries. A research in Iran reported comparative similarity of *blaTEM* (74.7%) and *blaCTXM* (41%) as the most frequent plasmid-mediated resistance genes supports our finding of *blaTEM* (75%) at TASH and *blaCTXM* (38.5%) at ZMH (Hoseinzadeh et al., 2024).

At TASH and ZMH hospitals, the plasmid-mediated AmpC genes showed diverse prevalence. At TASH, *blaCITM* (25%), *blaFOXM* and *blaMOXM* (6.25% each) underscore

the complexity of ARGs diversity being discharged into the WW drainage; whereas, *blaFOXM* (23.1%) and *blaEBCM* (7.7%) were detected from bacterial isolates from ZMH WW. This demonstrates a broader variety of AmpC genes found in hospital environments and patients in these national referral hospitals from all over the country.

Unlike ESBLs, these AmpC enzymes are specifically not inhibited by clavulanic acid; this makes them difficult to control clinically. Fadare and Okoh (2021) reported the *blaCITM* (12.9%) from the hospital WW in Eastern Cape Province, South Africa, which is less frequent than the finding from TASH (25%), indicating the diverse spread of AmpC genes in developing countries. Additionally they reported *blaFOXM* (9.7%) and *blaEBCM* (6.5%), which were consistent with our findings at TASH.

The 60% *blaFOXM* which was detected in *E. coli* from TASH WW isolates was nearly similar with a previous study of 65.2% *blaFOXM* (Esther et al., 2025). This exhibits that the selective pressure favoring *FOX* genes is persistent and widely disseminating within the capital's healthcare facilities and the signaling risk of these plasmid-mediated genes entering into urban water systems like the Akaki River.

In TASH and ZMH WW, the *E. cloacae* were found to most frequently harbor plasmid-mediated CP genes, a 100% co-carriage of *blaNDM* and *blaOXA-48* genes. Additionally, the *blaTEM* gene was identified in 100% of *E. cloacae, E. coli, P. aeruginosa, S. aureus, E. faecalis* and *B. cereus* isolates. The presence of this resistance gene in both GNB and GPB indicates its prevalence of resistance characteristics. Particularly, the prevalence of *blaTEM* (100%) resistance gene in *B. cereus* is concerning, as these bacteria form spores that support them preventing traditional disinfectants and WW treatments (Bianco et al., 2021). Our finding indicates more *blaTEM* (86.5%) prevalence than the Chaudhary et al. (2023) report on *E. coli* and *P. aeruginosa*, suggesting the alarming saturation of the hospitals' WW with resistance genes. In contrast, previous study revealed less *blaTEM* identification (50%) from *E. faecalis* in Bangladesh (Fahim et al., 2025); the finding of *blaTEM* (84.2%) detected in *B. cereus* isolates by Ghazaei (2021) was nearly similar with our result. *S. aureus* showed 100% *tetA* and *sul1* genes prevalence from TASH WW samples, suggesting a high status of interspecies genetic exchange, since *tetA* was commonly found in GNB (Chin et al., 2024).

The 100% frequency of both *blaOXA-48* and *blaKPC* in *K. pneumoniae* isolates from ZMH IS suggests the existence of the most successful disseminated resistance clones that have

colonized the hospital IS which was more frequent than the report from Lucknow, India, *blaOXA-48* (65%) (Muneer et al., 2025). From ZMH WW bacterial isolates, the plasmid-mediated *blaNDM* gene was identified from 100% of *C. freundii,* suggesting the capacity of this opportunistic pathogen to shield diverse MGEs (Elrahem et al., 2023). The plasmid-mediated resistance from TASH WW, *K. pneumoniae* was found to harbour *blaNDM* (100%), carbapenemase genes. This finding is consistent with the previous report (Awoke et al., 2022).

Concordance of phenotypic and molecular antimicrobial resistance

A significant discrepancy was observed between phenotypic and genotypic profiles, as 62.3% of isolates that were phenotypically negative for the ESBL-production were found to harbor ESBL-related ARGs upon PCR analysis. This suggests a high prevalence of silent resistance genes due to inefficient initiation sites, gene repression, or missing particular environmental stimuli in the hospital environment that may evade detection by standard laboratory susceptibility testing (Stasiak et al., 2021). Thus, this underscores the limitations of relying solely on phenotypic methods for surveillance and suggests a large reservoir of cryptic resistance at TASH and ZMH.

The Enterobacteriaceae showed different association hierarchy of ESBL production across the bacterial isolates. For examples, *K. pneumoniae* and *R. ornithinolytica* demonstrated perfect (100%) concordance, possibly due to high levels of gene expression and stability that make the ESBL production at the point of easily detectable by DDST. The high phenotypic resistance to β-lactam antibiotics corresponds with the detection of *blaTEM* and *blaCTX-M* genes, suggesting the presence of ESBL-producing strains. The lower concordance in *E. coli* (50%) and *E. cloacae* (35.7%) indicates a silent resistance effect (Deekshit and Srikumar, 2022), but do not express to the level of detectable in phenotypic tests (Bhattarai et al., 2024). This probably occurs because of weak promoters or environmental factors where genes are only expressed under specific conditions (Greco et al., 2021). Additionally, AmpC β-lactamases masking effect can be attributed to the low concordance which might interfere with phenotypic DDST, leading to false negatives notwithstanding the presence of ESBL genes (Kaur, 2016). In this study 15.1% of isolates exhibited phenotypic resistance to meropenem despite the absence of the targeted carbapenemase genes. This condition is typically driven by presence of another gene rather than the designed primer, porin loss or

mutation (Gashaw, et al., 2024), hyper-production of ESBLs or AmpC (Kaur, 2016) and efflux pump overexpression (Muderris et al., 2018).

The 49.3% of isolates carried the *tetA* gene but remained susceptible to tetracyline. These isolates likely carry other genes that didn't screen for, such as *tetB*, *tetM*, or *tetG* (Perewari et al., 2022) or they are using general multidrug efflux pumps that happen to expel tetracycline along with other toxins (Siasat & Blair, 2023).

Limitation of the study

While this study provided important insights about the antimicrobial resistance profiles and molecular landscape of AMR in Addis Ababa, there are a number of limitations that should be acknowledged. The study concentrated on two of Addis Ababa tertiary hospitals. The results could not accurately reflect the circumstances in private clinics in Ethiopia.

Another limitation of the study was that although the resistance genes were detected using PCR, WGS was not used in this investigation. WGS would have made it possible to follow clonal lineages more effectively by providing more detailed information on plasmid incompatibility groups and the precise genetic neighborhood of the resistance genes. Furthermore, the sampling was limited to particular wards and effluent sites and this affected the sample size due to lack of resource and time given for the study. Limitations of this study include the relatively small sample size, cross-sectional design, and lack of temporal sampling, which limit generalizability.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The phenotypic and molecular findings of AMR in WW and IS of tertiary hospitals in Addis Ababa highlight a significant public health concern. This reveals that clinical settings are not only points of care but also hotspots for the selection and dissemination of resistant bacteria. The contamination of IS within hospitals highlights gaps in infection prevention and control (IPC) protocols, facilitating the spread of resistance between the environment, healthcare workers, and patients. Since hospital effluents in Addis Ababa are often discharged into municipal systems with minimal treatment, these facilities act as point sources for disseminating MDR strains into the broader community and ecosystem. It is apparent that these bacteria originate in high-risk settings where selective pressure from extensive antimicrobial usage is constant, as evidenced by the fact that 33.3-100% of isolates were recognized as MDR, and majority of species recorded MAR score ≥ 0.2.

The widespread presence of chromosomal and plasimid-mediated ARGs such as ESBL genes (*blaCTXM* and *blaTEM*), AmpC β-lactamase genes, (*blaCITM*, *blaFOXM*, *blaEBCM*, *blaMOXM* and *blaDHAM*), carbapenamase genes (*blaKPC, blaNDM, blaOXA-48* and *blaOXA-24/143* groups), and non β-lactamases such as tetracycline resistance gene (*tetA*), sulphonamide resistance gene (*sul1*) and aminoglycosides resistance gene (*ermB*) underscores the complexity of treating MDR infections and confirms a MDR landscape.

Since these last-resort resistance genes are located on plasmids, they can be easily transferred between different bacterial species, including those that have not yet been exposed to carbapenem antimicrobials. Overall, these findings indicate that hospital effluents and surfaces in Addis Ababa are high-risk zones for the formation and dissemination of MDR superbugs into the community sewage and environment.

6.2. Recommendations

All relevant bodies should act quickly to reduce the disastrous consequences of hospital WW being dumped into community drainage systems without proper treatment.

- The Environmental Protection Authority (EPA) and Ethiopia's Ministry of Health ought to provide adequate programs and regulations for hospital waste management as well as antimicrobial therapy.

- Ethiopia's medical and educational systems require attention and enough number of trained staff in hospital waste management and the prohibition of Antimicrobial resistance.
- Finally, to mitigate the risks identified in this research, we would like to recommend the hospitals (Tikir Anbessa Specialized Hospital and Zewuditu Memorial Hospital) to focus on on-site WW treatment, enhanced surface disinfection, capacity building, One Health surveillance and practice a public health policy.
- For upcoming researchers, we would like to suggest additional studies to take place on the whole genome sequence, bacteriophages mediated resistance, effect of drug residues and characterization of plasmids in health facilities to add insightful data to the present AMR.

REFERENCES

1. Abamecha, A., Wondafrash, B., & Abdissa, A. (2015). Antimicrobial resistance profile of Enterococcus species isolated from intestinal tracts of hospitalized patients in Jimma, Ethiopia. *BMC Research Notes*, *8*, 213. https://doi.org/10.1186/s13104-015-1200-2
2. Abban, M. K., Ayerakwa, E. A., Mosi, L., & Isawumi, A. (2023). The burden of hospital acquired infections and antimicrobial resistance. *Heliyon*, *9*(10), e20561. https://doi.org/10.1016/j.heliyon.2023.e20561
3. Abebe, M. A. (2017). Study of Hazardous Biomedical Waste Management Practices and Development of Hazardous biomedical Waste Management Guidelines in Addis Ababa. . . *ISSN*, *1*(8).
4. Abosse, J. S., Assefa, S., Teketel, Z., Efa, D. A., Wolde, B., & Eregno, F. E. (2025). Water pollution and cattle health risks in the Akaki River Catchment, Central Ethiopia: Implications for one-health. *Frontiers in Water*, *7*, 1642296. https://doi.org/10.3389/frwa.2025.1642296
5. Abosse, J. S., Megersa, B., Zewge, F., & Eregno, F. E. (2024). Healthcare waste management and antimicrobial resistance: A critical review. *Journal of Water and Health*, *22*(11), 2076–2093. https://doi.org/10.2166/wh.2024.232
6. Addae-Nuku, D. S., Kotey, F. C., Dayie, N. T., Osei, M.-M., Tette, E. M., Debrah, P., & Donkor, E. S. (2022). Multidrug-Resistant Bacteria in Hospital Wastewater of the Korle Bu Teaching Hospital in Accra, Ghana. *Environmental Health Insights*, *16*, 11786302221130613. https://doi.org/10.1177/11786302221130613
7. Adugna, C., & Sivalingam, K. M. (2022). Prevalence of Multiple Drug-Resistant Bacteria in the Main Campus Wastewater Treatment Plant of Wolaita Sodo University, Southern Ethiopia. *International Journal of Microbiology*, *2022*, 1781518. https://doi.org/10.1155/2022/1781518
8. Ahmed, T., Islam, Md. S., Haider, N., Elton, L., Hasan, B., Nuruzzaman, M., Rahman, Md. T., Kabir, S. M. L., & Khan, Md. S. R. (2023). Phenotypic and Genotypic Characteristics of Antimicrobial Resistance in Citrobacter freundii Isolated from Domestic Ducks (Anas platyrhynchos domesticus) in Bangladesh. *Antibiotics*, *12*(4), 769. https://doi.org/10.3390/antibiotics12040769

9. Al-Zahrani, I. A. (2018). Routine detection of carbapenem-resistant gram-negative bacilli in clinical laboratories. A review of current challenge. *Saudi Medical Journal*, *39*(9), 861–872. https://doi.org/10.15537/smj.2018.9.22840
10. Amangelsin, Y., Semenova, Y., Dadar, M., Aljofan, M., & Bjørklund, G. (2023). The Impact of Tetracycline Pollution on the Aquatic Environment and Removal Strategies. *Antibiotics (Basel, Switzerland)*, *12*(3), 440. https://doi.org/10.3390/antibiotics12030440
11. Amsalu, G., Moges, F., Bayu, G., & Gelaw, B. (2024). Magnitude and antimicrobial susceptibility profile of bacteria isolated from pediatric sepsis cases at University of Gondar Hospital, Northwest Ethiopia. *BMC Pediatrics*, *24*(1), 491. https://doi.org/10.1186/s12887-024-04969-8
12. Arnold, B. J., Huang, I.-T., & Hanage, W. P. (2022). Horizontal gene transfer and adaptive evolution in bacteria. *Nature Reviews. Microbiology*, *20*(4), 206–218. https://doi.org/10.1038/s41579-021-00650-4
13. Asante, J., Hetsa, B. A., Amoako, D. G., Abia, A. L. K., Bester, L. A., & Essack, S. Y. (2021). Multidrug-Resistant Coagulase-Negative Staphylococci Isolated from Bloodstream in the uMgungundlovu District of KwaZulu-Natal Province in South Africa: Emerging Pathogens. *Antibiotics*, *10*(2), 198. https://doi.org/10.3390/antibiotics10020198
14. Asfaw, T. (2018). Review on hospital wastewater as a source of emerging drug resistance pathogens. *Journal of Research in Environmental Science and Toxicology*, *7*(2). https://doi.org/10.14303/jrest.2018.020
15. Assefa, M., Tigabie, M., Amare, A., Girmay, G., Geteneh, A., Ayalew, G., Biset, S., & Almagharbeh, W. T. (2025). Emergence of extensively and pan-drug resistance in clinical bacterial isolates: A systematic scoping review from Ethiopian public health perspective. *PLOS Neglected Tropical Diseases*, *19*(8), e0013363. https://doi.org/10.1371/journal.pntd.0013363
16. Atalay, Y. A., Gebeyehu, N. A., & Gelaw, K. A. (2024). The prevalence of hospital acquired infection and associated factors among patients admitted at Wolaita Sodo University Comprehensive Specialized Hospital, in Ethiopia. *IP Journal of Nutrition, Metabolism and Health Science*, *7*(1), 43–50. https://doi.org/10.18231/j.ijnmhs.2024.008

17. Awad, Y. M., Kim, K. R., Kim, S.-C., Kim, K., Lee, S. R., Lee, S. S., & Ok, Y. S. (2015). Monitoring Antibiotic Residues and Corresponding Antibiotic Resistance Genes in an Agroecosystem. *Journal of Chemistry*, *2015*, 1–7. https://doi.org/10.1155/2015/974843
18. Awoke, T., Teka, B., Aseffa, A., Sebre, S., Seman, A., Yeshitela, B., Abebe, T., & Mihret, A. (2022). Detection of blaKPC and blaNDM carbapenemase genes among Klebsiella pneumoniae isolates in Addis Ababa, Ethiopia: Dominance of blaNDM. *PLOS ONE*, *17*(4), e0267657. https://doi.org/10.1371/journal.pone.0267657
19. Awoke, T., Teka, B., Seman, A., Sebre, S., Yeshitela, B., Aseffa, A., Mihret, A., & Abebe, T. (2021). High Prevalence of Multidrug-Resistant Klebsiella pneumoniae in a Tertiary Care Hospital in Ethiopia. *Antibiotics*, *10*(8), 1007. https://doi.org/10.3390/antibiotics10081007
20. Ayalew, W., Mulu, W., & Biadglegne, F. (2019). Bacterial contamination and antibiogram of isolates from health care workers' fomites at Felege Hiwot Referral Hospital, northwest Ethiopia. *Ethiop. J. Health Dev.*
21. Bardossy, A. C., Zervos, J., & Zervos, M. (2016). Preventing Hospital-acquired Infections in Low-income and Middle-income Countries: Impact, Gaps, and Opportunities. *Infectious Disease Clinics of North America*, *30*(3), 805–818. https://doi.org/10.1016/j.idc.2016.04.006
22. Başaran, S. N., & Öksüz, L. (2025). Newly developed antibiotics against multidrug-resistant and carbapenem-resistant Gram-negative bacteria: Action and resistance mechanisms. *Archives of Microbiology*, *207*(5), 110. https://doi.org/10.1007/s00203-025-04298-z
23. Belay, W. Y., Getachew, M., Tegegne, B. A., Teffera, Z. H., Dagne, A., Zeleke, T. K., Abebe, R. B., Gedif, A. A., Fenta, A., Yirdaw, G., Tilahun, A., & Aschale, Y. (2024). Mechanism of antibacterial resistance, strategies and next-generation antimicrobials to contain antimicrobial resistance: A review. *Frontiers in Pharmacology*, *15*, 1444781. https://doi.org/10.3389/fphar.2024.1444781
24. Berhe, T. T., Feleke, A. K., Bekele, G. E., Gebrihiwot, E. M., Ali, Y. H., & Tedila, G. W. (2025). Facilitators and barriers of infectious waste management practice in public and private health facilities of Addis Ababa, Ethiopia: A qualitative phenomenological

study. *Frontiers in Public Health*, *13*, 1538124. https://doi.org/10.3389/fpubh.2025.1538124

25. Beshah, D., Desta, A. F., Woldemichael, G. B., Belachew, E. B., Derese, S. G., Zelelie, T. Z., Desalegn, Z., Tessema, T. S., Gebreselasie, S., & Abebe, T. (2023). High burden of ESBL and carbapenemase-producing gram-negative bacteria in bloodstream infection patients at a tertiary care hospital in Addis Ababa, Ethiopia. *PloS One*, *18*(6), e0287453. https://doi.org/10.1371/journal.pone.0287453
26. Bhattarai, R. K., Basnet, H. B., Dhakal, I. P., & Devkota, B. (2024). Antimicrobial resistance of avian pathogenic Escherichia coli isolated from broiler, layer, and breeder chickens. *Veterinary World*, 480–499. https://doi.org/10.14202/vetworld.2024.480-499
27. Bhaurao, B. C., Rajendra, A. S., & Sarita, L. S. (2022). Isolation and characterization of multidrug resistance bacteria from hospital sewage samples, Maharashtra, India. *African Journal of Biotechnology*, *21*(1), 16–25. https://doi.org/10.5897/AJB2021.17394
28. Bianco, A., Capozzi, L., Monno, M. R., Del Sambro, L., Manzulli, V., Pesole, G., Loconsole, D., & Parisi, A. (2021). Characterization of Bacillus cereus Group Isolates From Human Bacteremia by Whole-Genome Sequencing. *Frontiers in Microbiology*, *11*, 599524. https://doi.org/10.3389/fmicb.2020.599524
29. Bill Simpson, John Deatrick, Hunter Johnson. (2017). Wastewater Sampling. *SESD Operating Procedure*.
30. Bodena, D., Teklemariam, Z., Balakrishnan, S., & Tesfa, T. (2019). Bacterial contamination of mobile phones of health professionals in Eastern Ethiopia: Antimicrobial susceptibility and associated factors. *Tropical Medicine and Health*, *47*, 15. https://doi.org/10.1186/s41182-019-0144-y
31. Bogaerts, P., Rezende De Castro, R., De Mendonca, R., Huang, T.-D., Denis, O., & Glupczynski, Y. (2013). Validation of carbapenemase and extended-spectrum -lactamase multiplex endpoint PCR assays according to ISO 15189. *Journal of Antimicrobial Chemotherapy*, *68*(7), 1576–1582. https://doi.org/10.1093/jac/dkt065
32. Bonso, M., Bedada, D., & Dires, S. (2023). Bacterial Contamination and Antimicrobial Resistance in Drinking Water From Food and Drinking Establishments in Shashemane Town, Ethiopia. *Environmental Health Insights*, *17*, 11786302231216864. https://doi.org/10.1177/11786302231216864

33. Bose, P., Sobur, K. A., Lijon, B., Rahman, Z., Ahamed, T., Sultana, P., Muktaruzzaman, M., Khatun, M., & Islam, A. (2025). Characterization of enterotoxin, antibiotic resistance genes, and antimicrobial susceptibility profiling of Staphylococcus aureus isolated from table eggs: Implications for food safety and public health. *Open Veterinary Journal*, *15*(3), 1187–1205. https://doi.org/10.5455/OVJ.2025.v15.i3.11
34. Brepoels, P., De Wit, G., Lories, B., Belpaire, T. E. R., & Steenackers, H. P. (2025). Selective pressures for public antibiotic resistance. *Critical Reviews in Microbiology*, *51*(3), 417–426. https://doi.org/10.1080/1040841X.2024.2367666
35. Caliskan, O., & Alocilja, E. C. (2023). A Review of Carbapenem Resistance in Enterobacterales and Its Detection Techniques. *Microorganisms*, *11*(6), 1491. https://doi.org/10.3390/microorganisms11061491
36. CDC. (2019). *National Infection & Death Estimates for Antimicrobial Resistance*. https://www.cdc.gov/drugresistance/national-estimates.html
37. Chaudhary, M. K., Jadhav, I., & Banjara, M. R. (2023). Molecular detection of plasmid mediated blaTEM, blaCTX−M,and blaSHV genes in Extended Spectrum β-Lactamase (ESBL) Escherichia coli from clinical samples. *Annals of Clinical Microbiology and Antimicrobials*, *22*(1), 33. https://doi.org/10.1186/s12941-023-00584-0
38. Chen, J., Yu, Z., Michel, F. C., Wittum, T., & Morrison, M. (2007). Development and Application of Real-Time PCR Assays for Quantification of *erm* Genes Conferring Resistance to Macrolides-Lincosamides-Streptogramin B in Livestock Manure and Manure Management Systems. *Applied and Environmental Microbiology*, *73*(14), 4407–4416. https://doi.org/10.1128/AEM.02799-06
39. Chin, J.-J., Lee, H.-M., Lee, S.-Y., Lee, Y.-Y., & Chew, C.-H. (2024). High Carriage of tetA, sul1, sul2 and bla TEM Resistance Genes among the Multidrug-resistant Uropathogenic Escherichia coli (UPEC) Strains from Malaysian Patients. *Tropical Life Sciences Research*, *35*(2), 211–225. https://doi.org/10.21315/tlsr2024.35.2.10
40. Christaki, E., Marcou, M., & Tofarides, A. (2020). Antimicrobial Resistance in Bacteria: Mechanisms, Evolution, and Persistence. *Journal of Molecular Evolution*, *88*(1), 26–40. https://doi.org/10.1007/s00239-019-09914-3
41. CLSI. (2024). *Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100.* Clinical and Laboratory Standards Institute.

42. Cobrado, L., Silva-Dias, A., Azevedo, M. M., & Rodrigues, A. G. (2017). High-touch surfaces: Microbial neighbours at hand. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology*, *36*(11), 2053–2062. https://doi.org/10.1007/s10096-017-3042-4
43. Cruz, S., Abreu, D., Gomes, R., Martins-Oliveira, I., Silva-Dias, A., Perez-Viso, B., Cantón, R., & Pina-Vaz, C. (2024). An improved protocol for bacteria identification by MALDI-TOF MS directly from positive blood cultures. *European Journal of Clinical Microbiology & Infectious Diseases*, *43*(3), 605–610. https://doi.org/10.1007/s10096-023-04725-3
44. Cusack, T. P., Ashley, E. A., Ling, C. L., Rattanavong, S., Roberts, T., Turner, P., Wangrangsimakul, T., & Dance, D. a. B. (2019). Impact of CLSI and EUCAST breakpoint discrepancies on reporting of antimicrobial susceptibility and AMR surveillance. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *25*(7), 910–911. https://doi.org/10.1016/j.cmi.2019.03.007
45. Dafaalla, A. A., Ahmed Ali, A. S. M., & Osman Jubara, A. (2023). Bacterial Contamination of Surfaces in The Surgical Rooms at Al-Mak Nimer University Hospital, Shendi Town- Sudan, 2023. *Journal of Surgical Case Reports and Reviews*, *2*(1). https://doi.org/10.59657/2993-1126.brs.23.005
46. Das, S., & Dash, H. R. (2015). *Microbial Biotechnology- A Laboratory Manual for Bacterial Systems*. Springer India. https://doi.org/10.1007/978-81-322-2095-4
47. Dawodu, O. G., & Akanbi, R. B. (2021a). Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus. *PLOS ONE*, *16*(8), e0254658. https://doi.org/10.1371/journal.pone.0254658
48. Dawodu, O. G., & Akanbi, R. B. (2021b). Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus. *PLOS ONE*, *16*(8), e0254658. https://doi.org/10.1371/journal.pone.0254658
49. Deekshit, V. K., & Srikumar, S. (2022). 'To be, or not to be'—The dilemma of 'silent' antimicrobial resistance genes in bacteria. *Journal of Applied Microbiology*, *133*(5), 2902–2914. https://doi.org/10.1111/jam.15738
50. Dhengesu, D., Lemma, H., Asefa, L., & Tilahun, D. (2022). Antimicrobial Resistance Profile of Enterobacteriaceae and Drinking Water Quality Among Households in Bule

Hora Town, South Ethiopia. *Risk Management and Healthcare Policy*, *15*, 1569–1580. https://doi.org/10.2147/RMHP.S370149

51. Diriba, K., Awulachew, E., Tekele, L., & Ashuro, Z. (2020). Fecal Carriage Rate of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae Among Apparently Health Food Handlers in Dilla University Student Cafeteria. *Infection and Drug Resistance*, *13*, 3791–3800. https://doi.org/10.2147/IDR.S269425
52. Egbenyah, F., Udofia, E. A., Ayivor, J., Osei, M.-M., Tetteh, J., Tetteh-Quarcoo, P. B., & Sampane-Donkor, E. (2021). Disposal habits and microbial load of solid medical waste in sub-district healthcare facilities and households in Yilo-Krobo municipality, Ghana. *PloS One*, *16*(12), e0261211. https://doi.org/10.1371/journal.pone.0261211
53. Elbehiry, A., Marzouk, E., & Abalkhail, A. (2025). Fluoroquinolone resistance in ESKAPE pathogens: Evolutionary pathways, one health transmission, and clinical surveillance. *Frontiers in Microbiology*, *16*, 1719066. https://doi.org/10.3389/fmicb.2025.1719066
54. Elrahem, A. A., El-Mashad, N., Elshaer, M., Ramadan, H., Damiani, G., Bahgat, M., Mercuri, S. R., & Elemshaty, W. (2023). Carbapenem Resistance in Gram-Negative Bacteria: A Hospital-Based Study in Egypt. *Medicina*, *59*(2), 285. https://doi.org/10.3390/medicina59020285
55. Endalamaw, K., Tadesse, S., Asmare, Z., Kebede, D., Erkihun, M., & Abera, B. (2024). Antimicrobial resistance profile of bacteria from hospital wastewater at two specialized hospitals in Bahir Dar city, Ethiopia. *BMC Microbiology*, *24*(1), 525. https://doi.org/10.1186/s12866-024-03693-8
56. Esbelin, J., Santos, T., & Hébraud, M. (2018). Desiccation: An environmental and food industry stress that bacteria commonly face. *Food Microbiology*, *69*, 82–88. https://doi.org/10.1016/j.fm.2017.07.017
57. Esther Chigbaziru Nwojiji, Ijeoma Onyinye Okolo, Chigoziri Akudo Osuji, Ugonna Cassandra Aniokete, Sikiru Oladimeji Akomolafe, Uchechukwu Okekeaji, Christiana Inuaesiet Edemekong, Ikemesit Udeme Peter, & Ifeanyichukwu Romanus Iroha. (2025). Molecular Detection of Ampicillinase blaEBCM, blaFOX and blaDHAM resistant genes in multi-drug resistant Gram-negative bacteria. *GSC Biological and*

Pharmaceutical Sciences, *31*(2), 113–122. https://doi.org/10.30574/gscbps.2025.31.2.0181

58. Fadare, F. T., & Okoh, A. I. (2021). Distribution and molecular characterization of ESBL, pAmpC β-lactamases, and non-β-lactam encoding genes in Enterobacteriaceae isolated from hospital wastewater in Eastern Cape Province, South Africa. *PLOS ONE*, *16*(7), e0254753. https://doi.org/10.1371/journal.pone.0254753
59. Fahim, N. A. I., Rana, M. L., Hasan, M. A. E., Salam, S., Masud, R. I., Huda, N., Saha, S., & Rahman, M. T. (2025). Biofilms and antibiotic resistance profile of Enterococcus faecalis in selected dairy cattle farm environments in Bangladesh. *PloS One*, *20*(5), e0323667. https://doi.org/10.1371/journal.pone.0323667
60. Fonton, P., Grant, R., Gasser, M., Buetti, N., Kronenberg, A., & Harbarth, S. (2025). Incidence and Resistance Patterns of Citrobacter spp. in Switzerland: A Nationwide, Retrospective Surveillance Study (2010–2022). *Microorganisms*, *13*(4), 786. https://doi.org/10.3390/microorganisms13040786
61. Gamuya, E., Salum, M. S., Mtewele, B. A., Minja, B., Damiano, P., Mtemisika, C. I., Mnibi, K. P., Abednego, R. N., Okamo, B. C., Silago, V., Mshana, S. E., Claus, H., & Seni, J. (2025). Contamination of hospital surfaces by third-generation cephalosporin-resistant gram-negative bacteria in district hospitals in Mwanza, Tanzania: Urgent need for enhanced infection prevention and control. *Infection Prevention in Practice*, *7*(3), 100475. https://doi.org/10.1016/j.infpip.2025.100475
62. Gashaw, M., Gudina, E. K., Ali, S., Gabriele, L., Seeholzer, T., Alemu, B., Froeschl, G., Kroidl, A., & Wieser, A. (2024). Molecular characterization of carbapenem-resistance in Gram-negative isolates obtained from clinical samples at Jimma Medical Center, Ethiopia. *Frontiers in Microbiology*, *15*, 1336387. https://doi.org/10.3389/fmicb.2024.1336387
63. Gashaw, M., Gudina, E. K., Tadesse, W., Froeschl, G., Ali, S., Seeholzer, T., Kroidl, A., & Wieser, A. (2024). Hospital Wastes as Potential Sources for Multi-Drug-Resistant ESBL-Producing Bacteria at a Tertiary Hospital in Ethiopia. *Antibiotics (Basel, Switzerland)*, *13*(4), 374. https://doi.org/10.3390/antibiotics13040374
64. Geleta, D., Abebe, G., Tilahun, T., Gezahegn, D., Workneh, N., & Beyene, G. (2024). Phenotypic bacterial epidemiology and antimicrobial resistance profiles in neonatal

sepsis at Jimma medical center, Ethiopia: Insights from prospective study. *PloS One*, *19*(9), e0310376. https://doi.org/10.1371/journal.pone.0310376

65. Geta, K., & Kibret, M. (2022). Antibiotic Resistance Profiles of Bacteria Isolated from Hotspot Environments in Bahir Dar City, Northwestern Ethiopia. *Journal of Multidisciplinary Healthcare*, *15*, 1403–1414. https://doi.org/10.2147/JMDH.S364324
66. Ghazaei, C. (2021a). *Molecular Detection of blaTEM, blaCTX-M and blaSHV Beta-Lactamase Genes in Bacillus cereus Strains Isolated from Infant Dry Milk Samples*. *10*(1).
67. Ghazaei, C. (2021b). Molecular Detection of blaTEM, blaCTX-M and blaSHV Beta-Lactamase Genes in Bacillus cereus Strains Isolated from Infant Dry Milk Samples. *Journal of Medical Bacteriology*, *10*(1), pp.1-10.
68. Gobezie, M. Y., Hassen, M., Tesfaye, N. A., Solomon, T., Demessie, M. B., Kassa, T. D., Wendie, T. F., Andualem, A., Alemayehu, E., & Belayneh, Y. M. (2024). Prevalence of meropenem-resistant Pseudomonas Aeruginosa in Ethiopia: A systematic review and meta-analysis. *Antimicrobial Resistance and Infection Control*, *13*(1), 37. https://doi.org/10.1186/s13756-024-01389-2
69. GRAM. (2023). *The burden of antimicrobial resistance (AMR) in Ethiopia*. Global Research on AMR. Available: https://www.healthdata.org/sites/default/files/2023-09/Ethiopia.pdf
70. Greco, F. V., Pandi, A., Erb, T. J., Grierson, C. S., & Gorochowski, T. E. (2021). Harnessing the central dogma for stringent multi-level control of gene expression. *Nature Communications*, *12*(1), 1738. https://doi.org/10.1038/s41467-021-21995-7
71. Guarddon, M., Miranda, J. M., Rodríguez, J. A., Vázquez, B. I., Cepeda, A., & Franco, C. M. (2014). Quantitative detection of tetracycline-resistant microorganisms in conventional and organic beef, pork and chicken meat. *CyTA - Journal of Food*, *12*(4), 383–388. https://doi.org/10.1080/19476337.2014.892030
72. Hafezi, A., & Khamar, Z. (2024). The Method and Analysis of Some Biochemical Tests Commonly Used for Microbial Identification: A Review. *Comprehensive Health and Biomedical Studies*, *3*(2). https://doi.org/10.5812/chbs-160199
73. Hailemichael, T., Girma, L., Fissiha, P., Geteneh, A., & Kassa, T. (2023). Isolation of virulent phages against multidrug-resistant Acinetobacter baumannii recovered from

inanimate objects of Jimma Medical Center, Southwest Ethiopia. *BMC Infectious Diseases*, *23*(1), 820. https://doi.org/10.1186/s12879-023-08823-7

74. Hassoun-Kheir, N., Stabholz, Y., Kreft, J.-U., de la Cruz, R., Romalde, J. L., Nesme, J., Sørensen, S. J., Smets, B. F., Graham, D., & Paul, M. (2020). Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. *The Science of the Total Environment*, *743*, 140804. https://doi.org/10.1016/j.scitotenv.2020.140804
75. He, S., Ding, Q., Wu, W., Zhang, Y., Kang, Y., Meng, Y., Zhu, S., & Wu, J. (2025). Functional potential and applications of Ureibacillus massiliensis based on whole-genome sequencing analysis. *BMC Microbiology*, *25*(1), 507. https://doi.org/10.1186/s12866-025-04225-8
76. Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., & Piddock, L. J. V. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet (London, England)*, *387*(10014), 176–187. https://doi.org/10.1016/S0140-6736(15)00473-0
77. Hoseinzadeh, M., Sedighi, M., Yahyapour, Y., Javanian, M., Beiranvand, M., Mohammadi, M., Zarei, S., Pournajaf, A., & Ebrahimzadeh Namvar, A. (2024). Prevalence of plasmid-mediated quinolone resistance genes in extended-spectrum beta-lactamase producing Klebsiella pneumoniae isolates in northern Iran. *Heliyon*, *10*(18), e37534. https://doi.org/10.1016/j.heliyon.2024.e37534
78. Hussain, S. M., Sharif, A., Bashir, F., Ali, S., Javid, A., Hussain, A. I., Ghafoor, A., Alshehri, M. A., Naeem, A., Naeem, E., & Amjad, M. (2025). Polymerase Chain Reaction: A Toolbox for Molecular Discovery. *Molecular Biotechnology*. https://doi.org/10.1007/s12033-025-01390-z
79. Jabeen, N., Ullah, W., Khalid, J., & Samad, Z. (2023). Estimating antibiotics consumption in a tertiary care hospital in Islamabad using a WHO's defined daily dose methodology. *Antimicrobial Resistance and Infection Control*, *12*(1), 132. https://doi.org/10.1186/s13756-023-01311-2
80. James, D. O. O. (2025). *Investigating the Impact of Antimicrobial Resistance on Global Health and Combating Strategies*. *9*(3).
81. Kaur, H., Kaura, R., Tiirik, K., Truu, M., Truu, J., Kook, M., Danilian, D., Kisand, V., Mehraliyeva, L., Ahonen, M., Kivisaari, M., Tamminen, J., Semjonov, A., & Ivask, A.

(2026). *Context-dependent effects of antimicrobial coatings on microbial load and bacterial community composition on public high-touch surfaces*. Microbiology. https://doi.org/10.64898/2026.02.05.703978

82. Kaur, J. (2016). Enhancing Phenotypic Detection of ESBL in AmpC co-producers by using Cefepime and Tazobactam. *Journal of Clinical and Diagnostic Research*. https://doi.org/10.7860/JCDR/2016/15264.7041
83. Kebede, A. A., Bedada, T. L., Teklu, D. S., Beyene, D., & Tullu, K. D. (2022). Occurrence and anti-microbial susceptibility pattern of extended spectrum beta-lactamase producing Enterobacteriaceae in governmental hospitals wastewater in Addis Ababa, Ethiopia. *Tropical Medicine and Health*, *50*(1), 57. https://doi.org/10.1186/s41182-022-00437-0
84. Khan, Baig, F. K., & Mehboob, R. (2017). Nosocomial infections: Epidemiology, prevention, control and surveillance. *Asian Pacific Journal of Tropical Biomedicine*, *7*(5), 478–482. https://doi.org/10.1016/j.apjtb.2017.01.019
85. Khan, Z. A., Siddiqui, M. F., & Park, S. (2019). Current and Emerging Methods of Antibiotic Susceptibility Testing. *Diagnostics (Basel, Switzerland)*, *9*(2), 49. https://doi.org/10.3390/diagnostics9020049
86. Kiledal, A., & A Maresca, J. (2023). *Chromosomal DNA extraction from Gram-positive bacteria, V3 v3*. https://doi.org/10.17504/protocols.io.5jyl85119l2w/v3
87. Kindu, M., Moges, F., Alemu, A., Hailu, D., Joseph, R., Besufikad, E., Tsehay, D., Bashah, D., Tigabu, Z., Mihret, A., & Gelaw, B. (2025). Carbapenemase-encoding genes in critical gram-negative bacteria isolated from ICU patients with infections and/or gastrointestinal carriage, and environmental samples in the amhara National Regional state, Ethiopia. *PLOS One*, *20*(9), e0330613. https://doi.org/10.1371/journal.pone.0330613
88. Kindu, M., Moges, F., Ashagrie, D., Tigabu, Z., & Gelaw, B. (2023). Multidrug-resistant and carbapenemase-producing critical gram-negative bacteria isolated from the intensive care unit environment in Amhara region, Ethiopia. *PloS One*, *18*(11), e0295286. https://doi.org/10.1371/journal.pone.0295286
89. Kiros, T., Damtie, S., Eyayu, T., Tiruneh, T., Hailemichael, W., & Workineh, L. (2021). Bacterial Pathogens and Their Antimicrobial Resistance Patterns of Inanimate Surfaces

and Equipment in Ethiopia: A Systematic Review and Meta-analysis. *BioMed Research International*, *2021*, 5519847. https://doi.org/10.1155/2021/5519847

90. Kitaba, A. A., Bonger, Z. T., Beyene, D., Ayenew, Z., Tsige, E., Kefale, T. A., Mekonnen, Y., Teklu, D. S., Seyoum, E., & Negeri, A. A. (2024). Antimicrobial resistance trends in clinical Escherichia coli and Klebsiella pneumoniae in Ethiopia. *African Journal of Laboratory Medicine*, *13*(1), 2268. https://doi.org/10.4102/ajlm.v13i1.2268
91. Korakot Chansareewittaya. (2021). Existence of bacterial contamination on inanimate surfaces and equipment in sub-district health-promoting hospitals in Chiang Rai, Thailand. *Journal of Current Science and Technology*, *11*, 355366. https://doi.org/10.14456/JCST.2021.36
92. Kumar, M., Jaiswal, S., Sodhi, K. K., Shree, P., Singh, D. K., Agrawal, P. K., & Shukla, P. (2019). Antibiotics bioremediation: Perspectives on its ecotoxicity and resistance. *Environment International*, *124*, 448–461. https://doi.org/10.1016/j.envint.2018.12.065
93. Laizer, Z. L., Philbert, I. N., Charles, A. S., Musa, K., Tibendarana, J. R., Mabula, P. L., Maghembe, R. S., Shayo, A., Mkumbaye, S. I., Kassam, N. A., & Kajeguka, D. C. (2025). *Bacterial Contamination of Inanimate Surfaces and Equipment, Distribution and Susceptibility Patterns in Pediatric Wards at a Northern Tanzania Zonal Referral Hospital*. Infectious Diseases (except HIV/AIDS). https://doi.org/10.1101/2025.07.08.25331103
94. Lee, J.-H. (2019). Perspectives towards antibiotic resistance: From molecules to population. *Journal of Microbiology (Seoul, Korea)*, *57*(3), 181–184. https://doi.org/10.1007/s12275-019-0718-8
95. Legese, M. H., Asrat, D., Mihret, A., Hasan, B., Mekasha, A., Aseffa, A., & Swedberg, G. (2022). Genomic Epidemiology of Carbapenemase-Producing and Colistin-Resistant *Enterobacteriaceae* among Sepsis Patients in Ethiopia: A Whole-Genome Analysis. *Antimicrobial Agents and Chemotherapy*, *66*(8), e00534-22. https://doi.org/10.1128/aac.00534-22
96. Legese, M. H., Asrat, D., Swedberg, G., Hasan, B., Mekasha, A., Getahun, T., Worku, M., Shimber, E. T., Getahun, S., Ayalew, T., Gizachew, B., Aseffa, A., & Mihret, A. (2022). Sepsis: Emerging pathogens and antimicrobial resistance in Ethiopian referral

hospitals. *Antimicrobial Resistance & Infection Control*, *11*(1), 83. https://doi.org/10.1186/s13756-022-01122-x

97. Marembo, T., & Chirenda, C. (2025). Antimicrobial resistance and molecular characterization of ESBL-producing Enterobacterales from Parirenyatwa Hospital wastewater in Harare. *JAC-Antimicrobial Resistance*, *7*(5), dlaf170. https://doi.org/10.1093/jacamr/dlaf170
98. Matloko, K., Fri, J., Ateba, T. P., Molale-Tom, L. G., & Ateba, C. N. (2021). Evidence of potentially unrelated AmpC beta-lactamase producing Enterobacteriaceae from cattle, cattle products and hospital environments commonly harboring the blaACC resistance determinant. *PLOS ONE*, *16*(7), e0253647. https://doi.org/10.1371/journal.pone.0253647
99. Mbanga, J., Sibanda, A., Rubayah, S., Buwerimwe, F., & Mambodza, K. (2018). Multi-Drug Resistant (MDR) Bacterial Isolates on Close Contact Surfaces and Health Care Workers in Intensive Care Units of a Tertiary Hospital in Bulawayo, Zimbabwe. *Journal of Advances in Medicine and Medical Research*, *27*(2), 1–15. https://doi.org/10.9734/JAMMR/2018/42764
100. Mekengo, B. M., Hussein, S., & Ali, M. M. (2021). Distribution and antimicrobial resistance profile of bacteria recovered from sewage system of health institutions found in Hawassa, Sidama Regional State, Ethiopia: A descriptive study. *SAGE Open Medicine, 9*, 20503121211039097. https://doi.org/10.1177/20503121211039097
101. Melariri, H., Freercks, R., van der Merwe, E., Ham-Baloyi, W. T., Oyedele, O., Murphy, R. A., Claasen, C., Etusim, P. E., Achebe, M. O., Offiah, S., & Melariri, P. E. (2024). The burden of hospital-acquired infections (HAI) in sub-Saharan Africa: A systematic review and meta-analysis. *EClinicalMedicine*, *71*, 102571. https://doi.org/10.1016/j.eclinm.2024.102571
102. Mogessie, H., Legesse, M., Feleke Haile, A., Tesfaye Beyene, G., T/haimanot, T., Alemu, A., & Ashenafi, M. (2025). Genetic analysis of virulence, extended spectrum beta lactamase, and carbapenem resistance in *Escherichia coli* O157:H7 isolates from drinking water and wastewater in Addis Ababa, Ethiopia. *Journal of Environmental Science and Health, Part A*, *60*(7), 342–350. https://doi.org/10.1080/10934529.2025.2596531

103. Mou, T. J., Nupur, N. A., Haque, F., Islam, M. F., Rahman, Md. S., Huq, Md. A., & Parvez, A. K. (2023). Characterization of Extended Spectrum Beta-Lactamases (ESBL) Producing *Escherichia coli* Isolates from Surface Water Adjacent to Pharmaceutical Industries in Bangladesh: Antimicrobial Resistance and Virulence Pattern. *Microbiology and Biotechnology Letters*, *51*(3), 268–279. https://doi.org/10.48022/mbl.2305.05006
104. Muderris, T., Durmaz, R., Ozdem, B., Dal, T., Unaldı, O., Aydogan, S., Celikbilek, N., & Acıkgoz, Z. C. (2018). Role of efflux pump and OprD porin expression in carbapenem resistance of Pseudomonas aeruginosa clinical isolates. *The Journal of Infection in Developing Countries*, *12*(01), 001–008. https://doi.org/10.3855/jidc.9486
105. Muneer, F., Raj, N., Das, A., Singh, V., Sen, M., & Agarwal, J. (2025). The Rising Menace: Carbapenem-Resistant Klebsiella pneumoniae in a Tertiary Care Center and Co-dominance of blaNDM, blaOXA-48 Along With the Emergence of blaVIM. *Cureus*. https://doi.org/10.7759/cureus.94303
106. Murray, A. K., Stanton, I., Gaze, W. H., & Snape, J. (2021). Dawning of a new ERA: Environmental Risk Assessment of antibiotics and their potential to select for antimicrobial resistance. *Water Research*, *200*, 117233. https://doi.org/10.1016/j.watres.2021.117233
107. Nagaraja, R, M., Hiremath, I., & Am, A. P. (2025). Biochemical diversity profiling of Ralstonia solanacearum (L.) isolates causing bacterial rhizome rot of ginger (Zingiber officinale Rosc.) under in vitro conditions. *International Journal of Advanced Biochemistry Research*, *9*(11S), 743–753. https://doi.org/10.33545/26174693.2025.v9.i11Sj.6310
108. Naghavi, M., Vollset, S. E., Ikuta, K. S., Swetschinski, L. R., Gray, A. P., Wool, E. E., Robles Aguilar, G., Mestrovic, T., Smith, G., Han, C., Hsu, R. L., Chalek, J., Araki, D. T., Chung, E., Raggi, C., Gershberg Hayoon, A., Davis Weaver, N., Lindstedt, P. A., Smith, A. E., … Murray, C. J. L. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. *The Lancet*, *404*(10459), 1199–1226. https://doi.org/10.1016/S0140-6736(24)01867-1
109. Naseer, F. (2017). Phenotypic Cofirmatory Disc Diffusion Test (PCDDT), Double Disc Synergy Test (DDST), E-Test Os Diagnostic Tool for Detection of Extended Spectrum

Beta Lactamase (ESBL) Producing Uropathogens. *Journal of Applied Biotechnology & Bioengineering*, *3*(3). https://doi.org/10.15406/jabb.2017.03.00068

110. Obayiuwana, A., Ogunjobi, A., Yang, M., & Ibekwe, M. (2018). Characterization of Bacterial Communities and Their Antibiotic Resistance Profiles in Wastewaters Obtained from Pharmaceutical Facilities in Lagos and Ogun States, Nigeria. *International Journal of Environmental Research and Public Health*, *15*(7), 1365. https://doi.org/10.3390/ijerph15071365
111. O'NEILL, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations*. Available from: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf
112. Östholm Balkhed, Å. (2014). *Extended-Spectrum ß-Lactamase-Producing Enterobacteriaceae: Antibiotic consumption, Detection and Resistance Epidemiology*. Linköping University Electronic Press.
113. Patience Wagido, E., Tom, A., Prosper, N., Alfred, T., Genevieve, T. M., Wagido, O. C., Langoya, A. S., Brian, S., Ritah, K., Nalumaga, P. P., Yekosani, M., Raymond, A., Bagenda, C., Peter, R. S., & Abraham, B. (2025). Bacterial contamination and infection control practices in the mortuary at Mbarara Regional Referral Hospital, Uganda. *Antimicrobial Stewardship & Healthcare Epidemiology*, *5*(1), e214. https://doi.org/10.1017/ash.2025.10131
114. Perewari, D. O., Otokunefor, K., & Agbagwa, O. E. (2022). Tetracycline-Resistant Genes in Escherichia coli from Clinical and Nonclinical Sources in Rivers State, Nigeria. *International Journal of Microbiology*, *2022*, 9192424. https://doi.org/10.1155/2022/9192424
115. Pérez-Pérez, F. J., & Hanson, N. D. (2002). *Detection of Plasmid-Mediated AmpC -Lactamase Genes in Clinical Isolates by Using Multiplex PCR*.
116. Poirel, L., Potron, A., & Nordmann, P. (2012). OXA-48-like carbapenemases: The phantom menace. *The Journal of Antimicrobial Chemotherapy*, *67*(7), 1597–1606. https://doi.org/10.1093/jac/dks121
117. Prasek, K., Kiersnowska, I., Wójkowska-Mach, J., Różańska, A., Romaniszyn, D., Foryciarz, E., Kwiećkowska, L. B., & Krzych-Fałta, E. (2025). Microbial Contamination on High-Touch Surfaces in Outpatient Clinics: Identification of

Bacterial Strains from Areas of Patient and Medical Staff Occupancy. *Microorganisms*, *13*(3), 698. https://doi.org/10.3390/microorganisms13030698

118. Pruden, A., Alcalde, R. E., Alvarez, P. J. J., Ashbolt, N., Bischel, H., Capiro, N. L., Crossette, E., Frigon, D., Grimes, K., Haas, C. N., Ikuma, K., Kappell, A., LaPara, T., Kimbell, L., Li, M., Li, X., McNamara, P., Seo, Y., Sobsey, M. D., … Zhou, Z. (2018). An Environmental Science and Engineering Framework for Combating Antimicrobial Resistance. *Environmental Engineering Science*, *35*(10), 1005–1011. https://doi.org/10.1089/ees.2017.0520
119. Ramu, S. V., Babitha, K. C., Shailesh, K. S., Nataraja, K. N., & Udayakumar, M. (2010). *Modification of alkaline lysis method for rapid plasmid DNA isolation*. *4*(3).
120. Ranjbar, R., & Alam, M. (2024). Antimicrobial Resistance Collaborators (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Evidence Based Nursing*, *27*(1), 16–16. https://doi.org/10.1136/ebnurs-2022-103540
121. Raoofi, S., Pashazadeh Kan, F., Rafiei, S., Hosseinipalangi, Z., Noorani Mejareh, Z., Khani, S., Abdollahi, B., Seyghalani Talab, F., Sanaei, M., Zarabi, F., Dolati, Y., Ahmadi, N., Raoofi, N., Sarhadi, Y., Masoumi, M., Sadat Hosseini, B., Vali, N., Gholamali, N., Asadi, S., … Ghashghaee, A. (2023). Global prevalence of nosocomial infection: A systematic review and meta-analysis. *PloS One*, *18*(1), e0274248. https://doi.org/10.1371/journal.pone.0274248
122. Ratna, T. A., Sharon, B. M., Velin, C. A. B., & Palmer, K. L. (2025). *Factors affecting CRISPR-Cas defense against antibiotic resistance plasmids harbored by Enterococcus faecalis laboratory model strains and clinical isolates* (2025.03.10.642232). bioRxiv: The Preprint Server for Biology. https://doi.org/10.1101/2025.03.10.642232
123. Regasa, B., Girma, E., Tesfaye, M., & Seid, M. (2021). Assessment of the Bacteriological Profile and Antibiotic Susceptibility Patterns of Wastewater in Health Facilities of Ethiopia. *International Journal of Microbiology*, *2021*, 9969479. https://doi.org/10.1155/2021/9969479
124. Reynoso, E. C., Laschi, S., Palchetti, I., & Torres, E. (2021). Advances in Antimicrobial Resistance Monitoring Using Sensors and Biosensors: A Review. *Chemosensors*, *9*(8), 232. https://doi.org/10.3390/chemosensors9080232
125. Richer-Fortin, A., Veillette, M., Rossi, F., Longtin, Y., Larrotta, A., Paquet-Bolduc, B., & Duchaine, C. (2025). Characterization of the environment of patients colonized with

carbapenemase-producing organisms: Role of air and surfaces in the dissemination of key resistance genes. *Journal of Hospital Infection*, *164*, 55–63. https://doi.org/10.1016/j.jhin.2025.07.003

126. Robert, S. B., Murray, E. G. D., & Smith, N. R. (1957). Bergey's manual of determinative bacteriology. 7th edition. *The Williams & Wilkins Company*.
127. Sabri, N. A., van Holst, S., Schmitt, H., van der Zaan, B. M., Gerritsen, H. W., Rijnaarts, H. H. M., & Langenhoff, A. a. M. (2020). Fate of antibiotics and antibiotic resistance genes during conventional and additional treatment technologies in wastewater treatment plants. *The Science of the Total Environment*, *741*, 140199. https://doi.org/10.1016/j.scitotenv.2020.140199
128. Sahle, B., & Merid, Y. (2024). Prevalence and antibiotic resistance of Staphylococcus aureus in wound infections: A hospital study in Hawassa, Ethiopia. *Journal of Infection in Developing Countries*, *18*(10), 1530–1538. https://doi.org/10.3855/jidc.19023
129. Sathitakorn, O., Jantarathaneewat, K., Weber, D. J., Apisarnthanarak, P., Rutjanawech, S., & Apisarnthanarak, A. (2023). Efficacy of Environmental Cleaning Protocol Featuring Real-Time Feedback with and without PX-UV in Reducing the Contamination of Gram-Negative Microorganisms on High-Touch Surfaces in Four Intensive Care Units in Thailand. *Antibiotics*, *12*(3), 438. https://doi.org/10.3390/antibiotics12030438
130. Sebre, S., Abegaz, W. E., Seman, A., Awoke, T., Desalegn, Z., Mihret, W., Mihret, A., & Abebe, T. (2020). Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates from Inanimate Hospital Environments at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia. *Infection and Drug Resistance*, *Volume 13*, 4439–4448. https://doi.org/10.2147/IDR.S286293
131. Siasat, P. A., & Blair, J. M. A. (2023). Microbial Primer: Multidrug efflux pumps. *Microbiology*, *169*(10), 001370. https://doi.org/10.1099/mic.0.001370
132. Silago, V., Mruma, E. C., Msemwa, B., Mtemisika, C. I., Phillip, S., Ndagula, R. A., Said, M. M., Mushi, M. F., & Mshana, S. E. (2022). Predominance of Acinetobacter spp., Harboring the blaIMP Gene, Contaminating the Hospital Environment in a Tertiary Hospital in Mwanza, Tanzania: A Cross-Sectional Laboratory-Based Study. *Pathogens*, *11*(1), 63. https://doi.org/10.3390/pathogens11010063

133. Silva, K. P. T., & Khare, A. (2024). Antibiotic resistance mediated by gene amplifications. *Npj Antimicrobials and Resistance*, *2*(1), 35. https://doi.org/10.1038/s44259-024-00052-5
134. Sitotaw, B., Ayalew, F., Girma, A., Geta, K., Tadesse, B., & Birhanu, A. G. (2024). High Prevalence of Antibiotic Resistance Bacteria Isolated From Bahir Dar City Municipal Solid Waste Dumpsite, North West Ethiopia. *Environmental Health Insights, 18*, 11786302241260508. https://doi.org/10.1177/11786302241260508
135. Smith, H. Z., Hollingshead, C. M., & Kendall, B. (2025). Carbapenem-Resistant Enterobacterales. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK551704/
136. Song, H.-J., Moon, D. C., Mechesso, A. F., Kang, H. Y., Kim, M. H., Choi, J.-H., Kim, S.-J., Yoon, S.-S., & Lim, S.-K. (2020). Resistance Profiling and Molecular Characterization of Extended-Spectrum/Plasmid-Mediated AmpC β-Lactamase-Producing Escherichia coli Isolated from Healthy Broiler Chickens in South Korea. *Microorganisms*, *8*(9), 1434. https://doi.org/10.3390/microorganisms8091434
137. Sorescu, I., & Stoica, C. (2021). Online Advanced Bacterial Identification Software, an Original Tool for Phenotypic Bacterial Identification. *Romanian Biotechnological Letters*, *26*(6), 3047–3053. https://doi.org/10.25083/rbl/26.6/3047-3053
138. Stasiak, M., Maćkiw, E., Kowalska, J., Kucharek, K., & Postupolski, J. (2021). Silent Genes: Antimicrobial Resistance and Antibiotic Production. *Polish Journal of Microbiology*, *70*(4), 421–429. https://doi.org/10.33073/pjm-2021-040
139. Szymański, M., Skiba, M. M., Piasecka, M., & Olender, A. (2024). Synergistic Effect of Ceftazidime-Avibactam with Aztreonam on Carbapenemase-Positive Klebsiella pneumoniae MBL+, NDM. *Infection and Drug Resistance*, *17*, 2307–2313. https://doi.org/10.2147/IDR.S459695
140. Tadesse, M. L., & Dolamo, B. L. (2022). Assessment of healthcare waste management practices and associated factors in Addis Ababa City Administration Public Health Facilities. *PLOS ONE*, *17*(11), e0277209. https://doi.org/10.1371/journal.pone.0277209
141. Tao, S., Chen, H., Li, N., Wang, T., & Liang, W. (2022). The Spread of Antibiotic Resistance Genes In Vivo Model. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2022*, 1–11. https://doi.org/10.1155/2022/3348695

142. Tesema, M. Y. (2025). Alternative approaches to combat antimicrobial resistance a silent pandemic outbreak concerns. *Discover Medicine*, *2*(1), 196. https://doi.org/10.1007/s44337-025-00439-x

143. Tesema, M. Y., & Birhanu, A. G. (2024). One health initiative to mitigate the challenge of antimicrobial resistance in the perspectives of developing countries. *Bulletin of the National Research Centre*, *48*(1), 19. https://doi.org/10.1186/s42269-024-01176-4

144. Teshome, A., Alemayehu, T., Deriba, W., & Ayele, Y. (2020). Antibiotic Resistance Profile of Bacteria Isolated from Wastewater Systems in Eastern Ethiopia. *Journal of Environmental and Public Health*, *2020*, 2796365. https://doi.org/10.1155/2020/2796365

145. Thapa Shrestha, U., Shrestha, S., Adhikari, N., Rijal, K. R., Shrestha, B., Adhikari, B., Banjara, M. R., & Ghimire, P. (2020). Plasmid Profiling and Occurrence of β-Lactamase Enzymes in Multidrug-Resistant Uropathogenic Escherichia coli in Kathmandu, Nepal. *Infection and Drug Resistance*, *Volume 13*, 1905–1917. https://doi.org/10.2147/IDR.S250591

146. Tilahun, M., Shibabaw, A., & Adane, M. (2025). Prevalence and multidrug resistance patterns of bacterial pathogens in wastewater and drinking water systems from hospital and non-hospital environments in Ethiopia: A systematic review and meta-analysis. *BMC Infectious Diseases*, *25*(1), 250. https://doi.org/10.1186/s12879-025-10660-9

147. Toribio-Celestino, L., Calvo-Villamañán, A., Herencias, C., Alonso-del Valle, A., Sastre-Dominguez, J., Quesada, S., Mazel, D., Rocha, E. P. C., Fernández-Calvet, A., & San Millan, A. (2024). A plasmid-chromosome crosstalk in multidrug resistant enterobacteria. *Nature Communications*, *15*(1), 10859. https://doi.org/10.1038/s41467-024-55169-y

148. Trung, N. T., Hien, T. T. T., Huyen, T. T. T., Quyen, D. T., Binh, M. T., Hoan, P. Q., Meyer, C. G., Velavan, T. P., & Song, L. H. (2015). Simple multiplex PCR assays to detect common pathogens and associated genes encoding for acquired extended spectrum betalactamases (ESBL) or carbapenemases from surgical site specimens in Vietnam. *Annals of Clinical Microbiology and Antimicrobials*, *14*(1), 23. https://doi.org/10.1186/s12941-015-0079-z

149. Tufa, K. F., & Birhanu, A. G. (2025). Antimicrobial Resistance Profile of *Escherichia coli* Isolated From Hospital and Industrial Wastewater Systems. *Environmental Health Insights*, *19*, 11786302251339254. https://doi.org/10.1177/11786302251339254
150. UNEP. (2022). *Environmental Dimensions of Antimicrobial Resistance: Summary for Policymakers*. United Nations Environment Programme. https://wedocs.unep.org/bitstream/handle/20.500.11822/38373/antimicrobial_R.pdf
151. Ur, S., Ali, T., Ali, I., Khan, N. A., Han, B., & Gao, J. (2018). The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. *BioMed Research International*, *2018*, 9519718. https://doi.org/10.1155/2018/9519718
152. Wang, S., Ma, X., Liu, Y., Yi, X., Du, G., & Li, J. (2020). Fate of antibiotics, antibiotic-resistant bacteria, and cell-free antibiotic-resistant genes in full-scale membrane bioreactor wastewater treatment plants. *Bioresource Technology*, *302*, 122825. https://doi.org/10.1016/j.biortech.2020.122825
153. Wassie, B., Gintamo, B., Mekuria, Z. N., & Gizaw, Z. (2022). Healthcare Waste Management Practices and Associated Factors in Private Clinics in Addis Ababa, Ethiopia. *Environmental Health Insights*, *16*, 11786302211073383. https://doi.org/10.1177/11786302211073383
154. Weerakkody, L. R., & Witharana, C. (2024). A rapid, inexpensive and effective method for the efficient isolation of genomic DNA from Gram-negative bacteria. *Molecular Genetics and Genomics*, *299*(1), 26. https://doi.org/10.1007/s00438-024-02120-x
155. WHO. (2015). *Global action plan on antimicrobial resistance*. World Health Organization. https://iris.who.int/handle/10665/193736
156. WHO. (2021). *Global antimicrobial resistance and use surveillance system (GLASS) report: 2021*. World Health Organization. https://apps.who.int/iris/handle/10665/341666
157. WHO. (2022). *Strategic framework for collaboration on antimicrobial resistance – together for One Health.* Geneva. https://www.who.int/publications/i/item/9789240045408
158. WHO. (2023). *Antimicrobial resistance: Accelerating national and global responses*.
159. WHO. (2024a). *High-level messaging on the HAI and AMR burden*. World Health Organisation. https://www.who.int/campaigns/world-hand-hygiene-day/key-facts-and-figures

160. WHO. (2025). *The fourth National Action Plan on Antimicrobial Resistance*. Available at https://www.afro.who.int/countries/ethiopia/news/ethiopia-launched-fourth-national-action-plan-antimicrobial-resistance-during-waaw-2025
161. WHO, W. (2024b). *WHO Bacterial Priority Pathogens List, 2024*. World Health Organization. https://www.who.int/publications/i/item/9789240093461
162. World Bank. (2017). *Drug-Resistant Infections: A Threat to Our Economic Future*. World Bank, Washington, DC. https://openknowledge.worldbank.org/handle/10986/26707
163. Xue, J., Lei, D., Zhao, X., Hu, Y., Yao, S., Lin, K., Wang, Z., & Cui, C. (2022). Antibiotic residue and toxicity assessment of wastewater during the pharmaceutical production processes. *Chemosphere*, *291*(Pt 2), 132837. https://doi.org/10.1016/j.chemosphere.2021.132837
164. Yao, S., Ye, J., Yang, Q., Hu, Y., Zhang, T., Jiang, L., Munezero, S., Lin, K., & Cui, C. (2021). Occurrence and removal of antibiotics, antibiotic resistance genes, and bacterial communities in hospital wastewater. *Environmental Science and Pollution Research*, *28*(40), 57321–57333. https://doi.org/10.1007/s11356-021-14735-3
165. Yenew, C., Kebede, M., & Mulat, M. (2022). Drinking Water Antimicrobial Resistance Enteric Bacterial Load and Public Health Risk in Northwest, Ethiopia: A Laboratory-Based Cross-Sectional Study. *Ethiopian Medical Journal*, *60*(2).
166. Yosef, H. L., Yohannes, M., Diriba, R., & Legese, M. H. (2025). Multidrug-resistant bacterial profiles of inanimate objects at Zewditu Memorial Hospital, Addis Ababa, Ethiopia. *PLOS One*, *20*(10), e0333361. https://doi.org/10.1371/journal.pone.0333361
167. Yu, Y., Zhang, Y., Wang, Y., Liao, M., Li, B., Rong, X., Wang, C., Ge, J., Wang, J., & Zhang, Z. (2023). The Genetic and Phenotypic Diversity of Bacillus spp. From the Mariculture System in China and Their Potential Function against Pathogenic Vibrio. *Marine Drugs*, *21*(4), 228. https://doi.org/10.3390/md21040228
168. Zhang, F., Jiang, X., Chai, L., She, Y., Yu, G., Shu, F., Wang, Z., Su, S., Wenqiong, W., Tingsheng, X., Zhang, Z., Hou, D., & Zheng, B. (2014). Permanent draft genome sequence of Bacillus flexus strain T6186-2, a multidrug-resistant bacterium isolated from a deep-subsurface oil reservoir. *Marine Genomics*, *18 Pt B*, 135–137. https://doi.org/10.1016/j.margen.2014.09.007

169. Zhang, X., Tong, J., Dong, M., Akhtar, K., & He, B. (2022). Isolation, identification and characterization of nitrogen fixing endophytic bacteria and their effects on cassava production. *PeerJ*, *10*, e12677. https://doi.org/10.7717/peerj.12677
170. Zhen, X., Stålsby Lundborg, C., Sun, X., Zhu, N., Gu, S., & Dong, H. (2021). Economic burden of antibiotic resistance in China: A national level estimate for inpatients. *Antimicrobial Resistance and Infection Control*, *10*(1), 5. https://doi.org/10.1186/s13756-020-00872-w
171. Zheng, D., Yin, G., Liu, M., Chen, C., Jiang, Y., Hou, L., & Zheng, Y. (2021). A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. *The Science of the Total Environment*, *777*, 146009. https://doi.org/10.1016/j.scitotenv.2021.146009

APPENDICES

Appendix 1. Identification codes of bacterial isolates categorized by hospital and sample source type

Hospital	Sample type	Sample site	Sample ID	Isolate ID	Bacterial isolates
Tikur Anbessa Specialized Hospital	Inanimate surface	Neonates Intensive Care Unit Room 1	TS1	TS11	*R. ornithinolytica*
				TS12	*E. cloacae*
				Ts13	*B. flexus*
				TS14	*E. coli*
		Neonates Intensive Care Unit Room 2	TS2	TS21	*A. baumannii*
				TS22	*S. aureus*
				TS23	*E. coli*
		Neonates Intensive Care Unit Room 3	TS3	TS31	*E. cloacae*
				TS32	*E. coli*
				TS33	*B. cereus*
		Operation Room 1	TS4	TS41	*A. baumannii*
				TS42	*E. cloacae*
		Operation Room 2	TS5	TS51	*E. cloacae*
				TS52	*B. cereus*
				TS53	*E. coli*
		Operation Room 3	TS6	TS61	*U. massiliensis*
				Ts62	*S. caprae*
				Ts63	*S. aureus*
				TS64	*A. lwoffii*
		Operation Room 4	TS7	TS71	*E. cloacae*
				TS72	*E. coli*
		General Intensive Care Unit Room 1	TS8	TS81	*E. coli*
		General Intensive Care Unit Room 2	TS9	TS91	*L. adecarboxylata*
		General Intensive Care Unit Room 3	TS10	TS101	*E. coli*
				TS102	*S. epidermidis*

		General Intensive Care Unit Room 4	TS11	TS111	*S. epidermidis*
		Pediatrics Room 1	TS12	Ts121	*K. oxytoca*
		Pediatrics Room 2	TS13	TS131	*E. cloacae*
		Delivery Room 1	TS14	TS141	*S. epidermidis*
		Delivery Room 2	TS15	TS151	*B. cereus*
		Mainlab-Room 1	TS16	Ts161	*S. caprae*
		Mainlab-Room 2	TS17	TS171	*E. asburiae*
	Wastewater	Ememegrgency Ward	TW1	Tw11 TW12 TW13 TW14	*S. aureus* *E. coli* *K. oxytoca* *S. epidermidis*
		Operation Ward	TW2	TW21 Tw22 TW23 TW24	*E. coli* *B. flexus* *B. cereus* *S. aureus*
		Pediatrics	TW3	TW31 TW32	*K. oxytoca* *E. coli*
		Intensive Care Unit	TW4	TW41 TW42 TW43 TW44 TW45	*K. pneumoniae* *E. coli* *E. cloacae* *B. cereus* *B. flexus*
		Composite	TW5	TW51	*E. coli*
Zewditu Memorial Hospital	Inanimate surface	Intensive Care Unit Room 1	ZS1	ZS11 ZS12 ZS13	*S. epidermidis* *B. cereus* *E. coli*
		Intensive Care Unit Room 2	ZS2	ZS21 ZS22 ZS23	*E. coli* *B. cereus* *E. faecalis*

		Intensive Care Unit Room 3	ZS3	ZS31 ZS32 ZS33 ZS34 ZS35	*S. aureus* *E. cloacae* *B. cereus* *E. faecalis* *E. faecium*
		Neonates Intensive Care Unit Room 1	ZS4	ZS41 ZS42 ZS43 ZS44	*P. aeruginosa* *E. cloacae* *B. cereus* *E. coli*
		Neonates Intensive Care Unit Room 2	ZS5	ZS51 ZS52	*E. cloacae* *S. aureus*
		Delivery Room 1	ZS6	ZS61	*E. coli*
		Operation Room 1	ZS7	ZS71	*E. hormaechei*
		Operation Room 2	ZS8	ZS81 Zs82 ZS83 ZS84	*S. haemolyticus* *S. aureus* *E. cloacae* *E. faecium*
		Pediatrics Room 1	ZS9	ZS91	*K. pneumoniae*
		Pediatrics Room 2	ZS10	ZS101 ZS102	*K. pneumoniae* *B. flexus*
		Medical Room 1	ZS11	ZS111 ZS112 ZS113	*S. aureus* *E. coli* *B. cereus*
		Medical Room 2	ZS12	Zs121 ZS122	*E. coli* *E. cloacae*
	Wastewater	Medical Ward	ZW1	Zw11 ZW12 ZW13	*K. oxytoca* *B. cereus* *E. cloacae*

				ZW14	*E. coli*
		Pediatrics Ward	ZW2	ZW21 ZW22 ZW23	*C. freundii* *E. coli* *E. faecalis*
		Intensive Care Unit	ZW3	ZW31 ZW32 ZW33	*B. cereus* *E. coli* *P. aeruginosa*
		Composite Site	ZW4	Zw41 Zw42 ZW43	*E. cloacae* *S. aureus* *E. coli*

Appendix 2. Detailed biochemical profiles

Isolate ID	Biochemical Tests													
GNB	GS	Ca	OF	OX	MR	VP	I	CI	H_2S	M	U	G	TSI	Bacteria
TS101	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TS14	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TS23	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TS32	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TS53	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TS72	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TS81	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TW12	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TW21	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TW32	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
Tw42	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
TW51	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZS112	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZS121	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*

ZS13	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZS21	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZS44	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZS61	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZW14	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZW22	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZW32	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZW43	-	+	F	-	+	-	+	-	-	+	-	+	A/A	*E. coli*
ZS101	-	+	F	-	-	+	-	+	-	-	+	+	A/A	*Klebsiella* spp.
ZS91	-	+	F		-	+	-	-		-	+	+	A/A	*Klebsiella* spp.
TW41	-	+	F		-	+	-	-		-	+	+	A/A	*Klebsiella* spp.
TS91	-	+	F	-	+	+	+	-	-	+	-	+	A/A	*Enterobacter* spp.
ZS41	-	+	O	+	-	-	-	-	-	+	-	-	K/NC	*P. aeruginosa*
ZW33	-	+	O	+	-	-	-	-	-	+	-	-	K/NC	*P. aeruginosa*
TW13	-	+	F		-	±	-	-		-	+	+	A/A	Klebsiella spp.
TS121	-	+	F		-	±	-	-		-	+	+	A/A	*Klebsiella* spp
ZW11	-	+	F		-	±	-	-		-	+	+	A/A	*Klebsiella* spp
TW31	-	+	F		-	±	-	-		-	+	+	A/A	*Klebsiella* spp
TS12	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter* spp.
TS131	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
TS31	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
TS42	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
TS51	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
TS71	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
TW43	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
ZS122	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
ZS32	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
ZS42	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
ZS51	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*

ZS83	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
ZW13	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
Zw41	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
ZS71	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
TS171	-	+	F	-	-	+	-	+	-	+	±	+	A/A	*Enterobacter spp.*
ZW21	-	+	F	-	+	-	-	+	+	+	±	+	K/A	*Citrobacter* spp.
TS11	-	+	F		-	+	+	-		-	+	+	A/A	*Raoultella* spp
TS21	-	+	O	-	-	-	-	-	-	-	-	-	K/K	*Acinetobacter* spp
TS41	-	+	O	-	-	-	-	-	-	-	-	-	K/K	*Acinetobacter* spp
TS64	-	+	O	-	-	-	-	-	-	-	-	-	K/K	*Acinetobacter* spp
GPB	GS	Cat	OF	OX	CoA	MR	VP	I	He	MSA	U	G	TSI	
TS22	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
TS63	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
TW11	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
TW24	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
ZS111	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
ZS31	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
ZS52	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
Zs82	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
Zw42	+	+	F	-	+	+	+	-	β	+	+	-	A/A	*S. aureus*
TS102	+	+	F	-	-	+	+	-	+	-	+	-	A/A	*CoNS* spp
TS111	+	+	F	-	-	+	+	-	γ	-	+	-	A/A	*CoNS* spp
TS141	+	+	F	-	-	+	+	-	γ	-	+	-	A/A	*CoNS* spp
TW14	+	+	F	-	-	+	+	-	γ	-	+	-	A/A	*CoNS* spp
ZS11	+	+	F	-	-	+	+	-	γ	-	+	-	A/A	*CoNS* spp
ZS81	+	+	F	-	-	+	+	-	γ	-	+	-	A/A	*CoNS* spp
TS161	+	+	F	-	-	+	+	-	γ	-	+	-	A/A	*CoNS* spp
TS62	+	+	F	-	-	+	+	-	γ	-	+	-	A/A	*CoNS* spp
ZS23	+	-	F	-	NA	+	+	-	γ	NA	-	-	A/A	*Enterococcus* spp

ZS34	+	-	F	-	NA	+	+	-	γ	NA	-	-	A/A	*Enterococcus* spp
ZW23	+	-	F	-	NA	+	+	-	γ	NA	-	-	A/A	*Enterococcus* spp
ZS35	+	-	F	-	NA	+	+	-	γ	NA	-	-	A/A	*Enterococcus* spp
ZS84	+	-	F	-	NA	+	+	-	γ	NA	-	-	A/A	*Enterococcus* spp
TS13	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TW22	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TW45	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZS102	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TS61	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TS151	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TW23	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TS33	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TS52	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
TW44	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZS113	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZS12	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZS22	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZS33	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZS43	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZW12	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp
ZW31	+	+	F	±	NA	+	±	-	β	NA		±	A/A	*Bacillus* spp

Abbreviations: +, positive; -, negative; ±, variable; O, oxidative; F, fermentative; NA, not applicable; K/K, alkali; A/A, acidic; β, beta hemolysis; γ, gama hemolysis; GS, Gram staining; Ca, catalase test; OF, oxidative fermentative; OX, oxidase test; CoA, coagualase test; MR, methylred test; VP, Vogues Prousger; I, indole test; He, hemolysis; MSA, mannitol salt agar; U, urase test; G, gas production; TSI, triple sugar iron.

Appendix 3. Tabular summary of MALDI-TOF MS report

SN	Isolate ID	Bacteria species	Score	SN	Isolate ID	Bacteria species	Score
1.	TS101	*E. coli*	2.55	2.	TW45	*B. flexus*	2.63
3.	TS102	*S. epidermidis*	2.88	4.	TW51	*E. coli*	2.68
5.	TS11	*R. ornithinolytica*	2.56	6.	ZS101	*K. pneumoniae*	2.63
7.	TS111	*S. epidermidis*	2.75	8.	ZS102	*B. flexus*	2.55
9.	Ts121	*K. oxytoca*	2.65	10.	ZS11	*S. epidermidis*	2.65
11.	TS12	*E. cloacae*	2.88	12.	ZS111	*S. aureus*	2.67
13.	TS131	*E. cloacae*	2.78	14.	ZS112	*E. coli*	2.50
15.	Ts13	*B. flexus*	2.85	16.	ZS113	*B. cereus*	2.50
17.	TS14	*E. coli*	2.78	18.	ZS12	*B. cereus*	2.42
19.	TS141	*S. epidermidis*	2.88	20.	Zs121	*E. coli*	2.82
21.	TS151	*B. cereus*	2.83	22.	ZS122	*E. cloacae*	2.82
23.	Ts161	*S. caprae*	2.85	24.	ZS13	*E. coli*	2.82
25.	TS171	*E. asburiae*	2.78	26.	ZS21	*E. coli*	2.73
27.	TS21	*A. baumannii*	2.55	28.	ZS22	*B. cereus*	2.82
29.	TS22	*S. aureus*	2.55	30.	ZS23	*E. faecalis*	2.82
31.	TS23	*E. coli*	2.85	32.	ZS31	*S. aureus*	2.73
33.	TS31	*E. cloacae*	2.73	34.	ZS32	*E. cloacae*	2.42
35.	TS32	*E. coli*	2.53	36.	ZS33	*B. cereus*	2.42
37.	TS33	*B. cereus*	2.73	38.	ZS34	*E. faecalis*	2.42
39.	TS41	*A. baumannii*	2.73	40.	ZS35	*E. faecium*	2.73
41.	TS42	*E. cloacae*	2.55	42.	ZS41	*P. aeruginosa*	2.73
43.	TS51	*E. cloacae*	2.73	44.	ZS42	*E. cloacae*	2.62
45.	TS52	*B. cereus*	2.73	46.	ZS43	*B. cereus*	2.42
47.	TS53	*E. coli*	2.50	48.	ZS44	*E. coli*	2.42
49.	TS61	*U. massiliensis*	2.55	50.	ZS51	*E. cloacae*	2.52
51.	Ts62	*S. caprae*	2.43	52.	ZS52	*S. aureus*	2.42
53.	Ts63	*S. aureus*	2.55	54.	ZS61	*E. coli*	2.73

55.	TS64	*A. lwoffii*	2.43	56.	ZS71	*E. hormaechei*	2.42
57.	TS71	*E. cloacae*	2.43	58.	ZS81	*S. haemolyticus*	2.73
59.	TS72	*E. coli*	2.43	60.	Zs82	*S. aureus*	2.73
61.	TS81	*E. coli*	2.43	62.	ZS83	*E. cloacae*	2.63
63.	TS91	*L. adecarboxylata*	2.43	64.	ZS84	*E. faecium*	2.73
65.	Tw11	*S. aureus*	2.55	66.	ZS91	*K. pneumoniae*	2.33
67.	TW12	*E. coli*	2.55	68.	Zw11	*K. oxytoca*	2.53
69.	TW13	*K. oxytoca*	2.55	70.	ZW12	*B. cereus*	2.73
71.	TW14	*S. epidermidis*	2.73	72.	ZW13	*E. cloacae*	2.82
73.	TW21	*E. coli*	2.73	74.	ZW14	*E. coli*	2.72
75.	Tw22	*B. flexus*	2.73	76.	ZW21	*C. freundii*	2.82
77.	TW23	*B. cereus*	2.73	78.	ZW22	*E. coli*	2.82
79.	TW24	*S. aureus*	2.63	80.	ZW23	*E. faecalis*	2.62
81.	TW31	*K. oxytoca*	2.73	82.	ZW31	*B. cereus*	2.86
83.	TW32	*E. coli*	2.73	84.	ZW32	*E. coli*	2.55
85.	TW41	*K. pneumoniae*	2.63	86.	ZW33	*P. aeruginosa*	2.57
87.	TW42	*E. coli*	2.63	88.	Zw41	*E. cloacae*	2.47
89.	TW43	*E. cloacae*	2.43	90.	Zw42	*S. aureus*	2.38
91.	TW44	*B. cereus*	2.63	92.	ZW43	*E. coli*	2.81

Appendix 4. Antimicrobial resistance profiling of bacterial isolates from the wastewater drainage of TASH wards

Bacterial		Antimicrobials										
Gram negative	Site	AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	NIT	CXM	CTX
K. pneumoniae (n = 1)	ICU	NA	+	-	+	+	+	+	+	-	+	-
	Em	NA	-	-	-	-	-	-	-	-	-	+
K. oxytoca (n = 2)	Ped	NA	-	-	-	-	-	-	-	-	-	+
	OR	+	+	+	+	+	+	+	-	+	-	NA
E.coli (n = 5)	Ped	+	-	-	-	-	-	-	-	-	-	NA

	ICU	-	-	-	-	-	+	-	-	-	-	NA
	Com	+	-	-	-	-	+	-	-	-	-	NA
E. cloacae (n = 1)	ICU	NA	+	+	+	+	-	+	+	+	-	NA
Gram positive	Site	CIP	TE	SXT	NIT	OX	FOX	ERY	NOR	DOX	CD	VAN
	Em	-	-	-	+	+	-	+	-	+	-	+
S. aureus (n = 2)	OR	-	+	-	-	-	-	-	-	-	-	-
S. epidermidis (n = 1)	Em	-	+	-	-	+	-	-	-	-	+	+

Key: +, 1; -,0; NA, not applicable.

Appendix 5. Antimicrobial resistance profiles of bacterial isolates from the IS of TASH wards

Bacterial	Antimicrobials											
Gram negative	site	AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	SXT	NIT	AK	CTX
	NICU2	+	+	-	-	-	+	-	-	-	-	NA	NA
	NICU3	-	-	+	+	+	-	+	-	-	-	NA	NA
	OR2	+	-	-	+	+	+	-	-	+	+	NA	NA
E. coli (n = 7)	GICU1	-	-	-	-	-	+	-	-	-	-	NA	NA
R. ornithinolytica (n = 1)	NICU1	NA	-	+	+	+	+	-	-	+	+	NA	NA
	NICU1	NA	+	+	+	+	-	+	+	-	-	NA	NA
	OR7	NA	+	-	+	-	+	-	-	-	-	NA	NA
E. cloacae (n = 6)	Ped2	NA	+	-	-	+	+	+	+	-	-	NA	NA
	NICU2	NA	-	-	+	NA	-	-	-	-	-	-	+
A. baumannii (n = 2)	OR1	NA	-	-	-	NA	+	-	-	-	-	-	-
A.Iwoffii (n = 1)	OR3	NA	-	-	-	NA	+	-	-	-	NA	+	-
Gram positive		CIP	TE	SXT	NIT	OX	FOX	ERY	NOR	VAN			CD
	NICU2	-	-	-	-	+	+	-	-	+			-
S. aureus (n = 2)	OR3	-	-	-	+	+	-	-	-	+			-
S. caprae (n = 2)	OR3	+	+	+	-	+	+	-	-	+			+

	ML1	-	-	-	-	+	-	-	-	+	+
	GICU3	-	+	-	-	+	+	+	-	+	-
	GICU4	-	-	-	-	+	+	+	+	+	-
S. epidermidis (n = 3)	Del1	-	-	-	-	+	+	+	-	+	-

Appendix 6. Antimicrobial resistance profiling of bacterial isolates from wastewater drainage of ZMH wards

Bacteria		Antimicrobials									
Gram negative	Ward	AMP	PI	GEN	CAZ	AT	MRP	TE	NIT	AK	CTX
C. freundii (n = 1)	Ped	NA	-	NA	-	-	-	NA	NA	+	+
K. oxytoca (n = 1)	Med	NA	-	-	-	-	+	-	-	NA	-
	Med	+	-	-	+	+	+	-	+	NA	NA
	Ped	-	-	-	-	-	-	-	+	NA	NA
	ICU	-	+	-	-	-	-	-	-	NA	NA
E. coli (n = 4)	Com	+	-	-	-	-	+	-	-	NA	NA
	Med	NA	-	-	-	+	+	+	+	NA	NA
E. cloacae (n = 2)	Com	NA	-	-	+	-	+	-	-	NA	NA
P. aeruginosa (n = 1)	ICU	NA	+	-	-	-	-	NA	NA	NA	+
Gram positive		AMP	GEN	CIP	TE	SXT	NIT	ERT	DOX	VAN	
S. aureus (n = 1)	Com	NA	-	-	+	-	+	-	-	+	

Appendix 7. Antimicrobial resistance profiles of bacterial isolates from the inanimate surfaces of ZMH wards

Bacterial	Antimicrobials											
Gram negative		AMP	PI	GEN	CAZ	AT	MRP	CIP	TE	SXT	NIT	CXM	CTX
	Ped1	NA	-	-	+	+	+	+	-	-	-	-	-
K. pneumoniae (n = 2)	Ped2	NA	+	+	-	+	+	+	+	-	+	-	+
E. coli (n = 6)	ICU1	+	-	-	+	+	+	-	-	-	+	-	NA

	NICU1	-	-	-	-	-	-	-	+	-	-	-	NA
	Del1	+	+	-	+	+	+	+	-	+	-	-	NA
	Med1	+	-	-	-	+	+	-	+	-	+	-	NA
	Med2	+	-	-	+	-	-	-	-	-	+	-	NA
E. hormaechei (n = 1)	OR1	NA	-	-	-	-	-	-	+	-	-	-	NA
	ICU3	NA	-	-	-	-	+	-	-	-	-	+	NA
	NICU1	NA	-	-	-	+	+	+	+	-	+	-	NA
	NICU2	NA	-	-	-	-	-	-	-	-	-	-	NA
	OR2	NA	-	-	-	+	+	-	-	-	-	-	NA
E. cloacae (n = 5)	Med2	NA	+	+	+	+	+	+	-	+	-	+	NA
P. aeruginosa (n = 1)	NICU1	NA	-	-	-	-	+	-	NA	NA	NA	NA	-

Gram positive		AMP	GEN	CIP	TE	SXT	NIT	OX	ERY	NOR	VAN
	ICU3	NA	-	-	-	-	-	+	+	-	+
	NICU2	NA	+	-	+	+	+	-	-	+	-
	OR2	NA	-	-	-	-	+	+	-	-	+
S. aureus (n = 4)	Med1	NA	-	-	-	-	+	+	+	-	-
S. epidermidis (n = 1)	ICU1	NA	-	-	-	-	-	+	-	-	+
S. haemolyticus (n = 1)	OR2	NA	-	-	-	-	-	+	-	-	-
	ICU3	-	NA	-	+	NA	-	NA	-	+	-
E. faecium (n = *2)*	OR2	+	NA	-	-	NA	+	NA	-	-	+
	ICU2	-	NA	-	-	NA	+	NA	+	-	+
E. faecalis (n = *2)*	ICU3	+	NA	-	+	NA	-	NA	-	-	-

Appendix 8. DNA isolation

Briefly, for chromosomal DNA isolation, each bacterial colony was inoculated into 5 mL of LB medium and incubated at 37 °C for 24 hours. The suspension culture was added into 2 mL microcentrifuge tube and centrifuged at 13000 rpm for 5 minutes, at 4 ℃ using micro centrifuge device (Globe Scientific Instruments, India) and the medium was removed by aspiration, leaving the bacterial pellet as dry as possible. The bacterial pellet was resuspended in 200 µL of ice-cold alkaline lysis solution I (lysis buffer I, 50 mM Tris pH 8.0 with HCl, 10 mM EDTA, autoclaved and stored at 4 °C) then vortexed vigorously. The 200 µL of freshly prepared alkaline lysis solution II (lysis buffer II, 200 mM NaOH, 1% SDS, freshly prepared just before the use) was added to the bacterial suspension and the tube rapidly inverted 5 times and left on ice for 1 minute. The 150 µL ice-cold alkaline lysis solution III (lysis buffer III, 3.0 M KOAc, pH 5.5, autoclave and store at 4 °C) was added to the microcentrifuge tube and inverted 5 times, then incubated on ice for 5 minutes. The bacterial lysate was centrifuged at 13000 rpm for 5 minutes and the supernatant was discarded. Then, 200 µL of TE buffer was added to the white pellet and heated up for 10 minutes in 70 °C. Equal volume of phenol: chloroform (1:1) was added to the lysate and vortexed vigorously for 30 seconds and centrifuged at 13000 rpm for 10 minutes. The aqueous phase was recovered, transferred to new microcentrifuge tube and 0.6 volumes of isopropanol, 2 volumes of 100% ethanol and 20 µL of sodium acetate were added and centrifuged for 5 minutes after gentle mixing. Then, the liquid was discarded and 1 mL of 70% Ethanol was added to wash away salts and centrifuged for 2 minutes. The ethanol was discarded and the pellet was exposed to air-dry. The pellet was resuspended in 200 µL nucleus free water and the solution was vortexed gently for few seconds.

Briefly, for chromosomal DNA isolation, each bacterial colony was inoculated into 5 mL of Luria-Bertani (LB) broth medium and incubated at 37 °C for 24 hours. The suspension culture was centrifuged at 13000 rpm for 5 minutes, at 4 ℃ using micro centrifuge device (Globe Scientific Instruments, India) and the medium was removed by aspiration, leaving the bacterial pellet as dry as possible. The bacterial pellet was resuspended in 100 µL of ice-cold alkaline lysis solution I (Lysis buffer I, 50 mM Tris pH 8.0 with HCl, 10 mM EDTA, autoclaved and stored at 4 °C) then vortexed vigorously. The 200 µL of freshly prepared alkaline lysis solution II (Lysis buffer II, 200 mM NaOH, 1% SDS, freshly prepared just before the use) was added to the bacterial suspension and the tube rapidly inverted 5 times

and left on ice for 1 minute. The 150 µL ice-cold alkaline lysis solution III (Lysis buffer III, 3.0 M KOAc, pH 5.5, autoclave and store at 4 °C) was added to the microcentrifuge tube and inverted 5 times, then incubated on ice for 5 minutes. The bacterial lysate was centrifuged at 13000rpm for 5 minutes at 4 °C and the supernatant was transferred to a new labelled microcentrifuge tube. Two and half (2.5) volume of isopropanol was added to precipitate the plasmid DNA and the repeated inversion was done thoroughly without vortexing. Then, centrifuged at 13000rpm for 20 minutes and the supernatant was removed by gentle aspiration and the tube was stood in an inverted position over a paper towel to allow all fluid to drain away. The supernatant was discarded to collect the pellet. The 20 µL of 70% ethanol was added, then the closed tube was inverted several times and centrifuged at 13000 rpm for 5 minutes and the supernatant was removed by gentle aspiration leaving open until residual ethanol has evaporated. The pellet was resuspended in 200 µL nucleus free water and the solution was vortexed gently for few seconds.

Appendix 9. PCR protocols

The identification of ESBL genes (*blaCTXM*, *blaSHV* and *blaTEM*) was executed via mPCR according to the protocol described by Trung et al. (2015) with minor modifications. The mPCR was performed in 25 µL of amplification reaction mixture. The reaction consisted the following components: 0.25 mM dNTP mix, 1X FIREPol@R 10x buffer B, 2 mM $MgCl_2$, 1.25 U FIREPol@R DNA polymerase. The primer concentrations were optimized at 0.4 µM *blaCTXM*, 0.48 µM *blaSHV*, and 0.08 µM *blaTEM*. The final volume was adjusted using nuclease-free water.

Multiplex PCR assays were performed to identify three ESBL genes (*blaPER*, *blaGES* and *blaVEB*) and eight carbapenemase-producing (CP) genes *blaVIM* and *blaNDM*, *blaIMP* and *blaKPC*, *blaOXA-48* and *blaOXA-58* group, *blaOXA-23* group and *blaOXA-24/143* group). The CP genes were paired into four duplex reactions. The protocols were adapted from Bogaerts et al. (2013) with some modifications. Each reaction was performed in a 25 µL PCR tube containing: 0.25 mM dNTP mix, 1X FIREPol@R 10x buffer B, 2 mM $MgCl_2$, 1 U FIREPol@R DNA polymerase and 2 µL of template DNA. The final concentration of each primer was 0.2 µM, with the exception of *blaIMP*, which was optimized at 0.6 µM. The final volume was adjusted using nuclease-free water.

The detection of AmpC genes was performed via mPCR following the classification scheme of Pérez-Pérez and Hanson (2002) with minor modifications. The bacterial isolates were screened for six gene families classified into 2 multiplex reactions (*blaMOXM, blaDHAM* and *blaACCM*) and (*blaCITM, blaEBCM* and *blaFOXM*). Both multiplex reactions were prepared in a 25 µL final volume, composed of: 0.2 mM dNTP mix, 1X FIREPol@R 10x buffer B, 1.5 mM $MgCl_2$, 1.25 U FIREPol@R DNA polymerase and 2 µL of template DNA. The specific primer concentrations were: 0.6 µM *blaMOXM*, 0.6 µM *blaDHAM*, 0.5 µM *blaACCM*, 0.6 µM *blaCITM*, 0.5 µM *blaEBCM*, and 0.4 µM *blaFOXM*. The final volume was adjusted using nuclease-free water.

The detection of the rare CP genes *blaGIM, blaAIM, blaSIM* and *blaDIM* was conducted by mPCR based on the protocol described by Poirel et al. (2012) with minor modifications. The four targets were amplified simultaneously in a single in 25 µL reaction mixture. The optimized reaction conditions consisted of: 0.25 mM dNTP mix, 1X FIREPol@R 10x buffer B, 2.5 mM $MgCl_2$, 1U FIREPol@R DNA polymerase and 2 µL of template DNA. Each primer (*blaSIM, blaDIM, blaGIM* and *blaAIM*) was added to a final concentration of 0.2 µM. The final reaction volume was reached using nuclease-free water.

The detection of macrolide resistance gene *ermB* was performed following Chen et al. (2007) with some modifications. The PCR was employed in 25 µL reaction. The amplification mixture consisted of: 0.2 mM dNTP mix, 1X FIREPol@R 10x buffer B, 2 mM $MgCl_2$, 1.25 U FIREPol@R DNA polymerase and 2 µL of DNA. The specific primers for the *ermB* gene were added at a final concentration of 0.5 mM each. The final reaction volume was adjusted using nuclease-free water.

The identification of *sul1* and *tetA* was done according to Awad et al. (2015) with minor modifications. A singleplex PCR (Spx) was executed for the detection of the *sul1* gene. The 25 µL reaction mixture which contained: 0.2 mM dNTP mix, 1X FIREPol@R 10x buffer B, 2 mM $MgCl_2$, 1.25 U FIREPol@R DNA polymerase and 2 µL of template DNA. Each specific primer was added in 0.4 µM concentration. Similarly, the *tetA* gene was detected in a 25 µL reaction volume. The components were the same to the *sul1* protocol, with the exception of the primer concentration (0.5 µM for the *tetA*). In both assays, the final reaction volume was adjusted using nuclease-free water. The PCR condition was set according to the protocol mentioned (**Error! Reference source not found.**). PCR mixtures with the addition of water in place of template DNA were used as negative controls.

Appendix 10. Polymerase chain reaction (PCR) amplification parameters for ARG screening

PCR	Primer pairs	Initial denaturation		Number of cycle	Denaturation		Annealing		Extension		Final extension	
		℃	min	S	℃	s	℃	s	℃	min	℃	min
	blaCTXM	95	4	35	94	25	58	45	72	1	72	10
	blaTEM	95	4	35	94	25	58	45	72	1	72	10
ESBL-1	*blaSHV*	95	4	35	94	25	58	45	72	10	72	10
	blaNDM	95	5	35	94	30	57	90	72	90	72	10
CARBA-1	*blaVIM*	95	5	35	94	30	57	90	72	90	72	10
	blaIMP	95	5	35	94	30	57	90	72	90	72	10
CARBA -2	*blaKPC*	95	5	35	94	30	57	90	72	90	72	10
	blaOXA-48	95	5	35	94	30	57	90	72	90	72	10
CARBA -3	*blaOXA-23* group	95	5	35	94	30	57	90	72	90	72	10
CARBA -4	blaOXA-24/143 Group	95	5	35	94	30	57	90	72	90	72	10
	blaOXA-58 group	95	5	35	94	30	57	90	72	90	72	10
	blaPER	95	5	35	94	30	57	90	72	90	72	10
	blaGES	95	5	35	94	30	57	90	72	90	72	10
	blaBEL	95	5	35	94	30	57	90	72	90	72	10
ESBL -2	*blaVEB*	95	5	35	94	30	57	90	72	90	72	10
	blaMOXM	94	3	30	94	30	58	45	72	60	72	7
	blaDHAM	94	3	30	94	30	58	45	72	60	72	7
AmpC -1	*blaACCM*	94	3	30	94	30	58	45	72	60	72	7
	blaCITM	94	3	30	94	30	58	45	72	60	72	7
	blaEBCM	94	3	30	94	30	58	45	72	60	72	7
AmpC -2	*blaFOXM*	94	3	30	94	30	58	45	72	60	72	7

PCR	Primer pairs	Initial denaturation		Number of cycle	Denaturation		Annealing		Extension		Final extension	
		℃	min	S	℃	s	℃	s	℃	min	℃	min
	blaAIM	94	5	36	94	30	52	40	72	50	72	5
CARBA -5	*blaGIM*	94	5	36	94	30	52	40	72	50	72	5
	blaSIM	94	5	36	94	30	52	40	72	50	72	5
CARBA -6	*blaDIM*	94	5	36	94	30	52	40	72	50	72	5
ermB	*ermB*	94	4	30	94	30	58	30	72	45	72	7
sul1	*sul1*	95	5	35	95	30	55.9	30	72	40	72	7
tetA	teA	94	5	35	94	30	58	30	72	45	72	5

Key: ESBL, extended broad spectram β-lactamase; CARBA, carbapenemase; AmpC, Ambler Class C β-lactamases; ℃, degree Celsius; s, second; min, minute

Appendix 11. Distribution of chromosomal-mediated ARGs in the wastewater drainage of TASH wards

Bacteria	Wards	Antimicrobial resistance genes							
		blaCTXM	*blaTEM*	*blaCITM*	*blaFOXM*	*blaOXA-48*	*ermB*	*tetA*	*sul1*
K. pneumoniae (n = 1)	ICU	+	+	-	-	+	-	-	-
	Em	+	+	-	-	-	-	-	-
K. oxytoca (n = 2)	Ped	+	+	-	-	-	-	-	-
	Em	-	+	+	+	-	+	-	+
	OR	-	+	+	-	-	-	+	-
	Ped	-	+	-	-	-	+	+	+
	ICU	+	+	-	+	-	+	-	-
E. coli (n = 5)	Com	+	+	+	+	-	-	+	+
E. cloacae (n = 1)	ICU	-	+	+	-	-	-	-	+
S. aureus (n = 2)	Em	-	+	-	-	-	-	-	-

	OR	-	+	-	-	-	-	-	+
S. epidermidis (n = 1)	Em	+	+	-	-	-	-	-	+
B. flexus (n = 2)	OR	-	+	-	-	-	-	-	-

Appendix 12. Distribution of chromosomal-mediated ARGs in the inanimate surfaces of TASH wards.

Bacteria	Wards	Antimicrobial resistance genes								
		blaCTXM	*blaTEM*	*blaCITM*	*blaFOXM*	*blaNDM*	*blaOXA-48*	*ermB*	*tetA*	*sul1*
K. oxytoca (n = 1)	Ped1	+	+	-	-	-	-	-	+	-
	NICU-	-	+	+	-	-	-	-	+	+
	NICU2	-	+	+	-	-	-	+	-	+
	NICU3	-	+	+	-	-	-	-	+	-
	OR2	+	+	-	-	-	-	+	+	+
	OR7	-	+	+	-	-	-	-	+	+
	GICU1	-	+	+	+	-	-	-	-	-
E. coli (n = 7)	GICU3	-	+	-	+	-	-	-	+	-
L. adecarboxylata (n = 1)	GICU2	-	+	-	-	-	-	-	-	+
E. asburiae (n = 1)	ML2	-	+	-	-	-	-	-	+	-
R. ornithinolytica (n = 1)	NICU1	-	+	-	-	-	-	-	-	+
	NICU1	-	+	-	-	+	+	-	+	+
	NICU3	-	+	+	-	+	+	-	+	-
	OR1	-	+	-	-	-	+	-	+	+
	OR2	-	+	+	-	+	-	-	+	-
	OR7	-	+	+	-	-	+	-	+	+
E. cloacae (n = 6)	Ped2	-	+	-	-	+	+	-	-	-
	NICU2	-	+	-	-	-	-	-	-	+
A. baumannii (n = 2)	OR2	-	+	-	-	-	-	-	-	-
A. Iwoffii (n = 1)	OR3	-	+	-	-	-	-	-	-	+
S. aureus (n = 2)	NICU2	-	+	-	-	-	-	-	-	-

	OR3	-	+	-	-	-	-	-	-	+
	OR3	-	+	-	-	-	-	-	-	+
S. caprae (n = 2)	ML1	-	+	-	-	-	-	-	-	+
	GICU3	+	+	-	-	-	-	-	-	+
	GICU1	+	+	-	-	-	-	-	-	+
S. epidermidis (n = 3)	Del1	-	+	-	-	-	-	-	-	+
B. cereus (n = *3)*	OR2	-	+	-	-	-	-	-	-	-
B. flexus (n = 1)	NICU1	-	+	-	-	-	-	-	-	-
U. massiliensis (n = 1)	OR3	-	+	-	-	-	-	-	-	-

Appendix 13. Distribution of chromosomal-mediated ARGs in the wastewater drainage of ZMH wards

		Antimicrobial resistance genes										
Bacterial Isolate	wards	*blaCTX M*	*blaTE M*	*blaCIT M*	*blaEBC M*	*blaFOX M*	*blaMOX M*	*BlaDHA M*	*blaND M*	*blaOXA-48*	*tetA*	*sul1*
C. freundii (n = 1)	Ped	+	+	+	+	-	+	+	+	-	-	-
K. oxytoca (n = 1)	Med	-	+	-	-	-	-	-	-	-	+	-
	Med	-	+	-	-	+	-	-	-	-	+	-
	Ped	+	+	+	-	+	-	-	-	-	-	-
	ICU	-	+	+	-	-	-	-	-	-	+	+
E. coli (n = 4)	Com	-	+	-	-	-	-	-	-	-	+	+
	Med	-	+	+	-	-	-	-	+	+	+	+
E. cloacae (n = 2)	Com	-	+	+	-	-	-	-	+	-	-	-
P. aeruginosa (n = 1)	ICU	-	+	-	-	-	-	-	-	-	-	-
S. aureus (n = 1)	Com	-	+	-	-	-	-	-	-	-	-	+
E. faecalis (n = 1)	Ped	-	+	-	-	-	-	-	-	-	-	-
B. cereus (n = 2)	Med	-	+	-	-	-	-	-	-	-	-	-

Appendix 14. Distribution of chromosomal-mediated ARGs in the IS of ZMH wards

Bacterial Isolate	Wards	Antimicrobial resistance genes								
		blaCTXM	*blaTEM*	*blaCITM*	*blaFOXM*	*blaNDM*	*blaOXA-48*	*ermB*	*tetA*	*sul1*
	Ped1	-	+	-	-	-	-	-	-	-
K. pneumoniae (n = 2)	Ped2	-	+	-	-	-	-	-	-	-
	ICU1	-	+	-	-	-	-	+	-	-
	ICU2	+	+	+	+	-	-	-	-	+
	NICU1	-	+	+	+	-	-	-	-	+
	Del1	-	+	+	-	-	-	-	+	+
	Med1	-	+	+	+	-	-	+	+	+
E. coli (n = 6)	Med2	+	+	-	+	-	-	-	+	+
E. hormaechei (n = 1)	OR1	-	+	-	-	-	-	-	-	-
	ICU3	-	+	+	-	-	+	-	+	+
	NICU1	-	+	-	-	-	+	-	-	-
	NICU2	-	+	+	-	-	-	-	+	+
	OR2	-	+	+	-	-	-	-	-	-
E. cloacae (n = 5)	Med 2	-	+	-	-	+	+	-	-	+
P. aeruginosa (n = 1)	NICU1	-	+	-	-	-	-	-	-	-
	ICU3	-	+	-	-	-	-	-	-	-
	NICU2	-	+	-	-	-	-	-	-	+
	OR2	-	+	-	-	-	-	-	-	-
S. aureus (n = 4)	Med1	-	+	-	-	-	-	-	-	-
S. epidermidis (n = 1)	ICU1	+	+	-	-	-	-	-	-	-
S. haemolyticus (n = 1)	OR2	+	+	-	-	-	-	-	-	+
	ICU3	-	+	-	-	-	-	-	-	-
E. faecium (n = *2)*	OR2	-	+	-	-	-	-	-	-	-
	ICU2	-	+	-	-	-	-	-	-	-
E. faecalis (n = *2)*	ICU3	-	+	-	-	-	-	-	-	-

B. cereus (n = *5)*	Med1	-	+	-	-	-	-	-	-	-
B. flexus (n = 1)	Med2	-	-	-	-	-	-	-	-	-

Appendix 15. Distribution of plasmid-mediated ARGs in the wastewater drainage of TASH wards

Bacteria	Ward	Antimicrobial resistance genes											
		blaCTXM	*blaTEM*	*blaFOXM*	*blaMOXM*	*blaDHAM*	*blaNDM*	*blaOXA-48*	*blaOXA-24/143*	*blaKPC*	*ermB*	*tetA*	*sul1*
K. pneumoniae (n = 1)	ICU	+	+	-	-	-	+	+	+	-	-	-	-
	Em	+	+	+	-	-	+	-	-	+	-	-	-
K. oxytoca (n = 2)	Ped	+	+	-	+	+	+	+	-	-	-	-	-
	Em	+	-	+	-	-	-	-	-	-	-	+	+
	OR	-	+	+	-	-	+	-	-	-	-	+	-
	Ped	-	+	-	-	-	-	-	-	-	+	+	+
	ICU	+	+	-	-	-	-	-	-	-	+	+	-
E. coli (n = 5)	Com	-	+	+	-	-	-	-	-	-	-	+	+
E. cloacae (n = 1)	ICU	+	+	-	-	-	+	+	-	-	-	+	+
	Em	+	+	-	-	-	-	-	-	-	-	+	+
S. aureus (n = 2)	OR	+	+	-	-	-	-	-	-	-	-	+	+
S. epidermidis (n = 1)	Em	-	+	-	-	-	-	-	-	-	-	+	+
B. cereus (n = *2)*	ICU	-	-	-	-	-	-	-	-	-	+	-	-
B. flexus (n = *2)*	ICU	-	+	-	-	-	-	-	-	-	-	-	-

Appendix 16. Distribution of plasmid-mediated ARGs in the inanimate surfaces of TASH wards

Bacteria	Wards	Antimicrobial resistance genes								
		blaCTXM	*blaTEM*	*blaFOXM*	*BlaNDM*	*blaOXA-48*	*blaKPC*	*ermB*	*tetA*	*sul1*
K. oxytoca (n = 1)	Ped1	+	+	+	+	-	+	-	-	-

	NICU1	-	+	-	+	-	-	+	+	+
	NICU2	+	+	-	+	-	-	+	+	+
	NICU3	-	+	-	+	-	-	+	+	-
	OR2	+	-	+	+	-	-	+	+	+
	OR7	+	+	-	-	-	-	+	+	+
	GICU1	-	+	-	+	-	-	+	+	-
E. coli (n = 7)	GICU3	-	+	-	-	-	-	-	+	-
L. adecarboxylata (n = 1)	GICU2	-	+	-	-	-	-	-	+	+
E. asburiae (n = 1)	ML2	-	+	-	-	-	-	-	+	-
R. ornithinolytica (n = 1)	NICU1	+	+	-	-	-	-	-	+	+
	NICU1	+	+	-	+	+	-	-	+	+
	NICU3	+	+	-	+	+	-	-	+	+
	OR1	-	-	-	+	-	-	-	-	+
	OR2	+	+	-	+	+	-	-	+	-
	OR7	-	+	-	+	+	-	-	+	+
E. cloacae (n = 6)	PED2	-	-	-	+	-	-	-	+	-
	NICU2	-	+	-	+	-	-	-	-	+
A. baumannii (n = 2)	OR1	-	+	-	+	-	-	-	-	-
A. Iwoffii (n = 1)	OR3	-	+	-	-	-	-	-	-	+
	NICU2	+	+	-	-	-	-	-	-	-
S. aureus (n = 2)	OR3	-	+	-	-	-	-	-	+	+
	OR3	-	+	-	-	-	-	-	+	+
S. caprae (n = 2)	ML1	-	+	-	-	-	-	-	+	+
	GICU3	-	+	-	-	-	-	-	+	+
	GICU4	-	+	-	-	-	-	-	+	+
S. epidermidis (n = 3)	Del1	-	+	-	-	-	-	-	+	-
	NICU3	-	-	-	-	-	-	-	-	-
	OR2	-	+	-	-	-	-	-	-	+
B. cereus (n = 3)	Del2	-	-	-	-	-	-	+	-	-

U. massiliensis (n = 1)	OR3	-	+	-	-	-	-	-	-	-

Appendix 17. Distribution of plasmid-mediated ARGs in the wastewater drainage of ZMH wards

Bacteria	Ward	Antimicrobial resistance genes								
		blaCTXM	*blaTEM*	*blaEBCM*	*blaFOXM*	*BlaNDM*	*blaOXA-48*	*ermB*	*tetA*	*sul1*
C. freundii (n = 1)	Ped	+	+	-	-	+	-	-	-	-
K. oxytoca (n = 1)	Med	-	+	+	+	-	+	-	-	-
	Med	+	+	-	-	-	-	+	+	-
	Ped	-	+	-	+	-	-	-	+	-
	ICU	+	+	-	+	+	-	+	+	+
E. coli (n = 4)	Com	-	+	-	-	-	-	-	+	+
	Med	+	+	-	-	+	+	-	+	+
E. cloacae (n = 2)	Com	+	+	-	-	+	+	-	+	-
P. aeruginosa (n = 1)	ICU	-	+	-	-	-	-	-	-	-
S. aureus (n = 1)	Com	-	+	-	-	-	-	-	-	+
E. faecalis (n = 1)	Ped	-	+	-	-	-	-	-	-	-
	Med	-	+	-	-	-	-	-	-	+
B. cereus (n = 2)	ICU	-	-	-	-	-	-	+	-	-

Appendix 18. Distribution of plasmid-mediated ARGs in the inanimate surfaces of ZMH wards

Bacteria	Wards	Antimicrobial resistance genes								
		blaCTXM	*blaTEM*	*blaFOXM*	*blaNDM*	*blaOXA-48*	*blaKPC*	*ermB*	*tetA*	*sul1*
	Ped1	+	+	-	-	+	+	-	-	-
K. pneumoniae (n = *2)*	Ped2	+	+	+	+	+	+	-	-	-
	ICU1	+	+	-	+	-	-	+	-	-
E. coli (n = 6)	ICU2	+	+	+	+	-	-	+	-	+

	NICU1	+	+	-	-	-	-	-	-	+
	Del1	+	+	-	+	-	-	-	-	+
	Med1	+	+	+	+	-	-	-	+	+
	Med2	-	+	+	-	-	-	-	+	+
E. hormaechei (n = 1)	OR1	-	+	-	-	-	-	-	+	-
	ICU3	+	+	-	+	-	-	-	+	+
	NICU1	-	+	-	-	+	-	-	+	-
	NICU2	+	+	-	-	+	-	-	+	+
	OR2	+	+	-	+	-	-	-	+	+
E. cloacae (n = 5)	Med2	+	+	-	-	-	-	-	-	+
P. aeruginosa (n = 1)	NICU1	-	+	-	-	-	-	-	-	-
	ICU3	+	+	-	-	-	-	-	+	-
	NICU2	+	+	-	-	-	-	-	+	+
	OR2	-	+	-	-	-	-	-	+	-
S. aureus (n = 4)	Med1	+	+	-	-	-	-	-	+	-
S. epidermidis (n = 1)	ICU1	-	+	-	-	-	-	-	+	+
S. haemolyticus (n = 1)	OR2	-	+	-	-	-	-	-	+	+
	ICU3	-	+	-	-	-	-	-	+	+
E. faecium (n = *2)*	OR2	-	+	-	-	-	-	-	-	+
	ICU2	-	+	-	-	-	-	+	-	-
E. faecalis (n = *2)*	ICU3	-	+	-	-	-	-	-	-	-
	ICU1	-	-	-	-	-	-	+	-	-
	ICU2	-	-	-	-	-	-	-	-	-
	ICU3	-	-	-	-	-	-	+	-	-
	NICU1	-	-	-	-	-	-	+	-	-
B. cereus (n = *5)*	Med1	-	+	-	-	-	-	-	-	+
B. flexus (n = 1)	Med1	-	-	-	-	-	-	-	-	-