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Masters in Food Safety

ASSESSMENT OF BACTERIAL CONTAMINATION AND ANTIBIOTIC RESISTANCE IN TRADITIONAL YOGURT MARKETED IN XAI-XAI AND CHOKWE DISTRICTS OF GAZA PROVINCE

DISSERTATION

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A dissertation

Submitted to the department of Animal production and Food Technology in partial fulfillment of the requirements for the degree of master (MSc.) in food safety

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DECLARATION

I, Marie Louise Iragena, hereby declare that this thesis entitled “assessment of bacterial contamination and antibiotic resistance in traditional yogurt marketed in Xai-Xai and Chókwè districts of Gaza Province” is entirely original with no submissions to other institutions for comparable reasons. It has not been submitted to any college or university for a degree or exam”. This dissertation is submitted in partial fulfillment of the requirements for the degree of Master of Science in food safety at the Faculty of Veterinary Medicine of Eduardo Mondlane University

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RECOMMENDATION

This dissertation has been submitted for examination with our approval as university and technical supervisors.

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DEDICATION

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ABBREVIATIONS AND ACRONYMS

ABR: Antibiotic resistance

AMR: Antimicrobial Resistance

ARB: Antibiotic resistant bacteria

CFU: Colony Forming Unit

DALYs: Disability-adjusted life years

DNA: Deoxyribonucleic Acid

EMB: Eosin Methylene Blue

FAO: Food and Agriculture Organization

GDP: Gross Domestic Product

GHP: Good Hygiene Practices

GMP: Good Manufacturing Practices

LAB: Lactic acid bacteria

LMI: Low and middle income

MAR: Multiple antibiotic resistance

MHA: Mueller hinton agar

MRS: De Man Rogosa and Sharpe agar

OECD: Organization for economic co-operation and development

PCA: Plate Count Agar

WHO: World Health Organization

RESUMO

A resistência antimicrobiana (RAM) é uma ameaça profunda à saúde e ao desenvolvimento em todo o mundo. Esta crise é impulsionada pelo uso excessivo de antibióticos na pecuária para promover o crescimento e prevenir doenças. Esse uso inadequado cria bactérias resistentes que podem ser transferidas para os seres humanos, tornando as infeções difíceis ou impossíveis de tratar. O objetivo desta pesquisa foi avaliar a contaminação bacteriana e o perfil de resistência aos antibióticos de iogurtes fermentados localmente. A contaminação bacteriana e a prevalência da RAM foram avaliadas através de 41 amostras coletadas no mercado informal dos distritos de Xai-Xai e Chókwè, na província de Gaza, Moçambique. As amostras de iogurte foram examinadas quanto à presença de bactérias ácido-lácticas e microrganismos de importância para a segurança dos alimentos (*Staphylococcus aureus* e *Escherichia coli*) utilizando meios seletivos. Os isolados purificados foram identificados e confirmados por coloração de Gram e testes bioquímicos. A suscetibilidade aos antimicrobianos foi avaliada utilizando o método de difusão em disco de Kirby-Bauer, seguindo as diretrizes do CLSI. A análise estatística foi realizada utilizando o STATA versão 15. A análise estatística foi realizada utilizando estatística descritiva e teste do qui-quadrado. Os resultados mostraram que foram obtidos um total de 60 isolados. Bactérias ácido-lácticas foram detetadas em todas as amostras de iogurte (100%), com uma contagem média logarítmica de 3,8 e 3,7 nos iogurtes naturais e doces, respetivamente. Bactérias patogénicas também foram detetadas, com *Staphylococcus aureus* presente em 39% (16/41) das amostras e *Escherichia coli* em 7,3% (3/41). O distrito de Chókwè apresentou a maior prevalência de contaminação, com 42,8% das amostras positivas para *S. aureus* e 14,3% positivas para *E. coli*. O teste de suscetibilidade aos antibióticos revelou elevada resistência entre os isolados de bactérias ácido-lácticas, acima de 90% para os antibióticos testados. Todos os isolados de *E. coli* foram resistentes à penicilina 3/3 (100%) e suscetíveis à ampicilina e à ciprofloxacina (100%). Notavelmente, 66,7% dos isolados demonstraram suscetibilidade intermediária à tetraciclina. Os isolados de *S. aureus* apresentaram a maior resistência à penicilina (68,8%), enquanto a maioria permaneceu suscetível à ampicilina, gentamicina e tetraciclina (75%). Em Chókwè, foi apresentada resistência em 66,7% dos isolados à penicilina e à tetraciclina, e em 50,0% dos isolados aos restantes antibióticos testados. Em Xai-Xai, foi observada resistência à penicilina em 70,0% dos isolados, enquanto a resistência aos restantes antibióticos testados foi de 10,0%. O índice médio de Resistência Múltipla aos Antibióticos (MAR) foi mais elevado nas bactérias ácido-lácticas (0,8), seguido por *S. aureus* (0,40) e *E. coli* (0,27). Concluiu-se que, em geral, a contagem de bactérias ácido-lácticas foi baixa, bactérias patogénicas estavam presentes e foi obtida uma elevada prevalência de resistência aos antibióticos nestes produtos, o que representa uma preocupação significativa para a saúde pública.

Palavras-chave: Resistência aos antibióticos, segurança microbiana, iogurte.

ABSTRACT

Antimicrobial resistance (AMR) is a profound threat to worldwide health and development. This crisis is driven by the overuse of antibiotics in livestock to boost growth and prevent disease. This misuse creates resistant bacteria that can transfer to humans, making infections difficult or impossible to treat. The objectives of this research was to assess the bacterial contamination and antibiotic resistance profiling of locally fermented yogurts. Bacterial contamination and AMR prevalence was assessed through 41 samples collected from the informal market in Xai-Xai and Chókwè districts of Gaza provinces of Mozambique. Yogurt samples were examined regard to lactic acid bacteria and microorganisms of food safety concern (*Staphylococcus aureus* and *Echerichia coli)* using selective media. The purified isolates were identified and confirmed by gram-staining and biochemical assay. Antimicrobial susceptibility was evaluated using the Kirby-Bauer disk diffusion method following CLSI guidelines. Statistical analysis was performed using STATA version 15. Statistical analysis was performed using descriptive statistics, chi-square test. The findings showed that a total of 60 isolates were obtained. Lactic acid bacteria were detected in all yogurt samples (100%), with mean log count of 3.8 and 3.7 in natural and sweet yogurt, respectively. Pathogenic bacteria were also detected, with *Staphylococcus aureus* present in 39% (16/41) of samples and *Escherichia coli* in 7.3% (3/41). Chókwè district showed the highest prevalence of contamination, with 42.8% of samples positive for *S. aureus* and 14.3% positive for *E. coli*. Antibiotic susceptibility assay revealed high resistance among LAB isolates above 90% in tested antibiotics. All *E. coli* isolates were resistance to penicillin 3/3 (100%) and susceptible to both ampicillin and ciprofloxacin (100%). Notably, 66.7% of the isolate demonstrated intermediate susceptibility to tetracycline. *S. aureus* isolates indicated the highest resistance to penicillin (68.8%), while most remained susceptible to ampicillin, gentamicin, and tetracycline (75%). In Chókwè, resistance was presented in 66.7% of isolates for penicillin & tetracycline, 50.0% of isolates for lest of tested antibiotics. In Xai-Xai, penicillin resistance was observed in 70.0% of isolates, whereas resistance to rest tested antibiotics was 10.0%. The mean Multiple Antibiotic Resistance (MAR) index was highest in LAB (0.8), followed by *S. aureus* (0.40) and *E. coli* (0.27). It was concluded that in general LAB count was low, pathogenic bacteria was present and high prevalence of resistance profile was obtained in these products which pose a significant public health concern.

Keywords: Antibiotics resistance, microbial safety, yogurt

I. INTRODUCTION

1.1 Motivation of the study

Antimicrobial resistance (AMR) presents a critical threat to global health and sustainable development. Tackling this challenge needs a cooperated effort between health, economic and environmental sectors. World Health Organization (WHO) has reported that AMR is approximately on the 10^{th} position of greatest concern to public health to humanity. The bothersome aspect is that AMR is something that happens naturally with time, usually through genetic changes. Antimicrobial-resistant organisms are increasingly prevalent in humans, animals, food, plants, and environmental reservoirs. The interconnectedness of these reservoirs facilitates the transmission and dissemination of antimicrobial resistance through direct contact, the food chain and environmental pathways (WHO, 2023).

Antimicrobial agents are classified according to the type of microorganism; include antibacterials (antibiotics), antivirals, antifungals, and antiparasitic agents. Antimicrobial resistance (AMR) occurs when microorganisms reduced susceptibility to the medicines, making infections more difficult to treat and increasing the risk of disease transmission, severe illness and death. (Vanderwall & Strine, 2022).

The administration of antibiotics in food producing animals is escalatingly seen as problematic, due to its link to antibiotic resistance that complicates infection treatment in humans. Given that the indiscriminate use of antimicrobials in food-producing animals can turn livestock populations into reservoirs for antibiotic-resistant bacteria. Prolonged administration of antibiotics in animal creates ideal conditions for the development and spread of resistant strains (Ma *et al*., 2021).

In fermented foods, microbial and enzymatic conversions determine and maintain food safety and quality, microorganisms play a significant role in the fermentation process of traditional foods, which can contribute to palatability, wholesomeness, improved nutritional quality, organoleptic characteristics, extended shelf-life, and used as food preservatives, as well as help to maximize and standardize fermentation (Dekeba Tafa & Abera Asfaw, 2020).

Traditional fermented dairy products are widely consumed across Africa, especially in livestock-keeping communities where cattle rearing serves as a primary source of livelihood (Omore *et al*., 2002). These foods, like "cheese" and "yoghurt," are essential to cultural customs in addition to being dietary staples (Kariyawasam *et al*., 2021). But in many African nations,

dairy production is done informally, which frequently leads to insufficient food safety regulations and increased risks of microbial contamination (Quigley *et al*., 2013)

Mozambique has a long history of producing dairy products, especially in rural regions where people depend on animals for their livelihood. The local economy and cuisine depend heavily on traditional fermented dairy products. However, the nation has serious problems with antibiotic resistance and food safety (Koide & Tinga, 2021). Smallholder farms that frequently lack access to contemporary veterinary care and appropriate hygienic practices define Mozambique's dairy industry. This circumstance raises the risk of microbiological infection and antibiotic abuse. Effective public health measures are further hampered by Mozambique's inadequate ability to conduct laboratory tests and monitor foodborne pathogens and antibiotic resistance (Rosse *et al*., 2024).Therefore the objective of this research was to evaluate the antimicrobial resistance profiles and microbiological safety of traditionally fermented yogurts in order to inform policies in relation to fermented yoghurt safety and resistance and as well as to create public awareness of traditional fermented yoghurt safety.

1.2 Problem statement

AMR is a significant public health concern that occurs when bacteria develop mechanisms that enables them to withstand the effects of drugs intended to eliminate them. This resistance can develop through various pathways, a contributing factor is the widespread use of antibiotics in agriculture, particularly in livestock production, where they are often administered to promote growth and prevent disease. This practice can lead to the emergence of Antibiotic resistant bacteria (ARB) within the food supply chain, especially in dairy products derived from treated (Mwasinga *et al*., 2023).

In 2023, antimicrobial resistance (AMR) was estimated to be responsible for approximately 1.27 million deaths worldwide (WHO, 2023). One in 5 deaths from AMR was in children of under 5 years age where they often died from previously treatable infections and 6 Priority bacterial pathogens are responsible for more than 70 percent of the deaths due to AMR (WHO, 2023). The World Bank projects that antimicrobial resistance could place a substantial economic burden on countries, potentially increasing healthcare costs by US$1 trillion by 2050 and causing annual GDP losses of between US$1 trillion and US$3.4 trillion by 2030. A substantial share of these costs is associated with ABR in bacterial infections (World Bank, 2017).

The Majority of studies indicate that treating patients with resistant infections is more expensive than treating those with non-resistant infections, due to longer illness duration, additional diagnostic tests, prolonged hospital stays, the need for more costly drugs, and higher mortality rates (Naylor *et al*., 2025).

Despite its critical importance, AMR doesn't receive enough attention as it should be in LMI countries due to lesser diagnostic, surveillance and legislative capabilities, especially in Sub-Saharan Africa. There are no real-time data and monitoring which increases the gap. A survey across fourteen Sub-Saharan African countries revealed a staggering 50,000 medical laboratories, out of which just 1.3% performed even basic bacteriology testing. In the entire African continent, there are 23 LMI countries' 134, where 43% completely lack data regarding AMR prevalence. Regardless, the current findings confirm that there is AMR resistance to antibiotics that are not subjected to recent standards (da Costa *et al*., 2024).

Mozambique in 2021 alone, an estimated 5,760 deaths were directly attributable to AMR, while 24,500 deaths were associated with resistant infections, placing Mozambique among the top ten countries with the highest age-standardized AMR mortality rates across 204 countries (Institute for Health Metrics and Evaluation, 2019). Children under five remain the most vulnerable group, accounting for the largest share of AMR-related deaths. Without urgent and coordinated interventions, projections suggest that Mozambique could face even higher mortality by 2030, further straining healthcare resources and undermining progress in infectious disease control (Robredo *et al*., 2022).

The growing use of antibiotics in human healthcare and livestock production in Mozambique has raised concerns about the emergence and transmission of antibiotic-resistant bacteria throughout the food supply chain. Inadequate regulation and limited monitoring of antimicrobial use in agriculture, particularly among small-scale and subsistence farmers, may further contribute to the occurrence and spread of antimicrobial resistance (AMR) in fermented dairy products. Furthermore, traditional fermentation processes may not always provide sufficient control over harmful pathogens, potentially leading to the contamination of dairy products with antibiotic-resistant and susceptible microorganisms, makes it unsafe to be consumed (Amir *et al*., 2024).

In Mozambique, a dual public health challenge has emerged, characterized by high levels of chronic malnutrition (44%) and the widespread occurrence of chronic diarrhea in both children and adults. Despite efforts to address these issues, micronutrient deficiencies remain prevalent

across both urban and rural populations. These conditions highlight the critical need for improved food safety and nutrition, particularly in traditional food sources like dairy products, which are integral to the local diet (Sambo *et al*., 2022).

Nhassengo *et al.* (2022), reported that among 18 samples of masi collected in southern Mozambique, 17 showed growth of green colonies with a metallic sheen on EMB agar. These samples were also positive in the Indole test, confirming the presence of *E. coli* as described by Mishra *et al*., (2024), the presence of pathogenic microorganisms occurs due to the use of primitive methods of production of fermented foods, as well as non-compliance with good hygiene and processing practices. The high prevalence of Enterobacteriaceae are related to the presence of acid-resistant *E. coli* strains (Hassani *et al*., 2022).

Staphylococcus aureus prevalence in milk products may be influenced by the hygiene of udders, teats, milking utensils, bulk milk tanks, drying towels, and milk handling and transportation to collections centers however its prevalence can be reduced by heat treatment and fermentation during dairy processing , but differences in farmers' and processors' practices across regions contribute to variability in prevalence (Borena *et al*., 2023).

Milk contamination represents a significant risk to public health, in particular for children, the elderly and the sick, who are the majority of people in the community who consume these products as dietary supplements (Dekeba Tafa & Abera Asfaw, 2020). Despite the potential risks, there is a lack of comprehensive data on the prevalence of antibiotic resistance and the microbial safety of traditional fermented milk products in Mozambique and many other African countries. This research essential for assessing the prevalence of AMR within the food supply chain, particularly in dairy products, and for informing the development of effective strategies to reduce the associated risks of antibiotic-resistant pathogens in Gaza region.

1.3 Objectives

1.3.1 General Objective

To assess the microbiological safety and antibiotic resistance profiles of bacteria isolated from locally fermented yogurts marketed in Gaza province.

1.3.2 Specific Objectives

a. To isolate and enumerate bacterial strains from traditional yogurt samples.

b. To phenotypically characterize and identify lactic acid bacteria isolated from traditional yogurt samples based on their macroscopical, microscopical and biochemical characteristics.

c. To determine the occurrence of selected pathogenic bacteria including *Escherichia coli* and *Staphylococcus aureus* among the bacterial isolates.

d. To determine the antimicrobial susceptibility profiles of the bacterial isolates.

1.4 Research Questions

a. What bacterial strains are present in traditional yogurt samples, and how can they be identified?
b. Are pathogenic microorganisms, such as *Escherichia coli* and *staphylococcus aureus* present in the fermented milk samples?
c. Are there any antibiotic-resistant microorganisms among the bacterial strains isolated from the fermented yogurt?
d. What are the antibiotic resistance profiles of bacteria in fermented yoghurt?

1.5 Contribution of the study

This study contributes to a better understanding of food safety and antimicrobial resistance (AMR) in traditional fermented dairy products marketed in Maputo and Gaza provinces. By assessing both microbial safety and antibiotic resistance this will highlights potential public health risks associated with the consumption of these products especially in vulnerable populations with limited access to healthcare and also it will improve food safety standards through microbial analysis by evaluating the hygienic quality of traditional dairy products, identifying contamination risks linked to production, handling, and marketing practices. This information can be used to develop and implement improved food safety protocols, ensuring safer food for consumers.

Traditional fermented dairy products play a vital role in food security and diet across many African communities. Traditional dairy producers, vendors, and other stakeholders within the value chain can benefit from the study's recommendations. The study will create public microbial safety awareness which can drive behavior change toward safer production and storage practices. It also supports the development of targeted interventions, such as training for local dairy producers and vendors, and encourages the implementation of regulations to control antibiotic use in livestock. Overall, the study supports a One Health approach by linking human, animal, and food system health, and offers a foundation for future research and public health strategies (Yamada *et al*., 2014).

II. LITERATURE REVIEW

2. 1 Traditional Fermented Dairy Products

Milk is a nutrient-rich and readily digestible food, containing substantial amounts of protein, minerals, carbohydrates, and various growth-promoting factors. Milk and its products act as a good environment for microbial growth which constitutes biological hazards for consumers (El-Toukhy *et al*., 2022). Fermentation is an ancient food-processing method that helps inhibit spoilage and pathogenic microorganisms, contributing to extend the shelf life of perishable foods. Fermentation can contribute to preservation of food, actively participate in the development of their texture, flavor and aroma, help to eliminate pathogens, allergens (Nhassengo *et al*., 2022).

Recent data from the Food and Agriculture Organization of the United Nations (FAO) showed that in 2021 the total milk production in Africa was about 50 million tons, representing 5.38 % of global milk production (FAO, 2022). Dairy products are an essential part of the world's population diet, including in Africa (Abdqani, 2016), where an increase in per capita consumption of milk has been observed (FAO, 2022). According to the Organization for Economic Co-operation and Development (OECD) and FAO, the global per capita consumption of dairy products is expected to increase by 1 % annually by 2030 (Simbine-Ribisse *et al.*, 2024).

There is a wide variety of artisanal dairy products, and their denomination and manufacturing processes differ between regions (Melini *et al*., 2019). Fermented dairy products exhibit variations in taste and consistency and may be classified into different categories, such as drinkable yogurt, fermented milk, butter, and cheese. These products are made by fermenting raw milk at room temperature by using spontaneous fermentation, back-slopping fermentation, or a specific starter and/or rennet addition. Fermentation is a simple process that does not require intensive labor; it extends shelf life and improves organoleptic features (Zinno *et al*., 2022).

2.1.1 Yogurt

Yogurt is a fermented dairy product produced through the bacterial fermentation of lactose in milk. During fermentation, lactic acid is produced, which lowers the pH of the milk, promotes protein coagulation, and contributes to the characteristic texture, flavor, and sensory properties of yogurt. The milk is typically incubated at a controlled temperature of approximately 40–45°C for several hours, allowing the fermentation process to occur and the milk to develop its

characteristic thickness. For the fermentation process, specific bacterial cultures are used, mainly *Lactobacillus bulgaricus* and *Streptococcus thermophiles* (Malawati, 2020)

Yogurts are highly appreciated, and since they are regarded as important in human diet, also help with certain gastrointestinal conditions, such as lactose intolerance, constipation, diarrheal diseases, colon cancer they are produced and consumed massively in many countries (Teagasc, 2020). Dairy products generally contain relatively low level of bioactive compounds. To improve their nutritional value, coloring, flavoring, texturizing plant or fruit-based additives can be used to fortify yogurt. Yogurts fortified with antioxidants from natural origin are an interesting food to satisfy consumer demands for healthy products (Caleja *et al*., 2016).

During milk fermentation, naturally occurring lactic acid bacteria (LAB) metabolize components of the milk and produce compounds that create conditions unfavorable to many harmful microorganisms. The resulting decrease in pH can limit the growth of pathogens such as *Staphylococcus aureus*, coliforms, *Escherichia coli*, *Campylobacter jejuni*, and *Vibrio cholerae*. Fermentation also breaks down part of the lactose present in milk, potentially improving its tolerance and digestibility among people who have difficulty digesting lactose (Hossain & Mozumder, 2022).

In Mozambique, raw cow's milk is used to produce masi, an unsweetened curdled milk, with a strong acidic taste and a firm semi-fluid consistency, to be consumed within seven days (Mattiello *et al*., 2018).

In traditional masi production, raw cow's milk is transferred into plastic containers and left sealed indoors for about two to three days, during which spontaneous fermentation takes place. The process relies on the indigenous microbial population of the milk, together with microorganisms present on or within the container, making fermentation an important control point in masi production. During the fermentation, two layers are formed, a liquid layer (whey) that is decanted and a thick clot (masi) that is kept in the bucket for consumption (Nhassengo *et al*., 2022). The presence of beneficial microorganisms also contributes to the drink's probiotic properties, which can enhance gut health, aid digestion and enhance immune function (Abdul Manan, 2025).

2.2 Antibiotic Resistance in fermented dairy products

Antibiotics are widely used in veterinary medicine for therapeutic (to treat sick animals), prophylactic (to prevent diseases), as well as for growth promotion (to improve feed utilization

and production) purposes (Simbine-Ribisse *et al*., 2024). Bacteria become antibiotic-resistant when they develop mechanisms that enable them to survive exposure to antibiotics that are normally effective against them. In other words, the bacteria become resistant to the effects of the antibiotics that would typically treat the infections they cause. This can happen through natural evolution, but more often it is driven by human actions, such as overuse or misuse of antibiotics in healthcare, agriculture, and animal farming (Brüssow, 2024).

Antimicrobial agents at low doses (sublethal or subtherapeutic) in the form of residues in feed might also be factors influencing bacterial antibiotic resistance, as they are able to induce genetic and phenotypic variability in exposed bacteria (Adamski *et al*., 2023). LAB, which are commonly used in the fermentation of dairy products such as (*Lactobacillus*, *Streptococcus*, *Lactococcus*, and *Bifidobacterium*), can harbor resistance genes, potentially originating from either the dairy production environment or from the antibiotics used in animal farming. It has been reported the presence of antibiotic-resistant LAB in various fermented dairy products, such as yogurt, cheese, and kefir (Lemma *et al*., 2021).

In food safety, recent years, antimicrobial resistance (AMR) has emerged as a major public health concern, particularly concerning the role of foodborne pathogens and the potential spread of antibiotic-resistant bacteria through the food chain. Fermented foods, such as dairy products including yogurt and cheese, as well as fermented vegetables, may serve as potential vehicles for the transmission of these microorganisms that are integral to their production and preservation. However, the presence of antibiotic-resistant bacteria in fermented foods poses unique concerns for public health and food safety (Kim & Ahn, 2022).

The link between antibiotic resistance and fermented foods arises from several factors including the use of antibiotics in livestock production for disease prevention and growth promotion. These antibiotics can end up in animal products, such as milk or meat, which can then be used in the production of fermented foods (Urban-Chmiel *et al*., 2022). Even with the use of pasteurization or other microbial control methods, traces of antibiotic-resistant bacteria may persist in the final product. Bacteria become resistant through several mechanisms including the enzymatic breakdown or modification of antimicrobial agents (e.g., β-lactamase production), alteration to antibiotics target sites, efflux pumps, biofilm formation, and horizontal gene transfer through plasmids and transposons. These mechanisms make infections harder to treat and facilitate spread within food chains (Obioha *et al*., 2023).

Recent studies show high levels of multidrug-resistant bacteria in African dairy products. (Mwasinga *et al*., 2023) reported multidrug-resistant *E. coli* in raw milk in Zambia, while Borena et al. (2023) documented resistant *S. aureus* in Ethiopian dairy products. Obioha et al. (2023) demonstrated that lactic acid bacteria in fermented milk can carry resistance genes transferable to pathogens.

In Mozambique, systematic AMR surveillance in dairy products is almost nonexistent. Limited studies suggest emerging risks: Rosse et al. (2024) found bacteria harboring virulence and resistance genes in foods sold in Maputo. Since traditional dairy is widely consumed and often produced informally, understanding AMR within these products is crucial

2. 3 Microbial Safety of Fermented Dairy Products

Food safety hazards encompass biological, chemical, and physical agents, as well as conditions associated with food, that may negatively affect consumer health. Within the dairy supply chain, potential hazards can be introduced throughout the production and handling process, from primary production and milking to processing, packaging, transportation, storage, preparation, and serving. From a healthy animal, raw milk is expected to be free from pathogens at the point of collection (Owusu-Kwarteng *et al*., 2020).

Biological hazards include bacteria, viruses, fungi, and parasites that can contaminate food and cause disease. Bacteria are microscopic, single-celled organisms, while viruses can replicate only inside living host cells. Bacteria and viruses are major causes of foodborne illnesses worldwide. Example of foodborne viruses include norovirus, hepatitis A virus, astrovirus, sapovirus, and rotavirus (Olaimat *et al*., 2024). Beneficial microorganisms, such as lactic acid bacteria can improve food digestibility and safety by inhibiting spoilage and pathogenic microorganisms but pathogenic microorganisms can cause serious illness and even death (García-Cano *et al*., 2019).

Generally, pathogenic bacteria including the genera *Staphylococcus, Streptococcus, Bacillus, Micrococcus, and Corynebacterium* can found on the mammary glands of dairy animals even without any sign of illness. While most foodborne pathogens such *as E. coli, Salmonella* spp. *and Campylobacter* spp inhabit the ruminant intestinal tract, others including *Listeria monocytogenes and Bacillus* spp are widespread in nature and live in soils and plant environments (Chedid *et al*., 2025).

Fungi are eukaryotic organisms that may exist as either single-celled or multicellular forms. Their cells contain a membrane-bound nucleus and lack chlorophyll and chloroplasts. Some pathogenic fungi can contaminate food and agricultural products, posing a potential risk to human health. Certain fungi produce toxic compounds known as mycotoxins, including aflatoxins and ochratoxins, patulin, fumonisins, trichothecenes, and zearalenone which can cause adverse health effects (Silva *et al*., 2023)

Parasites are organisms that depend on a host for survival and obtain nutrients from it. Foodborne parasites are commonly transmitted when people consume food or water contaminated with parasitic eggs or other infectious stages. Transmission can also occur through contact with the feces of an infected host, particularly when proper hand hygiene is not practiced before touching the mouth. Examples of foodborne parasites include *Toxoplasma gondii, Giardia intestinalis, Sarcocystis* spp*, Taenia saginata*, etc (De France *et al*., 2022).

Although microbial contamination of milk during milking cannot be completely prevented, maintaining high standards of hygiene and regularly cleaning and disinfecting the teats are essential for promoting udder health, preserving milk quality, and ensuring consumer safety (Kumari & Tripathi, 2025). In other hand environmental microorganisms such as psychrotrophic bacteria (*Pseudomonas fluorescens*, *Pseudomonas fragi*, *Acinetobacter spp.*, *Aeromonas spp*) and Spore-Forming Bacteria (*Bacillus cereus*, *Bacillus polymyxa*, *Clostridium spp*) can contaminate the milk by direct contact or through milking equipment and personnel on the farm if good hygienic practices (GHP) are not followed (Patil, 2019). Traditional African fermented milk products may be susceptible to contamination with human pathogens of public health concern due to the lack of proper control measures and adherence to good manufacturing practices (GMP) in the traditional fermentation processes (Owusu-Kwarteng *et al*., 2020).

The microbial contamination of fermented dairy products by pathogens such as *Salmonella* spp., *E. coli*, and *Listeria monocytogenes* can have serious public health implications. For healthy individuals, contamination typically results in gastrointestinal illnesses, which, while unpleasant, may resolve without long-term consequences. However, these effects can be particularly serious among vulnerable groups, including infants, older adults, pregnant women, and individuals with weakened immune systems. These individuals are at a higher risk of developing complications, including life-threatening infections, kidney failure (in the case of *E. coli*), and miscarriage or stillbirth (in the case of *Listeria*) (Ntuli *et al*., 2023).

2.4. Detection of pathogens and resistant strains in dairy products

The identification of pathogenic and antibiotic-resistant bacteria in milk products is critical for ensuring food safety and public health. As the prevalence of antibiotic-resistant strains continues to rise, it becomes increasingly important to have reliable methods for identifying both pathogenic microorganisms and those exhibiting resistance to common antibiotics (Saboktakin-Rizi *et al*., 2021). Several techniques are employed to detect these bacteria in milk products, starting from traditional culturing methods to advanced molecular and genomic techniques. This section provides an overview of the current methods used for detecting pathogens and resistant strains in dairy products, with an emphasis on their accuracy, speed, and application (Zhang *et al*., 2022).

Traditional microbiological methods, such as culture-based techniques and antimicrobial susceptibility testing, remain important tools for identifying pathogens and resistance profiles (Arreguin-Campos *et al*., 2023). However, molecular methods like PCR, real-time PCR, and next-generation sequencing offer more rapid, sensitive, and specific detection capabilities, allowing for quicker identification and monitoring of harmful microorganisms. As antimicrobial resistance continues to rise, these advanced techniques will play an increasingly critical role in the detection and management of resistant strains in dairy products. Continuous advancements in biosensors and other novel technologies may further improve the speed, accessibility, and efficiency of pathogen detection in the dairy industry, contributing to enhance food safety (Khater *et al*., 2021).

III. THEORETICAL FRAMEWORK

The theoretical behind of this research on the assessment of antibiotic resistance and microbial safety in traditional yogurt is fixed in several complementary models. The Germ Theory of Disease establishes that specific microorganisms are responsible for particular diseases, making it essential to monitor pathogens such as *Escherichia coli*, *Salmonella spp.*, *staphylococcus aureus* and *Listeria monocytogenes* in dairy products (Bintsis, 2017).

In Mozambique, the indiscriminate use of antibiotics by livestock farmers often without veterinary prescription has led to the presence of antibiotic residues in milk and dairy products, facilitating the development of antimicrobial resistance (WHO, 2021). This aligns with the One Health Framework, which emphasizes the interconnectedness of human, animal, and environmental health (Urban-Chmiel *et al*., 2022) and the AMR Ecology Theory, which explains how resistance genes persist and spread through agricultural practices and environmental reservoirs (Destoumieux-Garzón *et al*., 2018).

The Risk Assessment Theory further supports this research by offering a structured methodology for hazard identification, risk evaluation, and risk management in food safety monitoring (WHO, 2021). It is vital for evaluating the extent of microbial contamination and antibiotic resistance in yogurt and their consequences on human health. Moreover, environmental factors such as the persistence of resistance genes in soil, water, and animal gut microbiota create additional risks for the food chain. Effective risk mitigation strategies include proper fermentation practices, hygienic handling, and implementation of safety regulations and surveillance systems, which fall under the Food Safety and Public Health Theory (Paramasivam *et al.*, 2023).

IV. MATERIAL AND METHODS

4. 1. Study area

The study was done in Gaza province of Mozambique (figure1), with latitude - 25.38265° and 34.47319°longititude with elevation of 215 meters above the sea level, it has tropical savanna climate with temperatures between 20°C and 28°C (Hunter *et al*., 2020), samples were collected from two districts (Xai-Xai and Chókwè) targeting producers and vendors of traditional yogurt.

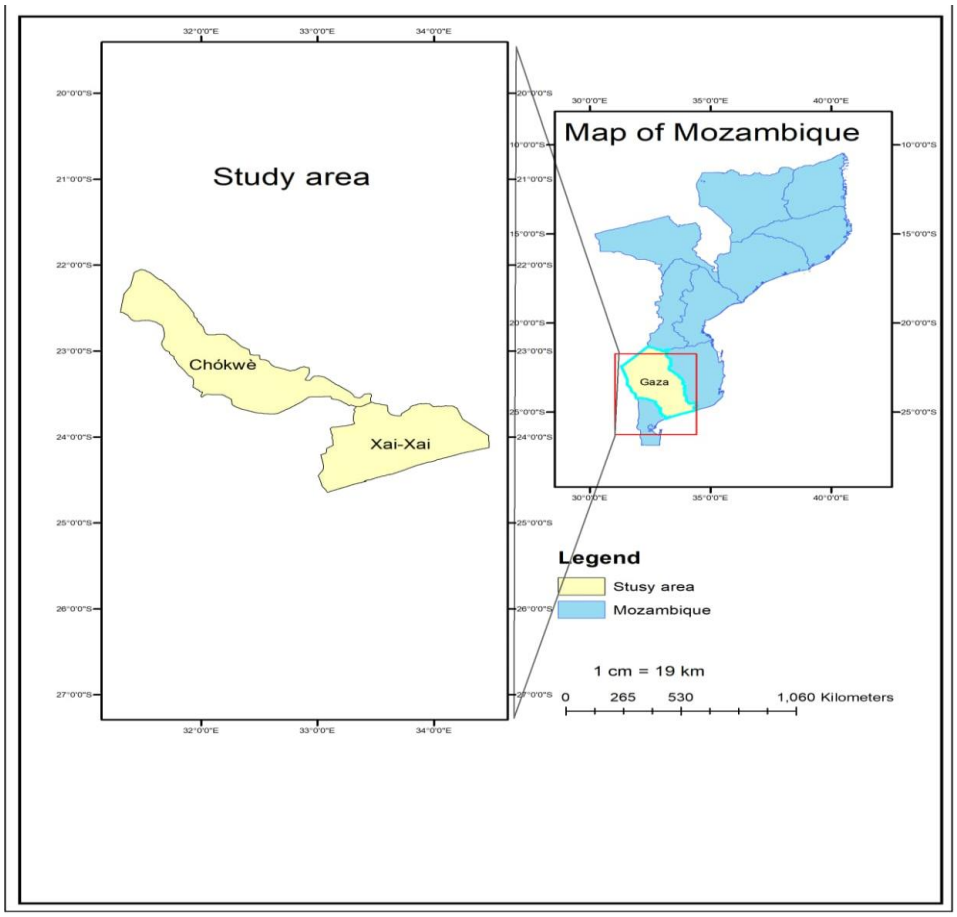


Figure 1: Map of Mozambique and location of Gaza study areas

Geographical location of the study area, Gaza provinces in the southern region of Mozambique. The left panel provides a national overview, highlighting the study region within the context of Mozambique's territorial boundaries. These two districts were selected due to their significance in livestock farming and dairy production. The scale (1 cm = 173 km) and orientation indicators support accurate spatial understanding of the study area.

4.2. Sample design

This study employed a laboratory-based cross-sectional study design to isolate, phenotypically characterize, and identify lactic acid bacteria (LAB), *Escherichia coli*, and *Staphylococcus aureus* from traditional yogurt samples. The laboratory analysis was conducted in a period of three months (March- May, 2026).

4. 3. Sample collection

4. 3. 1. Sample Size

A total of 41 traditional yogurt samples were collected from various local producers in household and vendors in informal markets of Xai-Xai and Chókwè districts and transported in microbiology laboratory for analysis.

4. 3. 2. Sampling procedure

A convenience sampling technique was employed to select traditional yogurt samples based on their accessibility and availability during the period of collection. A total of 250 ml of yogurt sample was collected to ensure the sample is sufficient for analysis. Samples were transferred into the sterile container, sealed tightly, and labeled. Samples were collected aseptically to prevent cross-contamination, all the collected samples were stored in cooler boxes maintained at 4±1°C. For subsequent analysis the collected samples were taken immediately to the microbiology of the Faculty of Veterinary Medicine at Eduardo Mondlane University (FAVET-UEM).

4.4. Microbiological analysis

4. 4.1. Sample preparation and Serial dilution

Ten mL of yogurt sample was aseptically transferred into to a sterile stomacher bag containing 90 mL of sterile 0.1% peptone water and homogenized in a stomacher machine (BBB company, USA) for 2 minutes at 230 rpm to obtain mother dilution, Subsequently, 1 mL of the homogenized suspension was transferred into a test tube containing 9 mL of sterile 0.1% peptone water for further serial dilutions. Fresh sterile pipette tips were used for each transfer.

4. 4.2. Isolation, identification and quantification of lactic acid bacteria

From prepared serial dilutions (10^{-1} to 10^{-3}) pipette 1ml of inoculum and place on corresponding petri dishes. Pour 15ml of de man, rogosa, and sharpe agar (MRS), The plate was gently rotated to distribute the inoculum evenly, allowed to solidify, and subsequently

incubated anaerobically with anaerobic generating sachets (Sigma - Aldrich) at 30ºC for 48h. After incubation the colony was counted using the colony count machine and results were expressed in CFU/mL. Presumptive LAB were identified based on their colony morphology, including characteristics such as colour, size, and shape. The isolates were purified by successive streaking on the MRS and incubated anaerobically at 30ºC for 24h. The isolates were Gram-stained and tested for catalase reaction, oxidase test (Levin, 2005).

To calculate colonies forming unit the following formula was used after counting and the results will be converted to $\log_{10}$ CFU/ml (Roberts & Greenwood, 2019).

$$CFU = \frac{C}{V(n1+0.1n2)*d} \quad \text{.....eq(1)}$$

Where:

C: sum of all colonies from all plates

V: volume of the sample added to all each plate

N1: number of plates counted in first dilution

N2: number of the plate counted in the second dilution

D: the dilution from which the first count was obtained

4.4.3. Isolation and identification of *Escherichia coli*

Isolation and identification of *E. coli* was performed according to ISO 7251:2005 (ISO, 2005). 1ml of the sample was placed onto petri dishes (6 plates were used 2 plates for each dilution), 15 mL of Eosin Methylene Blue (EMB) agar was added, and the plates were incubated under aerobic conditions for 24 hours at 37°C. After 24 hrs, total bacteria count was taken. Positive metallic green colored presumptive *E. coli* colonies were counted with colony count machine and sub cultured onto 15ml plate count agar (PCA) and incubate at 37°C for 24 hours to obtain pure isolated colonies. Purified colonies were inoculated in tube containing 4 ml of tryptone broth and incubate at 37°C for 24 hrs. 3 drops of Kovacs reagent were added in tube to conform presence of *E. coli.* Tubes are considered positive *E.coli* by the presence of pinkish-red ring on the top layer (Roberts & Greenwood, 2019).

4.4.4. Isolation and Identification of *Staphylococcus aureus*

A 0.1 mL aliquot from the initial dilution was aseptically dispensed onto the surface of pre-solidified Mannitol Salt Agar (MSA) plates using a micropipette with sterile tip. The inoculum was streaked across the medium using a sterile disposable plastic loop. Duplicate plates were prepared and incubated in an inverted position at 37°C for 24-48 hours. Presumptive *S.*

aureus colonies were identified by their characteristic cream, yellow, or white morphology and the fermentation of mannitol (yellowing of the medium) and were selected for further confirmation. Identification was achieved through a series of biochemical tests, including Gram staining (to observe Gram-positive cocci in clusters), as well as oxidase, catalase, and coagulase tests to conform presence of *staphylococcus aureus* (Roberts & Greenwood, 2019).

4.4.5. Quality control procedures

For each isolation procedure, an uninoculated plate containing the respective culture medium was incubated under the same conditions as the inoculated plates and used as a negative control to assess the sterility of the culture media and detect possible contamination during the isolation procedure. The absence of growth on the control plates indicated that the culture media and incubation procedures were free from detectable contamination.

For antibiotic susceptibility testing, *staphylococcus aureus* ATCC 25923 strain was used as a positive control to ensure the accuracy and reliability of the susceptibility testing procedure. The results obtained for the quality control strain were compared with the expected susceptibility ranges to verify the performance of the culture media, antibiotic discs, and testing conditions.

4. 5. Antibiotic Susceptibility testing

According to Sowunmi et al. (2022), the Bauer–Kirby disc diffusion technique, originally described by Bauer, Kirby, Sherris, and Turk (1966), is a recommended method for assessing antimicrobial susceptibility. This method was applied to evaluate the antimicrobial susceptibility of *Escherichia coli*, *Staphylococcus aureus*, and lactic acid bacteria. 4 isolated colony of *E. coli, staphylococcus aureus* or *LAB* colonies was purified in nutrient agar and incubated at 37 ºC for 24h. 4-5 isolated pure colonies was selected in each isolated bacterial strain and suspend in 4ml sterile saline (0.85% NaCl) for each. The bacterial suspension was adjusted to turbidity equivalent to the 0.5 McFarland standard (1.5×10^8 CFU/mL). A sterile cotton swab was immersed in the bacterial suspension, and excess fluid was removed by gently pressing the swab against the inner wall of the tube. After that, the cotton swab was streaked evenly on Mueller-Hinton agar surface three times, rotating plate 60°. The plates were allowed to dry for 3-5 minutes; antibiotic disks were placed on MHA using sterile forceps 24mm apart of each other and 15mm from plate edge and incubated for 24h at temperature of 37^0C. After 24 hrs, the diameter was measured using mm ruler and the results were interpreted and then

translated into Sensitive (S), Intermediate (I), and Resistant (R) categories according to the interpretation table of the CLSI 2024 (***Table 1***). The choice of antibiotics was based on the commonly used in Veterinary and public health in Mozambique which are ampicillin, ciprofloxacin, tetracycline, Penicillin and gentamicin treatments (Nhatsave *et al*., 2021).

Table 1*:* Disk diffusion test of antibiotic break point in *E. coli, Staphylococcus aureus* and Lactic Acid Bacteria

Antibiotic Agent	Disk content	*E. coli*			*Staphylococcus aureus*			Lactic Acid Bacteria		
		S(mm)	I(mm)	R(mm)	S(mm)	I(mm)	R(mm)	S(mm)	I(mm)	R(mm)
Tetracycline	30 µg	≥18	15-18	≤14	≥19	15-18	≤14	≥19	15-18	≤14
Gentamicin	10 µg	≥15	13-14	≤12	≥15	13-14	≤12	≥13	-	≤12
Ciprofloxacin	5 µg	≥21	16-20	≤15	≥21	16-20	≤15	≥19	14-18	≤13
Ampicillin	10 µg	≥17	14-16	≤13	≥18	-	<18	≥16	13-15	≤12
Penicillin	10 µg	-	-	-	≥29	-	≤28	≥28	20-27	≤19

The multiple antibiotic resistance index (MAR) was used as an epidemiological indicator to assess the risk of antibiotic resistance. It was determined by dividing the number of antibiotics to which an isolate was resistant by the total number of antibiotics tested as it illustrated in following formula.

$$\textbf{MAR Index} = \frac{a}{b} \quad \text{.....(eq 2)}$$

Where a represents the number of antibiotics to which the isolate showed resistance, and b denotes the total number of antibiotics tested. Generally, MAR index value higher than 0.2 indicates the isolate is coming from area of high-risk contamination (frequent antibiotic use), while less than 0.2 indicates low-risk sources (Choushette & Satpute, 2022)

4.6. Statistical analysis

Data from the Laboratory study was entered into Microsoft Excel version 16 and analyzed using Stata version 15.0. Descriptive statistics was used to calculate percentage of distribution of bacteria isolates across markets and bacterial group, to calculate the percentage prevalence of co-isolation patterns across the markets, to calculate percentage prevalence of pathogenic bacteria across sample type and markets as well as to calculate the percentages of bacterial

isolates classified as resistant, intermediate, or sensitive to each of the antibiotics across the two markets. The paired sample t test was used to compare the mean counts of lactic acid bacteria among the markets. The Fisher's exact test was used to compare the presence or absence of pathogens between sample types across the two markets.

For all analyses, a $P \leq 0.05$ was used as the cut-off point for the significance difference.

V. RESULTS

5.1. Prevalence of bacteria strain in traditional yogurt

A total of 41 sample was collected in two districts. Twenty seven samples were collected in Xai-Xai district, 14 samples were collected in Chókwè districts. Each sample was isolated and screened against three bacterial strains *(Escherichia coli, Staphylococcus aureus*, and Lactic Acid Bacteria), No bacterial growth was observed on the uninoculated control plates during the isolation of all bacterial strains indicating that the culture media and incubation conditions were free from detectable contamination. The findings presented in **Figure 2** showed that lactic Acid Bacteria (LAB), which serve as the baseline fermentative microflora, achieved a 100% presence rate across all sampled locations. However, the presence of foodborne contaminant indicators varied heavily by districts. Yogurt samples sourced from the Chókwè district exhibited the poorest microbiological profile, yielding a 42% presence rate for *Staphylococcus aureus* and a 14% presence rate for *Escherichia coli*.

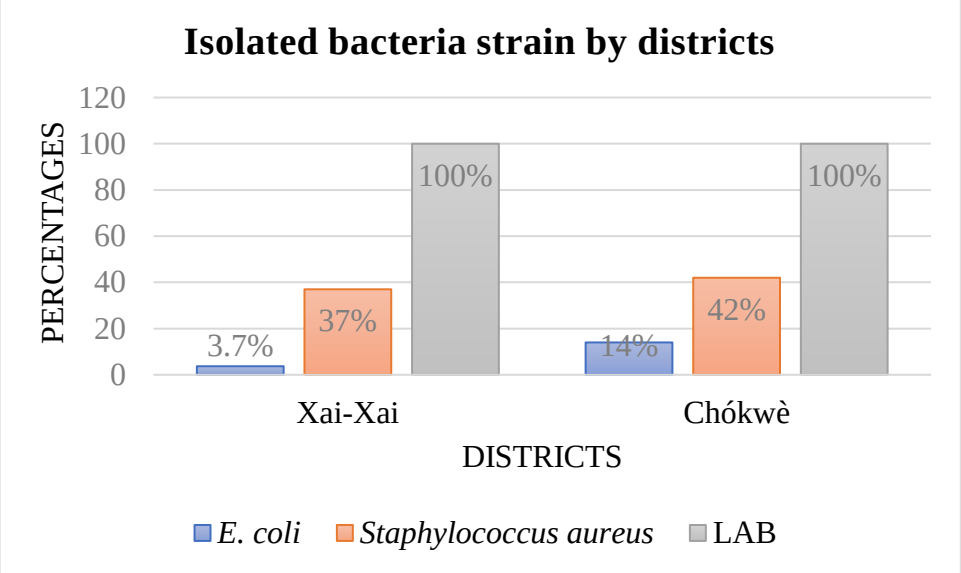


Figure 2: Bacteria strain distribution by Xai-Xai, Chókwè districts. In all district a high occurrence of lactic acid bacteria was observed, followed by *Staphylococcus aureus* and *Escherichia coli*, respectively

A total of 60 bacterial isolates were obtained from 41 samples, 14 sweet yogurt and 27 natural yogurts. Each isolate was selected based on distinct colony morphology characteristics including size, shape, color, and texture observed on the culture media. The distribution of bacterial isolate obtained from fermented yogurt shows that. Lactic acid bacteria prevalence (100%) was highest among the three bacteria strain and *E. coli* prevalence was lowest (7.3 %) (Figure 3).

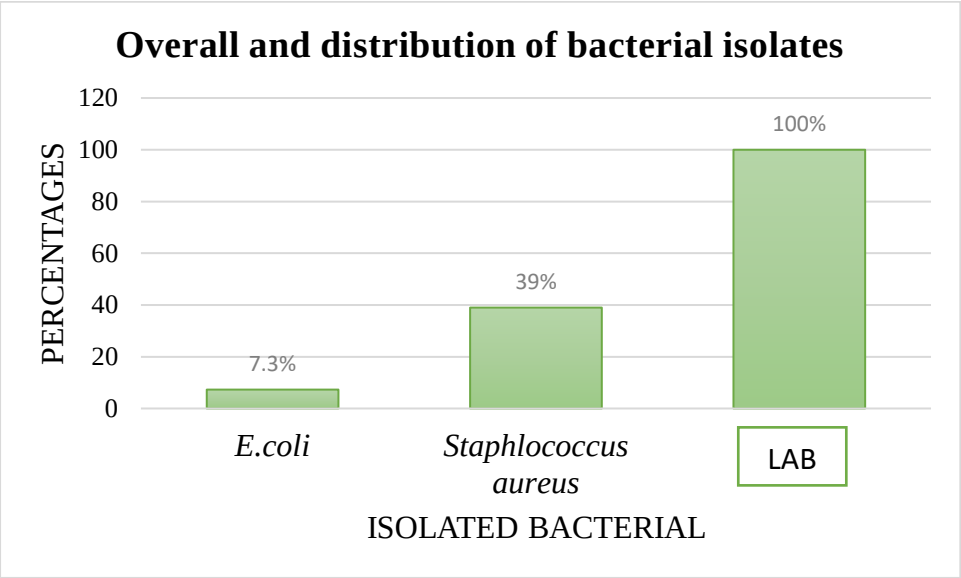


Figure 3: Overall distribution of bacterial isolates showing the percentage occurrence of *E. coli* (7.3%), *Staphylococcus aureus* (39%), and lactic acid bacteria (100%)

The comparative occurrence of bacterial isolates across districts (**Table 2**), co-isolation analysis revealed that out of 41 yogurt samples tested, 57.1% (n = 24) were completely containing exclusively Lactic Acid Bacteria without pathogenic co-contamination. Multi-strain contamination profiles were prominently localized within specific regions. Xai-Xai exhibited a strong dual-contamination profile, with 37.0% (n = 10) of samples testing positive for both LAB and *Staphylococcus aureus*. Chókwè displayed the highest complexity of contamination, where 35.7% (n = 5) of yogurt samples carried dual LAB + *Staphylococcus strains*, 7.1% (n = 1) carried dual LAB + *E. coli* strains, and an additional 7.1% (n = 1) demonstrated a complete triple-strain co-isolation pattern harboring LAB, *Staphylococcus aureus*, and *E. coli* simultaneously.

Table 2: Prevalence and co-isolation patterns of isolated bacterial stain in yogurt samples across two districts (N = 41).

Districts	Total samples	1 stain only (LAB)	2 stain co- isolated (LAB+ *S.aureus)*	2 Strains co-Isolated (LAB + *E. coli*)	3 Strains co-isolated (LAB + *S.aureus* + *E. coli)*
Xai-Xai	27	16 (59.2%)	10 (37%)	1 (3.7%)	0 (0%)
Chókwè	14	7 (50%)	5 (35.7%)	1 (7.1%)	1 (7.1%)
Overall total	41	23 (56%)	15 (36%)	2 (4.8%)	1 (2.4%)

5.1.1. Prevalence and microbial load of lactic acid bacteria

A One-way ANOVA was performed to compare microbial load of yogurt type. The analysis revealed that there was no statistically significant difference in mean LAB counts between the two types with P= 0.304 **(Table 3)**. Since the p-value was greater than the significance level of 0.05, indicating that the number of colony-forming units of lactic acid bacteria does not vary with the addition or non-addition of sugar to the samples tested.

Table 3: Mean log LAB counts, microscopic morphological characteristics of bacterial isolates from natural and sweet yogurt samples across districts

Sample type	**Mean LAB count (Log10 CFU/ml)**	**Mean log count in Xai-Xai**	**Mean log count in Chokwe**	**Morphological characteristics of microorganisms in microscopic**
Natural yogurt	3.8 ± 0.35	3.9± 0.04	4± 0.10	Catalase negative, Gram positive straight to slightly curved bacilli.
Sweet yogurt	3.7± 0.36	3.7 ± 0.37	3.7 ± 0.36	Catalase-negative, Gram-positive cocci exhibiting characteristic chain-like and paired cellular morphology
P -value	0.304	0.08		

5.1.2. Prevalence of pathogenic bacteria in traditionally fermented yogurt

To determine microbial safety, 41 yogurt samples were evaluated for the presence and distribution of *Escherichia coli* and *Staphylococcus aureus* across two distinct product types (natural and sweet yogurt). The results shows that the higher prevalence of *S. aureus* in both categories compared to *E. coli*. Specifically, sweet yogurt samples exhibited the highest overall pathogen burden, yielding a 50% prevalence rate for *S. aureus*. *E. coli* was absent in sweet yogurt samples (0%) but present in natural yoghurt (11%)

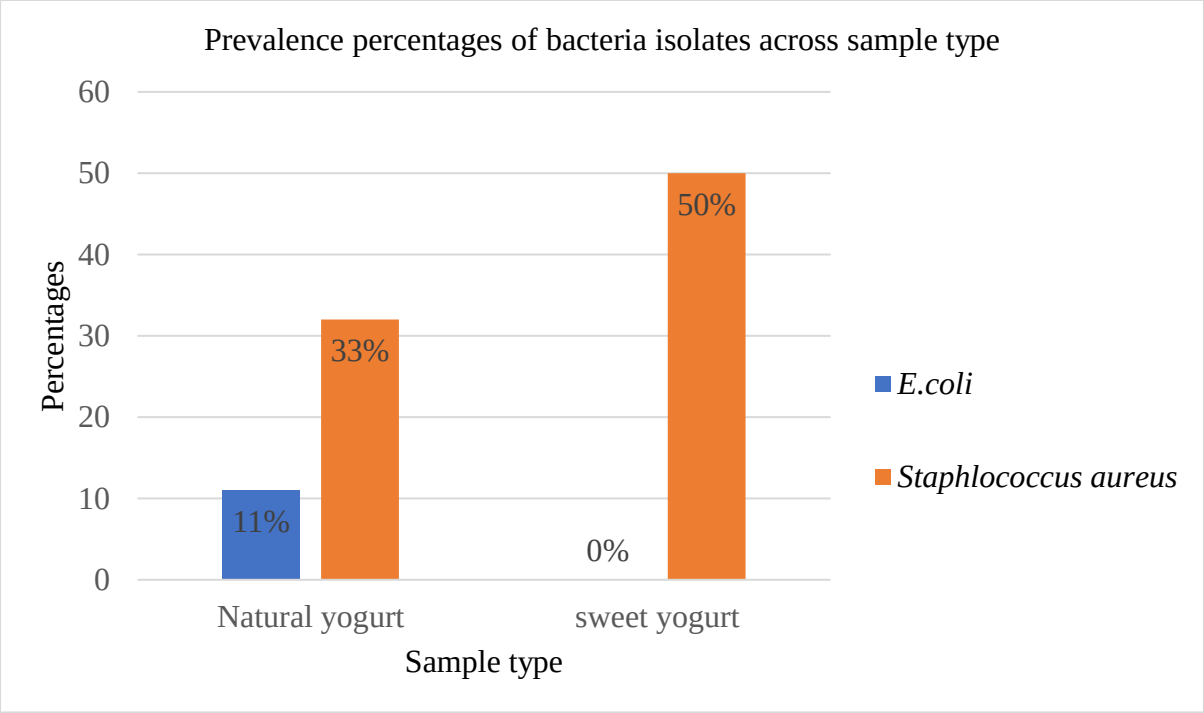


Figure 4: Percentage prevalence of *E. coli* and *S. aureus* isolated from natural and sweet yogurt samples

A detailed summary of prevalence rates and their corresponding statistical values shows that Chókwè had highest prevalence of both *E.coli* (14.3%) and *S. aureus* (42.8%). Occurrence of *E.coli* and *S. aureus* in Xai-Xai was 3.7 % and 37 % respectively ***(*Table 4).**

Table 4: Prevalence of *Staphylococcus aureus* and *Escherichia coli* in yogurt samples collected from Chókwè, Xai-Xai

Location	*S. aureus* prevalence %	*E. coli* prevalence %
Chókwè	42.8% (6/14)	14.3% (2/14)
Xai-Xai	37.0% (10/27)	3.7% (1/27)
Total	**39 % (16/41)**	**7.3 % (3/41)**

5.2. Antibiotic resistance profiles of Lactic acid bacteria, *E.coli* and *Staphylococcus aureus*

To determine the resistance profile of the isolated microorganisms, susceptibility assay was performed on various antibiotics against each microorganism.

5.2.1. Antibiotic resistance profile of Lactic acid bacteria

The phenotypic antimicrobial susceptibility profiles of the bacterial isolates collected from 42 samples are summarized in (**Table 5).** In general, a widespread prevalence of antibiotic resistance was reaveled among the isolates, the highest rates of resistance were observed against ampicillin (95%) and tetracycline (95%), followed closely by gentamicin (93%) and penicillin (91%). Ciprofloxacin exhibited the minimum resistance rate among the antibiotics tested. Intermediate the maximum rate recorded for gentamicin at 4.7%. Penicillin and ciprofloxacin were more susceptible with rate of 9 % and 7.5 % respectively. The observed resistance patterns suggest a high prevalence of multidrug resistance among the LAB isolates.

Table 5: Antimicrobial susceptibility profile of lactic acid bacteria isolates against selected antibiotics

Antibiotics	Resistance (%)	Intermediate (%)	Susceptible (%)
Ampicillin	40(95 %)	0%	2(4.7%)
Ciprofloxacin	38(90 %)	1(2.3 %)	3(7.5 %)
Gentamicin	39(93%)	2(4.7%)	1(2.3 %)
Penicillin	38(91 %)	0 %	4(9 %)
Tetracycline	40(95 %)	1(2.3 %)	1(2.3 %)

5.2.2. Prevalence of antibiotic resistance profile in *E. coli*

A total of 3 identified *E.coli* isolate was tested to various type of antibiotics include ampicillin, ciprofloxacin, gentamicin, penicillin and tetracycline. The results indicated highest resistance

to penicillin (100%) and lowest resistance to gentamicin 33.3%. Ampicillin, ciprofloxacin was 100% sensitive and tetracycline was 66.7% intermediate **(Table 6)**.

Table 6: Antimicrobial susceptibility profile of *E.coli* isolates against selected antibiotics

Antibiotics	Resistance (%)	Intermediate (%)	Susceptible (%)
Ampicillin	0%	0 %	3(100%)
Ciprofloxacin	0%	0%	3(100%)
Gentamicin	1(33.3%)	1(33.3%)	1(33.3%)
Penicillin	3(100%)	0 %	0%
Tetracycline	0%	2(66.7 %)	1(33.3 %)

5.2.3. Prevalence of antibiotic resistance in *staphylococcus aureus*

A total of 16 *staphylococcus aureus isolate* was tested to antibiotic resistance, a result showed that high resistance to penicillin 68.8% and resistance rate to other antibiotics was 25% **(Table 7)**. The isolates tested intermediate to ciprofloxacin at rate of 12.5% and were sensitive at rate of 75% to ampicillin, gentamicin and tetracycline.

Table 7: Antimicrobial susceptibility profile of *Staphylococcus aureus* isolates against selected antibiotics

Antibiotics	Resistance (%)	Intermediate (%)	Susceptible (%)
Ampicillin	4 (25%)	0 %	12 (75%)
Ciprofloxacin	4 (25%)	2 (12.5%)	10 (62.5%)
Gentamicin	4 (25%)	0 %	12 (75 %)
Penicillin	11 (68.8%)	0 %	5 (31.2 %)
Tetracycline	4 (25 %)	0 %	12 (75 %)

Antibiotic resistance profile in *staphylococcus aureus* by study location indicated that Chókwè demonstrated a more concerning and uniform resistance profile across several antibiotics. In Chókwè, resistance was observed in 66.7% (4/6) of isolates for penicillin and tetracycline, 50.0% (3/6) of isolates for ciprofloxacin, gentamicin, and ampicillin. In Xai-Xai, penicillin resistance was observed in 70.0% (7/10) of isolates, whereas resistance to ciprofloxacin, gentamicin, and ampicillin was 10.0% (1/10), and no tetracycline resistance was detected. Fisher's exact test showed that tetracycline resistance differed significantly between the two

locations (p = 0.008), whereas no significant differences were observed for penicillin (p = 1.000), ciprofloxacin (p = 0.118), gentamicin (p = 0.118) and ampicillin (p = 0.118).

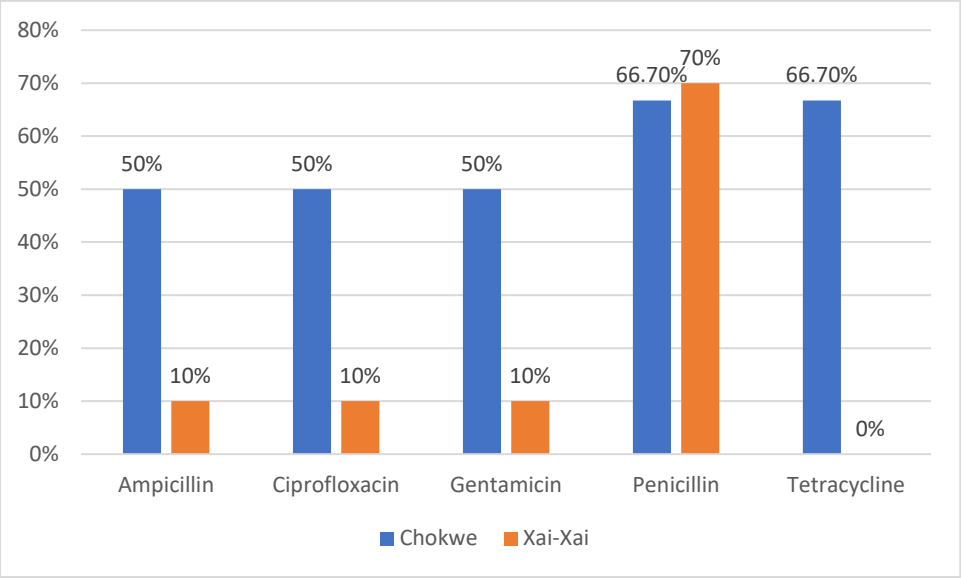


Figure 5: Comparison of antibiotic resistance rates among *Staphylococcus aureus* isolates from Chókwè and Xai-Xai

Antibiotic Resistance (MAR) indices for *S. aureus* were calculated against five antibiotics. The overall mean MAR index between two areas were 0.33, Chókwè has high mean MAR (0.57) and Xai-Xai has (0.18) (Table 8).

Table 8: Mean multiple antibiotic resistance (MAR) index profiles and risk levels of *S. aureus* isolates from Xai-Xai and Chókwè

Locations	Isolate count	Mean MAR index	Maximum MAR	Resistance risk level
Xai-Xai	10	0.18	0.6	Low risk
Chókwè	6	0.57	1	High risk
Total	16	**0.33**	1	**High risk**

Overall, the comparative analysis revealed highly distinct resistance patterns driven by both bacterial group and geographic location **(Figure 6)**. Lactic Acid Bacteria (LAB) resistance was critically high across both regions, remaining uniform at 93% for all five tested drugs in Chókwè and rising slightly to 96% for Ampicillin, Penicillin, Ciprofloxacin, and Gentamicin in Xai-Xai. However, a major geographical variance occurred for Tetracycline, where

resistance plummeted to just 8% in Xai-Xai compared to the 93% observed in Chókwè. In contrast, *Escherichia coli* displayed identical profiles at both study sites, exhibiting 100% resistance to Penicillin and 33.3% to gentamicin. *Staphylococcus aureus* showed a consistently higher resistance burden in Chókwè, ranging from 50% for Ampicillin, Ciprofloxacin, and Gentamicin, up to 67% for Penicillin and Tetracycline.

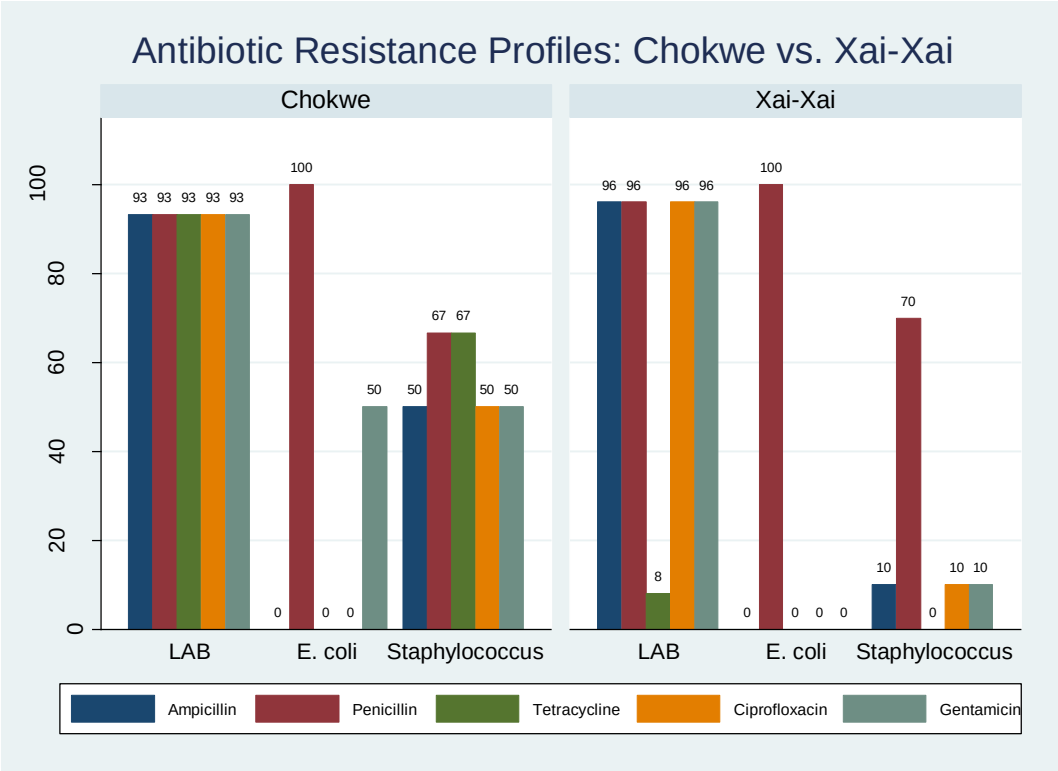


Figure 6: Comparative phenotypic antibiotic resistance profiles (%) of Lactic Acid Bacteria, *Escherichia coli* and *Staphylococcus aureus* isolated from Chókwè and Xai-Xai districts. Bar heights and values denote the percentage prevalence of phenotypic resistance.

5.3 Multiple antibiotic resistance index of *E. coli, Staphylococcus aureus* and lactic acid bacteria

To calculate MAR index, the antibiotics resistant by isolates were divided by total number of antibiotics tested. The results show that in a total of 60 isolates, lactic acid bacteria isolates have high MAR index 0.8, followed by *staphylococcus aureus* with MAR index of 0.4 **(Figure 7)**.

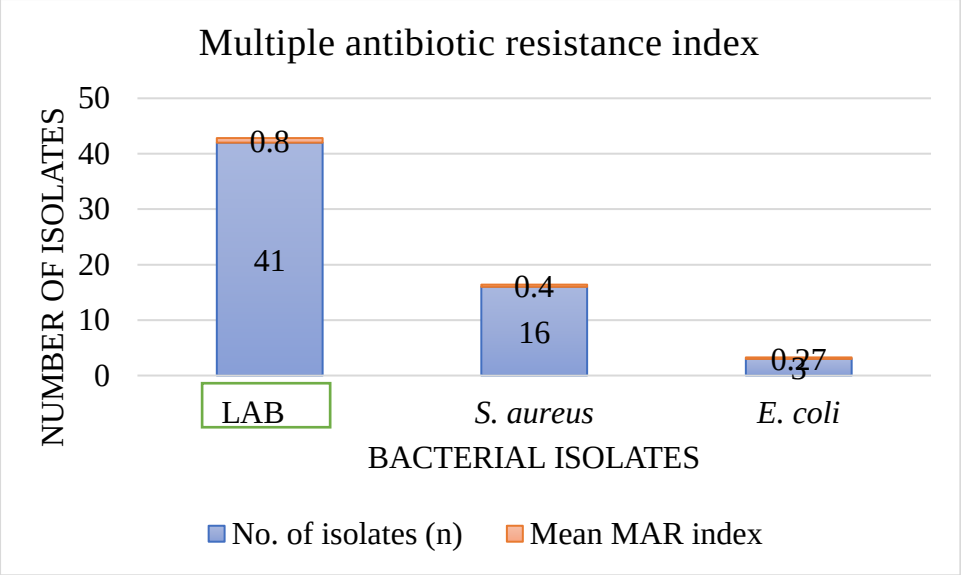


Figure 7: Multiple antibiotic resistance index by bacterial group

The distribution of MAR index among bacterial isolates ***(figure 8).*** LAB showed the highest proportion of high-risk isolates, while *S. aureus* displayed a mixed pattern of low and high-risk isolates. In contrast, *E. coli* was predominantly classified within the low-risk category, indicating lower exposure to antimicrobial selective pressure.

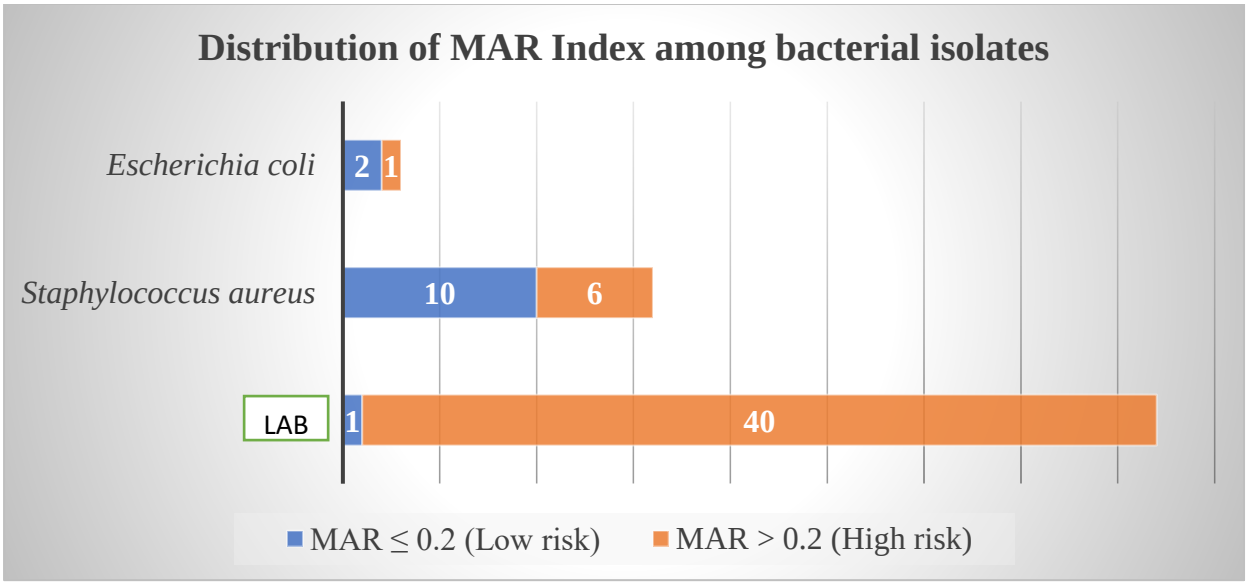


Figure 8: Distribution of Multiple Antibiotic Resistance (MAR) index among LAB, *Staphylococcus aureus* and *Escherichia coli* isolates. The bars represent the number of isolates with MAR index ≤ 0.2 (low risk) and > 0.2 (high risk).

To evaluate the consumer biosafety risks associated with the analyzed yogurt products, the isolated bacterial groups were categorized based on their strict multidrug resistance (MDR) profiles. Following international surveillance criteria, an individual isolate was classified as

multidrug-resistant if it exhibited phenotypic resistance to three or more distinct antimicrobial classes. The localized distribution of MDR prevalence across Chókwè and Xai-Xai is summarized in Figure 9.

The data reveals highly contrasting multidrug resistance patterns that are heavily dependent on both the bacterial and the location origin of the samples. Lactic Acid Bacteria demonstrated critically high MDR rates across both regions, with 93% of LAB isolates in Chókwè and 96% in Xai-Xai qualifying as multidrug-resistant. Conversely, *Escherichia coli* isolates exhibited a complete absence of multidrug resistance (0% MDR) at both study sites due to their susceptibility to all modern classes tested. *Staphylococcus aureus* geographical variance was observed: isolates recovered from yogurts in Chókwè displayed a significantly higher multidrug resistance burden at 50% MDR, compared to the minor 10% MDR prevalence recorded among strains from the Xai-Xai market.

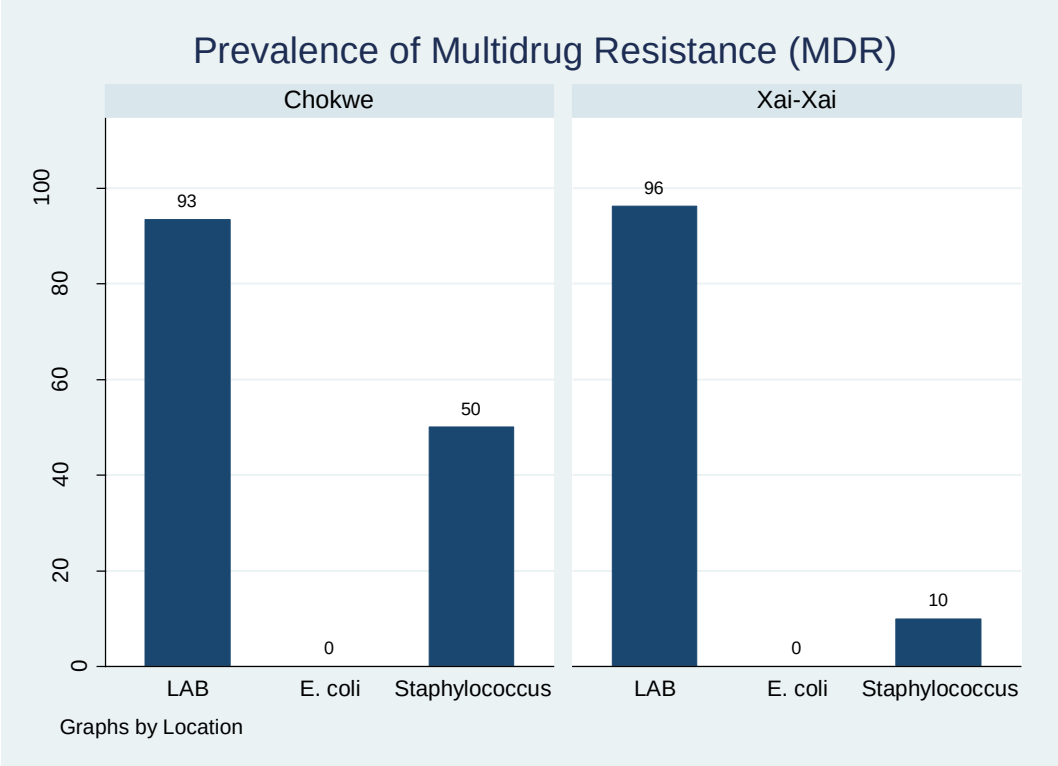


Figure 9: Comparative phenotypic prevalence (%) of multidrug resistance (MDR) among Lactic Acid Bacteria, *Escherichia coli* and *Staphylococcus aureus* isolated from marketed yogurt samples in Chókwè and Xai-Xai

VI. DISCUSSION

The present study assessed the microbiological quality of 41 samples of traditional yogurt sold in two informal markets, comprising two product types: natural yogurt and sweetened yogurt. The sample size is sufficient for statistical analysis and can provide useful information about the microbiological quality of the samples studied, conclusions can be drawn from the analyzed data, regarding the microbiological quality and antibiotic resistance profile of the samples studied and across study areas

The ubiquitous 100% recovery of Lactic Acid Bacteria across all three markets was expected, since LAB play a fundamental role in yogurt fermentation. Through fermentation process LAB convert lactose into lactic acid, promoting pH reduction, milk protein coagulation, and the development of the product's organoleptic characteristics, such as viscosity & flavor. Furthermore, LAB product bacteriocins and other antimicrobial compounds (Kang *et al*., 2019). However, the co-isolation of *Staphylococcus aureus* and *E. coli* in Chókwè and Xai-Xai indicates significant hygiene failure in the local dairy value chains (Garofalo *et al*., 2023).

The results of this study demonstrated a consistency in LAB populations across the tested yogurt types, as evidenced by the non-significant p-value (0.304). This uniformity suggests that the variations did not exert a selective pressure on the starter cultures or the natural microflora present during fermentation. However the quantification of LAB obtained were lower (3.8 ± 0.36 Log CFU/ml) than the results reported by Beukes et al. (2001) (5.7 to 9.1 Log CFU/ml); Nyambane et al. (2014) (7.86 Log to 8.32 Log CFU/ml) and Nhassengo et al. (2022) (4.23 ± 1.09 Log CFU/ml) in masi produced in South Africa, Kenya and Mozambique respectively.

The LAB species identified in this study are similar with those previously reported in similar fermented products across different African countries. Studies by Beukes et al. (2001), Nhassengo et al. (2022), and Kayitesi et al. (2017) also identified *Lactobacillus plantarum* and *Lactobacillus fermentum* as dominant species, along with other LAB such as *Leuconostoc mesenteroides* subsp. *mesenteroides* and *Lactococcus spp.* This similarity suggests that the microbial composition of the product studied shares common characteristics with those reported in related traditional fermented foods in the region (Nyambane *et al.*, 2014).

E. coli contamination was identified at a rate of 11% of the natural yogurt samples, whereas all sweet yogurt samples remained completely free of the bacteria. These findings suggest a clear failure to meet the strict sanitation standards set by the Codex Alimentarius. According to these guidelines for fermented milks, *E. coli* must be entirely absent (c=0, m=0) to guarantee

consumer safety (Codex, 2002). The overall *E. coli* detected was 7% which was lower compare to study of Nhassengo et al. (2022) (94%); Kandil et al. (2018) (88%) and Omola et al. (2014) (34%) in massi and yoghurt samples. The presence of these organisms in samples from Chókwè and Xai-Xai suggest a breakdown in hygiene, either during the raw milk collection or the post-pasteurization handling phase.

However the low prevalence of *E. coli* in present study directly linked to the rapid pH drop (to 4.0–4.6) caused by the lactic acid production of starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) *(*Deddefo *et al*., 2024). Most *E. coli* strains are highly acid-sensitive, causing their populations to decline to undetectable levels during refrigerated storage *(*Deddefo *et al*., 2024).

The contamination with *E.coli* in Chókwè and Xai-Xai is a public health concern. Because yogurt is an acidic environment (usually pH < 4.6), the survival of *E. coli* suggests that these samples may have been recently contaminated or that the strains possess acid-resistance mechanisms. Presence of *E.coli* suggested post-pasteurization contamination of the product prior to or during packaging, these results highlight an urgent need for training traditional producers in Good Manufacturing Practices (GMP) to eliminate fecal coliforms and ensure the safety of the final product (Ogwaro *et al*., 2023).

Staphylococcus aureus was detected in 16 out of 41 (39%) samples, which is lower than the 84.6% (88/104) reported by Abdulrahman & Sanmi, (2021), and the rates found in plain yogurt (72%), mish (72%), fruit yogurt (48%), and laban rayeb (48%) by El Latif *et al*. (2022). This 38.1% detection rate was higher than the 36% found in small-scale yogurt by Kandil *et al*. (2018**)** and higher than the 0% detection rate in a Nigerian study by (Omola *et al*., 2014).

Presence of *S. aureus* in Chókwè and Xai-Xai yoghurt indicates contamination from food handlers, post-processing cross-contamination, handling, and packaging, milk from cows suffering from subclinical mastitis (Hameed & Mohammed, 2013). While heat treatments and fermentation typically suppress pathogens *S. aureus* demonstrated enhanced survival and growth in sweetened yogurts compared to natural yogurt, this survival is facilitated by added sugars, increased pH buffering capacity, and reduced probiotic competition in sweetened formulations (Kadariya *et al*., 2014). *Staphylococcus aureus* is a prolific producer of heat-stable enterotoxins that cause acute food poisoning, even if active fermentation reduces live

bacterial counts over time, these robust toxins remain a persistent threat to consumer health (Nhatsave *et al*., 2021).

The contrasting prevalence rates of these two pathogens are directly linked to their primary sources of introduction. *Staphylococcus aureus* is a natural inhabitant of human skin, hair, and nostrils (Musa *et al*., 2023). In the traditional settings of Chókwè and Xai-Xai where yogurt is manually handled, packaged, or sold in open containers, the risk of contamination from handlers is exceptionally high, *E. coli* is an environmental and fecal pathogen typically introduced much earlier in the production chain through contaminated raw milk or poor water quality (Nadi *et al*., 2024). The lower incidence of *Escherichia coli* across both locations implies fecal contamination, while present, is not the primary microbiological threat (Lemma *et al*., 2021).

The distribution of pathogenic bacteria in the two sampling locations was evaluated across (Chókwè and Xai-Xai) to identify potential geographical hotspots for contamination. While bacterial presence was detected at all sites, prevalence rates varied significantly by both location and pathogen type. Based on the Pearson chi-square test there was no statistically significant association between sampling location and the detection of *S. aureus* ($x^2(2) = 0.7628$, $Pr = 0.683$), and *E. coli* ($x^2(2) = 1.6353$, $Pr = 0.441$). This suggests that the risk of *S. aureus* and *E.coli* contamination was relatively uniform across the study area.

Regarding resistance, the present study demonstrated a high prevalence of antibiotic resistance among LAB isolates detected within tested samples, with resistance rates exceeding 90% for ampicillin, tetracycline, gentamicin, and penicillin. These findings indicate the widespread occurrence of antimicrobial resistance among LAB and support previous reports suggesting that food-associated LAB may serve as reservoirs of antibiotic resistance determinants (Hummel *et al*., 2007). Chouanguep & Oda, (2012), reported that 100% of their strains were susceptible to Penicillin G and Ampicillin, 50% resistance to Gentamicin and 50% intermediate to Tetracycline.

The antibiotic susceptibility profiles for LAB of yogurt isolates reveal a critical mix of intrinsic traits and acquired resistance risks. The critical high resistance to Gentamicin (90–95%) and Ciprofloxacin (91–100%) is primarily intrinsic, driven by a natural lack of cytochrome-mediated electron transport systems and active efflux pumps (Sherif *et al.*, 2025). However, the parallel high resistance to β-lactams (90–100% for Penicillin and Ampicillin) and

Tetracycline (93–99%) strongly suggests acquired, plasmid-mediated traits, positioning starter cultures like *Streptococcus thermophilus* as potential environmental reservoirs for horizontal gene transfer to consumers (Campedelli *et al.*, 2019).

The results of this work reported AMR profiles of the *E. coli* isolates was 0 % resistant on ampicillin, tetracycline and ciprofloxacin which was very lower compare to study of Mwasinga *et al*. (2023) reported that the AMR profiles of the *E. coli* isolates from raw milk revealed resistance to commonly used antibiotics, including ampicillin (50%), tetracycline (40.1%). Salam *et al*.(2021) showed 11.8% resistance on ciprofloxacin, tetracycline demonstrated 0% resistance in the present study compared to 4.4% resistance was observed by Salam *et al*., (2021).

Escherichia coli displayed a uniform 100% resistance to Penicillin, representing standard, outer-membrane-driven intrinsic resistance, this intrinsic resistance is primarily due to the low permeability of the outer membrane, which prevents Penicillin G from reaching its target penicillin-binding proteins effectively. All isolates were susceptible to ampicillin, although ampicillin belongs to the penicillin class, it is an aminopenicillin with improved penetration through the outer membrane of Gram-negative bacteria. The observed susceptibility suggests that the isolates did not possess acquired resistance mechanisms, such as β-lactamase production. Emerging intermediate traits for Tetracycline (66.7%) and fragmented resistance patterns for Gentamicin highlight low-level, chronic exposure to veterinary drug residues within the local dairy agricultural supply chain (El-halem & Attia, 2021).

The findings of the current study shows that 25 % of *staphylococcus aureus* isolates were resistant on ampicillin, tetracycline, ciprofloxacin, gentamicin and 68.8% was resistant on penicillin. These results are lower than those reported by Nadi *et al*.(2024) who reported that 76.4% of the tested strains were resistant to ampicillin. El-halem (2019) revealed 0% of *S. aureus* were resistant to ciprofloxacin, 92.6 % was resistant to penicillin G, 65.4 % resistant to tetracycline and 9.9 % resistant to gentamycin.

Antibiotic resistance profiles of *S. aureus* isolates from raw milk and yogurt samples, the results, indicated susceptibility to ciprofloxacin, gentamicin alongside resistance to penicillin as reported by Pervaiz *et al*. (2024) which is similar to the results obtained in present study.

The elevated rate of resistance to penicillin obtained among *Staphylococcus aureus i*solates in both Xai-Xai (70%) and Chókwè (66.7%) may indicate frequent or prolonged use of β-lactam antibiotics in dairy production systems, particularly for the treatment of bovine mastitis. The

observed resistance to penicillins in *S. aureus* is primarily enzymatic. Unlike Gram-negative bacteria, *S. aureus* produces extracellular beta-lactamase (penicillinase) which hydrolyzes the antibiotic before it reaches its target (Sionek *et al*., 2024).

Multiple Antibiotic Resistance (MAR) index found in present study suggest the possible presence of the mecA gene, which encodes the altered penicillin-binding protein PBP2a, rendering standard beta-lactams ineffective (Miruka & Nyakundi, 2026). All three evaluated bacterial categories crossed the threshold (> 0.2), revealing a widespread risk profile spanning food-associated starter cultures and opportunistic contaminant pathogens.

High risk multi drug resistance in lactic acid bacteria as illustrated in Figure 8, the lactic acid bacteria (n = 42) exhibited an exceptionally high mean MAR index of 0.83. This indicates that, on average, the isolated LAB strains were phenotypically resistant to approximately 83% of the tested antimicrobial agents. In fermented dairy products like yogurt this high index is a major biosecurity threat (Floris *et al*., 2025).

Escherichia coli presented a mean MAR index of 0.27. Although this was the lowest index value recorded across the study it remains above the critical risk barrier and proves that even transient sanitary indicators carry resistant properties that could complicate potential foodborne illness treatments (Drugea et al., 2025)

Staphylococcus aureus exhibited a mean MAR index of 0.40 this value significantly exceeds the 0.20 baseline, it implies that the *S. aureus* isolates were selected from herds frequently subjected to mastitis treatments or unhygienic condition of farmers during production process and milk supply chain (González-Puetate *el al*., 2025).

The findings reveal that the resistance load between Xai-Xai (Mean MAR = 0.18) and Chókwè (Mean MAR = 0.57) on *S. aureus* isolates. An MAR index greater than 0.20 is universally recognized as a threshold signifying that pathogens originate from high-risk exposure environment where antibiotics are heavily commercialized, misused, or routinely administered to livestock. Despite identical access to pharmaceuticals in both peri-urban locations, the higher resistance load in Chókwè is likely driven by the intensive nature of its local dairy farming practices and indicates the alarming presence of at least one isolate in Chókwè that was completely resistant to every single antibiotic tested.

Traditional fermented dairy products serve as direct vectors for antibiotic-resistant (AR) bacteria from animal flora to the human gut (Erginkaya *et al*., 2018). This microbiological risk

is primarily driven by traditional backslopping which utilizing a portion of a previously fermented batch to inoculate and initiate new production runs. Because artisanal producers continually recycle unmonitored cultures, this practice creates a continuous contamination loop, propagating and selecting for AR bacterial strains throughout subsequent production cycles (Sherif *et al.*, 2025)

The prevalence of AMR in traditional yogurt is probably due to frequent use or improper use of different antibiotics in the study areas contributing to selection for resistant strains. Similarly high levels of resistance could be due to overuse of these antimicrobials for treatment and prevention of various animal infections in the study area (Kembo *et al.*, 2025). A potential reason why high levels of resistance to several antimicrobials was recorded in this study could be due to the fact that dairy farmers in urban and peri-urban areas have relatively high access to antimicrobials exposing their animals to antimicrobials frequently *(Raul et al.*, 2024).

6.1. Limitation of the study

The findings of this study provide valuable evidence on bacterial contamination and antibiotic resistance of traditional yogurt sold in Gaza. However there was several limitations as the may have affected results interpretation and conclusions. These limitations are:

- ✓ Small sample size and the restriction of the study to only two districts, which limited the geographical coverage of the study. The small sample size was influenced by floods in the study area, which reduced milk production and caused financial losses among farmers, thereby affecting the availability of samples. Consequently, although the results provide useful information on the microbiological quality of traditional yogurt in the sampled districts, they may not fully represent the situation in other districts of Gaza Province.
- ✓ The study focused only on traditional yogurt and did not include other traditional dairy products; therefore, the findings cannot be extended to the microbiological quality of other traditional dairy products.
- ✓ Molecular techniques were not used to detect antimicrobial resistance genes. Therefore, the study could not confirm the genetic basis of the observed antimicrobial resistance or identify the specific resistance genes carried by the isolates.

VII. CONCLUSION

The present study sought to evaluate the bacterial contamination and antibiotic resistance profile of traditional yogurt collected in two districts. Based on findings the following conclusion were made:

- The study confirmed the successful isolation and identification of lactic acid bacteria in all yogurt samples, confirming their widespread occurrence in the traditional yogurt examined. However quantitative counts were consistently low across the collected samples regardless of whether it is natural and sweet traditional yogurt.
- The study successfully identified the presence of pathogenic bacterial, *Escherichia coli* and *Staphylococcus aureus*. The occurrence of these bacteria suggest the possible microbiological contamination and suggests the need for improved hygiene during the production, handling, storage, and/ or marketing of traditional yogurt.
- High levels of phenotypic resistance were observed among LAB isolates against all tested antibiotics, *E. coli* and *S. aureus* isolates also exhibited resistance to several of the tested antibiotics, with the highest resistance observed against penicillin, while susceptibility patterns differed according to the bacterial group (LAB, *E.coli*, *S. aureus*) and antibiotic tested.

VIII. RECOMMENDATIONS

8.1. Recommendations to future researchers

a. Furthermore, to conduct the study with large sample size and more different dairy products
b. Conduct research on antibiotic residues

8.2. Recommendations to Producers

a. Producers should implement good hygienic practices during the preparation and processing of traditional yogurt to minimize microbial contamination.
b. Producers should ensure that equipment, utensils, containers, and processing surfaces are properly cleaned and sanitized before and during yogurt production.

8.3. Recommendations to vendors

a. To Maintain Strict Cold Chain Management During Storage and Sale
b. Implement hygienic practices
c. Vendors should strictly use food-grade plastic or stainless-steel containers and utensils

8.4. Recommendations to government agencies

a. Relevant government agencies should strengthen the implementation and monitoring of regulations concerning the responsible use of antibiotics in animal production.
b. Training and awareness programs should be strengthened to educate farmers and other stakeholders about responsible antibiotic use and antimicrobial resistance

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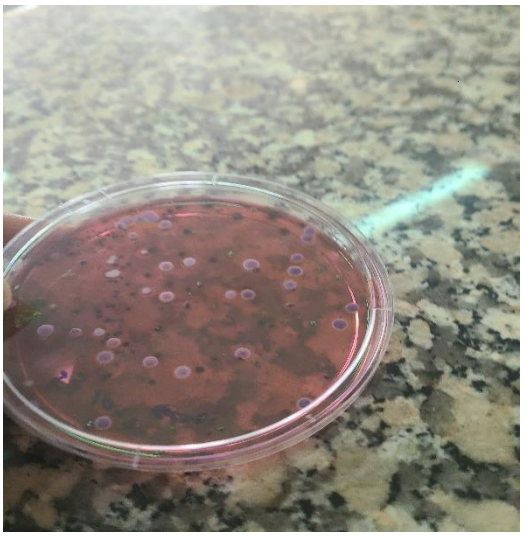
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X. APPENDICES

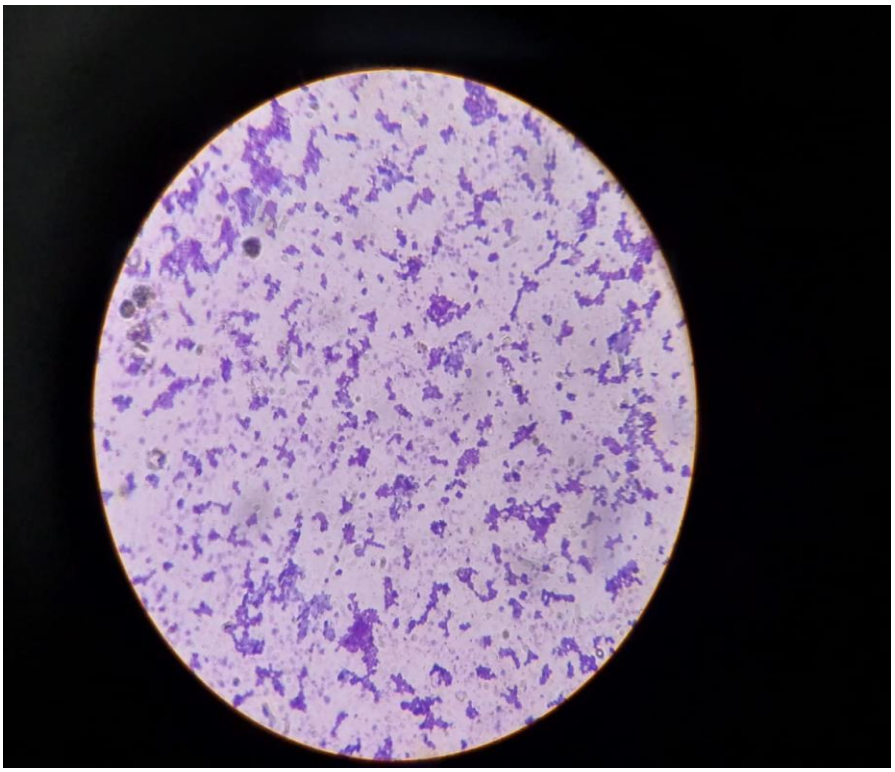
Appendix 1: *Staphylococcus aureus* and *E. coli* on specific isolated media

***Staphylococcus aureus* on mannitol salt agar** ***E.coli* on EMB**





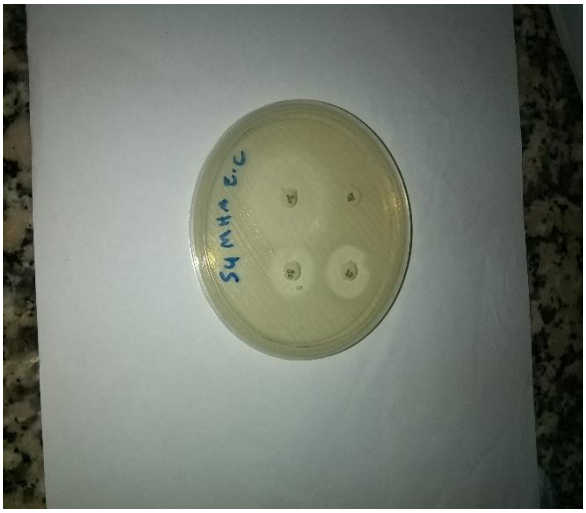
Appendix 2: Gram-staining – Microscopic gram positive bacteria



b

Appendix 3: Antibiotic Susceptibility test

AST on *E.coli*



AST on *Staphylococcus aureus*

