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COLLEGE OF HEALTH SCIENCES

MICROBIAL CONTAMINATION OF FOOD MILLING MACHINES IN TWO MAJOR MARKETS IN ACCRA

BY

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DECLARATION

I, Isaura Namen-Arikum Yelkebono, do hereby declare that the work presented in this thesis entitled, "Microbial Contamination of Food Milling Machines in Two Major Markets in Accra" is my original work and that this research has neither in part nor whole been presented to the University or elsewhere for any degree. I further declare that the authors whose works were referred to have been duly acknowledged.

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DEDICATION

Firstly, to the Almighty God, for His divine provision, protection, direction, and endless love

Secondly, to my beloved family

Miss. Augustina Anaba (Mother)

Miss. Isabella Kanyir Yelkebono (Sister)

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LIST OF ABBREVIATIONS

MPT	Makola Pepper/Tomatoes
APT	Agbogbloshie Pepper/Tomatoes
MC	Makola Corn
AC	Agbogbloshie Corn
MK	Makola Kokonte
AK	Agbogbloshie Kokonte
MFU	Makola Fufu
AFU	Agbogbloshie Fufu
MF	Makola Fish
AF	Agbogbloshie Fish
MG	Makola Groundnut
AG	Agbogbloshie Groundnut
MM	Makola Millet
AM	Agbogbloshie Millet
MA	Makola Agushie
AA	Agbogbloshie Agushie
MP	Makola Palmnut
AP	Agbogbloshie Palmnut
MH	Makola Herbs
AH	Agbogbloshie Herbs
AK/AMK/AN	Amikacin
AMP/AM	Ampicillin
AMC	Amoxicillin Clavulanic acid
C	Chloramphenicol
CAZ	Ceftazidime
CIP	Ciprofloxacin
CRO	Ceftriaxone
E	Erythromycin
FEP	Cefepime
FOX	Cefoxitin
GM/CN	Gentamicin
LVX	Levofloxacin
MEM	Meropenem
P	Penicillin

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TE/T	Tetracycline
TEI	Teicoplanin
TS/SXT	Cotrimoxazole
VA	Vancomycin
MALDI-TOF	Matrix Assisted Laser Desorption Ionization-Time of Flight

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ABSTRACT

Background: Food safety remains a critical public health concern, particularly in urban markets where unsanitary practices prevail. Some undesired implications of food contamination include foodborne illnesses, economic losses, and damage to public trust in the food supply chain. Although food milling machines are becoming increasingly common in Ghana, very little is known about their role in food safety.

Aim: This study aimed to assess the microbial contamination of food milling machines and its effect on milled food in Accra.

Methods: This was a descriptive cross-sectional study carried out at two major markets in Accra, Agbogbloshie and Makola. A total of 134 food milling machines were sampled in this study. Three swabs from various components of every milling machine were obtained using sterile swab sticks pre-moistened in peptone water. Additionally, food samples, before and after milling, were collected into sterile containers. All the samples were analyzed using standard microbiological methods, including antimicrobial susceptibility testing. A structured questionnaire was administered to machine operators to gather information on hygiene practices, cleaning routines, knowledge of food safety, and conditions around the milling machines.

Results: A total of 98 bacterial species were identified in the swab samples from the machines, with 13 species being of public health importance. The predominant organisms isolated were *Klebsiella pneumoniae* (39%), *Pseudomonas aeruginosa* (12.5%), *Enterobacter hormaechei* (10%), and *Enterococcus faecium* (8%). The mean total plate count (TPC), coliform plate count (CPC), and fecal coliform count (FCC) before milling were 528.3 x 102, 208.2 x 102, and 43.1 x 102, respectively, while TPC, CPC, and FCC after milling were 1088.8 x 102, 814.6 x 102, and

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369.2 x 102, respectively. Comparing unmilled and milled food samples, it was observed that milling machines were a source of microbial contamination in all the milled food samples. Some bacterial isolates from the milling machines exhibited a high prevalence of multidrug resistance (MDR). These included *E. cloacae* (61.5%), *E. hormaechei* (28.6%), *E. coli* (20.8%), and *K. pneumoniae* (4.4%). The hygienic and safety practices of the milling machines were poor, with all the machines not being, thoroughly cleaned after use.

Conclusion: The study identified a wide spectrum of bacteria that contaminated milling machines at the two markets in Accra, some of which had a high prevalence of MDR. Hence, there is a need for infection control practices at the milling sites to protect public health.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Food safety is a critical component of health, with significant implications for global public health and economic development. Annually, over 420,000 deaths are attributed to foodborne infections worldwide, predominantly occurring in underdeveloped or developing countries, with the food processing chain being a major contributor to this issue (Balali *et al*., 2020; Yar *et al*., 2023). In Africa, it is estimated that foodborne hazards result in approximately 137,000 deaths and 91 million acute illnesses each year, and children under five years of age are the most affected. The foodborne hazard risks include harmful bacteria such as *Salmonella* and *Escherichia coli*, parasites like tapeworms, and naturally occurring toxins such as cyanide poisoning (WHO, 2020).

The food processing chain involves several stages, from harvesting and packaging to handling (Yar *et al*., 2023). The milling of food, especially grains, cereals, legumes, and other food products, constitutes a critical phase in the food processing chain, where microbial contamination of milling machinery poses significant risks to consumers. Furthermore, aging and corrosion of grinding discs in milling machines may contribute additional contaminants to the processing chain (Prasad *et al*., 2018).

In Accra, the capital city of Ghana, numerous small-scale food milling operations serve the needs of the local population. However, the potential for microbial contamination in these facilities raises concerns, as it can lead to the transmission of foodborne illnesses and compromise the quality and safety of milled products (Popa *et al*., 2019; Aboagye *et al*., 2023). The high population density and urbanization of Accra exacerbate complex food safety challenges. Therefore, ensuring the

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microbial safety of food milling machines is imperative for protecting public health and maintaining consumer confidence in local food products (Dzah, 2015).

1.1 Problem Statement

Food milling machines in Accra are often operated under suboptimal hygienic conditions, and may potentially lead to microbial contamination of milled foods (Dzah, 2015). Many small-scale milling operators do not adhere to proper hygienic and sanitation practices. (Dzah, 2015; Popa *et al*., 2019; Aboagye *et al*., 2023). Observational investigation, involving weeks of daily monitoring of market hygiene by researchers, revealed that containers (cups, bowls, and buckets) used for measuring grains, cereals, and vegetables are not cleaned before or after use (Azuonwu *et al*., 2019). This could promote the growth of microorganisms in the measuring containers, which can be transferred to the products being measured (Azuonwu *et al*., 2019). Since some of these food products get contaminated on the farms or while being transported to the markets, transfer of microbes from the food products to the measuring containers is plausible (Alum *et al*., 2016). It is reported that, milling operators do not regularly clean the machines before or after use, hence, food residues trapped in the mills could serve as breeding grounds for harmful microbes (Alum *et al*., 2016; Azuonwu *et al*., 2019). As a result, the risk of foodborne outbreaks, such as gastroenteritis and enteric disorders among food handlers, milling machine operators, and consumers, becomes imminent (Azuonwu *et al*., 2019; WHO, 2020).

Additionally, the lack of awareness among milling machine operators regarding food safety practices, and the potential health risks associated with microbial contamination worsens the problem (Yar *et al*., 2023).

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1.2 Justification

Ensuring food safety is a fundamental responsibility of the food industry and regulatory authorities. Microbial contamination of food products can have severe consequences, including foodborne illnesses, economic losses, and damage to public trust in the food supply chain (WHO, 2020). There is a lack of comprehensive data on the microbial contamination of food milling machines in Accra. The outcome of this study will provide a better understanding of the potential risks and insights, for developing appropriate interventions and policies to improve food safety practices in the milling industry. Similar studies conducted by Dzah, (2015) in Accra and Yar *et al*., (2023) in Kumasi used biochemical tests for the identification of isolated microorganisms, however, did not perform antimicrobial susceptibility tests on the isolates. In this study, Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) was used to ensure a more accurate and robust identification of isolates, while antimicrobial susceptibility tests were performed on pathogenic isolates. Furthermore, the findings of this study can inform public health initiatives, and raise awareness among consumers and stakeholders about the importance of proper food handling and processing. By focusing on Accra, the study provides insights that can be generalized to other urban centers with similar practices, thereby contributing to broader food safety initiatives.

1.3 Aim

To evaluate microbial contamination of milling machines in Accra and its effect on milled food.

1.4 Objectives

1. To determine the microbial contamination of milled food and milling machines in Accra.
2. To evaluate the hygienic and safety practices related to the milling machines.

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3. To determine the antimicrobial susceptibility profiles of the predominant bacteria isolated from the milling machines.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Food Safety and Microbial Contamination

Food safety remains a vital public health concern globally, addressing practices aimed at preventing foodborne illnesses that impact millions of people each year. Ensuring food safety involves rigorous standards and procedures during all stages of the food chain, from production to consumption, to prevent contamination by harmful microorganisms, including bacteria, viruses, and fungi. These pathogens pose significant health risks, ranging from mild gastrointestinal symptoms to life-threatening conditions, such as listeriosis and botulism. These pathogens and their related foodborne illnesses are particularly dangerous for vulnerable populations like the elderly, young children, pregnant women, and individuals with weakened immune systems (Pittet *et al*., 2009; Scallan *et al*., 2011). According to the World Health Organization (WHO), foodborne diseases contribute to 600 million cases of illness annually, resulting in approximately 420,000 deaths globally, with a disproportionately high burden in low- and middle-income countries due to inadequate food safety infrastructure (WHO, 2022).

Microbial contamination arises from various factors, including poor hygiene practices, suboptimal cooking, and cross-contamination during food handling, processing, and storage (Rocourt *et al*., 2003). Poor handling practices, especially in environments with limited regulatory oversight or in informal markets, increase the risk of contamination by pathogens such as *Salmonella*, *E. coli*, and *Listeria monocytogenes*, which can survive under certain conditions even after cooking or processing (Schlundt, 2017; CDC, 2021). Cross-contamination remains a particular concern during food processing, as bacteria from raw food products or contaminated surfaces can easily spread to other foods if hygiene protocols are not strictly followed. Moreover, inadequate

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refrigeration and storage conditions can facilitate the growth of spoilage organisms and pathogens, emphasizing the need for stringent temperature control to preserve food quality and safety (Waldhans *et al*., 2024).

To address these risks, food safety measures include the implementation of strict hygiene standards, ongoing microbial testing, and advancements in food processing technologies designed to minimize contamination (Havelaar *et al*., 2015). Technologies such as high-pressure processing (HPP) and ultraviolet (UV) irradiation have gained attention as effective methods for reducing microbial loads in food products while preserving nutritional quality (Piyasena *et al*., 2019). Additionally, hazard analysis and critical control points (HACCP) systems have become foundational in food safety management, helping to identify, assess, and mitigate risks at critical stages of production and processing. Continuous education and training for food handlers also play an essential role, ensuring that personnel are aware of safe practices to prevent contamination and protect consumer health (Ababio, 2011).

The integration of such advanced food safety practices is essential, particularly as global food supply chains become more complex and food products increasingly cross international borders. Global initiatives, including those by the Food and Agriculture Organization (FAO) and WHO, emphasize the importance of collaborative efforts across nations to establish and maintain stringent food safety protocols (FAO/WHO, 2022). Ensuring food safety requires a multi-layered approach, combining regulatory oversight, technological innovation, and a commitment to public health standards to protect populations from the ongoing threat of foodborne illness.

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2.2 Food Milling Machines and Their Role in Food Processing

Food milling machines are fundamental components in the food processing industry, where they transform raw ingredients into fine, uniform particles essential for diverse food products. These machines support industries like cereal processing, spice grinding, flour milling, and even specialty food production, enabling mass production and uniformity in particle size that enhances texture, palatability, and cooking efficiency (Abdel-Aal, 2024). By reducing particle sizes, milling also increases the surface area of ingredients, optimizing subsequent processes such as mixing, hydration, and heat transfer during cooking. This is particularly advantageous in cereal and spice processing, where precise particle control can significantly impact product flavor and aroma (Garvey *et al*., 2023).

Advanced milling technologies, such as roller mills, hammer mills, and increasingly, cryogenic and jet mills, have revolutionized the food industry by enhancing consistency, efficiency, and precision in particle size reduction. Roller mills, for instance, provide consistent particle size through shear and compression, making them ideal for grain processing, while hammer mills are favored for coarser, high-capacity grinding needs (Singh *et al*., 2022). Recent technological advancements have also integrated automation and real-time monitoring, enabling food processors to maintain strict quality and safety standards. This has been particularly beneficial in maintaining consistent particle sizes, which, according to Lin, *et al.* (2020), significantly improves the structural quality and shelf life of food products.

However, while milling machines offer significant advantages, they also present contamination risks if not properly maintained. Particles left in milling equipment can harbor moisture, creating conditions for microbial growth, especially in warm or humid environments. Research by Compton *et al.* (2018) highlights that cross-contamination risks are pronounced in high-volume

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processing plants where machines are used continuously and may be insufficiently cleaned. Regular sanitation protocols, combined with proper equipment maintenance, are therefore essential to minimize bacterial growth and prevent foodborne pathogens from contaminating final products (Holah, 2014).

The adoption of these advanced milling technologies and stringent sanitation practices aligns with ongoing efforts in the food processing industry to enhance both product quality and safety. For instance, the International Association for Food Protection (IAFP) emphasizes that maintaining a clean milling environment not only safeguards consumer health but also prolongs machine life and reduces maintenance costs (IAFP, 2021). Consequently, as the demand for processed foods continues to grow, the importance of well-maintained and technologically advanced milling equipment remains pivotal in ensuring that food processing keeps pace with safety and quality expectations.

2.3 Microbial Contaminants in Food Milling Machines

Microbial contamination in food milling machines poses a significant challenge in food safety, with contaminants potentially originating from raw materials, environmental sources, and the surfaces of the machines themselves. Common bacterial contaminants, such as *Salmonella*, *Escherichia coli*, and *Bacillus cereus*, are particularly problematic due to their ability to survive on metal surfaces and under varied environmental conditions within milling equipment (Kusumaningrum *et al*., 2003; Forsythe, 2011; KuKanich, 2011). These bacteria can persist after routine cleaning, which increases the risk of cross-contamination in processed food products. Fungal species, including *Aspergillus* and *Penicillium*, are frequently detected in milling environments, particularly in humid conditions conducive to fungal growth (Lee & Magan, 2010).

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Such fungi not only reduce food quality but also pose health risks through mycotoxin production, which can lead to serious health issues upon consumption (Pitt & Hocking, 2009).

The design of milling machines—often with intricate parts and hard-to-reach areas—makes them particularly susceptible to biofilm formation. Biofilms, which are communities of microorganisms encased in a protective extracellular matrix, can develop on machine surfaces and provide a haven for bacteria and fungi to survive and proliferate. Biofilms are notably resistant to cleaning and sanitizing agents, often requiring specialized procedures to effectively eradicate them (Srey *et al*., 2013; Zhao *et al*., 2017). Biofilm formation in machine parts poses a major threat, as biofilm-associated resistant bacteria can periodically be released into food during processing, leading to recurring contamination events that compromise food safety.

Research has consistently demonstrated that improper sanitation and maintenance of milling machines lead to the accumulation of microbial contaminants, which can then be transferred to food products, causing spoilage or foodborne illness. Rajkovic *et al.* (2010) highlighted that inadequate sanitation protocols increase microbial load and transfer rates, with bacterial contaminants shown to survive in milling environments even under low-moisture conditions, where they tend to be more resilient (Swanson, 2011). This makes effective sanitation and hygiene critical components in food milling operations.

To mitigate these risks, regular and thorough cleaning schedules, combined with the use of food-safe sanitizers, are essential. Monitoring microbial loads through routine swab testing and microbial load assessment can help in identifying contamination hotspots within the machinery. Advanced approaches, such as incorporating antimicrobial surfaces or implementing automated cleaning systems, have also been suggested as effective control measures (Kusumaningrum *et al*., 2003; Hu *et al*., 2024). These strategies, alongside vigilant maintenance, are indispensable to

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prevent contamination, ensuring both the quality and safety of food products processed in milling machines.

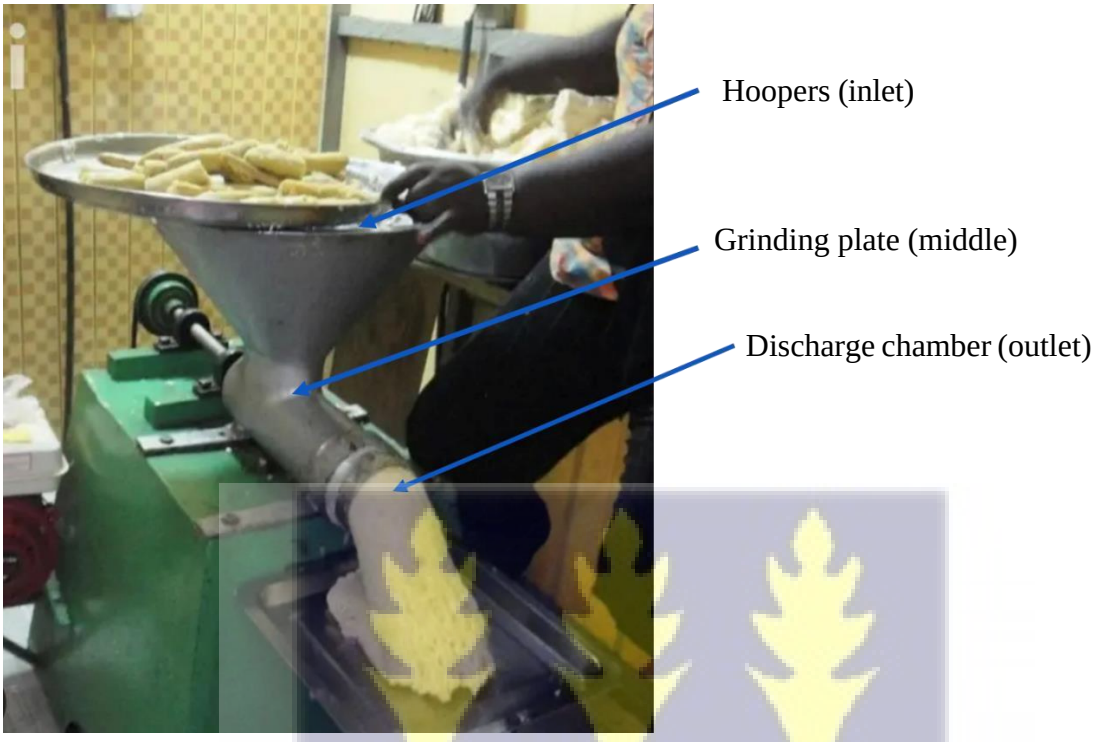

Figure 1: A picture of a fufu milling machine

2.3.1 Microbial Contamination of Food Milling Machines in Ghana

Food milling machines are central to Ghana's food processing landscape, especially in informal markets where they are used to grind staples such as maize, groundnuts, cassava, and pepper. However, studies have consistently highlighted these machines as key sources of microbial contamination. Dzah (2015) first identified a range of pathogenic organisms in milled tomatoes, attributing their presence to the lack of proper sanitation practices and poor awareness among machine operators. Similarly, Addo *et al*., (2020) reported that food contact surfaces in milling environments—particularly those in open markets—harbored high levels of coliforms and other bacteria due to inadequate cleaning and poor operator hygiene.

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Recent findings have reinforced these concerns. Yar *et al*., (2023) demonstrated the persistence of bacteria such as *Salmonella, E. coli, S. aureus,* and *Klebsiella pneumoniae* on milling machines, with biofilm formation playing a major role in their survival despite routine cleaning efforts. Their study found widespread contamination across different parts of the machines, including inlets, middles, and outlets. These findings align with global evidence showing how resistant pathogens can survive in food environments and pose serious health risks when transmitted through contaminated food products.

Aboagye *et al*., (2023) provided further evidence of cross-contamination during milling, showing high microbial loads in the fufu milling machines compared to traditional mortar and pestle. Collectively, these studies highlight the urgent need for improved food safety protocols, targeted hygiene interventions, and regulatory enforcement to mitigate contamination risks and protect public health in Ghana's milling sector.

2.4 Sources and Routes of Microbial Contamination in Milling Machines

Microbial contamination in milling machines arises from multiple interconnected sources and pathways throughout the milling process. Raw materials like grains, spices, and pulses can act as primary carriers of microorganisms, introducing contaminants from the agricultural environment directly into the processing facility. Field exposure to soil, animal waste, and untreated water is a common pathway through which raw ingredients become contaminated with pathogens such as *Salmonella* and *E. coli*, which then persist through transport and storage (Alegbeleye *et al*., 2018; Berger *et al*., 2022). Environmental factors in milling facilities, such as airborne dust particles, process water, and ambient air, can also introduce contaminants during milling operations, especially in facilities without proper ventilation and air filtration systems (Zhao *et al*., 2014;

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Clausen *et al*., 2020). Additionally, water used for cleaning and cooling equipment can harbor and spread microbial communities if not properly treated and monitored.

Human handlers represent another critical contamination vector, as poor hygiene practices, such as insufficient handwashing or wearing contaminated clothing, can transfer pathogens to food contact surfaces. Research by Montville and Schaffner (2011) demonstrates that handling practices are a significant factor in microbial transfer, with pathogens often spreading from unwashed hands to equipment surfaces and food products, posing a substantial contamination risk. To mitigate this, the use of personal protective equipment (PPE) and rigorous hygiene training for personnel has proven effective, though adherence remains essential for sustained food safety.

The structural design of milling machines also influences contamination routes. Many machines have complex parts, such as rollers, blades, and hoppers, which contain nooks, crevices, and hard-to-clean areas that can harbor biofilms. Biofilms—protective layers formed by microbial communities—are particularly resistant to standard cleaning and disinfection efforts, allowing bacteria and fungi to persist in milling environments over extended periods (Holah *et al*., 2014; Chmielewski & Frank, 2022). These biofilms can gradually release microorganisms into food products, leading to contamination and spoilage even with regular cleaning.

Cross-contamination is a major risk when pathogens from one batch of material or a machine surface come into contact with a new, uncontaminated batch of ingredients. Such contamination may occur directly through equipment or indirectly through poorly sanitized surfaces or tools within the facility. This highlights the importance of stringent sanitation protocols, including the use of sanitizers and physical barriers to limit contamination spread (Van Houdt & Michiels, 2005; Forsythe, 2011). Consequently, understanding and mitigating these diverse contamination routes is crucial for developing effective strategies that prevent microbial proliferation within milling

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operations (Sperber, 2007). Regular equipment cleaning, strict adherence to hygiene practices, and continuous monitoring for contaminants are necessary to enhance food safety in milling facilities.

2.5 Factors Influencing Microbial Growth in Milling Machines

Microbial growth in milling machines is influenced by a combination of environmental factors such as temperature, moisture, and nutrient availability, each playing a critical role in determining microbial survival and proliferation. Temperature control is particularly essential, as most pathogenic microorganisms grow optimally between 20-40°C, with some foodborne pathogens like *Salmonella* and *Listeria* able to survive in even broader temperature ranges under favorable conditions. Milling processes inherently generate frictional heat, which can elevate local temperatures on equipment surfaces and, if not controlled, create ideal conditions for microbial growth (Jay *et al*., 2005; Podolak *et al*., 2010). Thus, maintaining appropriate ambient temperatures and mitigating heat buildup during operation are vital strategies for microbial control in milling environments (Gould & Russell, 2003).

Moisture levels in milling environments also have a pronounced impact on microbial behavior. Elevated humidity within the facility or residual moisture on equipment surfaces can encourage microbial growth and biofilm formation, as biofilms tend to form more readily in moist environments where bacteria can adhere to surfaces and resist cleaning efforts (Sharma & Anand, 2002). Managing humidity through adequate ventilation, dehumidifiers, or air filtration systems is essential, as high moisture not only promotes microbial proliferation but can also lead to clumping of powdered ingredients, affecting product consistency and quality.

Nutrient availability is another significant factor that supports microbial persistence in milling machines. Organic residues left behind from grains, spices, and other materials processed in the machines can serve as nutrient sources, supporting microbial communities and facilitating growth.

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These residues are especially problematic in the nooks and crevices of machines where they can accumulate and become difficult to remove, providing a continuous source of nutrients for microbial growth (Storgårds *et al*., 2006). Food particles that accumulate in these areas can sustain microbial populations, which can then be transferred to subsequent batches of food products, emphasizing the need for thorough cleaning after each production cycle.

The physical condition of milling machines is a key determinant of contamination risk. Worn parts, scratches, and hard-to-reach crevices provide ideal environments for microbial colonization, as they are less accessible to cleaning agents and physical scrubbing. Studies have shown that worn or damaged surfaces harbor more bacteria due to the increased surface area and ability of microbes to attach and form biofilms (Holah *et al*., 2014; Kostaki *et al*., 2012). Therefore, regular maintenance and the timely replacement of machine parts are essential practices to reduce microbial harborage and ensure food safety.

Mitigating machine contamination requires rigorous sanitation protocols, including regular cleaning, disinfection, and maintenance schedules tailored to milling machine designs. Effective control measures also encompass environmental monitoring to track moisture and temperature levels and the implementation of routine microbial testing to identify contamination hotspots. These efforts help to address the physical and environmental factors influencing microbial growth, ultimately safeguarding the quality and safety of food products (Kusumaningrum *et al*., 2003; Mecca *et al*., 2025).

2.6 Health Implications of Microbial Contamination in Milled Foods

Microbial contamination in milled foods is a serious public health concern, posing risks to consumer health through foodborne illnesses that can vary from mild gastrointestinal distress to life-threatening conditions. Symptoms of foodborne illness, including nausea, vomiting, diarrhea,

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and abdominal pain, are often acute and can lead to severe dehydration, particularly in young children, the elderly, and individuals with weakened immune systems. In vulnerable populations, foodborne pathogens can cause severe complications, such as kidney failure from *E. coli* O157 or meningitis from *Listeria monocytogenes*, with potential for long-term health impacts or mortality (CDC, 2018; WHO, 2020).

Pathogens like *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157 are commonly associated with contaminated milled foods, given the diverse range of raw materials and environments these foods encounter during processing. These pathogens can persist on milling equipment surfaces, especially when cleaning and sanitation protocols are inadequate, increasing the risk of cross-contamination between batches (Forsythe, 2011). For example, *Salmonella* is known for its resilience in low-moisture environments, such as flour and dry spices, making milling equipment and facilities particularly vulnerable (Beuchat *et al*., 2013). *Listeria*, on the other hand, can survive and grow at refrigeration temperatures, posing a heightened risk for milled foods stored under cooler conditions (Swaminathan & Gerner-Smidt, 2007).

The economic impact of foodborne illnesses associated with contaminated milled foods extends beyond health implications. Medical expenses, lost productivity due to illness, and the substantial costs associated with product recalls and legal actions place a significant financial burden on both individuals and food companies. According to Scharff (2012), the economic losses associated with foodborne illnesses can reach billions annually in developed nations alone, factoring in healthcare costs, reduced consumer confidence, and costs of brand reputation damage. Furthermore, food recalls, as seen in recent incidents involving contaminated flour and baking mixes, underscore the substantial resources required to manage such crises and restore public trust (Scharff, 2012).

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To ensure the microbiological safety of milled foods, it is essential to implement rigorous monitoring and control measures throughout the production process. This includes regular microbial testing, adherence to stringent hygiene protocols, and swift responses to contamination events. The adoption of Hazard Analysis and Critical Control Points (HACCP) and other preventive frameworks, as well as advanced detection technologies, enables food producers to identify and mitigate contamination risks early in the process (Havelaar *et al*., 2015; Sperber, 2005). Emphasizing food safety training for workers, strict sanitation of milling equipment, and environmental monitoring are also crucial to maintain safe milling environments and reduce the incidence of foodborne illnesses associated with milled products.

2.7 Regulatory Framework and Food Safety Practices in Ghana

Ghana's regulatory framework for food safety is designed to protect public health through comprehensive laws, standards, and guidelines that regulate food production, processing, and distribution. The Food and Drugs Authority (FDA) serves as the primary regulatory body responsible for overseeing food safety, with mandates that cover the entire food supply chain, including the manufacture, import, export, distribution, and sale of food products (FDA Ghana, 2021). Established under the Public Health Act of 2012 (Act 851), the FDA is empowered to set and enforce food safety standards, ensuring that products comply with hygiene and safety requirements to prevent foodborne illnesses (Government of Ghana, 2012). This Act is fundamental to the country's food safety framework, setting strict penalties for violations and encouraging adherence to safety protocols among food businesses.

The Ghana Standards Authority (GSA) plays a crucial supporting role in this framework by working closely with the FDA to develop and implement food safety standards. Together, these agencies strive to align Ghana's food safety regulations with international benchmarks, including

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those from the Codex Alimentarius, to facilitate both domestic food safety and international trade (GSA, 2020). The collaboration between the FDA and GSA helps to establish quality and safety benchmarks for various food products, reinforcing consumer confidence in the market.

Despite these regulatory measures, Ghana faces several challenges in implementing effective food safety practices. Limited financial and human resources, coupled with gaps in technical capacity, hinder the FDA and GSA's ability to conduct widespread inspections and enforce compliance, particularly in rural and informal sectors where food processing and distribution often occur without adequate oversight (Cook *et al*., 2024). Moreover, informal food vendors and small-scale processors often operate without formal food safety training or licenses, raising risks of contamination and foodborne illnesses (Odonkor *et al*., 2020).

To address these issues, Ghana has focused on enhancing regulatory frameworks and building institutional capacity. Key initiatives include training programs for food handlers, public awareness campaigns on food safety practices, and efforts to improve inter-agency coordination to strengthen enforcement capabilities (Mensah & Julien, 2011). Additionally, the FDA has implemented food safety training and certification programs aimed at informal sector vendors to raise hygiene standards and foster safer food environments (FDA Ghana, 2021). Continuous investments in these areas, alongside international collaboration for technical assistance, are essential to advancing food safety in Ghana and reducing the prevalence of foodborne illnesses.

2.8 Standards Assessment of Microbiological Quality in Food Safety Evaluation

Microbiological quality assessment is an essential component of food safety evaluation, providing a quantitative measure of the microbial load present on food, food contact surfaces, or processing equipment. Internationally, standards for microbiological quality are guided by agencies such as the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the International Commission on Microbiological Specifications for Foods (ICMSF), and

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the Codex Alimentarius Commission. These standards provide acceptable limits for microbial indicators to ensure that food products and processing environments do not pose a health risk to consumers.

In Ghana, microbiological quality assessment of food processing equipment, including food milling machines, is generally guided by principles from the Ghana Standards Authority (GSA) and global food safety systems such as Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practices (GMPs). These frameworks emphasize the need to monitor indicator organisms such as coliforms and *E. coli* to evaluate hygiene, and to use total plate counts to measure overall microbial load on equipment or in food. Milling machines, which frequently come into contact with raw and processed foods, are critical points for such assessments due to their high potential for cross-contamination when sanitation practices are poor (Addo *et al*., 2020; Aboagye *et al*., 2023).

2.8.1 Microbiological Indicators Safety and Quality in Food Milling.

The Total Plate Count, also known as the Aerobic Plate Count (APC), measures the total number of viable aerobic bacteria present in a sample. It serves as a general indicator of the microbiological quality and cleanliness of a food or surface. In the context of food milling machines, a high TPC suggests poor cleaning practices, residual organic matter, or biofilm formation on machine surfaces that support bacterial growth. Acceptable limits vary depending on the item being tested, but for food contact surfaces, counts are generally expected to be less than 10^2–10^3 CFU/cm², while food samples typically should not exceed 10^5–10^6 CFU/g or CFU/ml (ICMSF, 2011).

The Coliform Plate Count quantifies bacteria belonging to the coliform group, which includes genera such as *Enterobacter spp., Klebsiella spp.*, and *Citrobacter spp*. Coliforms are used as hygiene indicator organisms, meaning their presence signifies environmental contamination,

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poor handling, or inadequate sanitation during processing. In milling machines, high CPC values indicate that equipment surfaces are contaminated with environmental or fecal bacteria, typically due to handling with unwashed hands or contact with contaminated food residues. For food or surfaces, coliform counts should ideally be below detectable limits (≤10 CFU/g or CFU/cm² or CFU/ml); anything above this indicates poor hygiene or sanitation failure (WHO, 2022).

The Fecal Coliform Count detects thermotolerant coliforms, primarily *Escherichia coli*, which originate from the intestinal tracts of humans and animals. Thus, they are used as a specific indicator of fecal contamination. In the context of milling machines, a positive FCC result means that the machine or the food being milled has come into contact with fecal material, possibly through contaminated water, unhygienic handling, or environmental exposure. High FCC levels are a serious red flag, as they indicate potential contamination with enteric pathogens such as *Salmonella spp*., *Shigella spp*., and *E. coli* O157:H7. Fecal coliforms should not be detectable in 1g or 1ml of food; any positive result is considered unsatisfactory for food intended for consumption (FAO/WHO,2020).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study sites: Makola and Agbogbloshie Markets

The study was conducted at two urban markets in the Greater Accra region: Makola and Agbogbloshie markets (Figure 1). The Makola market, located in the heart of Accra, is one of Ghana's largest commercial centers, known for its diverse goods and food processing activities. It is a dynamic environment where food safety practices are essential due to high foot traffic and dense vendor concentration.

The Agbogbloshie market, located in Accra's central district, is a significant trading center, known for fresh produce and informal food processing. It faces unique environmental and health challenges due to its electronic waste (e-waste) recycling activities. Selecting Makola and Agbogbloshie markets as study sites offered a comprehensive view of microbial contamination risks in different market environments. The Makola market represented a structured, high-traffic commercial hub, while Agbogbloshie reflected the challenges of informal markets with significant environmental health hazards.

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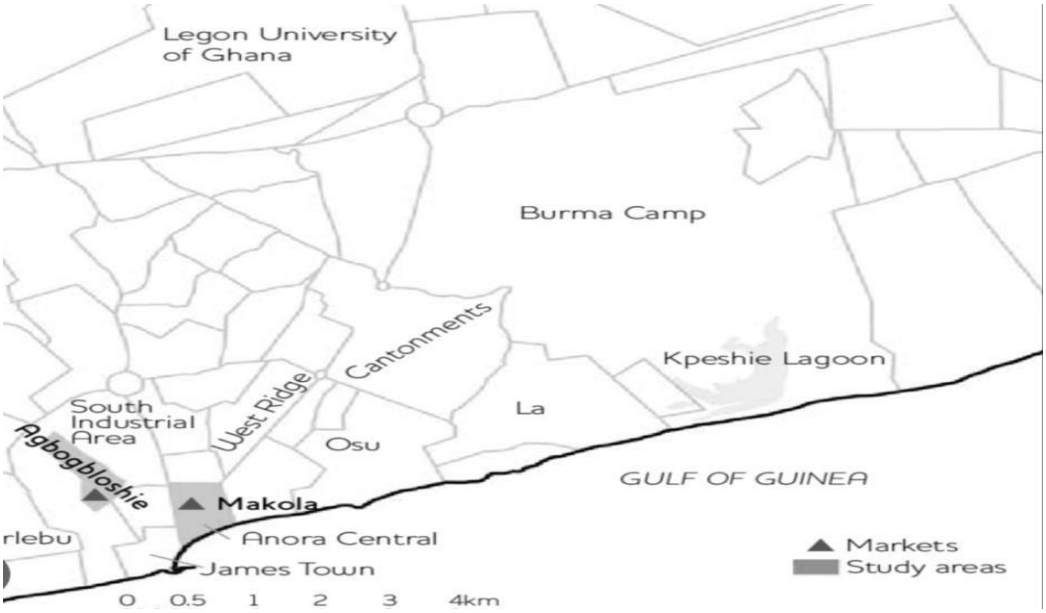

Figure 2: A map of the Accra Metropolitan Area showing the study locations.

3.2 Study Design

The study was a descriptive cross-sectional study. Samples were collected from food milling machines and foods to be milled in the two markets. Only functioning milling machines in both Makola and Agbogbloshie markets were included in the study, and broken-down milling machines in both markets, as well as operators who did not consent for their milling machines to be sampled, were excluded.

3.3 Sample Size:

All milling machines in the study sites available within the sampling period were sampled; 32 from Makola and 102 from Agbogbloshie. At both locations, the milling machines were separately sampled after milling different ingredients namely (1) tomato/pepper, (2) corn, (3) millet, (4) palm nut, (5) agushie (white-seed melon), (6) groundnut and (7) fufu (a meal made from pounding/milling cassava and unripe plantain). There was variation in the number of mills, due to the sample size being dependent on the number of mills present at the sample location. For example, if 15 corn mills were found in Makola, all 15 were sampled; if 5 tomato/pepper mills were found,

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all 5 were sampled (Yar *et al*., 2023). Food samples were collected into sterile containers before and after milling.

3.4 Data Collection Methods:

3.4.1 Microbiological Sampling:

Swab samples were taken from the hoppers (inlet), grinding plates (middle), and discharge areas (outlet). Sampling was performed during peak operation hours (9 am to 2 pm) to reflect typical usage conditions. Sterile swab sticks moistened in peptone water were used to swab components of the hoppers, pounding compartment, and discharge unit for fufu pounding machines, and hoppers, grinding compartment, and discharge ports for pepper/tomato and corn milling machines, respectively. Three different swab samples were taken from each milling compartment and discharge units of all selected milling machines that were included in the study. Food samples were taken before and after milling from a selected number of milling machines into sterile containers. All the samples were stored in sterile leak-proof tubes/containers with peptone water and transported on ice to the Department of Medical Microbiology laboratory for analysis using standard laboratory techniques.

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Figure 3: Pictures depicting the sampling process on site.

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3.4.2 Questionnaire and Interviews:

A structured questionnaire was administered to machine operators to gather information on hygiene practices, food safety practices, maintenance of milling machines, cleaning routines, and knowledge of food safety and microbial contamination. Direct observation of the operational practices and environmental conditions around the milling machines was performed.

3.5 Microbial Analysis

Swabs were cut into Falcon tubes containing two milliliters of peptone water, vortexed, and incubated for 12 to 18 hours at 37°C. Serial dilutions (from 10^0 to 10^4) followed by plate count, coliform count, and fecal coliform count were performed on the food samples using the pour plating method. Ten-fold serial dilutions ranging from 10^0 to 10^{-4} were performed by transferring 1 ml of the homogenized food suspension into 9 ml of sterile saline under aseptic conditions. From each dilution, 1 ml aliquots were inoculated into sterile Petri dishes, after which molten Plate Count Agar (PCA) was added for total viable count (TPC), MacConkay Agar for total coliform count (TCC), and Eosin Methylene Blue (EMB) Agar for fecal coliform count (FCC). The samples were mixed thoroughly by gentle rotation and allowed to solidify. The plates were incubated at 37°C for 12 to 18 hours. After incubation, colonies were counted manually, and results were expressed as colony-forming units per ml (CFU/ml) of sample. Plates containing 30–300 colonies were selected for enumeration, and the mean counts from duplicate plates were used for statistical analysis.

The calculation for TPC, TCC, and FCC is basically the number of colonies by the dilution factor divided by the volume plated, $\frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (ml)}}$

The food samples (10^0) were incubated for 12 to 18 hours, vortexed, and 1 ml pipetted into Rappaport and selenite broth, then re-incubated at 45°C for 48 hours to increase the chances of isolating *Salmonella* spp. The suspensions were vortexed and cultured on blood, chocolate,

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mannitol salt, MacConkey, Salmonella Shigella Agar, and Xylose Lysine Deoxycholate agar and incubated for 12 to 18 hours at 37°C. The cultures were purified, pure isolated colonies were picked with a sterile toothpick, and inoculated on a target plate. Formic acid and matrix were added to the isolated colonies on the target plate, and inserted into the Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) for identification.

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Figure 4: Pictures depicting stocking of pure isolates in the laboratory

3.6 Antimicrobial Susceptibility Test with the Kirby-Bauer Method

Adhering to the guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2024), susceptibility of pathogenic isolates to standard antimicrobials was tested using the Kirby-Bauer method. The antibiotics used were Tetracycline (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Rifampicin (5 µg), Cotrimoxazole (1.25 + 23.75 µg), Penicillin (10 µg), Clindamycin (2 µg), Cefoxitin (30 µg), Linezolid (30 µg), Vancomycin (30 µg), Ampicillin (10 µg), Aztreonam (30 µg), Meropenem (10µg), Chloramphenicol (30µg), Ceftriaxone (30µg), Cefepime (30µg), Amikacin (30µg), Ciprofloxacin (30µg), Levofloxacin (0.5µg), and Ceftazidime (30µg). Each test isolate was emulsified in normal saline to create a suspension similar in turbidity to that of a 0.5% McFarland standard, with the help of a nephelometer. A sterile cotton swab was dipped into the suspension, pressed against the interior walls of the container to drain excess fluid. It was swabbed evenly across the entire surface of a Mueller-Hinton agar plate, in three different dimensions, to obtain a semi-confluent growth following incubation. The plates were incubated at 37ºC for 18 to 24 hours,

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and the zones of inhibition around the antibiotic discs were measured and interpreted according to the breakpoints of CLSI (2024).

3.7 Data Analysis:

Data was entered into Microsoft Office Excel daily, and results were analyzed using Statistical Package for Social Sciences (SPSS) version 25. Error adjustments in measurements were performed using the Huber-White method. Data on the bacterial flora colonizing the milling machines and resistance to the antimicrobials tested were summarized using descriptive statistics. Comparison of contamination levels and hygiene practices between Makola and Agbogbloshie markets was tested using t-tests or chi-square tests, and for individual differences between groups, POSCT-HOC, and TURKEY comparisons were employed to determine a significant difference at a 95% confidence interval ($P \leq 0.05$).

3.8 Ethical Considerations:

Approval from the Ethical and Protocol Review Committee of the College of Health Sciences, UG (Protocol number: CHS-Et/M.4 - P4.6/2024-2015), Accra Metropolitan Assembly, and verbal consent from participants were obtained. A high level of respect for the rights and confidentiality of all the milling machine operators was maintained. The risks and benefits associated with the study were clearly explained to the prospective study participants for them to make an informed choice of enrolling in the study. All data, both electronic and physical, has been stored in secure password-protected archival systems.

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CHAPTER FOUR

4.0 RESULTS

4.1 Demographics of Milling Machine Operators and Their Sanitary Practices

Table 1 provides an overview of the demographics and sanitary practices of 92 milling operators. The majority (77.2%) were male, and 87% were aged between 31 and 65 years. Most participants (78.3%) had at least a primary, JHS, or SHS education. Milling machine ownership varied, with 41.3% operating two machines, and 95.7% having a license. Sanitary practices were generally poor, with only 1.1% conducting weekly maintenance and none washing machine compartments with soap. Awareness of microbial contamination risks was low (15.2%). Hand hygiene was inadequate, as 70.7% did not use soap to wash their hands before milling, 84.8% did not wear hair covers, and none used gloves, resulting in direct hand contact with food. Only 7.7% of the operation sites were rated good in terms of the sanitation of milling sites.

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Table 1: Demographics of Milling Machine Operators and their sanitary practices

Characteristics	Categories	Frequency	Percentage %
Gender	Male	71	77.2
	Female	21	22.8
Age range/yr	22 -30	12	13
	31 - 65	80	87
Level of Education	Uneducated	20	21.7
	Primary	26	28.3
	JHS	29	31.5
	SHS	17	18.5
Number of Milling Machines per operator	1	26	27.2
	2	18	41.3
	3	11	15.2
	4	6	13
	5	3	3.3
Operates with License	Yes	88	95.7
	No	4	4.3
Frequency of maintaining mills	Once a week	1	1.1
	Once a month	20	21.7
	When it breakdowns down	71	77.2
Wash machine compartments with soap	No	92	100
Aware of microbial contamination in mills	Yes	14	15.2
	No	78	84.8
Frequency of washing hands daily	Once	23	25
	Twice	16	17.3
	Often	12	13
	Occasionally	41	44.6
Wash hands with soap before operation	Yes	27	29.3
	No	65	70.7
Wear hair covers while milling	Yes	14	15.2
	No	78	84.8
Wear gloves while milling	No	92	100
Is there hand contact with food while milling	Yes	92	100
Sanitation of operation site	Satisfactory	45	48.9
	Good	7	7.6
	Bad	40	43.5

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4.2 Bacteria Identified in Food Milling Machines

Table 2 provides a list of bacteria of public health interest found in the milling machines; a total of 351 isolates were of public health interest. *Klebsiella pneumoniae* was the most frequently isolated pathogen, accounting for 137 isolates, with 86 (44.8%) from Agbogbloshie and 51 (20.14%) from Makola. Other prevalent organisms include *Escherichia coli* (24), *Enterobacter cloacae* (26), *Enterobacter hormaechei* (35), and *Pseudomonas aeruginosa* (44), with higher counts generally found in Agbogbloshie. Additionally, several other pathogens were isolated, including *Acinetobacter baumannii* (11), *Serratia marcescens* (4), and *Enterococcus faecium* (28), among others. A list of bacteria not of public health interest isolated can be found in Appendix 2.

Table 3 shows the distribution of bacterial isolates across different types of food milling machines sampled in Makola and Agbogbloshie, with a total of 887 isolates. Agbogbloshie accounted for the majority of isolates (65.3%), while Makola contributed 34.7%. The highest number of isolates was found in pepper/tomato milling machines (436 total, 33.5% from Agbogbloshie and 15.7% from Makola). Corn milling machines had the second-highest number of isolates (238 total), followed by kokonte machines (65 isolates, all from Agbogbloshie). Bacterial Isolates were also found in machines used for processing fufu, fish, groundnut, millet, agushie, palmnut, and herbs, with varying distribution between the two locations.

Table 4 shows the frequency of bacteria of public health interest isolated from the various milling machines. A total of 351 bacterial isolates were identified from various machine types, with pepper/tomatoes and corn milling machines having the highest contamination. *Klebsiella pneumoniae* was the most common isolate, especially in pepper/tomatoes and corn, followed by *Pseudomonas aeruginosa*, *Enterobacter hormaechei*, and *Enterococcus faecium*.

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Table 2: List of Bacteria of Public Health Interest Isolated from the Milling Machines

Isolates	Makola	Agbogbloshie	Total
Klebsiella pneumoniae	51(20.14%)	86(44.77%)	137(64.92%)
Escherichia coli	12(5.22%)	12(8.21%)	24(14.17%)
Enterobacter cloacae	9(5.97%)	17(9.70%)	26(15.67%)
Enterobacter hormaechei	12(5.97%)	23(14.17%)	35(20.15%)
Acinetobacter baumannii	9(6.71%)	2(0.76%)	11(7.46%)
Serratia marscens	-	4(2.24%)	4(2.24%)
Bordetella hinzi	-	1(0.76%)	1(0.76%)
Pseudomonas aeruginosa	12(7.46%)	32(20.15%)	44(27.61%)
Enterococcus faecium	7(5.22%)	21(14.18%)	28(19.40%)
Enterococcus faecalis	1(0.74%)	4(2.98%)	5(3.73%)
Staphylococcus aureus	-	1(0.74%)	1(0.74%)
Staphylococcus sciuri	4(2.98%)	12(7.46%)	16(10.45%)
Stenotrophomonas maltophilia	10(5.97%)	9(6.72%)	19(12.69)
TOTAL	127	224	351

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Table 3: Distribution of Isolates from Food Milling Machines in Makola and Agbogbloshie, 2024.

Type of Machine	Makola	Agbogbloshie	Total (n)
Pepper/Tomatoes (PT)	139(15.7%)	297(33.5%)	436
Corn (C)	96(10.8%)	142(16.0%)	238
Fufu (FU)	11(1.2%)	9(1.0%)	20
Fish (F)	-	5(0.6%)	5
Groundnut (G)	13(1.5%)	47(5.3%)	60
Millet (M)	17(1.9%)	-	17
Agushie (A)	12(1.4%)	14(1.6%)	26
Kokonte (K)	-	65(7.3%)	65
Palmnut (P)	10(1.1%)	-	10
Herbs (H)	10(1.1)	-	10
Total (N)	308(34.7%)	579(65.3%)	887

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Table 4: Frequency of Bacteria of Public Health Interest Isolated from Food Milling Machines in Makola and Agbogbloshie

Isolate	Pepper/tomatoes	Corn	Kokonte	Groundnut	Agushie	Fufu	Millet	Palmnut	Herbs	Fish	Total
Klebsiella pneumoniae	59 (31.3%)	41(19.4%)	12(6.7%)	8(3.7%)	8(2.2%)	2(0.7%)	3(0.7%)	2(0.7%)	1(0.7%)	1(0.7%)	137
Escherichia coli	11(6%)	10(6%)	0	1(07%)	1(0.7%)	1(0.7%)	0	0	0	0	24
Enterobacter cloacae	5(3%)	6(4.5%)	6(3.7%)	2(1.5%)	2(0.7%)	2(0.7%)	1(0.7%)	1(0.7%)	1(0.7%)	0	26
Enterobacter hormaechei	16(9.7%)	8(5.2%)	3(1.5%)	7(3.7%)	1(0.7%)	0	0	0	0	0	35
Acinetobacter baumannii	5(3.7%)	3(1.5%)	0	1(0.7%)	1(0.7%)	0	1(0.7%)	0	0	0	11
Serratia marscens	3(1.5%)	0	1(0.7%)	0	0	0	0	0	0	0	4
Bordetella hinzi	1(0.7%)	0	0	0	0	0	0	0	0	0	1
Pseudomonas aeruginosa	21(13.4%)	14(7.5%)	1(0.7%)	4(2.2%)	1(0.7%)	2(1.5%)	0	0	0	1(0.7%)	44
Enterococcus faecium	8(4.5%)	10(6.7%)	4(3%)	2(1.5%)	2(1.5%)	0	1(0.7%)	0	0	1(0.7%)	28
Enterococcus faecalis	2(1.5%)	0	2(1.5%)	1(0.7%)	0	0	0	0	0	0	5
Staphylococcus aureus	1(0.7%)	0	0	0	0	0	0	0	0	0	1
Staphylococcus sciuri	8(6%)	5(3.7%)	1(0.7%)	2(1.5%)	0	0	0	0	0	0	16
Stenotrophomonas maltophilia	11(8.2%)	5(3.7%)	1(0.7%)	1(0.7%)	0	0	0	0	0	1(0.7%)	19
Total	151	102	31	29	16	7	6	3	2	4	351

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4.3 Cross Contamination between Milling Machines and Foods.

Table 5 shows a significant (p-value < 0.05) increase in bacterial contamination after milling, with total plate counts, coliforms, and fecal coliforms rising across most samples. Several machines, such as pepper/tomatoes and kokonte milling machines, recorded extremely high bacterial loads post- milling, indicating that the milling process contributes to contamination. Coliforms and fecal coliforms that were absent in some food samples before milling appeared after processing.

Table 6 demonstrates microbial contamination during the milling process by comparing bacteria present in food samples before and after milling with those found in the milling machines. Several bacterial species, such as *Klebsiella pneumoniae, Enterobacter cloacae, and Pseudomonas aeruginosa*, appeared in both milling machines and milled food samples, indicating cross-contamination. In most cases, new bacteria were introduced after milling, suggesting the machines as a primary source of contamination.

Table 7 shows the frequency of bacteria in the inlet, middle, and outlet sections of milling machines, with contamination fairly evenly spread. The results indicate significant microbial contamination throughout all sections of the machines.

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Table 5: Average Microbial Loads in Food Samples Before and After Milling at Makola and Agbogbloshie

Food type	Mean plate count 10^2		Mean coliform count 10^2		Mean fecal count 10^2	
	Before milling	After milling	Before milling	After milling	Before milling	After milling
Pepper	169.0	350.0	118.0	333.3*	24.0	186.7*
Corn	123.0	292.8*	41.2	118.3	11.8	37.0
Kokonte	195.0	350.0*	49.0	275.0*	7.3	143.0
Groundnut	41.3	96.0	0.0	88.0	0.0	2.5

* Significantly higher counts after milling with p - value < 0.05

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Table 6: Cross-Contamination of Food During the Milling Process at Makola and Agbogbloshie

ID	Food sample before milling	Milling machine	Food sample after milling
MC 2	*Enterobacter hormaechei*	*Klebsiella pneumoniae*	*Enterobacter kobei*
	Klebsiella pneumoniae	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
		Acinetobacter baumannii	*Acinetobacter baumannii*
		Enterobacter roggenkampii	*Pseudomonas aeruginosa*
		Enterobacter kobei	
MG 4	*Pseudomonas aeruginosa*	*Acinetobacter variabilis*	*Enterococcus faecium*
	Klebsiella pneumoniae	*Klebsiella pneumoniae*	*Enterobacter cloacae*
	Acinetobacter baumannii	*Lysinibacillus xylanilyticus*	*Acinetobacter variabilis*
		Enterococcus faecium	*Klebsiella pneumoniae*
		Enterobacter cloacae	
MFU 6	*Enterobacter kobei*	*Enterobacter cloacae*	*Limosilactobacillus fermentum*
		Acinetobacter baumannii	*Enterobacter cloacae*
		Pseudomonas otitidis	*Enterobacter kobei*
		Enterobacter kobei	*Pseudomonas otitidis*
		Klebsiella pneumoniae	
		Limosilactobacillus fermentum	
AC 14	*Enterococcus faecium*	*Klebsiella pneumoniae*	*Enterococcus faecium*
	Klebsiella pneumoniae	*Citrobacter sedlakii*	*Enterococcus casseliflavus*
	Cronobacter sp	*Lysinibacillus xylanilyticus*	*Citrobacter sedlakii*
		Enterococcus casseliflavus	*Lysinibacillus xylanilyticus*
		Enterococcus faecium	*Klebsiella pneumoniae*
AK 15	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Enterobacter kobei*
	Weissella paramesenteroides	*Enterobacter roggenkampii*	*Klebsiella pneumoniae*
		Enterobacter kobei	*Enterobacter cloacae*
		Klebsiella pneumoniae	
		Enterobacter cloacae	
AG 16	*Enterococcus casseliflavus*	*Lysinibacillus boronitolerans*	*Staphylococcus capitis*
	Enterococcus faecium	*Pseudomonas stutzeri*	*Enterococcus casseliflavus*
		Klebsiella pneumoniae	
		Enterococcus casseliflavus	
		Acinetobacter variabilis	

MC – Makola Corn, MG Makola Groundnut, MFU – Makola Fufu, AC – Agbogbloshie Corn, AK – Agbogbloshie Kokonte, AG – Agbogbloshie Groundnut

Table 7: Frequency of Bacteria Contaminating the Various Parts of the Food Milling Machines at Makola and Agbogloshie

Isolate	Inlet	Middle	Outlet	Total
Klebsiella pneumoniae	46(34.32%)	48(35.82%)	43(32.09%)	137
Escherichia coli	4(2.98%)	10(7.46%)	10(7.46%)	24
Enterobacter cloacae	5(3.73)	9(6.72%)	12(8.96%)	26
Enterobacter hormaechei	9(6.72%)	10(7.46%)	16(11.94%)	35
Acinetobacter baumannii	4(2.98%)	4(2.98%)	3(2.24%)	11
Serratia marscens	1(0.74%)	2(1.49%)	1(0.74%)	4
Bordetella hinzi	1(0.74%)	0	0	1
Pseudomonas aeruginosa	22(16.42%)	8(5.97%)	13(9.70%)	43
Enterococcus faecium	11(8.21%)	8(5.97%)	9(6.72%)	28
Enterococcus faecalis	1(0.74%)	2(1.49%)	2(1.49%)	5
Staphylococcus aureus	1(0.74%)	0	0	1
Staphylococcus sciuri	8(5.97%)	4(2.98%)	4(2.98%)	16
Stenotrophomonas maltophilia	6(4.48%)	9(6.72%)	5(3.73%)	20
Total	119	114	118	351

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4.4 Antimicrobial Resistance Patterns of Pathogenic Bacteria Isolated from the Milling Machines

Table 8 summarizes the antimicrobial resistance patterns of pathogenic bacterial isolates against different antibiotics. *Acinetobacter baumannii* showed high resistance to chloramphenicol (81.8%) and cefuroxime (81.8%), while *Enterobacter cloacae* exhibited very high resistance to cefoxitin (96.2%) and cefuroxime (57.7%). Similarly, *Enterobacter hormaechei* displayed significant resistance to cefoxitin (97.1%) and meropenem (97.1%). *E. coli* demonstrated substantial resistance to ampicillin (70.8%) and cefuroxime (99.7%), whereas *Klebsiella pneumoniae* had an extremely high resistance rate to ampicillin (98.5%). *Pseudomonas aeruginosa* showed moderate resistance to ceftazidime (18.2%) and gentamicin but minimal resistance to meropenem. *Stenotrophomonas maltophilia* had high resistance to meropenem (88.9%) and moderate resistance to ceftazidime (11.1%) and cefepime (16.7%)

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Table 8: Antimicrobial-Resistant Pattern of Pathogenic Isolates from Milling Machines at Makola and Agbogbloshie

List of Antibiotics	*Acinetobacter baumannii (n = 11)*	*Enterobacter cloacae (n = 26)*	*Enterobacter hormaechei (n = 35)*	*E. coli (n = 24)*	*Klebsiella pneumoniae (n = 137)*	*Pseudomonas aeruginosa (n = 44)*	*Stenotrophomonas maltophilia (n = 18)*
TS/SXT	0	2 (7.7%)	7(20%)	1(4.2%)	1(0.75%)	NA	NA
GM/CN	0	3 (11.5%)	7(20%)	0	1(0.75%)	NA	NA
FOX	NA	25 (96.2%)	34(97.1%)	0	6(4.5%)	NA	NA
MEM	0	6 (23.1%)	34(97.1%)	0	0	0	16(88.9%)
CIP	0	0	7(20%)	2(8.3%)	1(0.75%)	1(2.3%)	0
AMP/AM	NA	NA	0	17(70.8%)	132(98.5%)	NA	NA
C	9(81.8%)	0	0	0	1(0.75%)	NA	NA
AK/AMK/AN	0	3 (11.5%)	0	0	2(1.5%)	NA	NA
LVX	0	0	0	2(8.3%)	0	0	0
CXM	9 (81.8%)	15(57.7%)	10(28.6%)	22(99.7%)	21(15.7%)	NA	NA
TE/T	NA	2(7.7%)	0	5(20.8%)	5(3.7%)	NA	NA
AMC	NA	13(50%)	3(8.6%)	0	9(6.7%)	NA	NA
CRO	4 (36.4%)	0	0	0	0	NA	NA
FEP	0	3(11.5%)	0	0	0	0	3(16.7%)
CAZ	0	NA	0	0	0	8(18.2%)	2(11.1%)

AK/AMK/AN - Amikacin, AMP/AM – Ampicillin, AMC - Amoxicillin Clavulanic acid, C – Chloramphenicol, CAZ – Ceftazidime, CIP – Ciprofloxacin, CRO – Ceftriaxone, E – Erythromycin, FEP – Cefepime, FOX – Cefoxitin, GM/CN – Gentamicin, LVX – Levofloxacin, MEM – Meropenem, P – Penicillin, TE/T – Tetracycline, TEI – Teicoplanin, TS/SXT – Cotrimoxazole, VA – Vancomycin, NA – Not applicable.

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CHAPTER FIVE

5.0 DISCUSSION

5.1 Demographics of Milling Machine Operators and Their Sanitary Practices

The age distribution revealed that the majority of the participants, were primarily composed of middle-aged adults (31-65 years old).

In terms of compliance with regulations, 95.7% of participants operated with a license, indicating a generally high level of regulatory adherence. However, the maintenance frequency of mills was concerning, as 77.2% only serviced their machines when they broke down. This suggests a potential neglect of routine upkeep that could compromise safety and hygiene.

None of the operators washed the machine compartments with soap. Additionally, only 15.2% were aware of microbial contamination risks in mills, indicating a significant knowledge gap that could compromise food safety. These findings correspond with patterns observed in the study by Yar *et al*. (2023), however, unlike Yar *et al*.'s study, these results include data from licensed milling operators and provide a broader perspective on the subject. Dzah (2015) and Alum *et al*. (2016) reported similarly but attributed their findings to a lack of formal education and training. Handwashing practices were similarly inadequate, with 70.7% of participants not washing their hands with soap before milling operations.

Despite the lack of hand hygiene practices, all participants reported hand contact with food during milling, highlighting a critical area of concern for microbial contamination. Furthermore, only 15.2% wore hair covers while milling, and none used gloves, which significantly increases the risk of contamination.

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The sanitation of the operation site also varied, with 48.9% rated as satisfactory, while a considerable 43.5% were deemed unsanitary. This inconsistency in perceived sanitation could correlate with the high levels of microbial contamination risks observed.

5.2 The Microbial Contamination of Food Milling Machines

An objective of the study was to determine the microbial contamination and spectrum of bacteria contaminating food milling machines across Makola and Agbogbloshie. Notable disparities were revealed in both the number and types of machines, as well as the associated bacterial isolates. These disparities were also observed in a study by Yar *et al*. (2023) in Kumasi.

In this study, a total of 134 machines were sampled, with a striking concentration in Agbogbloshie (102 machines) compared to Makola (32 machines). Although both locations are urban markets, the higher number of machines in Agbogbloshie may indicate a greater scale of food processing activity, possibly influenced by market size or demand. This aligns with findings by Amoah *et al*. (2024), which highlight that, intra-urban variations can significantly affect the distribution of food processing facilities and their impact on local food systems.

The distribution of bacterial isolates across these machines was also notable, with a total of 887 isolates identified. Agbogbloshie contributed 65.3% of these isolates, indicating a higher microbial load associated with food milling processes in that area. This finding is unsurprising, given that over 90% of the operation sites, especially in the Agbogbloshie market, exhibited poor sanitation conditions. The highest number of isolates came from pepper/tomato milling machines (436 in total), corroborating findings by Karanth et al. (2023) that certain food processing environments are more conducive to bacterial proliferation.

The study identified 13 different species of bacteria of public health importance, highlighting the diversity of microbial communities in both locations, with *Klebsiella pneumoniae* being the most

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frequently isolated pathogen (64.92%). This was followed by *Pseudomonas aeruginosa* and other pathogens (44 isolates, 27.61%) including various *Enterobacter species*. In contrast, other studies in Ghana and Nigeria have reported *Salmonella spp*. (24%) and *S. aureus* (24%) as the commonest bacteria isolated, and others like *Klebsiella pneumoniae* (7.32% - 8%) (Yar *et al*., 2023; Azuonwu *et al*., 2019). These studies may differ in their reporting of predominant bacteria due to geographical location, environmental factors, pH levels, among other factors, which support the growth of particular bacterial species. In addition to all the bacteria which were common to both Makola and Agbogbloshie, *Bordetella hinzi, Serratia marcescens*, and *S. aureus* were found only in Agbogbloshie. The predominance of pathogens, particularly in Agbogbloshie, emphasizes the need for strict sanitation practices in food processing and adherence to recommendations from the World Health Organization (2020) regarding food safety measures.

Overall, a complex interaction between food processing machinery, microbial populations, and location was identified. Agbogbloshie's higher concentration of machines and bacterial isolates suggests a need for targeted interventions to improve food safety, as emphasized by previous studies on the microbiological quality of street food and local processing environments (Salifu *et al*., 2023).

5.3 Cross Contamination between Milling Machines and Foods.

The microbial load observed in this study (10^2 CFU/ml) was lower than the acceptable limit set by the Ghana Standards Authority (10^5 CFU/ml and above). However, it remains important to discuss these findings, as even relatively low levels of contamination can pose potential health risks, particularly to vulnerable or immunocompromised individuals within the population.

The increase in microbial loads, specifically total plate counts, coliform counts, and fecal coliform counts across all food types after milling, suggests that the milling process contributes significantly to microbial contamination in food products. Pepper samples recorded the highest

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microbial loads post-milling. The steep rise, particularly in fecal coliforms (from 24 to 186.7 × 10^2 CFU/ml) before and after milling, indicates potential exposure to unhygienic conditions and possible fecal contamination during processing. This trend is consistent with findings by Yar *et al.* (2023), who noted that spices and vegetables are highly prone to contamination when handled or processed under poor sanitary conditions.

Corn samples also showed substantial increases, with plate counts rising from 123 to 292.8 × 10^2 CFU/ml, and fecal coliforms from 11.8 to 37 × 10^2 CFU/ml. These values, though lower than those of pepper, remain significant. The relatively high microbial load in post-milled corn may be attributed to the popularity of corn-based foods, and the frequent use of shared milling equipment that may not be adequately cleaned between uses (CRA, 2018).

Kokonte, a dried cassava-based food, exhibited high microbial loads after milling, with fecal coliform counts increasing from 7.3 to 143 × 10^2 CFU/ml, a more than 19-fold increase. Given that kokonte is often sun-dried and processed in open-air environments before milling, it is exposed to microbes. The milling process likely intensifies the contamination by introducing additional contaminants through contact surfaces, as supported by findings from Massamby *et al*., (2025), which emphasized the role of post-harvest handling and processing equipment in increasing microbial risks in traditional African foods.

Interestingly, groundnut samples exhibited the lowest microbial loads both before and after milling. That notwithstanding, there was a noticeable post-milling increase in coliform counts from 0.0 to 88 × 10^2 CFU/ml, and fecal coliform counts from 0.0 to 2.5 × 10^2 CFU/ml. This suggests that although groundnuts may begin as relatively clean, contamination is introduced during the milling process (Nkansah *et al*., 2021). This finding is in line with Delhalle *et al*. (2020) study, which reported that contact surfaces and inadequate cleaning routines in informal food processing units are critical contributors to microbial transfer.

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The consistent increase in all microbial parameters after milling, across all food types, strongly points to milling equipment as a major source of contamination. Previous studies have shown that milling machines, particularly in open markets and informal settings, are rarely washed with soap or disinfected, creating ideal conditions for bacterial growth and transfer (Azuonwu *et al*., 2019). This poses serious food safety risks, especially since many of the foods assessed are consumed without further cooking. The presence of fecal coliforms in post-milled products is particularly concerning, as these bacteria are indicators of contamination with human or animal excretal waste and pose a high risk of gastrointestinal infections if consumed.

A comparison of bacteria found in food samples before and after milling, along with those isolated from milling machines, clearly shows that several organisms detected in the post-milling samples were absent in the pre-milling stage but were present on the machines themselves. This pattern strongly indicates that the milling equipment acts as a reservoir for microbial contamination and potentially transfers microbes to food during processing.

In several cases, post-milling samples contained bacterial species not originally present in the food but found on the milling equipment. For example, in sample MC 2, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated from the machine and later detected in the food sample after milling, though they were absent before milling. Similarly, in MG 4, *Acinetobacter variabilis* and *Enterobacter cloacae* appeared only after milling and were previously isolated from the machine. These findings are consistent with the observations by Delhalle *et al*. (2020), who reported that food contact surfaces in milling operations, when inadequately cleaned, become a major source of microbial transfer to foods.

The detection of less common species such as *Lysinibacillus xylanilyticus*, *Enterobacter roggenkampii*, and *Citrobacter sedlakii* also highlights the diversity of microbial communities harbored within milling environments. These organisms, while not always primary pathogens,

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indicate poor sanitation and serve as markers for environmental and fecal contamination (Berendes *et al.*, 2018). Notably, in sample AC 14, *Lysinibacillus xylanilyticus* and *Citrobacter sedlakii* were found in both the machine and milled product but were absent before milling, further substantiating the role of the equipment in contamination.

Another important observation is the detection of probiotic or commensal organisms such as *Weissella paramesenteroides* and *Limosilactobacillus fermentum* in some pre- and post-milling samples. While these organisms are generally considered non-pathogenic or beneficial, their presence alongside pathogenic or opportunistic species reflects the complex microbial interactions that occur during food processing and indicates the importance of targeted hygiene interventions across all stages of milling (Salifu *et al*., 2023).

The near-equal distribution of bacterial contamination across the three machine parts assessed (119 in the inlet, 114 in the middle, and 118 in the outlet) shows that microbial contamination is not confined to specific machine parts but rather the whole milling machine.

Klebsiella pneumoniae emerged as the most dominant organism with 134 isolates, evenly distributed across the inlet (46), middle (48), and outlet (43). This organism is a well-known opportunistic pathogen associated with both hospital-acquired and community-acquired infections and has been linked to foodborne transmission in developing countries (Crippa *et al*., 2023). Its high prevalence in all parts of the milling machine strongly implicates the equipment as a consistent source of contamination. Moreover, *K. pneumoniae* has shown a strong ability to form biofilms, which can adhere to machine surfaces and resist routine cleaning, making it particularly difficult to eliminate without proper sanitation protocols (Crippa *et al*., 2023).

Pseudomonas aeruginosa, the second most prevalent isolate (43), was most frequently found at the inlet (22 isolates), which may indicate its introduction through initial contact with raw food material. However, its presence at the outlet (13) also suggests its persistence throughout the

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milling machines. This organism is known for its resilience in moist environments and its resistance to disinfectants; this is a critical concern in food processing (Tutun *et al*., 2023; Bai *et al*., 2021). The ability of *P. aeruginosa* to survive across all machine parts also aligns with its previously documented role in post-harvest contamination (Mohanapriya *et al*., 2024).

Other notable organisms included *Enterobacter hormaechei* (35 isolates), *Enterococcus faecium* (28), *Enterobacter cloacae* (26), and *Escherichia coli* (24). These bacterial species are common indicators of fecal or environmental contamination and have been associated with foodborne outbreaks in numerous studies. The presence of *E. coli*, especially in the outlet and middle parts of milling machines, is particularly concerning, as it points to the final stage of processing being exposed to harmful pathogens that may end up in the consumer's food. Previous research by Yar *et al*. (2023) emphasized that, microbial contamination at the outlet point of food milling equipment is one of the most direct threats to food safety because this is the point where the final product exits and is most vulnerable to contamination.

Interestingly, contamination levels were significantly higher in the inlet section compared to the middle and outlet, suggesting that cleaning and sanitizing are inadequate at all contact points. The near-uniform presence of isolates in each part of the machines reflects not only poor hygiene practices but also plausible cross-contamination within the system, where bacteria from one part of the machine can spread to other parts in continuous operation.

Additionally, the detection of less common or typically non-pathogenic organisms like *Staphylococcus sciuri*, *Stenotrophomonas maltophilia*, and *Bordetella hinzi* further illustrates the diverse microbial ecology that can develop in poorly maintained food machinery. While not all such organisms are primary pathogens, their presence in food processing environments can serve

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as indicators of general unhygienic conditions and the potential for harboring and transferring pathogenic strains (Berendes *et al.,* 2018).

5.4 Antimicrobial Resistance Patterns of Pathogenic Bacteria

A significant antimicrobial resistance pattern was recorded across the various pathogenic isolates. Notably, *Enterobacter hormaechei and Klebsiella pneumoniae* exhibited varying resistance rates to multiple antibiotics, particularly cefoxitin (FOX), with resistance rates of 97.1% and 4.5%, respectively. This finding aligns with existing literature that emphasizes the growing challenge of treating infections caused by these pathogens, often linked to extended-spectrum beta-lactamase (ESBL) production (Pitout & Laupland, 2008).

Acinetobacter baumannii showed remarkably low resistance to ceftriaxone (CRO), with 36.4% of isolates demonstrating resistance. This is inconsistent with studies that identified *Acinetobacter* as a notorious pathogen known for its multidrug resistance, complicating treatment options in healthcare settings (WHO, 2017; Karalewitz *et al*., 2018).

The resistance observed in *Pseudomonas aeruginosa* is particularly concerning, especially regarding ceftazidime (CAZ) (Hirsch & Tam, 2010). The resistance noted in *Stenotrophomonas maltophilia* (88.9%) indicates an alarming trend towards carbapenem resistance, which has been reported in several studies as a growing threat to effective treatment (Rhoads, 2021).

Regarding cephalosporins, cefuroxime (CXM) exhibited resistance among various Enterobacteriaceae, especially with a striking 99.7% in *E. coli*. This suggests an urgent need for surveillance on the use of cephalosporins, which have increasingly faced resistance challenges, as documented in recent literature (Handa *et al*., 2024).

In contrast, resistance rates for gentamicin and ciprofloxacin appear relatively lower. For ciprofloxacin (CIP), the majority of strains remain susceptible, indicating a retained effectiveness of fluoroquinolones against certain isolates. However, emerging resistance in other studies

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suggests that caution is warranted when prescribing these agents (Mintz *et al*., 2023).

In total, 11.4% (40/351) of the pathogenic isolates were multidrug resistant (MDR); *E. cloacae and hormaechei, E. coli,* and *Klebsiella pneumoniae*. A proportion of 61.5% (16/26) of *E. cloacae* were resistant to cephalosporins, tetracycline, carbapenem, sulfonamides, and aminoglycosides, which aligns with the trends reported by Elbehiry *et al*., 2024. *E. hormaechei* showed a 28.6% (10/35) resistance rate to fluoroquinolones, cephalosporins, carbapenems, sulfonamides, and aminoglycosides, of which Liu *et al*. (2022) reported similar patterns, closely matching these findings. *E. coli* exhibited a 20.8% (5/24) resistance rate to fluoroquinolones, cephalosporins, sulfonamides, and tetracycline, with similar resistance patterns reported by Poirel *et al.* (2018), supporting these findings. *K. pneumoniae*: 4.4% (6/137) was resistant to Chloramphenicol, cephalosporins, tetracycline, sulfonamides, aminoglycosides, and fluoroquinolones, which Setiawan *et al*., 2022 and Li *et al*., 2024 also confirmed a relatable pattern of results.

Notably, the study did not isolate common foodborne pathogens such as extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* and other Gram-negative bacteria from the food milling machines. The absence of ESBL-producing bacteria, which are typically associated with healthcare settings rather than foodborne transmission, suggests a reduced risk of antibiotic-resistant infections. This finding has positive implications for food safety, as it indicates a lower likelihood of contamination by pathogens that pose severe health risks, particularly for immunocompromised individuals.

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CHAPTER SIX

6.0 CONCLUSIONS, LIMITATIONS, AND RECOMMENDATIONS

6.1 Conclusion

A diverse range of bacteria was identified in milling machines in Accra, with *Klebsiella pneumoniae* 15.4% being the most prevalent species in both study sites. Key foodborne pathogens, such as *Salmonella,* and *Shigella* were absent, indicating that the milling machines pose no threat from these pathogens. However, compared to pre-milled foods, milled food samples had a higher number of bacterial contaminations. The antimicrobial resistance patterns among various bacterial isolates reveal a concerning prevalence of multidrug resistance (MDR) in *E. cloacae* (61.5%), *E. hormaechei* (28.57%), *E. coli* (20.8%), and *Klebsiella pneumoniae* (4.4%), which further contributes to the growing concern of MDR. The study found inadequate management practices and insufficient awareness among operators regarding microbial contamination in milling machines.

6.2 Limitations

This study could not examine seasonal influences on microbial contamination. Also, the water used and the palms of the operators were not sampled. Anaerobic incubation was not done to detect microbes that require such conditions. Molecular analysis on bacterial isolates found in both the milling machines and milled food samples was not carried out hence, the study could not confirm cross-contamination between the milling machines and the milled food samples. These limitations are attributed to time constraints.

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6.3 Recommendation

Based on the outcomes in this study, it is recommended that:

- Regular and thorough inspections of milling machines should be conducted to minimize contamination and prevent foodborne outbreaks.
- Increase awareness to ensure the adoption of proper hygiene practices in the milling industry.
- Enforce strict food safety regulations to enhance contamination control.
- Implement food safety education programs for food handlers and consumers to promote safe practices.

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Appendix 1

Questionnaire

a. Demographic and geographical data	
Id: ……	
1. Sex	□ M □ F
2. Age	___/___/____ □ Unknown: Age: …… (year)
3. Level of education	□ *Primary* □ *Junior High School* □ *Senior High School* □ *University* □ *Uneducated*
4. Residence (Location)	……..
b. General information	
1. Number of milling machines	…….
2. Operates mills with license	□ *Yes* □ *No*
3. Frequency of maintaining mills	□ *Once a week* □ *Once a month* □ *When it breaks down* *Other specify*: ……
4. Do you wash machine compartment with soap?	□ *Yes* □ *No* *If yes, what is used?* ……

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5. Have you had any of these signs or symptoms?	□ *Pain behind the eyes* □ *Vomiting/ nausea* □ *Swollen glands* □ *Rash* *If other, specify:* …….
6. Aware of microbial contamination in mills	□ *Yes* □ *No*
7. Frequency of hand washing in a day	□ *Once* □ *Twice* □ *Often*
8. Wash hands with soap before operation	□ *Yes* □ *No*
9. Wear hand gloves while milling	□ *Yes* □ *No*
10. Wear hair covers while milling	□ *Yes* □ *No*
11. Is there hand contact with food while milling	□ *Yes* □ *No*
12. Sanitation of operation site	□ *Bad* □ *Satisfactory* □ *Good*

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Appendix 2

Table 9: List of Bacteria not of Public Health Interest Isolated from the Milling Machines

Isolates	Makola	Agbogbloshie	Total
Klebsiella sp. (aerogenes, variicola, oxytoca)	11(2.1%)	18(3.4%)	29
Pseudomonas sp. (balearica, guariconensis, mendocina, otitidis, stutzeri, monteii)	14(2.6%)	22(4.1%)	36
Enterococcus sp. (avium, casseliflavus, gallinarum, hirae)	26(4.9%)	30(5.6%)	56
Enterobacter sp. (kobei, bugandensis, ludwigii, roggenkampii)	7(1.3%)	19(3.5%)	26
Bacillus sp. (cereus, pumilus, subtilis,velezensis)	13(2.4%)	20(3.7%)	33
Ochrobactrum sp. (intermedium, tritici)	15(2.8%)	51(9.5%)	66
Acinetobacter sp. (haemolyticus, indicus, parvus, radioresistens, variabilis)	3(0.6%)	5(1%)	8
Staphylococcus sp. (hominis, epidermidis, gallinarum, haemolyticus, warneri, xylosus, kloosii)	13(2.4%)	17(3.2%)	30
Comamonas sp. (aquatica, kerstersii)	9(1.7%)	15(2.8%)	24
Kerstersia gyiorum	3(0.6%)	15(2.8%)	18
Providencia sp. (alcalifaciens, rettgeri, stuartii, vermicola)	6(1.1%)	19(3.5%)	36
Cronobacter sp.	9(1.7%)	4(0.7%)	13
Micrococcus lutes	2(0.2%)	1(0.2%)	3
Achromobacter sp. (denitrificans, insolitus, mucicolens, spanius, xylosoxidans)	5(1%)	8(1.5%)	13
Alcaligenes faecalis	2(0.4%)	9(1.7%)	11
Bordetella trematum	-	7(1.3%)	7
Brevibacillus laterosporus	1(0.2%)	-	1

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Isolates	Makola	Agbogbloshie	Total
Brevundimonas diminuta	-	1(0.2%)	1
Citrobacter sp. (amalonaticus, braakii, farmeri, freundii, sedlakii)	2(0.4%)	6(1.1%)	8
Corynebacterium provencense	1(0.2%)	-	1
Delftia acidovorans		1(0.2%)	1
Escherichia hermannii	2(0.4%)	1(0.2%)	3
Franconibacter pulveris	1(0.2%)		1
Glutamicibacter creatinolyticus		1(0.2%)	1
Heydrickxia oleronia		1(0.2%)	1
Kluyrera georgiana		1(0.2%)	1
Kosakonia radicincitans	2(0.4%)	1(0.2%)	3
Kurthia gibsonii	1(0.2%)		1
Lysinibacillus sp. (boronitolerans, fusiformis, pakistanensis, sphaericus, xylanilyticus)	21(3.9%)	47(8.8%)	68
Mixta calida	1(0.2%)		1
Morganella morganii	2(0.4%)		2
Paenibacillis illinoisensis	1(0.2%)		1
Paenochrobactrum pullorum		1(0.2%)	1
Pandoraea nosoerga	1(0.2%)		1
Paracandidimonas soli		1(0.2%)	1
Proteus sp. (hauseri, mirabilis, vulgaris)	7(1.3%)	30(5.6%)	37
Stenotrophomonas acidaminiphila		2(0.4%)	2

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Total	183(34.1%)	353(65.9%)	536

Appendix 3

Table 10: Machine Type, Cross-Contamination and Microbial Counts

ID	Food sample before milling	Milling machine	Food sample after milling	Plate count		Coliform count		Fecal coliform count	
				Before milling	After milling	Before milling	After milling	Before milling	After milling
MPT 1	*Bacillus badius*	*Klebsiella pneumoniae* *Klebsiella variicola* *Escherichia coli* *Enterobacter hormaechei* *Ochrobactrum intermedium* *Enterococcus faecium* *Stenotrophomonas acidaminiphila* *Enterococcus faecium* *Enterobacter kobei* *Escherichia coli*	*Klebsiella pneumoniae* *Escherichia coli* *Enterococcus faecium* *Ochrobactrum intermedium*	350	350	300	350	1	100
MC 2	*Enterobacter hormaechei*	*Klebsiella pneumoniae*	*Enterobacter kobei*	218	350	150	210	50	70

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	Klebsiella pneumoniae	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*						
		Enterobacter roggenkampii	*Acinetobacter baumannii*						
		Acinetobacter baumannii	*Pseudomonas aeruginosa*						
		Enterobacter kobei							
MG 3	*Lysinibacillus xylanilyticus*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	150	350	0	350	0	8
	Klebsiella variicola	*Pseudomonas stutzeri*	*Pseudomonas stutzeri*						
		Acinetobacter baumannii	*Kurthia gibsonii*						
		Kurthia gibsonii	*Enterobacter cloacae*						
		Enterobacter cloacae							
MG 4	*Pseudomonas aeruginosa*	*Acinetobacter variabilis*	*Enterococcus faecium*	6	23	0	0	0	0
	Klebsiella pneumoniae	*Klebsiella pneumoniae*	*Enterobacter cloacae*						
	Acinetobacter baumannii	*Lysinibacillus xylanilyticus*	*Acinetobacter variabilis*						
		Klebsiella pneumoniae	*Klebsiella pneumoniae*						
		Enterococcus faecium							
		Enterobacter cloacae							
		Klebsiella pneumoniae							

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MK 5	*Citrobacter freundii*	*Escherichia coli*	*Pseudomonas aeruginosa*	145	350	66	350	10	350
	Klebsiella variicola	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*						
		Achromobacter xylosoxidans							
		Acinetobacter baumannii							
		Kurthia gibsonii							
		Klebsiella pneumoniae							
MF 6	*Enterobacter kobei*	*Enterobacter cloacae*	*Limosilactobacillus fermentum*	210	350	80	350	15	350
		Acinetobacter baumannii	*Enterobacter cloacae*						
		Pseudomonas otitidis	*Enterobacter kobei*						
		Enterobacter kobei	*Pseudomonas otitidis*						
		Klebsiella pneumoniae							
		Limosilactobacillus fermentum							
MP 7	*Bacillus subtilis*	*Acinetobacter baumannii*	*Klebsiella pneumoniae*	100	350	30	350	15	350
	Klebsiella variicola	*Klebsiella pneumoniae*	*Acinetobacter baumannii*						

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	Klebsiella pneumoniae	*Lysinibacillus boronitolerans*							
		Klebsiella variicola							
MPT 8	*Escherichia coli*	*Escherichia coli*	*Enterococcus casseliflavus*	212	350	100	350	45	100
	Acinetobacter baumannii	*Bordetella trematu*	*Escherichia coli*						
	Bordetella trematum	*Providencia stuartii*	*Bordetella trematu*						
		Kerstersia gyiorum							
		Bordetella trematum							
MC 9	*Providencia stuartii*	*Klebsiella aerogenes*	*Pseudomonas aeruginosa*	120	350	7	150	1	70
	Pseudomonas aeruginosa	*Klebsiella pneumoniae*	*Klebsiella aerogenes*						
	Citrobacter amalonaticus	*Citrobacter amalonaticus*	*Enterococcus faecium*						
		Enterococcus faecium							
		Pseudomonas aeruginosa							
MPT 10	*Bordetella trematum*	*Enterococcus casseliflavus*	*Escherichia coli*	250	9	39	0	14	0
	Escherichia coli	*Escherichia coli*	*Pseudomonas aeruginosa*						
		Pseudomonas aeruginosa	*Enterococcus casseliflavus*						

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		Enterococcus faecium							
AA 11	*Bacillus badius*	*Klebsiella pneumoniae*	*Enterobacter roggenkampii*	30	209	9	45	4	20
	Kerstersia gyiorum	*Enterobacter roggenkampii*	*Kerstersia gyiorum*						
	Enterobacter roggenkampii	*Kerstersia gyiorum*	*Enterobacter kobei*						
		Enterobacter kobei							
		Acinetobacter baumannii							
		Bacillus badius							
		Enterobacter kobei							
AC 12	*Achromobacter Xylosoxidans*	*Enterobacter cloacae*	*Enterobacter kobei*	30	350	0	0	0	0
	Enterobacter cloacae	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*						
	Klebsiella variicola	*Enterobacter kobei*	*Achromobacter xylosoxidans*						
		Stenotrophomonas maltophilia							
		Citrobacter amalonaticus							
APT 13	*Escherichia coli*	*Alcaligenes faecalis*	*Klebsiella pneumoniae*	100	350	60	250	20	90

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	Pseudomonas mendocina	*Klebsiella pneumoniae*	*Enterobacter hormaechei*						
	Enterobacter hormaechei	*Escherichia coli*							
		Lysinibacillus boronitolerans							
		Achromobacter xylosoxidans							
		Stenotrophomonas maltophilia							
		Escherichia hermannii							
AC 14	*Enterococcus faecium*	*Klebsiella pneumoniae*	*Enterococcus faecium*	250	350	0	0	0	0
	Klebsiella pneumoniae	*Citrobacter sedlakii*	*Enterococcus casseliflavus*						
	Cronobacter sp	*Lysinibacillus xylanilyticus*	*Citrobacter sedlakii*						
		Enterococcus casseliflavus	*Lysinibacillus xylanilyticus*						
		Citrobacter sedlakii	*Klebsiella pneumoniae*						
		Enterococcus faecium							
AK 15	*Acinetobacter baumannii*	*Acinetobacter baumannii*	*Enterobacter kobei*	215	350	50	350	4	200
	Weissella paramesenteroides	*Enterobacter roggenkampii*	*Klebsiella pneumoniae*						

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		Enterobacter kobei	*Enterobacter cloacae*						
		Klebsiella pneumoniae							
		Enterobacter cloacae							
AG 16	*Enterococcus casseliflavus*	*Lysinibacillus boronitolerans*	*Staphylococcus capitis*	10	13	0	2	0	2
	Enterococcus faecium	*Pseudomonas stutzeri*	*Enterococcus casseliflavus*						
		Klebsiella pneumoniae							
		Enterococcus casseliflavus							
		Acinetobacter variabilis							
APT 17	*Lysinibacillus Xylanilyticus*	*Klebsiella pneumoniae*	*Citrobacter sedlakii*	120	350	90	350	33	350
	Ochrobactrum tritici	*Lysinibacillus xylanilyticus*	*Acinetobacter baumannii*						
	Lysinibacillus xylanilyticus	*Ochrobactrum tritici*	*Ochrobactrum tritici*						
	Ochrobactrum tritici	*Citrobacter sedlakii*							
		Acinetobacter baumannii							
		Pseudomonas stutzeri							

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AA 18	*Enterobacter roggenkampii*	*Stenotrophomonas maltophilia*	*Klebsiella pneumoniae*	55	250	6	35	4	30
	Klebsiella pneumoniae	*Klebsiella pneumoniae*	*Enterobacter hormaechei*						
		Enterobacter hormaechei	*Acinetobacter baumannii*						
		Acinetobacter baumannii							
		Acinetobacter variabilis							
		Escherichia coli							
AG 19	*Klebsiella variicola*	*Acinetobacter variabilis*	*Staphylococcus capitis*	1	20	0	0	0	0
	Acinetobacter baumannii	*Klebsiella pneumoniae*	*Enterococcus casseliflavus*						
		Enterobacter cloacae	*Acinetobacter variabilis*						
		Lysinibacillus xylanilyticus							
		Enterococcus faecium							
AK 20	*Weissella paramesenteroides*	*Escherichia coli*	*Enterobacter kobei*	270	350	30	50	6	0
	Citrobacter freundii	*Pseudomonas aeruginosa*	*Enterobacter cloacae*						
		Enterobacter roggenkampii	*Pseudomonas aeruginosa*						
		Acinetobacter baumannii	*Klebsiella pneumoniae*						

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Kurthia gibsonii

Klebsiella pneumoniae