ANTIBIOTIC RESISTANCE PATTERN AND PHENOTYPIC VIRULENCE CHARACTERISTICS OF BACTERIA ISOLATED FROM STREET VENDED FOOD

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NOVEMBER, 2025

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A PROJECT REPORT WRITTEN AND SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (B.Sc) IN MICROBIOLOGY.

NOVEMBER 2025

CERTIFICATION

This is to certify that this research work was carried out by GODFREY Grace Udhedhe with matriculation number LSC2103943 under the supervision of Prof. S.E Omonigho in the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin city.

DEDICATION

This thesis is dedicated to the love almighty the author and the finisher of this phase and to my mum, whose unending support, love, words of encouragement also to my dad for his love and advice.

ACKNOWLEDGMENT

I could possibly not find the right words to express how grateful I am to the lord almighty for giving me the grace to successfully undertake and finish this project.

Firstly, I would like to thank my supervisor Prof. S.E Omonigho for his guidance, support and understanding towards me and my colleagues for ensuring we all have everything necessary for the completion of this project. I would also like to thank Dr. A.A Imoni (Mrs) for her love, care, patience, support and well corrective method during this project, her assistance really proved to me that she really want the best for me and my colleagues. To all my lecturers, thank you for the invaluable knowledge you have inculcated in me, .God bless you all.

To my parents, Mr and Mrs Godfrey Okoro thank you for your unending prayers, love, financial support, moral support and constant advice, God bless you in his safe hands. To my siblings Okoro Priscilla Oyome, Okoro Victory Oghogho and Okoro Favour Eloho for always looking out for me, financial support, love, care, gifts, I love you all.

To my friends and project colleagues, Alamele Romeo, Nuatin Favour, Nwaeze Emmanuella, Osamede covenant, Imoobe Bright, Omogbeja Toluwanimi, Enahoro Esther, Ojezuanbi Gift, Aliu Tabitha, Osasogie, Praise, Grace, Tracy, Mary, Martha, Annastasia, Jessica, Freda, Jennifer,Omoye, Oki, Orevaoghene, Vivan and Adedokun Toluwalope, I say thank you for all your unrelentless contributions, ideas, support and making the struggles as a student a little less difficult.

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CHAPTER ONE

INTRODUCTION

Street vended foods are significant reservoirs for enteric pathogens, posing a substantial public health risk due to their association with poor hygiene and the potential to transmit antimicrobial resistance (AMR). Studies on street foods in regions like Nigeria reveal high levels of multidrugs-resistant (MDR) bacteria, *Escherichia coli , Klebsiella* spp*.,* and *Staphylococcus* spp., which are linked to foodborne illnesses. Investigating their resistance patterns and virulence characteristics is crucial for developing effective food safety measures, protecting consumer health, and combating the global rise in antibiotic resistance infections (Sabuj *et al*., 2018).

Street food refers to a wide variety of foods and beverages that are usually prepared or sold by vendors in street o any public places for direct human ingestion immediately or later. These foods are widely consumed in many-low and middle-income countries due to their low cost, rich taste, variety, and easy accessibility. Popular street food in Nigeria include jollof rice, spaghetti, beans, rice and stew and various fast food items such as plantain bole and sandwiches (Biswas *et al*., 2010., Rahman *et al*., 2017).

Despite their popularity, street foods are increasingly associated with public health risks. Poor hygiene practices, limited awareness among vendors, and inadequate food safety education contribute to the contamination of these foods with various enteric pathogens (Birgen *et al*., 2020).these pathogens includes *Escherichia coli , Salmonella enterica***,** *Shigella* spp*., Listeria monocytogenes,* and *Staphylococcus aureus***,** which can cause serious food-borne illnesses.

Among these, *E.coli* is the most commonly reported and studied due to widespread occurrence and role as a hygiene indicator. It belongs to the Enterobacteriaceae family and is a facultative

anaerobic bacterium frequently found in contaminated food and water. Its presence in food suggests possible fecal contamination and an increased risk of other pathogenic organisms (Frisca *et al*., 2007; Sabuj *et al*., 2018). Molecular markers like *usp*A (encoding universal stress protein) and *uid*A (encoding beta-glucuronidase enzyme) are used for it confirmation through culture and PCR-based methods (Godambe *et al*., 2017).

Additionally, antimicrobial resistance (AMR) is becoming a significant challenge, as many *E. coli*_strains isolated from street foods have show resistance to commonly used antibiotics. This limit empirical treatment option and increases the burden of disease (Sabuj *et al*., 2018).the spread of resistance bacteria through contaminated food poses a serious risk to both consumers and food handlers, particularly in settings with limited healthcare resources.

Food-borne diseases now represent acritical global health concern, accounting of an estimated 2 million death annually in underdeveloped countries (WHO).therefore, ensuring the microbial safety of street food, particularly through the monitoring of *E. coli*_and other enteric pathogens, is essential to controlling foodborne outbreaks and reducing public health risks.

The pathogenic mechanisms of *E. coli*_include the ability to adhere to host cells and invade mucosal surfaces. For example, virulence genes such as *hyl*A, which encodes hemolysin A, are capable of creating pores in host cell membranes, leading to cell lysis and tissue damage (Sarowska *et al*., 2019). Other virulence traits include adhesion factors that facilitate binding to the mucosal receptors in the small intestine, contributing to diarrheal diseases and enteric infections (Mousa and Shama, 2020).

Several types of pathogens can cause food-borne illnesses, including viruses, bacteria, fungi and parasites (Bintsis, 2017). The majority of pathogens transmitted through food are bacteria, which

cause a wide array of illnesses in both humans and animals. Among the most common bacteria found in food are *Salmonella* spp*., Shigella* spp*., Bacillus* spp*., Vibrio cholerae* (Elbehiry *et al*., 2019). Several stages can lead to food contamination, including the environments in which the animals are raised and vegetables are cultivated and harvested. Contamination can also occur during the manufacturing process, such as shipment, processing, or handling, additionally, cross-contamination can occur due to the people who prepare or consume food (Kamala *et al*., 2018).

Food-borne infections caused by these resistant microorganisms have serious public health implications. The bacteria involved can express extended-spectrum beta-lactamases (ESBLs), enzymes that make them resistant to abroad range of antibiotics. This can lead to unsatisfactory treatment outcomes and increased risk of complications (Novovic *et al* 2015).

Preventive measures especially proper food handling and responsible antibiotics use are critical to reducing the risk of such infections. Meat, while a valuable source of nutrients like protein, B vitamins, phosphorus, and zinc can also be a vehicle for transmitting resistant bacteria if not handled or cooked properly (Obeid *et al* 2019).

Research has shown that resistance genes can be transferred among microorganisms both inside and outside of hosts. As a result, bacteria that are resistant to multiple antibiotics can enter the food chain, increasing the risk of foodborne illnesses that are difficult to treat these concerns are heightened by the growing consumption of ready-to-eat foods, which may be produced under poor hygiene conditions by individuals who can unknowingly serve as carriers of resistant bacteria (El-sayed *et al* 2019).

The presence of multidrug-resistant microorganisms in food products is closely linked to the spread of antibiotic resistance genes, particularly those responsible for extended-spectrum

resistance. This growing resistance to antibiotics is a recognized global health issue. In both animal husbandry and healthcare sectors, improper use of antimicrobials has contributed to the spread of resistant bacteria, making treatment of infections more difficult and less effective (Bastam *et al* 2021).

1.1 Aim and Objectives

The aim of this study was to determine antibiotics resistance pattern and phenotypic virulence characteristics of bacteria isolated from street vended foods sold in Benin city, Edo state, Nigeria.

The specific objectives of this study were to

1. enumerate and isolate bacteria from street vended food samples.
2. identify bacterial isolates using cultural, morphological and biochemical characteristics
3. assess the phenotypic expression of key virulence factors (such as heamolysin production, biofilm formation, gelatinase) in the bacterial isolates.
4. determine the antiboitics susceptibility profiles of the bacterial isolates.

CHAPTER 2

LITERATURE REVIEW

2.1 Concept of street vended food

Street-vended foods ready-to-eat foods sold in informal settings such as market stalls, kiosks, and roadside carts play a vital socio-economic and nutritional role in many low- and middle-income countries. They provide affordable, convenient meals for urban populations and generate livelihoods for millions of vendors (WHO, 2015). However, the informal nature of street food vending often limits access to regulated infrastructure (safe water, refrigeration, waste disposal) and formal food-safety oversight, creating conditions that favour microbial contamination and foodborne disease transmission (FAO/WHO, 2014).

Street-vended foods are ready-to-eat foods and beverages prepared and/or sold by vendors or hawkers in streets and other public places, intended for immediate consumption or later consumption without further processing (WHO, 1996). These foods form a critical part of the informal food sector, particularly in low- and middle-income countries, where they provide affordable meals and livelihood opportunities but are often produced under conditions that fall outside formal regulatory oversight (FAO/WHO, 2010; Rane, 2011).

Street-vended foods, defined by WHO as ready-to-eat meals sold in informal public spaces, are socio-economically important but often produced under conditions of limited infrastructure, variable hygiene practices, and high environmental exposure, which collectively heighten their vulnerability to microbial contamination and foodborne pathogens (WHO, 1996; FAO/WHO, 2010; Rane, 2011; Barro *et al*., 2007).

Street foods are consumed without further cooking, eliminating a critical safety barrier and increasing the risk of pathogen transmission (Rane, 2011).Vendors typically operate in unregulated environments with limited access to infrastructure such as potable water, refrigeration, and waste disposal, making them vulnerable to microbial contamination (FAO/WHO, 2010; von Holy and Makhoane, 2006).

Street-vended foods contribute substantially to urban food supply and serve as an affordable source of nutrition while generating income for millions of people worldwide (WHO, 1996; Muyanja *et al*., 2011). Preparation and vending often occur in open-air environments, exposing foods to dust, flies, unsafe handling, and contact with contaminated surfaces, which promotes colonization by bacteria such as *Escherichia coli , Salmonella, Shigella, and Staphylococcus aureus* (Rane, 2011; Barro *et al*., 2007). Knowledge and adherence to food safety practices differ widely among vendors, and studies frequently report inadequate hand hygiene, improper storage, and re-use of cooking oil all of which increase microbial risks (von Holy and Makhoane, 2006; Muyanja *et al*., 2011). Street foods range from traditional snacks and beverages to cooked meals, reflecting local food culture and consumer demand, but their perishability often makes them microbiologically hazardous if not handled properly (Barro *et al*., 2007).

The demand for street-vended foods is driven by convenience, affordability, and cultural preferences. Urban lifestyles characterized by long working hours, commuting, and limited cooking facilities encourage reliance on ready-to-eat foods (WHO, 1996). Their widespread availability in high-traffic locations such as schools, workplaces, and transportation hubs further boosts daily consumption (Muyanja *et al*., 2011).

Beyond convenience, street foods meet cultural and sensory expectations. They often reflect traditional cuisines and flavors that resonate with consumers' social identity, creating loyalty and

regular patronage (von Holy and Makhoane, 2006). The diversity of product types from cooked meals and snacks to beverages and desserts ensures that street-vended foods cater to a broad demographic, from schoolchildren to working adults (FAO/WHO, 2010). Healthier food choices are also increasingly influencing consumer demand, with some vendors adapting to offer fresh fruit juices, grilled foods, and less oily preparations. Yet, taste, affordability, and accessibility remain the dominant factors shaping consumer decisions (Rane, 2011). This high and consistent demand underscores why street-vended foods are central to both nutritional intake and public health concerns, especially when microbial contamination or antimicrobial resistance is present.

Street-vended foods contribute significantly to nutritional intake by supplying energy, protein, and micronutrients, while their affordability, cultural relevance, and convenient accessibility drive high consumer demand; however, this same popularity increases public health risks when poor hygiene facilitates microbial contamination (FAO/WHO, 2010., Muyanja *et al*., 2011).

2.2 Classification of Street-Vended Foods in Africa

Street-vended foods in Africa encompass a wide variety of ready-to-eat (RTE) items prepared and sold in public spaces, typically outside formal food establishments. Their classification is important for understanding nutritional contributions, consumer demand, and microbiological risks (Alimi, 2016; FAO/WHO, 2019). Broadly, they may be categorized as follows:

2.2.1. Cooked Staple Meals and Cereal-Based Foods

These include boiled rice, beans, maize porridge (“ogi” or “pap”), yam, cassava products (“gari”, “eba”, “fufu”), and “injera” (Ethiopia). They are often sold hot but sometimes stored at ambient temperatures, creating conditions for microbial proliferation when reheating and holding practices are inadequate (Mensah *et al*., 2002). Cooked staple meals and cereal-based foods

constitute a cornerstone of daily diets across African cities and towns, serving as affordable, culturally familiar, and energy-dense options that sustain millions of consumers examples include rice dishes ("jollof", "waakye"), maize products ("kenkey","banku", roasted or boiled maize, pap/ogi), cassava derivatives (gari, fufu) and wheat-based snacks ("chapatti", "mandazi", "puff-puff"); these foods supply the bulk of dietary carbohydrates and therefore are the primary source of caloric intake for many low-income households, while their protein and micronutrient content vary widely depending on formulation and accompaniments (Mensah *et al*., 2002; FAO, 2003). Processing methods (fermentation of porridges, drying of cereals, parboiling of rice) can improve bioavailability of some B-vitamins and reduce antinutritional factors, but nutrient density often remains limited unless cereal staples are combined with legumes, animal foods or fortificants to supply essential amino acids, iron, zinc and vitamin B12 (Barro *et al*., 2002; Muyanja *et al*., 2011).

Cooked cereal meals are both vulnerable and high-risk: they are typically prepared in large batches and sold over many hours, creating opportunities for post-cooking contamination, temperature abuse (allowing mesophilic bacteria to proliferate), and hand- or surface-borne transfer of enteric pathogens such as *Escherichia coli , Salmonella* spp*. and Staphylococcus aureus* organisms repeatedly recovered in surveys of street foods across African settings (Mosupye and von Holy, 1999; Paudyal *et al.,* 2017; Bereda *et al.,* 2016). Empirical studies combining microbial enumeration with observational assessments show elevated total aerobic counts and coliform levels in many rice, maize and composite dishes sold on streets; these findings implicate inadequate reheating, use of contaminated water for cooking or washing, unhygienic utensils and limited access to refrigeration as principal drivers of contamination (Mensah *et al*., 2002; Birgen *et al*., 2020). The public-health consequences are important:

systematic reviews estimate that informal food sectors, including cereal-based street foods, contribute substantially to the burden of foodborne disease in sub-Saharan Africa, particularly affecting children, pregnant women and immunocompromised persons, and thereby interacting with nutrition outcomes (Delia, 2023; Paudyal *et al*., 2017). Nevertheless, interventions that are context-appropriate vendor training in safe time-temperature practices, provision of potable water and clean utensils, simple cold-holding or rapid reheating procedures, and small-scale fortification or recipe modification to boost protein and micronutrient content have been shown in field studies to reduce microbial loads while preserving the foods' affordability and cultural acceptability (Rane, 2011; FAO, 2003).

Cooked staple meals and cereal-based foods are central to diets across Africa providing the bulk of daily calories, acting as affordable staples for urban and rural households, and delivering variable amounts of protein, fats and micronutrients depending on formulation and accompaniments examples include rice dishes (jollof, waakye), maize products (kenkey, banku, pap/ogi), cassava derivatives (gari, fufu), wheat products ("chapatti", "mandazi", "puff-puff") and composite stews served over these staples (Mensah *et al.,* 2002). Macronutritionally, these foods are overwhelmingly carbohydrate-rich and thus critical for energy security, but their protein content and quality are often limited: unless paired with legumes, fish or meat, cereals and tubers supply low levels of indispensable amino acids (notably lysine) and therefore provide incomplete protein profiles for populations depending on them as primary foods (Barro *et al*., 2002; Muyanja *et al*., 2011). Processing practices that are traditional to the region such as fermentation of maize and sorghum porridge (ogi, ogi-ferments), parboiling of rice, and germination/drying of cereals can improve bioavailability of certain B-vitamins, reduce phytate-related mineral chelation (improving iron and zinc absorption), and contribute beneficial

microbial metabolites, but nutrient gains vary with technique and are not a universal substitute for dietary diversification or fortification (FAO 2003; Rane 2011). Fat content in many street-sold cereal foods increases where deep-frying is common (e.g., :puff-puff", "mandazi", "akara" when combined with cereals), improving energy density but also elevating total and saturated fat intake for regular consumers; conversely, simple boiled or steamed staples remain low in fat and contribute primarily to satiety and caloric needs (Barro *et al*., 2002; Mensah *et al*., 2002). Micronutrients are a consistent concern: unfortified cereal staples are generally poor sources of bioavailable iron, zinc and vitamin B_{12} (the latter absent unless animal products are added), which helps explain persistent micronutrient deficiencies in populations with cereal-dominant diets; targeted interventions small-scale fortification of flour, promotion of legume pairings, or encouraging inexpensive animal-source accompaniments are repeatedly recommended to raise nutrient density without undermining affordability (FAO 2003; Delia 2023).

From public-health perspective, the nutritional benefits of cooked cereal meals can be undermined by unsafe handling: large-batch cooking, time temperature abuse, and post-cooking contamination allow rapid growth of mesophilic bacteria and increase the risk of toxin production (e.g., *Staphylococcus aureus* enterotoxins) or infection by enteric pathogens such as *E. coli* and *Salmonella* if reheating or storage are inadequate, meaning that the same foods that provide calories and some nutrients can become vectors for disease that worsen nutritional status (through diarrhoeal disease and nutrient loss) in vulnerable groups, especially children and pregnant women (Mosupye and von Holy, 1999; Paudyal *et al*., 2017). Empirical surveys across African cities report elevated aerobic plate counts and coliforms in many rice-, maize- and cassava-based street meals, corroborating that microbiological quality is a crucial determinant of whether cereal staples contribute positively or negatively to health outcomes (Mensah *et al*.,

2002; Bereda *et al*., 2016; Birgen *et al*., 2020). In practice, the optimal public-health approach is therefore dual: (1) preserve the undeniable role of cereal staples for energy security by maintaining affordability and cultural acceptability, and (2) implement pragmatic, context-adapted measures to improve nutrient density and microbiological safety for example, promoting simple fortification, pairing cereals with legumes or small amounts of animal source foods, improving vendor training on time–temperature control and hand hygiene, and expanding access to potable water and safe utensils so that cooked staple meals become a more reliable vehicle of nutrition rather than a contributor to the burden of foodborne illness and nutrition loss (Rane 2011; Delia 2023).

2.2.2. Fried and Baked Snacks

Snacks such as "puff-puff" (fried dough), akara (bean cakes), meat pies, fried yam, doughnuts, and roasted maize fall into this group. They are popular for their affordability and convenience but face contamination risks from unhygienic handling, reused frying oil, and exposure to environmental pollutants (Rane, 2011; Alimi, 2016). Fried and baked snacks are a highly visible and economically significant component of street-vended foods across Africa, consumed daily by diverse socio-economic groups because of their affordability, sensory appeal, portability, and contribution to satiety; popular examples include fried dough products such as puff-puff in West Africa, mandazi in East Africa, fat cakes in Southern Africa, as well as samosas, meat pies, buns, doughnuts, chin-chin, and baked pastries prepared with wheat, maize, or composite flours (FAO, 2003; Mensah *et al*., 2002). Nutritionally, these snacks are dense sources of carbohydrates from refined flours, supplying rapid energy, while deep frying often increases their fat content substantially, making them high in calories but sometimes low in micronutrient density unless enriched or fortified (Barro *et al*., 2002; Rane, 2011). Protein content is modest and largely

dependent on fillings, such as meat, fish, egg, beans, or vegetables, which when incorporated can enhance the nutritional profile by providing high-quality amino acids and essential vitamins (Muyanja *et al*., 2011).

From a microbiological standpoint, fried and baked snacks present distinct risks: though frying at high temperatures generally destroys vegetative microbial cells, post-frying handling, prolonged storage at ambient temperature, and cross-contamination from utensils, vendor hands, or contaminated fillings often lead to recontamination by organisms such as *Staphylococcus aureus, Escherichia coli* , and coliform bacteria, which have been reported in surveys of street-vended snacks in Burkina Faso, Ghana, Ethiopia, and Nigeria (Mosupye and von Holy, 1999; Bereda *et al*., 2016; Birgen *et al*., 2020). In addition, starchy fried snacks are susceptible to spoilage when moisture condenses during cooling or storage, promoting fungal growth or bacterial proliferation under favorable conditions (Paudyal *et al*., 2017). Food safety challenges are compounded by the widespread use of low-cost frying oils that are repeatedly reheated, leading to degradation and production of trans-fatty acids and polymeric compounds, which negatively affect both food quality and health outcomes (Rane, 2011; FAO, 2003). Despite these risks, fried and baked snacks remain nutritionally and socially important because they provide quick, accessible meals for schoolchildren, workers, and travelers, and generate significant income for vendors, particularly women. Therefore, research in food microbiology and public health emphasizes the dual approach of promoting better practices such as using potable water, clean utensils, hygienic handling of fillings, improved oil management, and protective packaging while recognizing their socio-economic role in urban food systems (Mensah *et al*., 2002; Delia, 2023).

Fried and baked snacks occupy a prominent place in African foodscapes and, from a nutritional standpoint, present a distinct profile that is both beneficial and limiting depending on preparation

and consumption patterns. These snacks examples include puff-puff, mandazi, chin-chin, samosas, meat pies, doughnuts and various baked buns and pastries are typically made from refined cereal flours (wheat, maize or composite flours) and are therefore concentrated sources of rapidly available carbohydrate that supply quick energy and satiety for low-income urban consumers and schoolchildren (Mensah *et al*., 2002; FAO, 2003). Deep frying or the addition of fat in doughs raises their energy density substantially: total fat and caloric content increase markedly compared with boiled or steamed staples, which makes these snacks efficient calorie sources where food security is precarious but also elevates intake of total and often saturated fats when hydrogenated or palm oils are used (Rane, 2011; Barro *et al*., 2002). Protein content in plain fried/baked snacks is modest and of limited biological value when derived solely from cereal flours (often deficient in lysine), yet nutritional quality improves when snacks are enriched with legumes, eggs, milk or meat fillings examples being akara (bean-based fritters) or meat-filled samosas where amino-acid balance and micronutrient density (iron, zinc, B-vitamins) are meaningfully higher (Muyanja *et al*., 2011). Micronutrient density of typical fried/baked snacks is generally low unless fortification or enriched ingredients are used; routine consumption of unfortified refined snacks therefore contributes little to dietary iron, zinc or vitamin B_{12} needs and can exacerbate “empty-calorie” dietary patterns unless paired with nutrient-rich accompaniments (FAO, 2003; Delia, 2023). Processing practices influence nutrient quality: repeated reheating of oils degrades essential fatty acids and forms unhealthy oxidation products, and prolonged storage can lead to moisture migration and nutrient losses, whereas simple recipe modifications (partial substitution with wholegrain flours, incorporation of legume flours, or small-scale fortification) can raise fibre, micronutrient and protein content without large cost increases (Rane, 2011; Barro *et al*., 2002). Importantly, the nutritional benefit of these snacks

may be undermined by foodborne illness: post-production contamination or poor storage that leads to diarrhoeal disease will reduce nutrient absorption and contribute to negative health outcomes in vulnerable groups, particularly children and pregnant women linking microbiological safety directly to nutritional impact (Mosupye and von Holy, 1999; Paudyal *et al*., 2017). In public-health and programmatic terms, therefore, fried and baked snacks should be viewed as pragmatic vehicles for targeted nutrition improvements (fortification, use of blended flours, portion guidance) and for food-safety interventions (better oil management, hygienic handling and packaging) so that their role as accessible energy sources does not come at the expense of micronutrient adequacy or longer-term cardiometabolic and infectious disease risks (Mensah *et al*., 2002; Birgen *et al*., 2020; Delia, 2023).

2.2.3. Animal-Origin Foods

This group covers roasted and grilled meats (suya in Nigeria, nyama choma in Kenya and Tanzania), boiled eggs, fried fish, and poultry products. They are nutrient-rich and thus highly susceptible to bacterial growth if improperly cooked or stored (Barro *et al*., 2006; Igbinosa and Beshiru, 2019).Animal-origin foods form an essential component of street-vended and household diets across Africa, providing high-quality proteins, essential amino acids, micronutrients, and bioavailable minerals, but also presenting unique microbiological safety challenges when sold in informal settings. Common animal-origin foods include meat and meat products (beef, goat, chicken, suya, kebabs, fried meats), fish and seafood (grilled, smoked, fried, or dried fish), eggs, and dairy products such as milk, yoghurt, and locally processed cheese (wara in Nigeria), which are widely consumed for their nutritional benefits (FAO, 2003; Mensah *et al*., 2002). Nutritionally, these foods are superior to many plant-based street foods because they supply complete proteins rich in essential amino acids, readily absorbed iron (heme iron), zinc, vitamin

B12, and calcium in the case of dairy products, thereby addressing common deficiencies prevalent in African populations (Barro *et al*., 2002; Muyanja *et al*., 2011). For instance, grilled meats and fish sold by street vendors serve as affordable protein sources for urban consumers who may lack regular access to animal products through formal markets, while milk-based drinks and fermented dairy snacks provide probiotics and bioactive compounds that support gut health (Paudyal *et al*., 2017). However, these foods are also highly perishable and prone to microbial contamination if hygiene standards are not strictly maintained.

Several studies have reported the presence of *Salmonella* spp*., Escherichia coli , Listeria monocytogenes, Staphylococcus aureus,* and *Campylobacter* spp. in meat, fish, and dairy products sold by street vendors, often linked to inadequate refrigeration, prolonged ambient display, contaminated water, and poor handling practices (Mosupye and von Holy, 1999; Bereda *et al*., 2016; Birgen *et al.,* 2020). Smoked or dried fish, though nutritionally rich in protein and omega-3 fatty acids, may accumulate molds and aflatoxins if dried under unhygienic conditions, posing further public health risks (Rane, 2011). Moreover, repeated use of contaminated equipment such as knives, boards, and grilling racks contributes to cross-contamination, while lack of temperature control accelerates spoilage. Despite these risks, animal-origin street foods play a vital socio-economic role, offering accessible nutrition to millions of low-income urban residents and sustaining the livelihoods of vendors, especially women and small-scale producers. Therefore, food microbiology research highlights the importance of integrating safe processing techniques (adequate cooking, hygienic drying, use of clean water, improved packaging, and temperature control) with nutrition-sensitive interventions such as fortification or diversification, so that animal-origin foods continue to meet Africa's protein and micronutrient needs without heightening the burden of foodborne diseases (Delia, 2023; Mensah *et al*., 2002).

Animal-origin foods in Africa, which include meat, fish, eggs, milk, and dairy products, are nutritionally indispensable because they provide high-quality proteins, essential micronutrients, and bioactive compounds that are not easily available in plant-based staples. These foods are rich in complete proteins containing all essential amino acids in adequate proportions, which are necessary for growth, tissue repair, and immune function, particularly in vulnerable groups such as children, pregnant women, and the elderly (Mensah *et al*., 2002; FAO, 2003). Meat and offal are excellent sources of iron and zinc, both of which are highly bioavailable and help combat widespread deficiencies that contribute to anemia and impaired cognitive development in African populations (Barro *et al.*, 2002; Delia, 2023). Similarly, poultry and eggs provide not only digestible protein but also fat-soluble vitamins such as A, D, E, and K, as well as choline, which supports brain development (Muyanja *et al.*, 2011). Fish, especially small pelagic species consumed whole, are significant contributors of long-chain omega-3 fatty acids (EPA and DHA), calcium, iodine, and vitamin D, nutrients critical for cardiovascular health, skeletal development, and cognitive function (Paudyal *et al*., 2017). Dairy products such as milk, yoghurt, and traditional fermented cheese (wara) supply calcium, riboflavin, and probiotics that promote gut health and support bone mineralization (Bereda *et al*., 2016). From an energy standpoint, animal-origin foods are calorie-dense, providing fats that enhance satiety and aid in the absorption of fat-soluble vitamins, thereby complementing the carbohydrate-based staples common in African diets (Rane, 2011). Nutritional studies also highlight that incorporating modest amounts of animal products into diets dominated by cereals and tubers significantly improves overall nutrient adequacy scores and reduces the risk of stunting and micronutrient deficiencies (Mosupye and von Holy, 1999; Birgen *et al*., 2020). However, while nutritionally advantageous, the health benefits of these foods can be undermined by unsafe processing, microbial

contamination, and lack of preservation, which may lead to foodborne diseases that impair nutrient absorption and exacerbate undernutrition, underscoring the interconnection between nutritional value and food safety in African contexts (Mensah *et al*., 2002; Delia, 2023).

2.2.4. Fresh Fruits and Vegetables

Sliced fruits such as watermelon, pineapple, mango, cucumber, and vegetable salads are widely sold in African cities. While nutritious, they pose significant microbiological risks due to washing with unsafe water, contamination during cutting, and direct environmental exposure (Tambekar *et al*., 2008). Fresh fruits and vegetables are vital components of African diets supplying vitamin C, provitamin A (β-carotene), folate, potassium, dietary fibre and a range of phytonutrients that protect against micronutrient deficiencies and non-communicable diseases yet when sold through informal markets and street vendors they occupy a dual role as potent sources of nutrition and as frequent vehicles for microbiological contamination that can negate their health benefits (FAO, 2003; Mensah *et al*., 2002). Botanically diverse items fresh leafy vegetables (sukuma wiki/collards, spinach), fruit (mangoes, bananas, citrus), root vegetables (carrots, sweet potato), and salad items (tomato, cucumber, onions) deliver micronutrients that are either absent or poorly bioavailable in cereal-dominant diets and therefore are critical for preventing scurvy, vitamin A deficiency and anaemia when consumed regularly (FAO, 2003; Delia, 2023).

However, numerous field studies across African cities repeatedly document microbial hazards associated with fresh produce arising from cultivation with contaminated irrigation water, use of untreated manure,unhygienic handling at harvest and markets,inadequate washing,and contamination from vendor hands, cutting boards and display surfaces; common pathogens and indicators isolated include enteric bacteria (*Escherichia coli , Salmonella spp*.), *Shigella,*

coliforms, and occasionally protozoan parasites (e.g., Giardia, Cryptosporidium) and fungal contaminants, demonstrating that produce is frequently contaminated at multiple points of the value chain (Paudyal *et al*., 2017; Tambekar *et al*., 2008; Mosupye and von Holy, 1999). Microbiological assessments also show that ready-to-eat raw salads and cut fruit are particularly high-risk when sold without adequate cooling or protective packaging because cutting and slicing increases surface area, releases cellular fluids that support microbial growth, and provides routes for direct transfer of pathogens to the edible portion (Mensah *et al*., 2002; Birgen *et al*., 2020). Beyond microbial hazards, postharvest handling and prolonged ambient display accelerate physiological deterioration and nutrient loss (notably vitamin C), while residues from improper pesticide use can pose chemical safety concerns both of which undermine the nutritional quality of produce reaching consumers (FAO, 2003). Interventions shown to reduce risk in African contexts include good agricultural practices (safe water for irrigation and washing, composting of manure), hygienic harvesting and market handling, simple-onward processing (thorough washing with potable water, safe chlorinated dips where appropriate), improved cold-chain or shaded display, and vendor training on hand hygiene and cross-contamination avoidance; these measures both preserve nutrient content and substantially lower microbial loads, helping ensure that fruits and vegetables fulfill their potential as vehicles of micronutrient repletion rather than sources of foodborne illness (Rane, 2011; Bereda *et al*., 2016). In short, fresh produce in African food systems is nutritionally indispensable but also vulnerable: safeguarding its public-health value requires integrated, context-appropriate actions across farm, market and vending stages so that the clear dietary benefits improved vitamin and mineral intake, fibre and protective phytochemicals are realized without exposing consumers to avoidable microbiological or chemical hazards (FAO, 2003; Delia, 2023).

Fresh fruits and vegetables are among the most nutritionally valuable foods available across African diets, delivering a dense array of micronutrients, fibre and bioactive phytochemicals that are difficult to obtain in adequate amounts from cereal- and tuber-dominant diets alone: leafy greens (e.g., sukuma wiki, spinach) and orange-fleshed vegetables supply provitamin A (β-carotene) critical for vision and immune function, citrus and tropical fruits (mango, guava) provide plentiful vitamin C which enhances non-heme iron absorption and supports antioxidant defenses, while root vegetables (sweet potato, carrot) and fruits contribute potassium, folate and dietary fibre that support cardiovascular, neurodevelopmental and gut health (FAO, 2003; Delia, 2023). Regular consumption of small portions of fruits and vegetables substantially raises nutrient adequacy helping prevent vitamin A deficiency, scurvy and folate-related birth defects and supplies a range of polyphenols and other phytochemicals associated with lower risks of non-communicable diseases (Delia, 2023; FAO, 2003). From a macronutrient perspective these foods are low in energy but high in fibre, which promotes satiety and healthy glycemic responses when eaten with staples; fibre also supports a beneficial gut microbiome that can improve nutrient extraction and immune resilience (Mensah *et al*., 2002). However, the nutritional potential of fresh produce reaching urban consumers is moderated by post-harvest and vending realities in many African value chains: vitamin C and other labile nutrients degrade rapidly after harvest and with prolonged ambient storage or rough handling, and practices such as peeling, prolonged cutting or cooking can further reduce water-soluble vitamins unless careful minimal-processing methods are used (FAO, 2003; Rane, 2011). Importantly, microbiological and chemical safety issues intersect directly with nutritional outcomes produce irrigated or washed with contaminated water, or handled with soiled hands or cutting surfaces, can carry enteric pathogens (e.g., *Escherichia coli , Salmonella spp., Shigella* spp.) or protozoa that cause

diarrhoeal disease, which in turn reduces nutrient absorption and exacerbates undernutrition, particularly among children and pregnant women (Paudyal *et al*., 2017; Mosupye and von Holy, 1999). Field studies across African cities repeatedly document microbial contamination of raw salads, cut fruit and leafy vegetables at markets and street stalls, underscoring that without simple protective measures safe irrigation and washing water, hygienic harvesting and market handling, shaded display and rapid sale produce's benefits can be lost or even reversed (Tambekar *et al*., 2008; Birgen *et al*., 2020; Bereda *et al.,* 2016). Interventions that are feasible and evidence-backed in African contexts include promoting good agricultural practices (safe water, composting of manure), point-of-sale washing with potable water, shaded or cooled displays to slow nutrient loss, small-scale nutrient enhancement (e.g., encouraging consumption of vitamin-A rich varieties such as orange-fleshed sweet potato and biofortified maize), and vendor training in hygiene; together these actions both preserve micronutrient content and lower foodborne risk, enabling fruits and vegetables to realize their role as cornerstone foods for micronutrient repletion and long-term health in African populations (FAO, 2003; Delia, 2023).

2.2.5. Traditional Fermented Foods and Beverages

Fermented foods and drinks such as kunu (millet or sorghum drink), fura da nono (fermented milk blend), palm wine, and locally prepared fruit juices belong to this class. Although fermentation provides some microbial stability, post-fermentation handling, dilution with unsafe water, and poor storage compromise safety (Nkere *et al*., 2011; FAO, 2016).Traditional fermented foods and beverages constitute one of the most diverse and nutritionally significant categories of diets in Africa, representing not only a means of preserving perishable raw materials but also enhancing nutritional quality, digestibility, sensory attributes, and functional health benefits. These products include cereal-based fermented porridges and beverages such as

ogi in Nigeria, uji in Kenya, ting in Botswana, and tella in Ethiopia; dairy fermentations like nunu and wara in West Africa or amasi in Southern Africa; legume and root-based fermentations such as iru (fermented locust bean) and ogiri; as well as alcoholic beverages like palm wine, pito, and chibuku (FAO, 2003; Holzapfel, 2002). Nutritionally, fermentation increases protein digestibility by reducing anti-nutritional factors such as phytates and tannins, enhances bioavailability of minerals like iron and zinc, and enriches the vitamin profile through microbial synthesis of B-complex vitamins and vitamin K (Hounhouigan *et al*., 1993; Nout, 2009). Fermented dairy and cereal beverages also contribute probiotics lactic acid bacteria (*Lactobacillus, Leuconostoc, Streptococcus*) and yeasts (Saccharomyces) that improve gut microbiota balance, enhance immune function, and reduce diarrheal incidence, thereby linking fermented foods directly to improved public health (Tamang *et al.,* 2016; Chilton *et al*., 2015). From a microbiological standpoint, fermentation lowers pH, produces organic acids, alcohol, and antimicrobial metabolites that suppress pathogenic bacteria, thereby improving food safety and shelf-life compared to unfermented counterparts (Nout, 2009; Holzapfel, 2002). For example, lactic acid fermentation in cereal gruels creates conditions hostile to *Escherichia coli , Salmonella,* and *Staphylococcus aureus*, which are otherwise common contaminants in street-vended foods (Mensah *et al*., 2002). Moreover, fermented foods contribute social and economic value by providing affordable protein- and energy-rich diets to low-income households, supporting livelihoods of women vendors, and sustaining cultural identity across diverse African communities (FAO, 2003; Delia, 2023). Nevertheless, safety challenges remain when traditional fermentation relies on uncontrolled processes and unhygienic handling: mixed natural starter cultures, inadequate packaging, and ambient storage may lead to inconsistent quality and occasional survival of spoilage or pathogenic organisms, emphasizing the need for

microbiological monitoring and standardization (Nout, 2009; Rane, 2011). African fermented foods and beverages are nutritionally dense, culturally embedded, and microbiologically advantageous, but optimizing their safety and functional health benefits requires bridging traditional practices with modern food microbiology and hygiene principles (Holzapfel, 2002; Delia, 2023).

Traditional fermented foods and beverages across Africa deliver disproportionate nutritional benefits relative to their humble ingredients, because microbial fermentation improves digestibility, enriches vitamins, reduces antinutrients and supplies functional microbes that together enhance both micronutrient bioavailability and resilience to foodborne disease (Nout, 2009; Holzapfel, 2002). Cereal- and tuber-based ferments (e.g.," ogi", "uji", "ogi-riri", "kenkey" variants) undergo lactic-acid and yeast fermentation that lowers phytate levels and increases extractable iron and zinc, while microbial action and enzymatic breakdown raise free amino acids and improve protein digestibility effects repeatedly documented in laboratory and field studies and especially important where diets are otherwise cereal-dominated (Hounhouigan *et al*., 1993; Nout, 2009). Dairy fermentations (e.g., "nunu", "amasi", wara-derived products) concentrate high-quality animal protein, calcium and riboflavin and, through fermentation, develop probiotic lactic acid bacteria that can enhance gut barrier function, improve lactose tolerance in some populations, and reduce diarrhoeal incidence thereby protecting nutritional status in children and other vulnerable groups (Chilton *et al*., 2015; Tamang *et al*., 2016). Fermented legumes and condiments (e.g., "iru", "ogiri") both preserve protein-rich substrates and reduce tannins and oligosaccharides that impede nutrient absorption, making legume-derived amino acids more available when these products are used as relishes or ingredients in composite meals (Nout, 2009). Microbial synthesis during fermentation also produces B-group vitamins

(notably folate, niacin and certain B12-like activities in mixed fermentations), which can meaningfully raise micronutrient intake where industrial fortification is limited (Holzapfel, 2002; Tamang *et al*., 2016). Beyond specific nutrients, fermentation improves food safety and shelf-life by producing organic acids, bacteriocins and alcohol that inhibit pathogens this preservative effect not only reduces spoilage losses (supporting food security) but also lowers the risk of infection-driven nutrient loss (Mensah *et al*., 2002; Rane, 2011). The nutritional advantages vary with process control: uncontrolled or inconsistent fermentations may yield uneven vitamin synthesis or permit survival of spoilage organisms, and post-fermentation handling or adulteration can negate benefits (FAO, 2003). Consequently, public-health and nutrition interventions in African contexts increasingly view traditional fermentations as scalable nutrition platforms retaining their low-cost, culturally embedded role while improving starter-culture standardization, hygiene and small-scale fortification so that fermented foods reliably deliver protein, micronutrients, probiotics and improved mineral bioavailability to populations most at risk of undernutrition (Nout, 2009; Delia, 2023).

2.2.6. Milk and Dairy-Based Products

Street-vended dairy products such as fura da nono (fermented milk with millet), yogurt drinks, and fresh milk are common in West Africa. These foods are prone to contamination by *Listeria* spp*., Brucella* spp., and coliform bacteria, particularly when sold unpasteurized or stored without refrigeration (Nkere *et al*., 2011; Barro *et al*., 2006).Milk and dairy-based products in Africa encompass a wide spectrum of fresh, fermented and processed foods from raw and boiled milk, cream and traditional cheeses (“wara”, “nunu” derived curds), to fermented drinks such as amasi, cultured yoghurts and locally produced butters and they play a pivotal role in nutrition, food security and livelihoods while presenting specific microbiological and safety challenges..

Nutritionally, milk and dairy provide high-quality, complete protein (all essential amino acids), Highly bioavailable calcium and phosphorus for bone health, riboflavin and other B-group vitamins, and in animal-milk-consuming populations they are an important source of vitamin B_{12} and fat-soluble vitamins when full-fat varieties are consumed, making them critical for growth, cognitive development and maternal-child nutrition in many African settings (FAO, 2003; Mensah *et al*., 2002). Fermented dairy products such as nunu, amasi and spontaneously fermented yoghurts add probiotic lactic acid bacteria (*Lactobacillus, Streptococcus* and *Leuconostoc* species) that improve lactose tolerance, enhance gut barrier function and can reduce diarrhoeal disease incidence, thereby indirectly supporting nutritional status and micronutrient retention among vulnerable groups (Tamang *et al*., 2016; Chilton *et al*., 2015).

However, milk is highly perishable and vulnerable to contamination at many points: raw milk and poorly processed dairy products may carry pathogens including *Brucella* spp*., Salmonella* spp*., Listeria monocytogenes, Staphylococcus aureus (*including enterotoxin-producing strains), and *enterotoxigenic Escherichia coli ,* particularly when cold chains are absent, milking hygiene is suboptimal, water used for dilution or cleaning is contaminated, or when storage and distribution expose milk to temperature abuse (Mosupye and Von Holy, 1999; Bereda *et al*., 2016). A further chemical-food-safety concern is the occurrence of aflatoxin M1 in milk when dairy animals consume aflatoxin-contaminated feeds this metabolite is heat-stable and can pose long-term carcinogenic risks and reduce the safety of milk for infants and children (FAO/WHO reviews). Traditional processing (fermentation, smoking, sun-drying of cheeses) often improves shelf-life and reduces pathogen load through acidification and competitive microbial inhibition, yet uncontrolled or artisanal fermentations can be inconsistent in protective efficacy and may permit survival or growth of spoilage organisms when hygiene is poor (Holzapfel, 2002; Nout,

2009). Empirical surveys from urban African markets demonstrate frequent detection of elevated total viable counts, coliforms and occasional pathogenic isolates in raw or inadequately processed dairy sold by small-scale vendors, underlining the need for pragmatic interventions improved milking hygiene, rapid cooling or insulated transport, simple pasteurization or low-cost heat treatments, standardized starter cultures for reliable fermentation, and vendor training in sanitation to protect both the nutritive value and safety of dairy products without removing their economic and cultural benefits (Mensah *et al*., 2002; Rane, 2011; Delia, 2023). Policy and programmatic responses recommended in food-microbiology and public health therefore stress integrated value-chain approaches that preserve the essential nutritional contributions of milk and dairy to diets while reducing microbiological and chemical hazards through feasible, low-cost technologies and strengthened surveillance so that dairy products remain a safe, nutrient-dense pillar of food security in African communities (FAO, 2003; Bereda *et al*., 2016).

Milk and dairy-based products in Africa are nutritionally dense and play a critical role in meeting the protein, mineral and micronutrient needs of diverse populations, yet their value is intimately tied to how they are produced, processed and handled along informal value chains (Mensah *et al*., 2002; FAO, 2003). A single serving of milk or fermented dairy delivers high-quality "complete" protein with all essential amino acids needed for growth and tissue repair, highly bioavailable calcium and phosphorus for bone development, and vitamin B12 a nutrient largely absent from plant staples and crucial for neurodevelopment and hematologic health while full-fat varieties also provide fat-soluble vitamins that aid absorption of other micronutrients (Barro *et al*., 2002; Chilton *et al*., 2015). Fermentation of milk into products such as amasi, nunu and traditional yoghurts not only extends shelf-life but increases functional value by concentrating nutrients and introducing probiotic lactic acid bacteria (*Lactobacillus, Streptococcus* spp.) that can improve

gut microbiota composition, enhance lactose tolerance in some consumers and reduce the incidence and severity of diarrhoeal disease effects that indirectly preserve nutrient status in children and vulnerable adults (Tamang *et al*., 2016; Chilton *et al*., 2015). From a micronutrient perspective, regular small amounts of dairy improve population-level adequacy for calcium, riboflavin and B_{12} and contribute modestly to zinc and iron intakes when animal products are otherwise scarce (Muyanja *et al*., 2011; Delia, 2023). Importantly, milk's nutritive benefits are especially consequential where cereal-dominant diets predominate: even small daily additions of dairy to porridge or composite meals raise protein quality and the bioavailability of multiple micronutrients, thereby reducing stunting risk and improving growth outcomes in children (Mensah *et al*., 2002; Nout, 2009).

However, the public-health advantage of dairy can be compromised by microbial and chemical hazards common in informal systems: raw or poorly processed milk may harbor *Brucella, Listeria monocytogenes, Salmonella, enterotoxigenic, Staphylococcus aureus* and high coliform loads when milking hygiene, water quality and cold-chain are inadequate, and aflatoxin M1 a metabolite appearing in milk when animals consume contaminated feeds represents a persistent chemical risk that undermines dairy safety and long-term health (Mosupye & von Holy, 1999; Bereda *et al*., 2016; FAO/WHO reviews). Traditional low-cost interventions such as small-scale pasteurization or heat treatment, improved milking hygiene, rapid cooling or insulated transport, and promoting controlled starter cultures for fermentation have been shown to substantially reduce pathogen loads while preserving nutritional value and probiotic benefits, making these pragmatic measures for resource-constrained settings (Holzapfel, 2002; Rane, 2011). Finally, milk and dairy products are economically important supporting livelihoods, especially among pastoral and smallholder households and women vendors and therefore nutrition policy in Africa

increasingly emphasizes integrated value-chain solutions that protect and amplify dairy's nutritional contributions while minimizing microbiological and chemical hazards through feasible, context-appropriate technologies and surveillance (FAO, 2003; Delia, 2023).

2.2.7. Soups, sauces, and stews

Many African street vendors sell soups and stews such as egusi, okro, tomato-based sauces, and groundnut stews, which are eaten with staples. Because these are often prepared in bulk and reheated multiple times, they can harbor spore-forming bacteria such as *Clostridium perfringens* (Mensah *et al*., 2002).Soups, sauces, and stews are among the most widely consumed categories of traditional meals in Africa, serving as essential complements to staple foods such as rice, yam, cassava, maize, and plantain, while also acting as nutrient-dense vehicles that enhance both dietary diversity and palatability. They are typically prepared with a mixture of vegetables, spices, oils, legumes, meats, and sometimes fish, and their composition varies regionally, reflecting cultural heritage, ecological resources, and economic accessibility (FAO, 2003; Mensah *et al*., 2002). Nutritionally, soups and stews provide balanced macronutrients: proteins from meat, fish, legumes, or peanuts; carbohydrates from thickening agents like yam, cassava flour, or cereals; and fats from palm oil, groundnut oil, or sesame seeds, while also supplying vitamins (A, C, folate) and minerals (iron, zinc, magnesium, calcium) from leafy greens, tomatoes, peppers, and condiments (Barro *et al*., 2002; Delia, 2023).

Soups and sauces are highly perishable due to their high moisture content, nutrient richness, and neutral pH, making them excellent substrates for microbial proliferation if not properly handled or stored. Studies across African urban centers have reported contamination of soups and stews with coliforms, *Staphylococcus aureus, Salmonella enterica,* and *enterotoxigenic Escherichia coli*_, often linked to poor cooking hygiene, cross-contamination from utensils and water, and

prolonged holding at ambient temperatures during vending (Mensah *et al*., 2002; Mosupye and von Holy, 1999; Bereda *et al*., 2016). Although heat treatment during cooking inactivates most pathogens, post-cooking handling and storage remain critical points where microbial recontamination and growth occur, especially since many street vendors and households lack refrigeration or rely on repeated re-heating (Rane, 2011). In addition to microbiological safety, chemical hazards may occur when condiments and oils are adulterated, or when soup thickeners and seasonings contain aflatoxin-contaminated groundnuts, raising long-term carcinogenic risks (FAO, 2003). Nonetheless, soups and sauces play an indispensable role in improving nutrient intake and ensuring protein-energy sufficiency in diets dominated by starchy staples, while also contributing bioactive compounds such as antioxidants, flavonoids, and carotenoids from spices and vegetables that support immune function and reduce oxidative stress (Chilton *et al*., 2015; Tamang *et al*., 2016). Because of their central role in daily nutrition, interventions to improve food safety in soups, sauces, and stews focus on vendor training in hygienic preparation, safe water usage, proper storage, and consumer awareness, ensuring that these culturally rooted foods continue to serve as nutritious and safe components of African diets (Delia, 2023; Holzapfel, 2002).

Soups, sauces and stews are nutritionally powerful components of African diets because they act as concentrated, flexible vehicles for combining staples, vegetables, legumes, fish and meat into nutrient-dense meals that markedly improve dietary quality compared with staples eaten alone: by simmering vegetables, bones, pulses and animal tissues together, these preparations extract soluble vitamins, minerals (notably iron, zinc, calcium and B-vitamins) and collagenous proteins into the broth while retaining fibre and phytochemicals from plant ingredients, so a modest serving can deliver biologically important amounts of protein, bioavailable iron (especially when

animal-source ingredients are included), essential fatty acids from fish or cooking oils, and fat-soluble vitamins whose absorption is enhanced by the oils commonly used (FAO, 2003; Barro *et al*., 2002). When legumes (groundnuts, cowpeas, bambara nut) or animal proteins (meat, fish, offal, poultry) are incorporated, the amino-acid profile of the meal improves substantially lysine-poor cereals are complemented by legume or animal lysine, yielding a higher biological protein value that supports child growth and recovery from undernutrition (Muyanja *et al*., 2011; Mensah *et al*., 2002). Soups and stews that include bones or small fish provide accessible calcium, phosphorus and trace minerals (including iodine when marine species are used), and slow cooking increases mineral leaching into the broth, making commonly discarded cooking liquids an important source of micronutrients in low-resource settings (Nout, 2009; Delia, 2023). Simultaneously, the presence of vitamin C–rich ingredients (tomato, pepper, citrus) in many sauces enhances non-heme iron absorption from plant foods, helping reduce iron-deficiency anaemia that is otherwise common in cereal-based diets (FAO, 2003; Paudyal *et al*., 2017). Processing and culinary techniques used in soups and stews fermented condiments, prolonged simmering, and combination of diverse ingredients also reduce antinutritional factors (phytates, tannins) and improve protein digestibility and mineral bioavailability compared with raw or simply boiled staples (Hounhouigan *et al*., 1993; Nout, 2009). From a public-health perspective the nutritional advantages are manifest: routine small portions of nutrient-rich stews served with staples raise overall diet quality, reduce stunting risk and improve micronutrient adequacy at the population level, especially where animal-source foods are sporadic (Mensah *et al*., 2002; Delia, 2023). However, these benefits are conditional on safe preparation and handling time-temperature abuse, reuse of contaminated water, or unhygienic utensils can introduce pathogens or toxins that cause diarrhoeal disease and thereby impair nutrient absorption and retention,

reversing the very gains that soups and stews are intended to deliver (Mosupye and von Holy, 1999; Rane, 2011). Finally, modest, evidence-based modifications adding small amounts of animal protein or legume flour, using vitamin-rich vegetables, avoiding excessive oil reuse, and maintaining hygienic cooking and storage can substantially amplify the nutrient density and safety of these dishes without raising costs markedly, making soups, sauces and stews ideal, culturally acceptable platforms for nutrition improvement in African food systems (Muyanja *et al*., 2011; Barro *et al*., 2002; Delia, 2023).

2.3 Socio-Economic Relevance of Street-Vended Foods

Street-vended foods play an indispensable socio-economic role, particularly in urban centers of developing countries. They are widely recognized for their affordability, accessibility, and as a critical source of livelihood for millions of people globally.Street-vended foods in Africa hold immense socio-economic relevance, functioning not only as an indispensable component of daily diets but also as a critical pillar of urban and peri-urban economies. Their classification into groups such as cooked staple and cereal-based meals, fried and baked snacks, animal-origin foods, fresh fruits and vegetables, traditional fermented foods and beverages, soups, sauces, and dairy products underscores the wide range of affordable options they provide for consumers across socioeconomic strata (FAO, 2003; Mensah *et al*., 2002). From a consumer perspective, this classification highlights the role of street foods in food security, since they offer accessible, inexpensive meals that supplement household nutrition, particularly for low-income populations who cannot afford regular restaurant or packaged meals (Barro *et al*., 2002; Rane, 2011). On the vendor side, street food enterprises generate significant employment opportunities, especially for women, youth, and migrants with limited access to formal labor markets, thereby contributing to poverty alleviation and household income generation (Muyanja *et al*., 2011; Delia, 2023). The

classification also mirrors the economic diversity of vendors, ranging from small-scale hawkers selling simple snacks or fruits, to more established operators preparing complex dishes such as soups and sauces that require higher input and skill, reflecting varying levels of capital investment and economic return (Mosupye and von Holy, 1999; Bereda *et al*., 2016). In addition, street-vended foods stimulate local agricultural and livestock markets by creating steady demand for cereals, legumes, vegetables, meats, fish, and dairy, thus linking informal food economies to rural production systems and sustaining livelihoods along the value chain (Paudyal *et al*., 2017; Tamang *et al*., 2016). Thus, the socio-economic relevance of street-vended food, as revealed by its classification, lies in its dual role: on one hand, a low-cost, nutritionally significant, and culturally embedded food supply, and on the other, a dynamic informal sector that provides employment, sustains agricultural linkages, and supports urban food systems resilience, but requires targeted interventions in food safety and regulation to maximize its benefits while minimizing risks (FAO, 2003; Rane, 2011).

2.3.1. Affordability

Street foods are typically sold at lower prices compared to meals from formal establishments, making them accessible to low- and middle-income earners, students, and migrant workers who rely on such meals for daily sustenance (Rane, 2011). Their relatively low cost is due to minimal overheads, locally sourced raw materials, and the informal nature of the vending sector, which keeps prices within reach of economically disadvantaged populations (FAO/WHO, 2010). Affordability is one of the most critical dimensions of the socio-economic relevance of street-vended foods, as it ensures that even low-income populations can access ready-to-eat meals despite financial constraints. Street vendors typically sell food at significantly lower prices than formal restaurants because they operate with minimal overhead costs, rely on bulk preparation,

and source raw materials locally, making their products affordable to students, informal workers, and urban households struggling with economic limitations (Salamandane *et al*., 2023). This affordability not only provides daily sustenance but also contributes to food security, as many households in African and Asian cities rely on street food for the majority of their caloric intake due to its cost-effectiveness and convenience (Delia, 2023). Moreover, affordability acts as a coping strategy during periods of economic shocks, food price inflation, or limited access to formal food outlets, thereby helping to reduce hunger and malnutrition in vulnerable populations (FAO, 2003). However, the need to keep prices low may inadvertently encourage unsafe practices, such as the use of substandard raw materials, reusing frying oil, or inadequate storage, which increases the risk of microbial contamination (Mensah *et al*., 2002; Paudyal *et al*., 2017). Studies in food microbiology consistently show that pathogens such as *Escherichia coli , Salmonella,* and *Staphylococcus aureus* are more frequently detected in low-cost street foods prepared under poor hygiene conditions (Rane, 2011). Despite these risks, affordability remains indispensable, and research highlights that food safety can be improved through low-cost interventions such as hygiene training, potable water provision, and microcredit for equipment without substantially raising food prices (Salamandane *et al*., 2023; Delia, 2023). Thus, affordability is not only central to the accessibility of street foods but also to sustaining their role in urban food security, livelihoods, and public health.

2.3.2. Accessibility

Street foods are conveniently located in areas of high human traffic such as bus stops, markets, schools, and workplaces. Their ready-to-eat nature ensures immediate consumption without the need for further preparation, catering to the fast-paced lifestyles of urban dwellers (Muyanja *et al*., 2011). This accessibility not only provides a vital food source but also ensures dietary

diversity in urban diets, as street foods often reflect the traditional cuisines and cultural food preferences of the local population (Barro *et al*., 2007). Accessibility is another fundamental aspect of the socio-economic relevance of street-vended foods, as it ensures that diverse groups of consumers especially low-income populations can obtain ready-to-eat meals close to their places of work, residence, or travel. Unlike formal restaurants or supermarkets that may be geographically distant or financially restrictive, street food vendors operate in highly visible and convenient locations such as markets, bus stations, schools, construction sites, and roadside corners, thereby bridging the gap between food demand and availability in both urban and peri-urban settings (Mwangi *et al*., 2023). This widespread distribution increases physical access to meals for marginalized communities who often lack the time, facilities, or resources to prepare food at home (Alimi, 2016). Moreover, street-vended foods are culturally adaptive and locally embedded, offering familiar dishes prepared in portion sizes suited to individual purchasing power, which enhances social inclusivity in urban food systems (Omari *et al*., 2018). Food microbiology research has also shown that because of their accessibility, street-vended foods account for a large share of daily caloric intake among urban households in sub-Saharan Africa, with studies in Ghana and Nigeria reporting that up to 70% of working-class adults consume at least one street meal daily (Proietti *et al*., 2014). However, this same accessibility also increases exposure to microbial hazards, since food is frequently prepared and consumed in open-air environments without consistent access to potable water, refrigeration, or sanitation facilities, thereby creating conditions favorable for contamination by pathogens such as *Escherichia coli* and *Salmonella* (Feglo and Sakyi, 2012). Despite these risks, accessibility remains a vital characteristic of street foods because it aligns with urbanization trends, supports food security, and provides a functional response to the time–poverty faced by rapidly growing working

populations in Africa and beyond. To ensure that accessibility continues to support public health, experts recommend policies that integrate vendors into urban food safety systems by providing basic infrastructure such as clean water supply points, designated vending spaces, and waste management without undermining their capacity to serve dispersed and mobile consumers (Mwangi *et al*., 2023; Alimi, 2016).

2.3.3. Livelihood Source

Beyond consumer benefits, street food vending constitutes an important source of employment and income for millions of vendors, many of whom are women and primary household providers (von Holy and Makhoane, 2006). The sector contributes significantly to the informal economy, reducing unemployment while offering entrepreneurship opportunities in regions where formal job opportunities are scarce (WHO, 1996). In countries with high levels of poverty, this economic contribution is critical for sustaining households and promoting food security (FAO/WHO, 2010).

Street-vended foods are a crucial livelihood source, providing employment and income opportunities for millions of people across Africa and other developing regions where formal job markets remain limited. The street food sector is dominated by women and youth, who often use vending as a primary or supplementary income-generating activity to support household needs such as food, education, and healthcare (Chukuezi, 2010). Its importance lies in its low barriers to entry requiring minimal capital investment, reliance on locally available ingredients, and flexible working arrangements which makes it accessible to marginalized groups excluded from the formal economy (Mwangi *et al*., 2023). Beyond direct employment, the street food sector also sustains wider economic networks through linkages with farmers, transporters, utensil manufacturers, and small-scale suppliers, thereby amplifying its socio-economic significance

(Steyn *et al*., 2014). From a food microbiology perspective, however, the informality of this livelihood system can pose public health challenges: vendors often operate in environments without adequate infrastructure for safe storage, potable water, or waste disposal, creating conditions favorable for microbial contamination of food (Barro *et al*., 2006). Nevertheless, studies show that when vendors receive structured training in food hygiene and safety practices, the risks of contamination by pathogens such as *Escherichia coli* and *Salmonella* decrease significantly without undermining their earning capacity (Alimi, 2016). Thus, street food vending functions not only as a poverty alleviation mechanism but also as an adaptive strategy in contexts of rapid urbanization and unemployment, reinforcing its relevance as a livelihood source within the broader framework of urban food systems. Ensuring that interventions aimed at improving microbial safety are balanced with support for vendor livelihoods is therefore essential to preserve both economic and nutritional benefits of this critical sector (Mwangi *et al*., 2023; Delia, 2023).

2.3.4. Cultural and Social Value

Street foods frequently reflect traditional cuisines and cultural identities, preserving local food heritage and providing consumers with familiar tastes in rapidly urbanizing settings (von Holy and Makhoane, 2006). They also act as a medium of social interaction, as markets and roadside stalls serve as community hubs that foster social cohesion, cultural exchange, and a sense of belonging (Rane, 2011). Street-vended foods embody significant cultural and social value, serving as more than just sources of sustenance; they are deeply rooted in local traditions, identity, and community life. Across Africa and other developing regions, vendors often prepare meals using indigenous recipes and traditional cooking methods, thereby preserving culinary heritage and making culturally familiar foods accessible to diverse populations (Githiri *et al*.,

2022). For many urban dwellers, street food stalls function as social spaces where people from different socio-economic backgrounds interact, reinforcing social cohesion and cultural exchange (Proietti *et al*., 2014). The preparation and consumption of foods such as suya in Nigeria, nyama choma in Kenya, and fufu in Ghana illustrate how vending supports the transmission of cultural practices while meeting the nutritional and emotional needs of consumers (Mensah *et al*., 2002). Moreover, the affordability and proximity of these foods allow migrants and low-income populations to maintain connections with their cultural roots, even in rapidly modernizing urban environments (Mwangi *et al*., 2023). From a food microbiology standpoint, however, the same cultural value that sustains traditional preparation techniques may sometimes conflict with modern hygiene requirements; for instance, open-air cooking and communal dining, though culturally significant, can expose foods to contamination by pathogens such as *Salmonella* and *Escherichia coli* (Feglo and Sakyi, 2012). Nonetheless, these cultural practices remain vital because they provide not only nourishment but also a sense of belonging, identity, and continuity within fast-changing urban landscapes. Integrating food safety measures into traditional vending practices through community-based training, culturally sensitive interventions, and infrastructure support can therefore safeguard public health while preserving the cultural and social relevance of street foods (Githiri *et al*., 2022; Mwangi *et al*., 2023).

2.3.5. Gender Empowerment

Street food vending offers income-generating opportunities for women, who make up a large proportion of vendors worldwide. For many, vending is the primary means of providing for their households, covering school fees, health care, and other necessities (Muyanja *et al*., 2011). This informal employment fosters women's economic empowerment, giving them financial autonomy and a role in local food economies (Barro *et al*., 2007).

Street-vended foods play a critical role in gender empowerment across Africa and other developing regions, as women constitute the majority of vendors and rely on this sector as a primary source of income, economic independence, and social recognition. Street food vending offers women, particularly those with limited formal education or access to capital, a relatively low-barrier entry into entrepreneurship, enabling them to support household needs, pay school fees, and contribute to community welfare (Alimi, 2016). In many urban and peri-urban settings, women's participation in food vending enhances their decision-making power within households and strengthens their role in food security, thereby reshaping traditional gender dynamics (Hill *et al*., 2019). The preparation and sale of culturally significant foods such as akara in Nigeria, mandazi in Kenya, or vetkoek in South Africa not only preserve culinary traditions but also provide women with a socially valued identity linked to food and nutrition (Tinku *et al*., 2021). From a food microbiology perspective, the predominance of women in food vending highlights the importance of targeted training on hygiene and microbial safety practices, since poor handling may expose consumers to pathogens such as *Staphylococcus aureus* or *Escherichia coli* (Proietti *et al*., 2014). At the same time, initiatives aimed at improving women's access to resources such as microfinance, vendor associations, and food safety education have been shown to boost both economic resilience and public health outcomes (Osei-Asare and Eghan, 2014). Thus, street-vended foods represent not only a pathway for women's economic empowerment but also a platform for enhancing gender equity, public health, and cultural preservation within rapidly urbanizing societies.

2.3.6. Employment and Poverty Reduction

Beyond individual livelihoods, street-vended foods support employment for millions of people, including suppliers of raw materials, transporters, and equipment fabricators, thus creating

multiplier effects across local economies (von Holy and Makhoane, 2006). By offering low-barrier entry into entrepreneurship, the sector acts as a poverty reduction strategy in regions with high unemployment rates (FAO/WHO, 2010). Street-vended foods are not only vital for nutrition and food security but also represent a critical livelihood source for millions of people across Africa and other developing regions. The sector provides self-employment and income opportunities for individuals with limited education, formal skills, or capital, making it a significant driver of informal economies (Steyn *et al*., 2014). Women, in particular, constitute the majority of street food vendors, and for many households, earnings from vending represent the primary or supplementary source of income that supports daily living costs, schooling, and healthcare (Monney *et al*., 2013). The relatively low barriers to entry minimal start-up costs, flexible working hours, and reliance on locally available ingredients make vending an attractive option for marginalized groups excluded from formal employment (Mwangi *et al*., 2023). From a food microbiology perspective, however, the informal nature of street vending also presents challenges: vendors often operate without adequate food safety training or infrastructure, which increases the risk of microbial contamination and compromises consumer protection (Barro *et al*., 2006). Nevertheless, research has shown that when vendors receive even basic training in hygiene, storage, and preparation, contamination risks are significantly reduced without jeopardizing their livelihoods (Alimi, 2016).

Thus, the socio-economic relevance of street-vended foods extends beyond nutrition; they function as a safety net for low-income consumers and a sustainable livelihood strategy for vendors. However, these benefits must be weighed against the public health challenges posed by inadequate hygiene practices and microbial contamination, underscoring the need for balanced interventions that preserve economic gains while safeguarding food safety (Rane, 2011; Muyanja

et al., 2011). Street-vended foods are not only affordable and accessible to urban populations with limited income but also serve as a critical livelihood source for millions of vendors, particularly in developing countries, thereby contributing substantially to food security and informal economies (WHO, 1996; FAO/WHO, 2010; Rane, 2011; Muyanja *et al*., 2011; Von Holy and Makhoane, 2006).

2.4 Microbial contamination of street vended food

Microbial contamination remains one of the most critical public health concerns associated with street-vended foods, as inadequate hygiene, limited infrastructure, and exposure to environmental hazards create conditions favorable for the proliferation of foodborne pathogens. Numerous studies across Africa and other developing regions have shown that common microbial contaminants in street foods include *Escherichia coli , Salmonella* spp*., Staphylococcus aureus, and Listeria monocytogenes,* which are often linked to unsafe water use, improper storage, and cross-contamination during handling (Akabanda *et al*., 2017). In Accra, Ghana, for example, bacterial loads exceeding acceptable limits were detected in ready-to-eat foods due to insufficient vendor training and exposure to dust and flies (Mensah *et al*., 2012). Similarly, in Nigeria, street-vended foods such as roasted meat (suya) and fried snacks were found to harbor high counts of coliforms and enterotoxin-producing Staphylococcus aureus, reflecting risks from handling practices and ambient conditions (Chukuezi, 2010). From a food microbiology perspective, the survival and growth of these organisms are further facilitated by temperature abuse, as many vendors lack cold storage facilities, allowing pathogens to multiply rapidly in nutrient-rich foods like stews, rice, and dairy-based products (Kavitha *et al*., 2020). Beyond bacterial contamination, viral pathogens such as noroviruses and parasitic organisms like *Giardia lamblia* have also been reported in street foods, pointing to fecal-oral transmission

routes from contaminated water and poor sanitation infrastructure (Rane, 2011). The presence of these microorganisms not only threatens consumer health but also undermines the socio-economic relevance of the sector, since foodborne outbreaks can damage livelihoods and erode public trust in informal food markets. Addressing microbial contamination therefore requires a multifaceted approach, including enforcement of food safety regulations, provision of safe water, capacity-building for vendors, and community-level awareness campaigns to integrate microbiological safety with the cultural and economic importance of street foods (Alimi, 2016; Akabanda *et al*., 2017).

2. 4.1 Microbial contaminants associated with street vended foods

2.4.1.1 Pathogenic Bacteria

Pathogenic bacteria are the most critical contaminants in street-vended foods due to their direct role in foodborne diseases. Common pathogens include *Salmonella* spp*., Escherichia coli* O157:H7, *Listeria monocytogenes, Vibrio cholerae, and Staphylococcus aureus.* These organisms enter food mainly through poor hygiene, contaminated water, undercooked meals, and cross-contamination from utensils or hands. Their presence is strongly associated with outbreaks of diarrheal diseases in developing countries (Barro *et al*., 2006; Kavitha *et al*., 2020). Pathogenic bacteria represent the most critical classification of microbial contamination in street-vended foods, as they are directly responsible for foodborne illnesses that threaten consumer health worldwide. Foods sold by vendors, particularly ready-to-eat items such as cooked rice, meat products, salads, and dairy-based dishes, are highly susceptible to contamination by pathogenic bacteria including *Escherichia coli* O157:H7*, Salmonella* spp*., Staphylococcus aureus, Listeria monocytogenes, Shigella* spp*., and Vibrio cholerae* (Barro *et al*., 2006). These pathogens typically enter foods through multiple pathways such as poor hand hygiene, use of

contaminated water, inadequate cooking, temperature abuse, and cross-contamination from utensils or currency exchanged during food handling (Rane, 2011). For instance, *S. aureus* produces enterotoxins that are heat-stable, meaning that even reheating cannot eliminate their toxic effects, while *E. coli* O157:H7 is associated with severe gastroenteritis and hemolytic uremic syndrome when ingested in contaminated foods (Choudhury *et al*., 2011). Similarly, *Salmonella* is frequently isolated from meat-based street foods such as grilled chicken and suya in West Africa, reflecting both environmental exposure and poor food handling practices (Ghosh *et al*., 2007). From a food microbiology perspective, pathogenic bacteria are of greatest concern because of their ability to survive in diverse food matrices, adapt to stress conditions, and multiply rapidly when foods are left in the temperature danger zone (5–60°C). Their presence not only causes sporadic cases of illness but also contributes to large-scale outbreaks, undermining consumer confidence and imposing significant public health and economic burdens in low- and middle-income countries (Alimi, 2016). Effective mitigation requires improved training of vendors, access to potable water, adherence to hazard analysis critical control point (HACCP) principles, and continuous microbiological monitoring to reduce pathogenic contamination in this sector.

2.4.1.2 Indicator Organisms

Indicator organisms, such as total coliforms, fecal coliforms *(E. coli*), and enterococci, do not always cause illness directly but signal possible fecal contamination or poor sanitation in food preparation environments. High levels of indicator organisms in street foods suggest a potential risk for the presence of pathogenic bacteria. They are widely used in microbiological monitoring to assess hygiene quality (Tambekar *et al*., 2008). Indicator organisms are an important classification of microbial contamination in street-vended foods, as they do not always cause

illness directly but serve as markers of poor sanitary quality and the possible presence of enteric pathogens. The most common indicator organisms include total coliforms, fecal coliforms such as *Escherichia_coli_*, and enterococci, which are used to assess the hygienic conditions under which food is prepared, handled, and stored (Tambekar *et al*., 2008). Their detection in foods such as fresh salads, fruit juices, cooked rice, and meat products sold by vendors highlights contamination from fecal matter, unsafe water, or cross-contact with contaminated surfaces (Barro *et al*., 2006). For instance, the presence of *E. coli_*in street foods has been widely documented in Africa and Asia, and it strongly correlates with unsanitary practices such as handling money and food simultaneously, inadequate handwashing, or using unclean utensils (Chukuezi, 2010). High levels of indicator organisms in ready-to-eat foods are significant because they reflect an increased probability of pathogenic bacteria such as *Salmonella, Shigella, and Listeria* being present, even if those pathogens are not directly detected (Rane, 2011). From a food microbiology perspective, indicator organisms are useful because they are easier and cheaper to isolate and enumerate than pathogens, making them practical for routine monitoring of food safety in informal sectors (Kavitha *et al*., 2020). Therefore, controlling the contamination of street foods with these organisms is crucial, as they serve as an early warning system for microbial hazards, help in risk assessment, and guide interventions aimed at improving vendor hygiene, water quality, and sanitation practices.

2.4.1.3 Spoilage Microorganisms

Spoilage microbes include lactic acid bacteria, molds, and yeasts. While not typically pathogenic, these organisms degrade the sensory quality of food, leading to off-flavors, discoloration, gas production, and reduced shelf life. Their presence in street-vended foods reflects improper

storage or prolonged exposure to ambient conditions. Spoilage, though less threatening to health than pathogens, reduces consumer trust and contributes to food waste (Adams and Moss, 2008).

Spoilage organisms represent another critical classification of microbial contamination in street-vended foods, as they are responsible for reducing the sensory and nutritional quality of food products, thereby affecting consumer acceptability and safety. Unlike pathogenic or indicator organisms, spoilage microbes such as molds, yeasts, and certain bacteria including *Pseudomonas* spp*., Lactobacillus* spp*., and Bacillus* spp. primarily cause undesirable changes in taste, odor, texture, and appearance (Gram *et al*., 2002). In African and Asian cities where street vending is prevalent, improper storage, high ambient temperatures, and repeated reheating create favorable conditions for the proliferation of spoilage organisms, especially in foods like fried snacks, cooked rice, meat stews, and beverages (Mensah *et al*., 2002). For instance*, Pseudomonas* fluorescens has been frequently associated with slime formation and off-odors in meat and fish sold by vendors, while yeasts such as Candida and Saccharomyces contribute to souring of fruit juices and alcoholic beverages (Adams and Moss, 2008). Molds including *Aspergillus* and Penicillium can colonize baked goods and fried snacks, producing visible growth and sometimes secondary metabolites like mycotoxins, which present additional health risks beyond spoilage (Bankole and Adebanjo, 2003). Spoilage is particularly concerning in the context of street-vended food because consumers often lack access to refrigeration, and foods may be stored for prolonged periods under unhygienic conditions, accelerating microbial growth (Muinde and Kuria, 2005). Although spoilage organisms may not directly cause acute foodborne illness, their presence compromises food quality, shortens shelf life, and leads to economic losses for vendors, while also signaling lapses in hygiene and storage practices that could facilitate pathogenic contamination. Thus, addressing spoilage contamination requires improved vendor training,

stricter regulation of food handling environments, and better infrastructure to ensure safe storage and distribution.

2.4.1.4 Viral Contaminants

Enteric viruses such as noroviruses, rotaviruses, and hepatitis A virus are increasingly recognized in street foods, especially those prepared with contaminated water or handled with poor hygiene. Unlike bacteria, viruses cannot multiply in food but remain infectious, causing gastroenteritis and hepatitis outbreaks even at low doses (Mensah *et al*., 2012). Viral contaminants form an important classification of microbial hazards in street-vended foods, particularly because viruses are highly infectious at low doses and are often resistant to environmental stresses that would inactivate bacteria. Unlike bacteria, viruses cannot multiply in food but are transmitted through fecal–oral routes, poor hygiene, or the use of contaminated water during preparation and washing (Koopmans and Duizer, 2004). Among the most frequently reported foodborne viruses associated with street foods are human noroviruses, hepatitis A virus (HAV), and rotaviruses, all of which are linked to gastroenteritis outbreaks in urban and peri-urban populations (Mattison, 2011). For instance, noroviruses have been identified as leading causes of foodborne illness globally due to their extreme stability on food contact surfaces and their ability to persist in ready-to-eat items such as fresh produce, fruit juices, and salads sold by vendors (Patel *et al*., 2009). Similarly, HAV contamination in street-vended shellfish, raw vegetables, and dairy-based products has been documented, especially in low-resource settings where untreated water is used for food processing (Feldt *et al*., 2017). The informal food vending sector in Africa and Asia is particularly vulnerable to viral contamination because of inadequate sanitation infrastructure, lack of access to potable water, and limited awareness among vendors regarding viral transmission (Mensah *et al*., 2002). From a food microbiology perspective, viruses pose unique

challenges since conventional bacterial indicators like coliforms do not reliably predict their presence, necessitating more specific molecular detection methods such as RT-PCR (Bosch *et al*., 2018). Viral contaminants therefore represent not only a significant public health risk but also a critical gap in surveillance, as their outbreaks are often underreported compared to bacterial foodborne diseases. Strengthening vendor hygiene training, improving water quality, and implementing viral monitoring protocols are essential steps to mitigate their impact on street-vended foods.

2.4.1.5 Parasitic Contaminants

Protozoan parasites such as *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium* spp. have been reported in fresh produce, fruit juices, and minimally processed foods sold by street vendors. Contamination typically arises from washing with unsafe water or handling by infected individuals. These parasites contribute to diarrheal diseases, malnutrition, and poor health outcomes in vulnerable groups (Rane, 2011). Parasitic contaminants are a significant but often underreported category of microbial contamination in street-vended foods, posing major public health risks due to their ability to cause chronic infections and malnutrition in affected populations. Unlike bacteria and viruses, many parasites have environmentally resistant cysts, oocysts, or eggs that enable them to survive harsh conditions, including poor sanitation and inadequate food handling practices (Slifko *et al*., 2000). Street-vended foods commonly contaminated with parasites include fresh fruits, salads, juices, and undercooked meat or fish, particularly when vendors use untreated water for washing or preparation (Nyarango *et al*., 2008). Protozoan parasites such as *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium parvum* are frequently linked with foodborne outbreaks, causing diarrhea, abdominal pain, and in severe cases, long-term gastrointestinal disorders (Karanis *et al*., 2007). Helminthic parasites,

including *Ascaris lumbricoides, Trichuris trichiura,* and *Taenia* spp., are also of concern, as their eggs and larvae can contaminate street foods through fecal pollution, inadequate cooking, or unhygienic food handling (Duedu *et al*., 2014). In African cities, for example, high prevalence of intestinal parasites in street foods has been documented, with contamination traced to poor waste disposal systems, lack of potable water, and vendors' limited knowledge of food safety (Alimi, 2016). From a food microbiology standpoint, detection of parasitic contaminants is challenging, as their small size and morphological similarity to non-pathogenic species require specialized methods such as microscopy, immunoassays, or molecular diagnostics (Smith *et al*., 2007). The persistence of parasitic infections linked to street-vended foods not only reflects hygiene lapses but also highlights broader socio-economic vulnerabilities, as these infections disproportionately affect low-income urban populations with limited access to healthcare and safe food. Therefore, addressing parasitic contamination in street foods demands improved sanitation infrastructure, strict monitoring of water sources, and vendor education on safe preparation practices to reduce risks of transmission.

2.4.2 Sources of microbial contamination

The sources of microbial contamination in street-vended foods are diverse and interconnected, reflecting both environmental and human factors that compromise food safety in informal settings. One of the primary sources is the use of unsafe water for cooking, washing raw ingredients, or diluting beverages, as untreated water often harbors pathogens such as *Escherichia coli , Salmonella*, and protozoan cysts (Feglo and Sakyi, 2012). Contamination also arises from poor vendor hygiene, including inadequate handwashing, handling money while serving food, and neglecting protective clothing, all of which facilitate the transfer of enteric bacteria and viruses to ready-to-eat meals (Monney *et al*., 2014). Environmental exposure plays a

further role, as foods displayed in open-air markets are frequently exposed to dust, flies, and other vectors that introduce pathogens and spoilage organisms (Muyanja *et al*., 2011). Cross-contamination from utensils, cutting boards, and storage containers that are not properly sanitized is another major source, especially in high-turnover street vending environments (Alimi, 2016). Additionally, raw materials themselves can act as vehicles of contamination; for example, vegetables irrigated with wastewater, improperly processed meat, and unpasteurized dairy often contain pathogenic microorganisms before reaching the vendor (Mensah *et al*., 2002). Inadequate temperature control, such as prolonged holding of cooked foods at ambient conditions, further promotes microbial growth and toxin production by organisms like *Staphylococcus aureus* and *Bacillus cereus* (Rane, 2011). Collectively, these sources highlight systemic challenges in the street food sector, where limited infrastructure, vendor knowledge gaps, and socio-economic constraints intersect to create a high risk of microbial contamination. Addressing these issues requires an integrated food safety approach, including improved access to potable water, training programs for vendors, and stricter monitoring of food handling practices to break the chain of contamination at its sources.

2.4.2.1 Water

Unsafe or untreated water used in cooking, washing ingredients, or preparing beverages can introduce pathogens such as *E. coli, Salmonella*, and protozoan cysts into foods (Feglo and Sakyi, 2012). Water is one of the primary and most critical sources of microbial contamination in street-vended foods, particularly in developing countries where access to safe and potable water is limited. Contaminated water used for washing raw vegetables, fruits, or seafood, for cooking, or for preparing beverages can directly introduce pathogenic bacteria, viruses, and parasites into foods consumed by the public. Common microbial contaminants linked to unsafe water include

Escherichia coli , Salmonella spp*., Listeria monocytogenes, Giardia lamblia*, and hepatitis A virus, which are capable of causing severe gastrointestinal infections and foodborne outbreaks (Feglo and Sakyi, 2012; Mensah *et al*., 2002). Street food vendors often rely on untreated water from rivers, wells, or municipal supplies with intermittent treatment, and reuse water for multiple purposes, increasing the risk of microbial proliferation and cross-contamination (Alimi, 2016). Additionally, water used to rinse utensils, wash hands, or moisten food surfaces without prior treatment serves as a conduit for fecal contamination, highlighting the critical role of water quality in maintaining food safety (Monney *et al*., 2014). Microbiological analyses of street foods in African cities have consistently shown a strong correlation between the use of contaminated water and high levels of coliforms, *E. coli,* and other indicator organisms in ready-to-eat meals, reflecting both direct contamination and poor hygiene practices (Muyanja *et al*., 2011). From a food microbiology perspective, controlling waterborne contamination requires ensuring the use of potable water, proper storage in clean containers, and adherence to safe handling protocols during food preparation. Failure to address these issues not only increases the risk of foodborne disease but also undermines public confidence in street foods, which constitute a significant portion of daily dietary intake in urban populations.

2.4.2.2. Food Ingredients

Raw or improperly handled ingredients, including vegetables irrigated with wastewater, undercooked meat, unpasteurized milk, and seafood, may already harbor bacteria, viruses, or parasites before reaching vendors (Mensah *et al*., 2002). Food ingredients represent a major source of microbial contamination in street-vended foods, as raw materials often arrive at vending points already carrying pathogenic, spoilage, or indicator microorganisms. Vegetables, fruits, seafood, dairy products, and meat can harbor bacteria such as *Escherichia_coli ,*

Salmonella spp*., and Listeria monocytogenes, viruses* like hepatitis A, and protozoan parasites including *Giardia lamblia* and *Cryptosporidium parvum,* especially when grown, processed, or stored under unsanitary conditions (Mensah *et al*., 2002; Duedu *et al*., 2014). Contamination can occur at multiple stages, including irrigation of crops with untreated wastewater, improper slaughtering or handling of meat and poultry, inadequate pasteurization of milk, or prolonged storage under ambient temperatures, all of which enhance microbial survival and proliferation (Alimi, 2016). For instance, raw leafy vegetables and salad components sold by street vendors in African cities have consistently tested positive for fecal coliforms and Salmonella, indicating pre-existing contamination before preparation and vending (Feglo and Sakyi, 2012). Similarly, unpasteurized milk and soft cheese used in street foods provide a rich medium for bacterial growth, particularly under conditions of high ambient temperature and poor refrigeration (Monney *et al*., 2014). From a food microbiology perspective, the quality and handling of ingredients are critical determinants of the microbial safety of ready-to-eat foods. Interventions such as sourcing from certified suppliers, washing raw materials with potable water, and applying appropriate heat treatments can significantly reduce the microbial load and prevent foodborne illnesses. Failing to address ingredient contamination not only threatens consumer health but also contributes to outbreaks of foodborne diseases, economic losses, and undermines trust in street food vending.

2.4.2.3. Environment

Exposure to open-air conditions, dust, flies, rodents, and other vectors in street vending areas can deposit spoilage and pathogenic microorganisms on displayed foods (Muyanja *et al*., 2011). The environment in which street-vended foods are prepared, displayed, and sold constitutes a major source of microbial contamination, significantly influencing food safety outcomes. Open-air

vending sites are often located near roads, drains, waste disposal areas, or densely populated marketplaces, exposing foods to dust, aerosols, flies, rodents, and other environmental vectors that carry bacteria, viruses, and parasites (Muyanja *et al*., 2011). Flies, in particular, are known to transmit enteric pathogens such as *Salmonella, Shigella,* and *Escherichia coli*_ from fecal sources to ready-to-eat foods through mechanical transfer, while dust and aerosols deposit spores of spoilage organisms like *Bacillus* spp. and *Aspergillus* spp. onto food surfaces (Rane, 2011). Environmental contamination is exacerbated by poor drainage, stagnant water, and accumulated refuse in vending areas, which create breeding grounds for pathogenic microbes and increase the risk of food spoilage and foodborne infections (Mensah *et al*., 2002). Moreover, the lack of physical barriers, such as covers or protective display units, leaves foods directly exposed to environmental contaminants, while extreme temperatures further promote microbial growth on perishable items like cooked rice, meat stews, and dairy-based snacks (Alimi, 2016). Environmental contamination not only compromises the microbial quality of street foods but also complicates hygiene management, as even properly handled ingredients can be contaminated post-preparation. From a food microbiology perspective, addressing environmental contamination requires targeted interventions, including improved site selection, waste management, installation of protective display units, and periodic environmental monitoring to minimize exposure to pathogens and spoilage organisms. Such measures are essential to ensure that street-vended foods are safe for consumption and to reduce the burden of foodborne diseases in urban populations.

2.4.2.4. Equipment and Utensils

Contaminated knives, cutting boards, cooking pots, and serving containers that are poorly cleaned can transfer microbes between raw and cooked foods, leading to cross-contamination

(Alimi, 2016). Equipment and utensils play a pivotal role as sources of microbial contamination in street-vended foods, serving as vehicles for cross-contamination when not properly cleaned or sanitized. Knives, cutting boards, pots, serving spoons, and storage containers can harbor a wide range of bacteria, viruses, and parasites, particularly when used for multiple food types without adequate washing (Alimi, 2016). For instance, cutting boards used for raw meat or fish can retain *Salmonella, Listeria monocytogenes, or Escherichia coli,* which may then be transferred to cooked or ready-to-eat foods, leading to foodborne illness (Feglo and Sakyi, 2012). Similarly, improperly cleaned serving utensils and containers used for dairy products, sauces, or beverages can support the proliferation of spoilage organisms such as *Bacillus cereus* and yeasts, affecting both food quality and safety (Mensah *et al*., 2002). In many informal street food settings, vendors lack access to sufficient water or cleaning agents, and utensils may be rinsed in contaminated water or left at ambient temperature, which further encourages microbial growth (Monney *et al*., 2014). Moreover, repeated handling of utensils with bare hands or without gloves introduces additional microbial load, including pathogenic bacteria and viruses, which exacerbates contamination risks (Muyanja *et al*., 2011). From a food microbiology perspective, the sanitation of equipment and utensils is critical to breaking the chain of contamination, and strategies such as frequent washing with potable water, use of food-grade sanitizers, and separation of tools for raw and cooked foods can significantly reduce microbial hazards. Addressing contamination from utensils and equipment is therefore essential not only for maintaining the microbial quality of street-vended foods but also for preventing foodborne disease outbreaks in densely populated urban areas.

2.4.2.5. Vendor Handling Practices

Poor personal hygiene, such as inadequate handwashing, handling money while serving food, and lack of protective clothing, is a major source of microbial contamination in ready-to-eat foods (Monney *et al*., 2014). Vendor handling practices represent a critical source of microbial contamination in street-vended foods, as improper food handling directly facilitates the transfer of pathogenic and spoilage microorganisms to ready-to-eat meals. Street food vendors often handle money, raw ingredients, and cooked foods without adequate hand hygiene, creating opportunities for bacteria, viruses, and parasites to contaminate food surfaces (Alimi, 2016). Pathogenic microorganisms commonly associated with poor handling include *Escherichia coli , Salmonella* spp., *Staphylococcus aureus,* and enteric viruses such as hepatitis A and norovirus, which can cause gastrointestinal illnesses ranging from mild diarrhea to severe systemic infections (Mensah *et al*., 2002; Muyanja *et al*., 2011). In many informal vending settings, vendors lack access to clean water or handwashing facilities, fail to wear protective clothing such as gloves or aprons, and may store cooked and raw foods in close proximity, further increasing cross-contamination risks (Monney *et al*., 2014). The repeated touching of food items, utensils, and money without proper sanitation allows microbial transfer and rapid multiplication, particularly under tropical climates where high ambient temperatures favor bacterial growth (Feglo and Sakyi, 2012). Additionally, inadequate knowledge of safe food handling among vendors contributes to contamination, as observed in studies of urban markets in Ghana and Uganda, where a significant proportion of vendors did not follow recommended hygiene practices (Muyanja *et al*., 2011). From a food microbiology perspective, interventions such as structured hygiene training, provision of handwashing stations, use of gloves, separation of raw and cooked foods, and regular monitoring of vendor practices are essential to minimize

contamination and prevent foodborne disease outbreaks. Ensuring safe vendor handling practices is therefore fundamental to safeguarding public health, maintaining the microbiological quality of street-vended foods, and sustaining consumer confidence in informal food markets.

2.5 Food-borne pathogens associated with street vended food

Street-vended foods are frequently implicated in the transmission of diverse foodborne pathogens, which constitute a major public health concern, particularly in urban areas of developing countries. Bacterial pathogens such *as Escherichia coli , Salmonella* spp*., Shigella* spp*., Staphylococcus aureus, Listeria monocytogenes,* and *Bacillus cereus* are among the most commonly reported microorganisms isolated from ready-to-eat street foods, and they are responsible for outbreaks of diarrhea, gastroenteritis, and systemic infections (Feglo and Sakyi, 2012; Mensah *et al*., 2002). Viruses, including norovirus and hepatitis A virus, have also been detected in street-vended foods, particularly in items handled with bare hands or prepared with contaminated water, and can cause acute gastroenteritis and jaundice (Musa *et al*., 2017). Protozoan parasites such as *Giardia lamblia* and *Entamoeba histolytica* have been identified in salads, fresh fruits, and beverages sold by street vendors, reflecting fecal contamination from water or poor vendor hygiene (Duedu *et al*., 2014). Helminth eggs, including *Ascaris lumbricoides* and *Taenia* spp., are occasionally found in improperly cooked meat or unwashed vegetables, posing risks of intestinal parasitic infections (Alimi, 2016). Studies across African cities have shown that these pathogens are frequently linked to street-vended foods such as cooked rice dishes, fried snacks, sandwiches, and fresh produce, with microbial loads often exceeding acceptable safety limits (Muyanja *et al*., 2011). Contributing factors include inadequate cooking or reheating, improper storage at ambient temperatures, contaminated utensils, and insufficient personal hygiene among vendors. From a food microbiology standpoint,

the presence of these pathogens highlights the urgent need for surveillance, microbiological testing, and implementation of preventive measures such as proper cooking, safe water use, and vendor hygiene training to reduce foodborne disease incidence.

2.5.1. *Escherichia coli* (*E. coli*)

Commonly found in undercooked meat, raw vegetables, and improperly handled cooked foods. Pathogenic strains can cause diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (Feglo and Sakyi, 2012). *Escherichia coli* is one of the most frequently reported bacterial pathogens associated with street-vended foods, particularly in developing countries where hygiene practices and infrastructure are limited. While many strains are harmless commensals of the human intestine, pathogenic variants such as enterotoxigenic (ETEC), enterohemorrhagic (EHEC), and enteropathogenic (EPEC) *E. coli* are capable of causing severe gastrointestinal diseases, including diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (Mensah *et al*., 2002; Feglo and Sakyi, 2012). Street foods, including cooked rice, salads, fresh vegetables, sandwiches, and dairy-based snacks, are particularly vulnerable to *E. coli* contamination due to multiple factors such as use of untreated water, cross-contamination from raw meat or vegetables, and inadequate hand hygiene among vendors (Alimi, 2016). Microbiological surveys in African cities have revealed high counts of fecal coliforms and *E. coli* in ready-to-eat foods, indicating not only contamination from the ingredients themselves but also post-preparation contamination during handling, storage, and vending (Muyanja *et al*., 2011). The pathogen's persistence in foods is facilitated by its ability to survive under refrigeration, resist mild acidity, and adhere to surfaces such as cutting boards and utensils, which contributes to cross-contamination risks (Monney *et al*., 2014). From a food microbiology perspective, preventing *E. coli* contamination requires a combination of measures, including sourcing ingredients from safe suppliers, washing

and sanitizing vegetables with potable water, thorough cooking of foods, separating raw and cooked items, and enforcing strict hand hygiene and sanitation practices among vendors. Failure to address *E. coli*_contamination in street foods can lead to widespread foodborne outbreaks, posing significant public health risks and economic losses in urban communities heavily reliant on informal food markets.

2.5.2. *Shigella* spp.

Frequently transmitted via contaminated water or foods handled by infected vendors. Causes shigellosis, characterized by diarrhea and dysentery (Muyanja *et al*., 2011). *Shigella* species are significant bacterial pathogens associated with street-vended foods, particularly in developing countries, and are a leading cause of bacillary dysentery among urban populations. These bacteria are highly infectious, with as few as 10–100 organisms capable of causing disease, and are primarily transmitted through the fecal-oral route via contaminated water, food, or hands (Mensah *et al*., 2002; Muyanja *et al*., 2011). Street foods, such as salads, fresh vegetables, sandwiches, and ready-to-eat meals, are particularly vulnerable to *Shigella* contamination due to improper washing of raw ingredients, use of untreated water, inadequate hand hygiene among vendors, and cross-contamination from raw or undercooked foods (Feglo and Sakyi, 2012). Studies conducted in African cities have shown a considerable prevalence of *Shigella* in street-vended foods, reflecting the combined effects of poor sanitation infrastructure, lack of access to clean water, and insufficient vendor training in safe food handling (Alimi, 2016). Microbiologically, *Shigella* can survive on food surfaces and utensils, and under favorable environmental conditions, enabling it to persist in ready-to-eat foods sold in open-air markets. Preventive strategies include proper washing of vegetables and fruits with potable water, thorough cooking of potentially contaminated ingredients, separation of raw and cooked foods,

and rigorous adherence to personal hygiene practices among vendors. Addressing *Shigella* contamination in street foods is essential to reduce the incidence of foodborne dysentery, protect public health, and sustain consumer confidence in informal food markets.

2.5.3. *Listeria monocytogenes*

Associated with ready-to-eat foods like soft cheeses and cooked meats. Can cause listeriosis, especially dangerous in pregnant women, neonates, and immunocompromised individuals (Feglo and Sakyi, 2012). *Listeria monocytogenes* is a significant foodborne pathogen associated with ready-to-eat street-vended foods, notable for its ability to cause listeriosis, a serious infection with high morbidity and mortality rates among pregnant women, neonates, the elderly, and immunocompromised individuals. Unlike many bacteria, *L. monocytogenes* can grow at low temperatures, survive under high salt concentrations, and persist in moist environments, making it particularly resilient in food vending contexts where refrigeration is inadequate (Alimi, 2016; Feglo and Sakyi, 2012). Street-vended foods such as soft cheeses, dairy-based desserts, cooked meats, and salads are particularly vulnerable to contamination, especially when prepared with untreated water, handled by vendors without proper hygiene, or stored for extended periods under ambient conditions (Mensah *et al*., 2002). Microbiological surveys in urban African markets have reported sporadic isolation of *L. monocytogenes* from ready-to-eat foods, indicating that even low-level contamination can pose severe public health risks (Muyanja *et al*., 2011). Contamination can occur at multiple stages, including raw ingredient sourcing, food preparation, utensil use, and vendor handling. Preventive strategies include sourcing safe raw materials, maintaining cold chain storage, thoroughly cooking perishable items, separating raw and cooked foods, and implementing rigorous vendor hygiene and sanitation practices. Addressing *L. monocytogenes* contamination in street-vended foods is therefore critical to

preventing listeriosis outbreaks, safeguarding vulnerable populations, and ensuring the microbiological safety of informal food sectors.

2.5.4. Norovirus

Transmitted via contaminated water or food handled by infected vendors. Causes acute gastroenteritis and vomiting (Musa *et al*., 2017). Norovirus is a highly contagious viral pathogen and a major cause of foodborne gastroenteritis globally, including in street-vended foods, particularly in developing countries. This virus is commonly transmitted through the fecal-oral route, contaminated water, and improper handling of ready-to-eat foods, making street foods such as fresh salads, sandwiches, fruits, and beverages particularly vulnerable (Musa *et al*., 2017; Verhoef *et al*., 2015). Norovirus is resilient in environmental conditions, capable of surviving on surfaces, utensils, and food for extended periods, and is resistant to many routine disinfectants, which increases the risk of outbreaks (Mattison *et al*., 2007). Contamination often arises from infected food handlers who do not wash hands adequately after using the toilet or from the use of untreated water for washing ingredients or cleaning utensils (Baert *et al*., 2008). Studies conducted in urban African markets have documented the presence of norovirus in street-vended foods, particularly during peak vending hours when hygiene practices are compromised and high temperatures facilitate viral persistence (Musa *et al*., 2017). From a food microbiology perspective, preventive measures include strict personal hygiene among vendors, use of safe potable water for food preparation and cleaning, thorough washing of raw ingredients, and regular sanitation of utensils and surfaces. Implementation of these measures is critical to reducing the incidence of norovirus-related gastroenteritis, preventing large-scale foodborne outbreaks, and ensuring the safety of street-vended foods in urban and peri-urban settings.

2.5.5. Hepatitis A virus

Occurs in contaminated water, raw vegetables, or street foods handled by infected individuals. Causes liver inflammation, jaundice, and malaise (Musa *et al*., 2017). Hepatitis A virus (HAV) is a significant foodborne viral pathogen associated with street-vended foods, particularly in developing countries where sanitation and hygiene infrastructure are limited. HAV is transmitted primarily via the fecal-oral route, often through consumption of contaminated water, raw or undercooked foods, and ready-to-eat meals handled by infected vendors (Koffi-Nevry *et al*., 2017; Le Guyader *et al*., 2006). Street-vended foods such as fresh vegetables, fruits, salads, sandwiches, and beverages are especially susceptible to HAV contamination due to improper washing, use of untreated water, and inadequate hand hygiene practices among vendors. Studies in urban African and Asian markets have documented HAV presence in these foods, highlighting the risk of outbreaks in densely populated areas where street foods constitute a major component of the daily diet (Musa *et al*., 2017). HAV is highly resistant to environmental stress, capable of surviving on food surfaces and in water for prolonged periods, which facilitates transmission and persistence in informal food markets. From a food microbiology perspective, preventive measures include thorough washing of raw ingredients with potable water, proper cooking of perishable items, separation of raw and cooked foods, strict personal hygiene for food handlers, and public health education to raise awareness about contamination risks. Implementation of these measures is essential for preventing HAV-related outbreaks, safeguarding public health, and ensuring the microbiological safety of street-vended foods.

2.5.6. *Entamoeba histolytica*

Detected in improperly washed vegetables and water. Causes amoebic dysentery and liver abscesses (Duedu *et al*., 2014). *Entamoeba histolytica* is a protozoan parasite and an important foodborne pathogen frequently associated with street-vended foods, particularly in developing countries where sanitation and hygiene are often inadequate. This parasite causes amoebiasis, which may manifest as mild diarrhea, dysentery, or severe extraintestinal infections such as liver abscesses (Duedu *et al*., 2014; Alimi, 2016). Street foods such as salads, fresh fruits, beverages, and improperly washed vegetables are especially vulnerable to contamination due to the use of untreated water for washing, handling by infected food vendors, and exposure to unhygienic preparation environments. Studies conducted in African cities have reported the presence of *E. histolytica* cysts in street-vended foods, highlighting the risk of infection in urban populations that rely heavily on these foods for daily nutrition (Duedu *et al*., 2014). The cyst form of the parasite is highly resistant to environmental stress, capable of surviving on food surfaces and in water for extended periods, which facilitates transmission through the fecal-oral route. From a food microbiology perspective, preventive measures include thorough washing of raw ingredients with safe potable water, proper cooking of perishable foods, strict personal hygiene among food handlers, and education on safe food handling practices. Implementing these measures is crucial for reducing the prevalence of amoebiasis, preventing outbreaks associated with street-vended foods, and safeguarding public health in communities dependent on informal food markets.

2.6 Previous reports of contamination and outbreaks linked to street-vended foods in Nigeria

Several microbiological surveys and outbreak analyses indicate that street-vended foods in Nigeria frequently carry foodborne bacteria and have been implicated as a likely risk factor in foodborne and water-related outbreaks. Early and repeatedly cited microbiological assessments show high counts of indicator organisms and the presence of enteric pathogens in commonly vended ready-to-eat foods. For example, Umoh and Odoba's investigation of ready-to-eat street foods in Zaria found high total aerobic counts and significant contamination with *Bacillus cereus* and coagulase-positive *Staphylococcus aureus* among sampled items (Umoh and Odoba, 1999). A multi-city Nigerian survey also reported frequent isolation of *Escherichia coli* from street-vended foods and ready-to-eat vegetables an indicator of fecal contamination and poor handling practices (Oluwafemi *et al.,* 2013). More recent reviews and country-level analyses confirm that *E. coli, Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and other enteric organisms are commonly recovered from Nigerian street foods, reflecting ongoing contamination problems across settings (Mazi, 2023; Olabode and colleagues, 2013).

Beyond point prevalence studies, epidemiological investigations of large outbreaks in Nigeria emphasize setting-specific drivers where informal food markets and open-air food vending amplify transmission risk. The 2018 national cholera epidemic (43,996 cases; 836 deaths) affected many urban areas and investigations of drivers in Nigeria highlighted overcrowded markets, poor sanitation and unsafe food and water practices conditions in which street-vended foods and market food hygiene are plausible contributing factors to transmission, even when water contamination is the immediate vehicle (Elimian *et al*., 2019; Eneh *et al*., 2024). Several localized outbreak reports and public-health reviews similarly identify unregulated food vending

and inadequate vendor hygiene as recurring contributors to gastroenteritis clusters in Nigerian towns (Mazi, 2023).

A microbiological appraisal of street foods in Lagos and other cities documented *Salmonella* (including *S. typhimurium*) and other opportunistic pathogens in a sizable proportion of vended foods, suggesting that improperly cooked or post-cooking contaminated items can act as reservoirs for Salmonella transmission (Adebayo *et al.,* Mazi, 2023). Port Harcourt and other urban centers have produced similar findings: substantial fractions of sampled RTE foods exceeded acceptable microbial limits, and vendor practices (lack of potable water, hand hygiene, separation of raw/cooked food) were repeatedly associated with contamination (Oluwafemi *et al*., 2013).

In May–July 2011 a major outbreak of Shiga-toxin–producing *E. coli* O104:H4 caused thousands of cases of gastroenteritis and an unusually high number of hemolytic-uremic syndrome (HUS) cases in Germany and neighbouring countries; epidemiological and trace-back investigations identified fenugreek sprouts as the most likely vehicle of infection, highlighting how fresh, minimally processed plant foods can transmit highly virulent bacterial strains when contaminated at seed or irrigation stages. The outbreak underlined weaknesses in international seed/produce supply chains and the need for routine pathogen surveillance of sprouted seeds and fresh produce.

South Africa experienced the world's largest recorded listeriosis outbreak (Jan 2017–Jul 2018), with over 1,000 laboratory-confirmed cases. Whole-genome sequencing linked most clinical isolates to a single *Listeria monocytogenes* sequence type and traced the source to contaminated processed ready-to-eat meat (polony) produced at a food-processing facility. This case emphasized the severe risk posed by RTE meat products when *Listeria* gains access to

processing environments and persists in niches resistant to routine cleaning, and demonstrated the power of WGS for outbreak source attribution

Umoh and Odoba performed one of the earlier systematic microbiological surveys of ready-to-eat (RTE) street foods in Zaria, Nigeria. They sampled a range of commonly vended items and reported high total aerobic counts, frequent isolation of *Bacillus cereus* and coagulase-positive *Staphylococcus aureus*, and evidence that many samples exceeded acceptable microbiological limits. The authors attributed contamination to inadequate vendor hygiene, use of contaminated water, and environmental exposure at vending sites findings that established a baseline concern for RTE street foods in Nigeria.

Akinyemi and colleagues surveyed vended foods in Lagos and reported broad bacterial contamination (including *E. coli, Salmonella* spp. and *Staphylococcus aureus*) across many food types. Critically, the study documented multidrug resistance among several isolates: resistance to fluoroquinolones (ciprofloxacin, ofloxacin) and some third-generation cephalosporins was observed in *E. coli, S. Typhimurium* and *Klebsiella* isolates, while other isolates remained susceptible to certain cephalosporins and levofloxacin. The paper highlighted vended foods as potential reservoirs for dissemination of emerging drug-resistant Enterobacteriaceae in urban Nigeria.

A multi-city investigation that included several Nigerian urban centres examined RTE foods and ready-to-eat vegetables and repeatedly isolated fecal indicator bacteria *(E. coli)* and other enteric organisms, indicating widespread fecal contamination and poor handling practices. The study's findings reinforced that contamination is not limited to a single city but is a recurrent problem across Nigerian markets, with implications for foodborne disease risk among urban consumers.

Makinde *et al*. applied a modern approach (16S rRNA sequencing plus targeted biotoxin analysis) to 199 RTE food samples vended in Lagos (including bread, eko, shawarma, aadun, biscuits and kokoro). The study provided detailed bacteriological profiles, revealing a diverse bacterial community including taxa of public-health concern, and also screened for biotoxins. Their work not only confirmed frequent bacterial contamination of vended foods but also demonstrated the value of molecular tools for comprehensive surveillance of RTE foods in Nigerian urban settings.

Numerous smaller, city-level studies (Port Harcourt, Ibadan, Enugu and other locales) have repeatedly reported that substantial fractions of sampled street foods exceed microbiological safety limits and harbour *E. coli*, Salmonella spp., Staphylococcus aureus and other enteric organisms; vendor practices

2.7 Routes of Transmission of Enteric Pathogens

Enteric pathogens are primarily transmitted through the fecal–oral route, with contaminated food, water, and hands acting as key vehicles. In the context of street-vended foods, poor sanitation, inadequate cooking, and unhygienic handling practices create multiple opportunities for contamination and human infection (Barro *et al*., 2007; Rane, 2011). Transmission routes can be broadly grouped into foodborne, waterborne, person-to-person, and environmental pathways, often overlapping in informal food environments.

2.7.1. Food-borne Transmission

Food is the dominant transmission route for enteric pathogens, especially in urban settings where street-vended foods are widely consumed. Pathogens such as *Escherichia coli, Salmonella* spp*.,* and *Staphylococcus aureus* are introduced into foods through unsafe handling, cross-contamination from raw to cooked items, inadequate cooking, and prolonged storage at ambient

temperatures (Mensah *et al*., 2002; Umoh and Odoba, 1999). For example, *Salmonella* and *E. coli* have been frequently recovered from meat pies, rice, and suya (grilled meat) sold by vendors in Nigeria and Ghana, demonstrating the direct link between food handling practices and transmission risk (Akinyemi *et al*., 2013; Mensah *et al*., 2002).

2.7.2. Waterborne Transmission

Unsafe water used for food preparation, cooking, or washing utensils serves as a major transmission route for *Vibrio cholerae*, *Shigella* spp., and protozoan parasites like Giardia lamblia and Cryptosporidium parvum. Irrigation of fresh produce with contaminated water also facilitates transmission. Waterborne outbreaks in Nigeria and other African countries have often been linked to consumption of beverages or salads prepared with untreated water (Elimian *et al*., 2019; Thompson, 2000).

2.7.3. Person-to-Person Transmission

Direct person-to-person spread occurs when food handlers with poor personal hygiene contaminate food through unwashed hands or during serving. Pathogens such as *Shigella* and noroviruses require a low infectious dose, making them particularly prone to this mode of transmission (Kotloff *et al*., 1999; Patel *et al*., 2009). In many studies, the presence of coliforms on vendor hands and utensils has correlated with food contamination levels (Muyanja *et al*., 2011).

2.7.4. Environmental and Vector-Mediated Transmission

Environmental factors such as dust, flies, and unsanitary vending environments contribute significantly to contamination. Flies act as mechanical vectors, carrying enteric bacteria like *E. coli* and *Salmonella* from fecal matter to exposed food items (Barro *et al*., 2007). Open-air

vending close to refuse dumps or open drains increases the likelihood of environmental transmission. Additionally, improper waste disposal around vending sites exacerbates pathogen spread (Rane, 2011).

The transmission of enteric pathogens in street-vended foods is multifactorial, involving unsafe food handling, contaminated water, poor personal hygiene, and environmental exposure. Bacteria such as *E. coli*_and *Salmonella*, viruses such as noroviruses and HAV, and protozoa such as Giardia exploit these routes, leading to recurrent outbreaks and persistent foodborne illness burdens in developing countries (Mensah *et al*., 2002; Elimian *et al*., 2019; Patel *et al*., 2009). This underscores the urgent need for integrated interventions including hygiene education, improved water access, and stricter food safety monitoring in the informal food sector. Transmission of enteric pathogens is not restricted to a single pathway but often involves a combination of direct and indirect mechanisms that reinforce one another in settings where street-vended foods are prepared and consumed. These pathways have been widely documented in food microbiology and epidemiology research.

2.8 Concept of Virulence and Phenotypic Expression

In microbiology, virulence is defined as the degree of pathogenicity of a microorganism, describing its ability to invade a host, cause disease, and evade host defenses. Virulence is not a fixed property but rather a dynamic outcome of microbial genetic traits, host susceptibility, and environmental factors (Casadevall and Pirofski,1999). In food microbiology, virulence specifically refers to the attributes of foodborne pathogens such as *Escherichia coli , Salmonella enterica, Listeria monocytogenes* and *Staphylococcus aureus* that enable them to survive food environments, resist stress, and establish infection following ingestion (Scallan *et al*., 2011).

Virulence is mediated by virulence factors, which include toxins (e.g., *Staphylococcus aureus* enterotoxins), adherence molecules (e.g., fimbriae in *E._coli*), secretion systems (e.g., Type III secretion in *Salmonella*), and invasion proteins. These traits are often encoded on pathogenicity islands, plasmids, or phages, and their expression determines the severity of foodborne disease (Wagner and Waldor, 2002).

Virulence factors are molecular and structural traits that enable pathogens to colonize hosts, evade immune responses, and cause disease, and they play a crucial role in the public health risks posed by street-vended foods. Pathogens commonly isolated from street foods including *Escherichia coli , Salmonella* spp*., Shigella* spp*., Staphylococcus aureus,* and *Listeria monocytogenes* possess distinct virulence determinants such as adhesins, invasins, toxins, and secretion systems that enhance their pathogenic potential (Mensah *et al*., 2002; Alimi, 2016). For example, enterotoxigenic *E. coli* produces heat-labile and heat-stable toxins that induce diarrhea, while *S. aureus* synthesizes heat-stable enterotoxins capable of causing rapid-onset food poisoning (Feglo and Sakyi, 2012). *Salmonella* species utilize type III secretion systems to invade intestinal epithelial cells, and *Shigella* species produce *Shiga* toxins that disrupt protein synthesis in host cells, leading to dysentery (Muyanja *et al*., 2011). Street-vended foods, including salads, sandwiches, cooked rice, meat stews, and dairy-based snacks, are particularly vulnerable to contamination with virulent pathogens due to poor handling practices, inadequate cooking, cross-contamination, and storage under ambient conditions. The presence of virulence factors in pathogens not only increases the likelihood of symptomatic infections among consumers but also contributes to severe and sometimes systemic outcomes, especially in vulnerable populations such as children, pregnant women, and immunocompromised individuals. From a food microbiology perspective, mitigating virulence-associated risks requires integrated

interventions including proper cooking, separation of raw and cooked foods, rigorous hand hygiene, sanitation of utensils and equipment, and routine monitoring of microbial loads in street-vended foods. Addressing virulence in pathogens is therefore essential to prevent outbreaks, reduce morbidity and mortality, and ensure the safety of informal food markets in urban and peri-urban areas.

2.8.1. Adhesins (Attachment Factors)

Adhesion is a critical virulence factor that enables pathogens to attach to host epithelial cells, which is a prerequisite for colonization, persistence, and subsequent infection. In the context of street-vended foods, bacterial pathogens such as *Escherichia coli , Salmonella* spp., and *Listeria monocytogenes*_utilize adhesins specialized surface proteins or fimbriae to bind specifically to receptors on the intestinal mucosa of consumers (Mensah *et al*., 2002; Feglo and Sakyi, 2012). This attachment facilitates the establishment of infection by preventing the pathogen from being washed away by intestinal peristalsis or digestive fluids, thereby increasing the likelihood of disease. Street foods such as salads, sandwiches, cooked rice, meat stews, and dairy-based snacks are particularly vulnerable to contamination with adherent pathogens due to improper handling, cross-contamination, and the use of untreated water during preparation (Alimi, 2016). Microbiologically, adhesion not only supports colonization but also often triggers the expression of additional virulence determinants, including toxin production and invasion mechanisms, which exacerbate pathogenicity. Preventive strategies to mitigate adhesion-related risks in street-vended foods include thorough washing of raw ingredients with potable water, separation of raw and cooked foods, strict hand hygiene for vendors, and adequate cooking of perishable items. Adhesion as a virulence factor is therefore vital in food microbiology because it provides insight into how pathogens establish themselves in the host and informs effective food safety

interventions aimed at reducing the prevalence of foodborne illnesses linked to informal food markets.

2.8.2. Invasion Factors

Invasion is a critical virulence factor that allows pathogenic microorganisms to penetrate host epithelial cells, evade the immune system, and establish systemic or localized infections, making it highly relevant in the context of street-vended foods. Bacterial pathogens commonly associated with street foods, such as *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, and enteroinvasive *Escherichia coli*_, possess specialized invasion mechanisms that facilitate entry into intestinal epithelial cells and subsequent dissemination (Mensah *et al*., 2002; Alimi, 2016). For instance, *Salmonella* uses a type III secretion system to inject effector proteins into host cells, triggering cytoskeletal rearrangements that allow bacterial internalization, while *Listeria* expresses internalins that mediate uptake into epithelial cells and listeriolysin O that facilitates escape from phagosomes. Street-vended foods such as meat stews, cooked rice, sandwiches, salads, and dairy-based snacks are particularly susceptible to contamination with invasive pathogens due to poor handling, cross-contamination, and storage under ambient temperatures, which favor microbial survival. Invasion not only allows pathogens to establish infections more effectively but also enhances the severity and persistence of foodborne illnesses. Invasion as a virulence factor is essential for developing targeted interventions, including thorough cooking of perishable foods, proper hygiene practices by vendors, separation of raw and cooked foods, and environmental sanitation. Addressing invasion-mediated virulence is therefore crucial for reducing the risk of severe infections, protecting public health, and ensuring the safety of street-vended foods in urban and peri-urban communities.

2.8.3. Toxins

Toxin production is a pivotal virulence factor that enhances the pathogenic potential of microorganisms in street-vended foods by directly damaging host tissues or disrupting normal physiological processes. Bacterial pathogens such as *Staphylococcus aureus, Escherichia coli , Shigella* spp., and *Bacillus cereus* produce a range of toxins, including enterotoxins, *Shiga* toxins, and hemolysins, which are responsible for many foodborne illnesses (Mensah *et al*., 2002; Feglo and Sakyi, 2012). For instance, *S. aureus* synthesizes heat-stable enterotoxins that can induce rapid-onset vomiting and diarrhea even when the bacteria are no longer viable in the food, while enterotoxigenic *E. coli* produces heat-labile and heat-stable toxins that disrupt electrolyte balance in the intestines, causing diarrhea. *Shigella* species secrete Shiga toxins that inhibit protein synthesis in intestinal epithelial cells, leading to severe dysentery, and *B. cereus* produces emetic and diarrheal toxins in improperly stored rice and pasta dishes (Alimi, 2016). Street-vended foods, including cooked rice, meat stews, dairy-based snacks, and fried pastries, are particularly susceptible to toxin-producing pathogens due to improper cooking, prolonged storage at ambient temperatures, and cross-contamination during handling. From a food microbiology perspective, understanding toxin production is critical for assessing the risk associated with street-vended foods and implementing preventive strategies such as thorough cooking, rapid cooling, hygienic handling, and separation of raw and cooked foods. Addressing toxin-mediated virulence is essential for reducing foodborne disease outbreaks, protecting public health, and ensuring the safety of informal food markets.

2.8.4. Biofilm Formation

Biofilm formation is a critical virulence factor that allows microbial pathogens to adhere to surfaces, survive in hostile environments, and resist cleaning and disinfection, thereby increasing

the risk of contamination in street-vended foods. Pathogens commonly isolated from street foods, including *Escherichia coli , Staphylococcus aureus, Listeria monocytogenes*, and *Salmonella* spp., are capable of producing biofilms on food contact surfaces, utensils, and even on the foods themselves (Alimi, 2016; Feglo and Sakyi, 2012). These biofilms are structured communities of microorganisms embedded within an extracellular polymeric substance (EPS) matrix, which provides protection from environmental stressors such as heat, desiccation, and exposure to sanitizers. Street-vended foods such as salads, sandwiches, cooked rice, meat stews, and dairy-based snacks are particularly vulnerable because biofilm-forming pathogens can survive on utensils and preparation surfaces, leading to cross-contamination during handling and storage. The presence of biofilms complicates microbial eradication and increases the persistence of virulent pathogens in informal food markets. From a food microbiology perspective, understanding biofilm formation is essential for designing effective control measures, including thorough cleaning and sanitization of utensils, regular monitoring of preparation surfaces, proper storage of food items, and vendor training on hygiene practices. Addressing biofilm-mediated virulence is crucial for reducing microbial loads, preventing foodborne disease outbreaks, and ensuring the safety of street-vended foods consumed in urban and peri-urban communities.

2.8.5. Enzymes

Enzymes produced by microbial pathogens represent a major virulence factor that enhances their ability to colonize, invade, and damage host tissues, and they play a significant role in the contamination and pathogenicity of street-vended foods. Pathogens such as *Escherichia coli , Salmonella* spp*., Staphylococcus aureus, Listeria monocytogenes*, and *Shigella* spp. secrete various extracellular enzymes, including proteases, lipases, lecithinases, and hyaluronidases, which degrade host tissues and facilitate nutrient acquisition, tissue invasion, and dissemination

within the host (Mensah *et al*., 2002; Alimi, 2016). For example, proteases and lipases produced by *S. aureus* and *Listeria* break down proteins and lipids in host tissues, promoting colonization and foodborne disease, while lecithinases contribute to cell membrane disruption, enhancing bacterial spread. Street-vended foods, such as meat stews, cooked rice, sandwiches, dairy-based snacks, and fried pastries, are particularly vulnerable to contamination with enzyme-producing pathogens due to poor hygiene practices, cross-contamination, and storage under ambient conditions, which favor microbial survival and proliferation. From a food microbiology perspective, enzyme production is critical for pathogen virulence because it not only damages host tissues but also interacts synergistically with other virulence factors such as adhesins, toxins, and biofilm formation. Preventive strategies include thorough cooking, strict separation of raw and cooked foods, adequate sanitation of utensils and surfaces, and proper handling by vendors to minimize enzymatic activity and reduce the risk of foodborne infections. Understanding enzyme-mediated virulence is therefore essential for developing effective interventions to ensure the safety of street-vended foods and protect public health, especially in densely populated urban areas.

2.9 How Virulence Traits and Phenotypic Expression Enhance Pathogenicity

Street-vended foods create a unique ecological niche where pathogens survive, multiply, and express virulence traits that increase their disease-causing potential. The interaction between virulence determinants and phenotypic expression determines whether contamination results in harmless exposure or full-blown foodborne illness. Virulence traits significantly enhance the pathogenicity of microorganisms associated with street-vended foods by facilitating colonization, survival, invasion, and tissue damage within the host, thereby increasing the severity and incidence of foodborne illnesses. Pathogens commonly found in street foods, such as *Escherichia*

coli, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes,* express virulence factors including adhesins, toxins, hemolysins, enzymes, invasion proteins, and biofilm-forming capabilities that act synergistically to promote infection (Mensah *et al*., 2002; Alimi, 2016). For example, adhesins allow *E. coli* and *Salmonella* to attach to intestinal epithelial cells, establishing a foothold for colonization, while enterotoxins produced by *S.* *aureus* and enterotoxigenic *E.* *coli* disrupt normal host cell function, causing diarrhea and vomiting. Hemolysins lyse red blood cells to release nutrients, enzymes degrade host tissues to facilitate invasion, and biofilm formation enhances bacterial persistence on food contact surfaces, utensils, and within the foods themselves, making them resistant to environmental stressors and sanitization (Feglo and Sakyi, 2012). Furthermore, invasion and intracellular survival mechanisms employed by *Listeria* and *Salmonella* allow pathogens to evade host immune responses, increasing infection severity. Street-vended foods, including salads, cooked rice, meat stews, dairy-based snacks, and fried pastries, are particularly susceptible to contamination with virulent pathogens due to improper handling, ambient temperature storage, and cross-contamination. Understanding how these virulence traits collectively enhance pathogenicity is crucial in food microbiology for designing effective preventive strategies, such as thorough cooking, hygienic food handling, separation of raw and cooked foods, and routine monitoring of microbial loads. Addressing the combined effects of virulence factors in street-vended foods is essential for reducing the prevalence and impact of foodborne disease outbreaks in urban and peri-urban communities

2.9.1. Survival and Colonization in Food Matrice

Virulence traits such as biofilm formation and stress response systems allow pathogens to persist on utensils, raw ingredients, and food surfaces despite cleaning or refrigeration. In street-food

environments, poor hygiene and ambient temperatures facilitate phenotypic expression of these traits. *Listeria monocytogenes* expresses the stress regulator σB, enabling survival in acidic foods like suya or fried meat and later causing listeriosis in consumers (Chaturongakul and Boor, 2006). Salmonella enterica biofilm formation on food contact surfaces enhances persistence and transmission in ready-to-eat foods (Bridier *et al*., 2015).

2.9.2. Attachment and Invasion of Host Cells

Pathogens in street-foods often rely on adhesins and invasins to establish infection after ingestion. Phenotypic expression of these surface proteins is critical once pathogens reach the intestinal mucosa. Enteropathogenic *E. coli* (EPEC) expresses intimin (phenotypic adherence trait), forming attaching-and-effacing lesions that disrupt intestinal epithelial cells (Nataro and Kaper, 1998). *Shigella* spp. expresses invasion plasmid antigens (Ipa proteins) that enable epithelial penetration and dysentery (Kotloff *et al*., 1999).

2.9.3. Production of Enterotoxins and Cytotoxins

Street-foods held at unsafe temperatures provide conditions for pathogens to produce toxins, one of the most important virulence traits in food microbiology. The phenotypic expression of toxin genes often occurs during food storage or in the host intestine. *Staphylococcus aureus* phenotypically expresses enterotoxins SEA–SEE, leading to rapid food poisoning outbreaks linked to meat pies and rice in Nigeria (Argudín *et al*., 2010). *E. coli* O157:H7 expresses *Shiga* toxins (Stx1, Stx2), causing hemorrhagic colitis; detection requires both genotypic (PCR for stx) and phenotypic (Vero cell assays) confirmation (Scallan *et al.*, 2011).

2.9.4. Immune Evasion and Persistence in Hosts

Some pathogens carried in street-foods enhance pathogenicity by evading host immune defenses. *Klebsiella pneumoniae* in poorly cooked foods expresses capsule K antigen, making it resistant to phagocytosis and complement (Podschun and Ullmann, 1998).*Salmonella enterica* uses Type III secretion systems (T3SS) to manipulate host immunity and establish systemic infection (Galán *et al*., 2014).

2.9.5. Stress Response and Environmental Adaptation

Street-foods are often exposed to fluctuating temperatures, pH, and sanitation levels. Virulence gene regulators respond to these stresses, triggering phenotypic expression *E. coli* O157:H7 activates its acid tolerance response (ATR) in acidic foods (like fruit juices sold by vendors), ensuring survival through gastric acid to colonize intestines (Ryu and Beuchat, 1999).*Vibrio cholerae* phenotypically expresses toxin-coregulated pilus (TCP) in contaminated water or foods, essential for colonization and cholera outbreaks in unsanitary environments (Faruque *et al*., 1998).

2.10 Methods of Studying Virulence Traits and Phenotypic Expression in Street-Vended Foods

Understanding virulence traits and their phenotypic expression in pathogens isolated from street-vended foods is essential for assessing public health risks. Researchers employ a combination of classical microbiological, molecular, and phenotypic approaches to identify, characterize, and quantify virulence factors studying the virulence phenotype of microorganisms requires a combination of culture-based, biochemical, and molecular techniques, each providing complementary insights into pathogenic potential. The initial step often involves culture

characterization, where microorganisms are grown on selective or differential media to observe colony morphology, growth patterns, and environmental adaptability. For example, some pathogens demonstrate the ability to grow at elevated temperatures or under nutrient-limited conditions, which may suggest adaptation to host environments (Anoop *et al*., 2015). Additionally, colony morphology, hemolysis patterns on blood agar, and pigmentation can provide preliminary indications of virulence, although these characteristics alone are insufficient for definitive virulence profiling (Preda *et al*., 2021).Following culture characterization, biochemical tests are employed to assess metabolic and enzymatic activities associated with virulence. Common assays include hemolysin production, gelatinase activity, urease production, and biofilm formation. Hemolysin production, for instance, facilitates tissue invasion by lysing host cells, while biofilm formation enhances microbial persistence and resistance to host defenses (Sibley *et al*., 2012). These phenotypic traits provide functional evidence of pathogenic potential; however, some virulence factors may not be expressed under laboratory conditions, limiting detection using biochemical methods alone (Zhang *et al*., 2021).To achieve a more precise and comprehensive assessment, molecular techniques are utilized for the detection of specific virulence genes. Polymerase chain reaction (PCR) enables the identification of genes encoding toxins, adhesins, and secretion systems. For example, detection of hlyA (hemolysin) or fimH (type 1 fimbriae) genes confirms the pathogenic potential of strains (Preda *et al*., 2021). Molecular approaches can also identify antimicrobial resistance genes, which are frequently linked to virulence traits. Advanced methods such as whole-genome sequencing (WGS) and metagenomic analysis provide high-resolution insights into the genetic basis of virulence, allowing identification of both known and novel virulence factors (Sibley *et al*., 2012; Zhang *et al*., 2021).Integrating culture, biochemical, and molecular approaches offers a robust framework

for virulence assessment. This combination allows detection of a wide spectrum of virulence determinants, from phenotypic expression to underlying genetic traits, providing a more complete understanding of microbial pathogenicity and informing targeted therapeutic or control strategies (Anoop *et al*., 2015; Preda *et al*., 2021; Sibley *et al*., 2012).

2.11 Overview of Antimicrobial Resistance as a Global Concern

Antimicrobial resistance (AMR) has emerged as one of the most pressing public health challenges of the 21st century, threatening the effective treatment of a wide range of infectious diseases (World Health Organization [WHO], 2020). AMR occurs when microorganisms such as bacteria, viruses, fungi, or parasites evolve mechanisms to withstand the effects of antimicrobial agents that were previously effective against them. This resistance can arise through genetic mutations or acquisition of resistance genes via horizontal gene transfer, leading to the proliferation of drug-resistant strains (Laxminarayan *et al*., 2020). The rapid global dissemination of these resistant strains has been facilitated by factors such as overuse and misuse of antibiotics in human medicine, veterinary practices, and agriculture, as well as inadequate infection prevention and control measures (O'Neill, 2016; Ventola, 2015).The clinical consequences of AMR are profound, as infections caused by resistant pathogens are associated with increased morbidity, mortality, and prolonged hospital stays. For instance, multidrug-*resistant Escherichia coli , Klebsiella pneumoniae*, and *Staphylococcus aureus* have been linked to higher rates of treatment failure, particularly in low- and middle-income countries where healthcare infrastructure may be limited (Prestinaci *et al*., 2015). Moreover, AMR exacerbates the burden on healthcare systems by increasing the costs of treatment, as second- or third-line antibiotics are often more expensive, less effective, or more toxic (Laxminarayan *et al*., 2020). The global health and economic implications of AMR have led the World Health Organization to

designate it as a priority threat, urging international cooperation to curb its spread (WHO, 2020). From an epidemiological perspective, AMR is not confined to hospitals; it has become a community-level problem, largely due to the circulation of resistant pathogens in food, water, and the environment. The emergence of resistant strains in the food chain, particularly in street-vended and ready-to-eat foods, poses a significant public health risk, contributing to outbreaks of foodborne illnesses that are difficult to treat (Holmes *et al*., 2016). Environmental contamination with antibiotics and resistant bacteria, including from wastewater and agricultural runoff, further accelerates the dissemination of resistance genes among microbial populations (Martinez, 2018). This interconnectedness between humans, animals, and the environment underlines the importance of the One Health approach in tackling AMR, integrating public health, veterinary, and environmental strategies (Robinson *et al*., 2016).

Global surveillance and stewardship programs have been identified as key strategies in combating AMR. The implementation of antimicrobial stewardship programs aims to optimize antibiotic use by ensuring appropriate selection, dosing, and duration of therapy. Simultaneously, enhanced monitoring and surveillance systems facilitate early detection of emerging resistant pathogens and the evaluation of interventions (Prestinaci *et al*., 2015; Holmes *et al*., 2016). Despite these efforts, challenges remain, particularly in resource-limited settings where diagnostic capacities are inadequate, and over-the-counter availability of antibiotics perpetuates misuse (Ventola, 2015). Addressing AMR therefore requires coordinated international action, public education, and investment in research for new antibiotics and alternative therapies, such as bacteriophages and immunomodulators, to mitigate the looming threat of a post-antibiotic era (O'Neill, 2016). In conclusion, antimicrobial resistance represents a multifaceted global concern with profound implications for human health, economic stability, and food security. The

convergence of misuse in healthcare, agriculture, and environmental dissemination accelerates its spread, necessitating integrated approaches that combine surveillance, stewardship, research, and international policy frameworks. Without sustained action, AMR threatens to reverse decades of medical progress and undermine the ability to manage infectious diseases effectively (WHO, 2020; Laxminarayan *et al*., 2020).

Antimicrobial resistance (AMR) is one of the most pressing threats to public health, food safety, and global development in the 21st century. It occurs when microorganisms such as bacteria, viruses, fungi, and parasites evolve mechanisms that protect them against the action of antimicrobial agents, rendering standard treatments ineffective. The World Health Organization (WHO) recognizes AMR as a "silent pandemic" that undermines decades of progress in medicine, agriculture, and microbiology (WHO, 2020).

2.12 Mechanisms of Antimicrobial Resistance

Antimicrobial resistance (AMR) has emerged as one of the most pressing global health challenges of the 21st century. Bacteria have evolved diverse strategies to survive exposure to antibiotics, which compromise the efficacy of treatment and contribute to persistent infections (Santajit and Indrawattana, 2016). Among the primary mechanisms, enzymatic inactivation plays a critical role. Bacteria produce enzymes that chemically degrade or modify antibiotics, rendering them inactive. A prominent example is β-lactamases, which hydrolyze the β-lactam ring of penicillins and cephalosporins, thereby neutralizing their antibacterial activity (Wright, 2005). Similarly, aminoglycoside-modifying enzymes can acetylate, phosphorylate, or adenylate aminoglycosides, preventing them from binding to bacterial ribosomes and inhibiting protein synthesis (Egorov and Dzyuba, 2018). These resistance determinants are often located on plasmids, facilitating horizontal gene transfer and rapid dissemination among bacterial

populations, which significantly exacerbates the spread of resistance in both clinical and community settings (Varela *et al*., 2021).Efflux pump-mediated resistance constitutes another major survival strategy employed by bacteria. Efflux pumps are membrane-bound proteins that actively expel antibiotics from the bacterial cell, reducing intracellular drug concentrations and thereby decreasing drug efficacy (Gaurav *et al*., 2023). These pumps can be highly specific, targeting single antibiotics, or broad-spectrum, conferring multidrug resistance, as observed in many Gram-negative pathogens (Thakur *et al*., 2021). Overexpression of efflux pumps is frequently induced in response to antibiotic exposure, and the encoding genes may be located on plasmids or chromosomal DNA, enabling both intrinsic and acquired resistance. The activity of efflux pumps can also synergize with other resistance mechanisms, such as enzymatic inactivation, thereby amplifying the survival advantage of bacterial populations under antimicrobial pressure (Reygaert, 2018).

In addition to enzymatic degradation and efflux, bacteria often evade antibiotics through target modification. This mechanism involves structural alterations of the molecular targets of antibiotics, which diminishes drug binding and compromises antimicrobial efficacy (Lambert, 2005). For example, mutations in ribosomal RNA prevent aminoglycosides from binding to bacterial ribosomes, while modifications in penicillin-binding proteins (PBPs) confer resistance to β-lactam antibiotics, as exemplified by methicillin-resistant Staphylococcus aureus (MRSA) (Wilson *et al*., 2020). Target modification can be driven by point mutations, acquisition of alternative genes via horizontal transfer, or regulatory changes that alter target expression, and these adaptations are heritable, contributing to the long-term persistence of resistant strains in bacterial populations.

Biofilm-associated resistance further complicates the treatment of bacterial infections. Biofilms are structured communities of bacteria embedded in an extracellular polymeric matrix that forms on biotic and abiotic surfaces, including medical devices (Liu *et al*., 2024). Within biofilms, bacteria exhibit heightened tolerance to antibiotics due to multiple factors, including restricted drug penetration, altered metabolic states, and the presence of dormant persister cells (Singh *et al*., 2025). Additionally, the dense biofilm matrix promotes horizontal gene transfer, facilitating the spread of resistance determinants among resident bacterial populations (Liu *et al*., 2024). This multifaceted resistance makes biofilm-associated infections particularly difficult to eradicate, often requiring higher doses of antibiotics or combination therapies. Collectively, these mechanisms enzymatic inactivation, efflux pumps, target modification, and biofilm-associated resistance demonstrate the remarkable adaptability of bacteria and underscore the urgent need for novel therapeutic strategies and antimicrobial stewardship to mitigate the rising threat of AMR.

Antimicrobial resistance (AMR) in enteric pathogens is mediated through diverse molecular mechanisms that reduce or eliminate the efficacy of antimicrobial agents. These mechanisms may be intrinsic (naturally encoded in the bacterial genome) or acquired (via mutation or horizontal gene transfer). In food microbiology, understanding these mechanisms is critical because resistant strains in street-vended foods can spread rapidly to humans, animals, and the environment (Blair *et al*., 2015; Munita and Arias, 2016).

2.12.1. Enzymatic Inactivation of Antibiotics

One of the most common mechanisms is the production of enzymes that degrade or modify antibiotics. β-lactamases hydrolyze the β-lactam ring of penicillins and cephalosporins, rendering them inactive. Extended-spectrum β-lactamases (ESBLs) in *E. coli* and *Klebsiella pneumoniae* are widely reported in foodborne isolates (Paterson and Bonomo, 2005). Aminoglycoside-

modifying enzymes (AMEs) alter aminoglycosides through acetylation, phosphorylation, or adenylation, reducing binding to ribosomal targets (Ramirez and Tolmasky, 2010).

2.12.2. Alteration of Antimicrobial Targets

Mutations or modifications in bacterial targets prevent antibiotics from binding effectively.Alterations in penicillin-binding proteins (PBPs) confer resistance to β-lactams in *Streptococcus pneumoniae* and *enterococci* (Hakenbeck *et al*., 2012).Mutations in DNA gyrase (gyrA) and topoisomerase IV (parC) genes lead to fluoroquinolone resistance in *Salmonella and E._coli_*strains isolated from foods (Hooper and Jacoby, 2015).Modification of ribosomal RNA by methyltransferases confers resistance to macrolides, lincosamides, and streptogramins (Roberts, 2008).

2.12.3. Efflux Pump Systems

Efflux pumps actively expel antimicrobial agents out of the bacterial cell, lowering intracellular concentrations.Multidrug efflux pumps such as AcrAB-TolC in *E. coli* and *Salmonella* confer resistance to tetracyclines, fluoroquinolones, and chloramphenicol (Nikaido & Pagès, 2012). Overexpression of efflux pumps is particularly concerning in foodborne pathogens, as it contributes to multidrug resistance and enhances survival in harsh food environments.

2.12.4. Reduced Membrane Permeability

Alterations in outer membrane porins reduce antibiotic uptake.Loss or modification of OmpF/OmpC porins in *E._coli_and Enterobacter* spp. reduces β-lactam and quinolone entry (Delcour, 2009).This mechanism often acts synergistically with efflux pumps to strengthen resistance phenotypes. In street-vended foods, these resistance mechanisms are of major concern because they not only compromise treatment efficacy but also serve as reservoirs of resistance

determinants that can disseminate within the food chain and human gut microbiota. A comprehensive understanding of these mechanisms is crucial for developing effective antimicrobial stewardship and food safety intervention.

2.13 Antimicrobial Resistance in Enteric Pathogens

Street-vended foods have become an important part of the diet in many developing countries due to their affordability, convenience, and cultural value. However, they are increasingly recognized as reservoirs for antimicrobial-resistant (AMR) enteric pathogens, posing serious public health risks. Enteric bacteria such as *Escherichia_coli_*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, and *Vibrio* spp. have frequently been isolated from street-vended foods and shown resistance to multiple classes of antimicrobials (Okonko *et al*., 2010; Igbinosa and Beshiru, 2019).

2.13.1. Multidrug-Resistant *Escherichia coli*

E.coli, a common fecal indicator and enteric pathogen, is widely reported in street foods such as meat products, rice, salads, and beverages. Studies from Nigeria and Ghana revealed that *E. coli* isolates exhibited resistance to ampicillin, tetracycline, and cotrimoxazole, with some strains producing extended-spectrum β-lactamases (ESBLs) (Odu and Okonko, 2017; Beshiru *et al*., 2016). Such findings suggest that street-vended foods may act as vehicles for ESBL-producing Enterobacteriaceae, which are of global concern due to limited treatment options (Paterson and Bonomo, 2005).

2.13.2. Drug-Resistant *Salmonella* spp.

Food-borne *Salmonella* is a leading cause of gastroenteritis worldwide, and its presence in ready-to-eat foods is well documented. Street-food isolates often display resistance to first-line

antibiotics including ampicillin, chloramphenicol, and trimethoprim–sulfamethoxazole (Ajayi *et al*., 2016). Resistance to fluoroquinolones and third-generation cephalosporins has also been observed, raising concerns of treatment failures in invasive Salmonellosis (Fashae *et al*., 2018).

2.13.3. Antibiotic-Resistant *Shigella* spp.

Shigella species, frequently associated with food-borne dysentery, have shown increasing resistance when isolated from street-vended foods. Reports from sub-Saharan Africa indicate resistance to tetracyclines, sulfonamides, and ampicillin (Niyogi, 2005). Such resistance complicates treatment, especially in vulnerable populations such as children, who are most affected by shigellosis.

2.13.4. Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Street-vended foods, particularly meat and dairy products, have been implicated as sources of *S. aureus*, including methicillin-resistant strains (MRSA). In Nigeria, *S. aureus* isolated from street foods showed resistance to penicillin, erythromycin, and cefoxitin, with the presence of mecA-mediated resistance mechanisms (Adesetan *et al*., 2019). MRSA in foods is a dual threat, acting as both a toxin-producing pathogen and a reservoir of transferable resistance genes.

2.13.5. *Vibrio* Species

Pathogenic *Vibrio* spp., including *V. cholerae* and *V. parahaemolyticus*, are occasionally isolated from ready-to-eat seafood and beverages. Resistance to tetracycline, ampicillin, and fluoroquinolones has been reported in strains from African and Asian street foods, reflecting the impact of antibiotic misuse in aquaculture and environmental contamination (Igbinosa and Okoh, 2010).

2.13.6. Implications for Public Health

The detection of AMR enteric pathogens in street-vended foods highlights their role as vehicles of resistance genes across the food chain. Horizontal gene transfer via plasmids and integrons facilitates the dissemination of multidrug resistance among enteric bacteria (Wellington *et al*., 2013). This poses serious risks in developing countries, where access to effective antimicrobials is already limited, and food safety regulation is poorly enforced.

Antimicrobial resistance (AMR) in foodborne pathogens has emerged as a critical public health risk because it compromises the treatment of common gastrointestinal and systemic infections. Resistant strains of *Escherichia_coli_*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., and *Staphylococcus aureus* are increasingly isolated from contaminated food and food environments, reducing the effectiveness of essential antibiotics (WHO, 2021). The risks associated with AMR in foodborne pathogens can be classified into therapeutic challenges, epidemiological consequences, and socioeconomic impacts.

CHAPTER 3

METHODOLOGY

3.1 Sample Collection

A total of nine (9) samples of food consisting of three (3) types of food rice, beans, and spaghetti were collected from each of the three locations. The nature of the food collected were cooked vended foods. Each samples was collected using sterile gloves and stored in sterile polythene bags to avoid cross-contamination.

3.2 Determination of pH

The pH of the samples were determined using a pH meter. Five grams (5g) of sample were weighed and ground using porcelain mortar and pestle. The ground sample were transferred to a beaker and 50ml of distilled water was added. Samples were left for thirty minutes (30 mins) and then filtered. The value of each sample filtrate were taken after submerging the pH probe in the water sample and held for a couple of minutes to achieve a stabilized reading.

3.3 Preparation of Culture Media

The culture media used were prepared according to the manufacturer's instructions. The media used were Nutrient Agar, MacConkey Agar and Salmonella *Shigella* Agar.

3.3.1 Nutrient Agar

28g of Nutrient Agar (NA) powder was dissolved in 1 litre of distilled water in a conical flask with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121c for 15 minutes. The medium was cooled to 45-50c and then dispensed aspectically into sterile petri dish in the laminar flow.

3.3.2 MacConkey Agar

55g of MacConkey Agar (MCA) powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121c for 15 minutes. The medium was cooled to 45-50c and then dispensed aspectically into sterile petri dish in the laminar flow.

3.3.3 Salmonella *Shigella* Agar

63g of Salmonella *Shigella* Agar (SSA) powder was dissolved in 1 litre distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and boiled with frequent agitation to dissolve the medium completely. The medium was cooled to 50c and then dispensed aseptically into sterile petri dish in the laminar flow.

3.3.4 Mueller Hinton Agar

38 grams of Muller Hinton Agar (MHA) powder was dissolved in1litre of distilled water in a conical flask covered with cottin wool and aliminium foil paper.it was mixed thoroughly and Sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45c-50c and then dispensed aseptically into sterile petri dish and wait for the medium to solidify. The agar was prepared in a clean environment to prevent any contamination.

3.4 Isolation and characterization of bacteria

1g of sample was weighed and placed in 9ml sterile distilled water and allowed to stand for 30 minutes. The aliquot was then transferred aseptically to sterile petri dish plates. The prepared agar (for bacteria growth) was poured in aseptically and incubated at 37c for 24 hours. After successful growth of microorganisms the colonies were counted with a colony counter and the

results per dilution count were recorded. The number of colony forming unit per millimeter was calculated with the formula= Cfu/g

Pure Culture

One single colony was identified and re-streaked as a primary inoculant on the surface of a nutrient agar plate medium. Pure culture were checked from nutrient agar plates. After achieving a pure culture, the same colony was streaked onto a nutrient agar slant. These cultures were incubated at 37c for 24 hours.

3.5 Gram Staining

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides werevwashed off with distilled water, air dried and observed under oil immersion objectives.

3.6 BIOCHEMICAL TEST

3.6.1Catalase Test

This test was to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3.6.2 Urease Test

The urease test was use to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urea medium and incubated at 37ºC for 24-48 hours. Urease positive cultures produced a red-pink colour due to changes in the colour of the indicator

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

3.6.3 Citrate Utilization Test

This test was based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37ºC for 24 - 48 hours. The development of deep blue colour after incubation indicates a positive result.

3.6.4 Hydrogen Sulphide (H_2S) Test

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe2+) or lead (Pb2+) ion as H_2S indicator to a nutrient culture medium containing cysteine and sodium thiosulfate as the sulphur substrates. Hydrogen sulphide, a colourless gas, when produced reacts with sulphur metal salt (ferrous sulphate) forming a visible insoluble black sulphide precipitate.

3.6.5 Indole Test

Indole test was performed to determine the ability of the organism to split tryptophan molecule into indole. This test was performed to help differentiate species of the family enterobacteriaceae.

Kovac's reagent which contains hydrochloric acid, dimethylaminobenzaldehyde and amyl alcohol was used. Inoculate broth with the test organism and incubate for 18 – 24 hours at 370C.

Add 5ml of Kovac's reagent down the inner wall of the tube. Development of bright red colour at the interface of the reagent and the broth within seconds after adding the reagent is indicative of the presence of indole and is a positive test wile absence is negative

3.6.6 Sugar Fermentation Test

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. The growth medium comprised of peptone water, sugar (1%) and the indicator (bromocresol purple). The mixture was dispensed into test tubes and sterilized by autoclaving at 121°C for 15 minutes. After sterilizing, tubes were allowed to cool and then inoculated with the isolates and incubated at 37°C for 24hrs. Acid and gas production or acid only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from purple to yellow colour indicated a positive test.

Sugars used are: lactose, sucrose, glucose, fructose, maltose, starch and sorbitol.

3.7 Antibiotics Susceptibility Test

Antibiotics susceptibility testing is a laboratory procedure performed by medical technologists (clinical laboratory scientists) to identify which antimicrobial regimen is effective for individual patients. On a larger scale, the testing aids in evaluating treatment services provided by hospitals, clinics, and national programs to control and prevent infectious diseases (Lagier *et al*., 2015).

Test organisms were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion on prepared media. Ten (10) different commercial antibiotic discs were used. The antibiotic discs was carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates was inverted and incubated for 37°C for 24 hours. The diameter of the zone of inhibition will be

measured in millimeters (mm) using a meter rule. The experiments was carried out in triplicates to minimize probability of error.

3.8 Determination Of Virulence Characteristics

Virulence factors are molecules and traits produced by pathogens (like bacteria and viruses) that enable them to cause disease by colonizing a host, invading host cells, obtaining nutrients, and evading or subverting the host's immune system. These factors can include toxins, adhesins (for attachment), capsules (to evade immune detection), enzymes that break down host tissues, and structures that facilitate entry into host cells.

After biochemical test was done, virulent factor was performed to determine the enzyme utilization of the following: gelatinase, biofilm assay, hemolysin.

3.8.1 Haemolytic activity

Suspend 28 g of nutrient agar powder in 1 litre of distilled water and autoclave mixture at 121 degrees Celsius for 15 minutes. Once the nutrient agar has been autoclaved, allow to cool to about 45-50 °C then Add 5% (vol/vol) sterile defibrinated blood warmed to room temperature and mix gently. Avoid Air bubbles while dispensing into sterile plates while it's in molten form. Allow agar to solidify then streak isolates on agar. Incubate for 24hrs at 37°C.

Alpha-haemolysis (α) - the media is translucent with a greenish tinge around the colonies. This means is slight damage of the RBCs but absence of lysis. Beta-haemolysis (β) - the RBCs have been lysed and the media looks completely transparent around the colonies. Gamma-haemolysis (γ) - bacteria exhibit neither lysis nor clearing of any kind on agar plates. It is often referred to as non-haemolytic.

3.8.2 Gelatin hydrolysis test

An organism's capacity to create the gelatinases, extracellular proteolytic enzymes that liquefy gelatin, a component of vertebrate connective tissue, was assessed using this assay. Gelatin is hydrolyzed by the enzyme into individual amino acids and polypeptides. Gelatin becomes liquid and loses its structure as a result.

Gelatin medium was prepared by adding 10-15g/L gelatin to nutrient agar and sterilized. After sterilization, media was allowed to cool and dispensed into test and allowed to solidify in a slant position.

The test tube containing the nutrient gelatin medium was stabbed (half an inch) to inoculate a heavy inoculum of test bacteria (18 to 24 hours) and incubated at 37°C with an uninoculated medium (control). Tubes were placed in the fridge for 30 minutes, or until the control tube solidified. The tubes were gently tilted to monitor the test organism's liquefaction after 30 minutes of cooling. Even after refrigeration at 4°C, partial or entire liquefaction of the inoculation tube indicated a positive result; complete solidification of the inoculated tube after refrigeration indicated a negative test (Alnahdi, 2012).

3.8.3 Biofilm Assay

Isolates were subcultured by streaking on nutrient agar plates and incubated for 18-24hrs at 37°C. Isolates were then inoculated into test tubes containing 5-10ml sterilized peptone water and incubated overnight at 37°C. After incubation, the liquid supernatant was discarded and the tubes inverted on a test tube rack for 15-25mins to drain properly. 5-10ml of 1% w/v crystal violet solution was added to the tubes to stain the cells adhered to the walls forming a ring. After 25 min, the crystal violet solution was removed, and the tubes were washed with phosphate buffer saline (PBS) and inverted to dry.

The qualitative measurement of biofilm synthesis was based on color intensity and size of the adherent crystal violet ring with a score ranging from negative (−) to strong (++) Biofilm formation described by Fall *et al*(2014). Additionally, all samples were scored by the same person to minimize variability and maintain results consistency

CHAPTER FOUR

RESULTS

The total heterotrophic bacteria count from street vended food samples obtained from three different vendors (V1, V2, and V3), each selling beans, rice, and spaghetti in Benin City are represented in Table 4.1. The lowest bacterial count was observed in V3 (Beans) with 1.40 ±0.40 × 10^6 CFU/ml, while the highest count was recorded in V2 (Spaghetti) with 7.70±2.50 × 10^6 CFU/ml. Table 4.2 Shows Coliform Count on MacConkey Agar which ranged from 1.10 ± 0.20 × 10^6 CFU/ml in V3 (Beans) to 5.70 ± 3.10 × 10^6 CFU/ml in V2 (Beans). Bacteria counts on *Salmonella-Shigella* agar (SSA) is shown in Table 4.3. Ranged from 1.00 ± 0.00× 10^2 CFU/ml in V1 (Rice) to 5.30± 4.20 × 10^3 CFU/ml in V3 (Beans).

The cultural, morphological, and biochemical characteristics of nine bacterial isolates obtained from street vended foods are detailed in Table 4.4. The identified organisms include *Pseudomonas aeruginosa*, *Klebsiella* sp*., Escherichia coli* ,, *Shigella* sp*., Salmonella* sp[1]*., Salmonella* sp[2]*., Bacillus* sp*., Staphylococcus aureus* and *Staphylococcus* sp.

The distribution pattern of the bacteria isolates from street vended food samples is shown in figure 1 with *Staphylococcus* sp. having the highest frequency of occurrence, with a percentage of 28.57% while *Escherichia coli, Shigella* sp. and *klebsiella* sp. has the least percentage frequencies of 4.76%.

The pH values of street vended food samples (Table 4.5) ranged from 4.55±0.50 (vendor 3) to 5.86±0.05 (vendor 1).

Table 4.6 presents the antibiotic sensitivity patterns of the isolated Gram-negative bacteria. The isolates exhibited varying degrees of resistance and susceptibility to the ten antibiotics tested.

Most isolates showed sensitivity to tarivid and pefloxacin, with inhibition zones ranging from 16 mm to 22 mm. Resistance was particularly notable with amoxacillin, where several isolates including *Shigella* sp., *Klebsiella* sp., and *Salmonella* sp. showed resistance with zones of 0-10 mm. The resistance index (RI) varied from 0.2 (*Pseudomonas aureginosa*[1] , and *Escherichia coli*) to 0.4 (*Klebsiella* sp).

Table 4.7 Presents the antibiotic sensitivity patterns of the isolated Gram-positive bacteria. The isolates exhibited varying degrees of resistance and susceptibility to the ten antibiotics tested. Most isolates showed sensitivity to rocephin and ciprofloxacin, with inhibition zones ranging from 16 mm to 22 mm. Resistance was particularly notable with amoxacilin, zinnacef, and erythromycin, where isolates including *Staphylococcus* sp., *Bacillus* sp., and *Staphylococcus aureus* showed resistance with zones of 0-10 mm. The resistance index (RI) varied from 0.3 (*Staphylococcus* sp.) to 0.5 (*Bacillus* sp).

The phenotypic virulence characteristics of the bacterial isolates are shown in Table 4.8. six isolates demonstrated biofilm formation capability. Hemolytic activity varied among isolates, with *Pseudomonas aureginosa*[1] , *Escherichia coli , Pseudomonas aureginosa*[2], *Bacillus* sp. and *Staphylococcus aureus* showing β-hemolysis, while *Salmonella* sp[1]. Showed α-hemolysis. *Klebsiella* sp., *Shigella* sp., *salmonella* sp[2]. and *Stapylococcus* sp. showed γ-hemolysis (no hemolysis). None of the isolates demonstrated gelatinase activity.

Table 1: **Total Heterotrophic Bacteria counts (CFU/ml) of street vended foods sold in Benin city (mean±S.D)**

Samples	**Total bacteria count ($\times 10^6$)**
V1 (Beans)	4.40±0.40
V1 (Rice)	2.30±0.60
V1 (Spaghetti)	5.60±0.50
V2 (Beans)	3.60±0.40
V2 (Rice)	2.60±0.50
V2 (Spaghetti)	7.70±2.50
V3 (Beans)	1.40±0.40
V3 (Rice)	2.70±0.40
V3(Spaghetti)	4.70±0.40

key: V1=vendor 1; V2=vendor 2; V3=vendor 3

Table 2: **Total Coliform counts (CFU/ml) from street vended food sold in Benin city on MacConkey agar (mean±S.D)**

Samples	**Total coliform count ($\times 10^6$)**
V1 (Beans)	1.20±0.30
V1 (Rice)	4.00±0.30
V1 (Spaghetti)	2.10±0.30
V2 (Beans)	5.70±3.10
V2 (Rice)	1.20±0.20
V2 (Spaghetti)	1.40±0.20
V3 (Beans)	1.10±0.20
V3 (Rice)	1.80±0.20
V3 (Spaghetti)	2.50±0.40

key: V1=vendor 1; V2=vendor 2; V3=vendor 3

Samples	Total Bacteria Count ($\times 10^3$)
V1 (Beans)	1.33±0.61
V1 (Rice)	1.00±0.00
V1 (Spaghetti)	1.33±0.61
V2 (Beans)	4.70±3.10
V2 (Rice)	1.60±0.40
V2 (Spaghetti)	1.80±0.40
V3 (Beans)	5.30±4.20
V3 (Rice)	1.10±0.30
V3 (Spaghetti)	1.40±0.60

Table 3: **Total Bacteria counts (CFU/ml) from street vended food sold in Benin city on *Salmonella-Shigella* agar (mean±S.D)**

key: V1=vendor 1; V2=vendor 2; V3=vendor 3

	ISOLATE									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Characteristics										
Elevation	Raised	flat	Raised	raised	raised	raised	raised	flat	Flat	Flat
Margin	Undulate	undulate	Entire	Entire						
Color	Yellow	cream	Off white	cream	Cream	Cream	Off white	cream	Off white	cream
Transparency	Transparent	opaque	Transparent	Transparent	Opaque	opaque	Transparent	opaque	Transparent	Opaque
Shape	Irregular	irregular	Round	round	Round	round	round	round	Round	Round
Size	Small	large	Small	small	Small	small	Punctiform	small	Punctiform	small
Gram stain	-	-	-	-	-	-	-	+	+	+
Cell type	Rod	rod	Rod	rod	Rod	rod	rod	rod	Cocci	Cocci
Arrangement color(Gram reaction)	Single	pair	Chain	cluster	Pair	single	chain	cluster	Cluster	Pair
Fructose	-	+	+	-	+	-	-	+	+	+
Catalase	+	-	+	+	+	+	+	+	+	+
Maltose	-	+	+	-	-	+	-	+	+	+
Starch	-	+	-	-	-	-	-	+	-	-
Indole	-	-	+	-	+	-	-	-	-	-
Citrate	+	+	-	+	-	-	-	+	+	-
Urease	-	+	-	-	-	-	-	-	+	+
Glucose	-	+	+	-	+	+	+	+	+	+
Sucrose	-	+	+	-	-	-	-	+	+	+
Lactose	-	+	+	-	-	-	-	-	+	+
Sorbitol	-	+	+	-	+	+	+	+	-	-
H2S formation	-	-	-	-	-	+	+	-	-	+

Table 4: **Cultural, Morphological and Biochemical characteristics of Bacterial isolates.**

Coagulase	-	-	-	-	-	-	-	-	+	-
Oxidase	+	-	+	-	-	-	-	-	-	-
Probable	*Pseudomonas aeruginosa*[1]	*Klebsiella* sp.	*Escherichia coli*	*Pseudomonas aeruginosa*[2]	*Shigella* sp.	*Salmonella* sp[2].	*Salmonella sp*[1].	*Bacillus* sp.	*Staphylococcus aureus*	*Staphylococcus* sp.

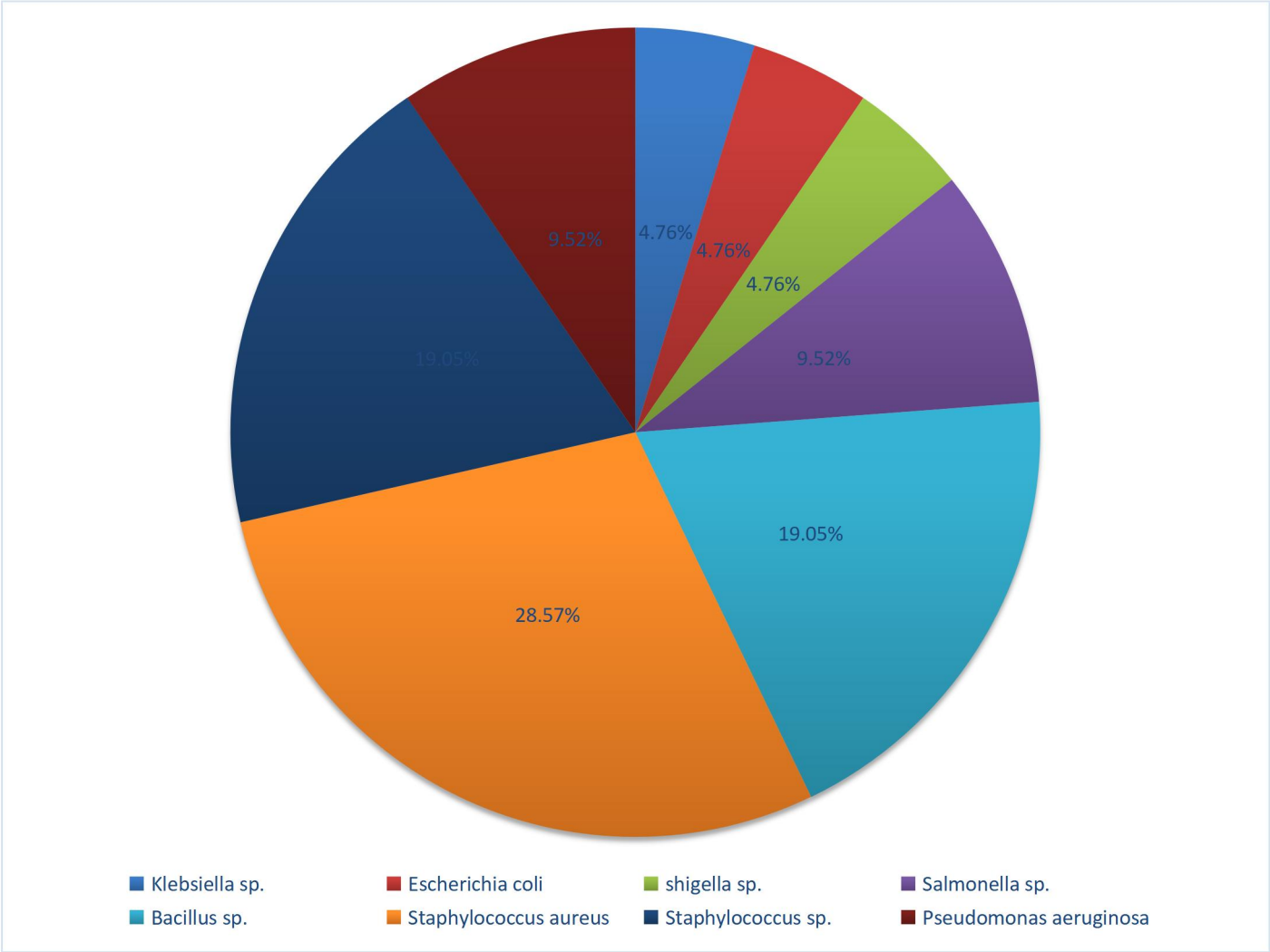

Figure 1: **Percentage frequency of occurrence of bacteria isolates from street-vended foods sold in Benin city**

Table 5**: pH values of street vended foods sold in Benin city.**

Sample name	**Mean±SD**
V1(Beans)	4.84±0.16
V1(Rice)	5.86±0.05
V1(Spaghetti)	4.99±0.06
V2(Beans)	4.86±0.08
V2(Rice)	5.30±0.09
V2(Spaghetti)	5.27±0.05
V3(Beans)	4.55±0.50
V3(Rice)	5.11±0.02
V3(Spaghetti)	5.30±0.04

key: V1=vendor 1; V2=vendor 2; V3=vendor 3

Table 6**: Antibiotic sensitivity pattern of Gram-negative bacteria isolates from street vended foods sold in Benin city.**

Isolates	LEV	CF	SP	CPX	AM	AU	CN	PEF	OFX	AZ	RI
***Shigella* sp.**	16(I)	18(S)	16(I)	14(I)	0(R)	O(R)	10(R)	18(S)	20(S)	16(I)	0.3
***Salmonella* sp[2]**	16(I)	14(I)	14(I)	14(I)	0(R)	0(R)	16(I)	20(S)	18(S)	18(S)	0.2
***Almonella* sp.**	18(S)	22(S)	14(I)	16(I)	0(R)	0(R)	8(R)	18(S)	18(S)	16(I)	0.3
***Klebsiella* sp.**	18(S)	20(S)	10(R)	10(R)	0(R)	0(R)	12(I)	24(S)	22(S)	12(I)	0.4
Escherichia coli	14(I)	12(I)	18(S)	8(R)	0(R)	12(I)	20(S)	22(S)	24(S)	20(S)	0.2
***Pseudomonas aeruginosa*[1]**	16(I)	16(I)	16(I)	16(I)	0(R)	6(R)	16(I)	16(I)	18(S)	18(S)	0.2
***Pseudomonas aeruginosa*[2]**	14(I)	14(I)	12(I)	16(I)	6(R)	0(R)	10(R)	18(S)	20(S)	16(I)	0.3

KEY: RESISTANCE INDEX (R.I) RESISTANCE (R) = 0-10MM INTERMEDIATE (I) =11-16MM SENSITIVE (S) =17MM AND ABOVE, **LEV**= LEVOFLOXACIN, **CF**= CEFOTAXIM, **SP**= SPARIFLOXACIN, **CPX**= CIPROFLOXACIN, **AM**= AMOXACILLIN, **AU**= AUGMENTIN, **CN**= GENTAMYCIN, **PEF**= PEFLOXACIN, **OFX**= TARIVID, **AZ**= AZITHROMYCIN

Table 7**: Antibiotic sensitivity pattern of Gram-positive bacteria isolates from street vended foods sold in Benin city.**

Isolates	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E	RI
Bacillus sp.	10(R)	12(I)	0(R)	0(R)	6(R)	12(I)	22(S)	16(I)	14(I)	10(R)	0.5
Staphylococcus aureus	20(S)	16(I)	12(I)	0(R)	4(R)	20(S)	16(I)	10(R)	18(S)	6(R)	0.4
Staphylococuss sp.	14(I)	12(I)	14(I)	10(R)	10(R)	20(S)	22(S)	14(I)	16(I)	10(R)	0.3

KEY: RESISTANCE INDEX (R.I) RESISTANCE (R) = 0-10MM INTERMEDIATE (I) =11-16MM SENSITIVE (S) =17MM AND ABOVE, PEFLOXACIN, **CN=**GENTAMYCIN, **APX**= AMPLICOX, **Z**= ZINNACEF, **AM**= AMOXACILLIN, **R**= ROCEPHIN, **CPX**= CIPROFLOXACIN, **S**= STREPTOMYCIN, **SXT**= SEPTRIN, **E**= ERYTHROMYCIN

Table 8**: Phenotypic virulence characteristics of bacteria isolates from street vended foods sold in Benin city**.

Isolates	**Hemolysis**	**Biofilm**	**Gelatinase**
Pseudomonas aeruginosa[1]	β	+	-
Klebsiella sp.	γ	-	-
Escherichia coli	β	+	-
Psedomonas aeruginosa[2]	β	+	-
Shigella sp.	γ	-	-
Salmonella sp[2].	γ	-	-
Salmonella sp[1].	α	+	-
Bacillus sp.	β	+	-
Staphylococcus aureus	β	+	-
Staphylococcus sp.	γ	-	-

CHAPTER FIVE

DISCUSSION

The bacterial enumeration results from this study revealed total heterotrophic counts ranging from 1.40 to 7.70 × 10^6 CFU/ml, substantially exceeding the internationally accepted threshold of 10^5 CFU/g for ready-to-eat foods established by the Codex Alimentarius Commission (2015). These elevated microbial loads provide unequivocal evidence of significant deficiencies in hygienic practices during food preparation, handling, and storage in the street foods sector. Investigations have corroborated these findings across multiple Nigerian urban centers and West African contexts. The pioneering work of Umoh and Odoba (1999) in Zaria established baseline concerns for ready-to-eat street foods in Nigeria, documenting high total aerobic counts of 3.67 to 4.29 $\log_{10}$/g. Frequent isolation of *Bacillus* cereus and coagulase-positive *Staphylococcus aureus*, with contamination attributed primarily to inadequate vendor hygiene, use of contaminated water, and environmental exposure at vending sites. Nearly three decades later, the persistence of similar contamination patterns observed in this study suggests that systemic improvements in street food safety have not materialized despite documented risks. The coliform counts documented in this investigation, ranged from 1.10 to 5.70 × 10^6 CFU/ml , serve as critical indicators of fecal contamination and deficient sanitation practices. Coliforms function universally as hygiene markers because their presence strongly suggests possible fecal contamination and inadequate food processing conditions. Recent work by Abalkhail (2023) had a mean value of 5.98 to 7.32 logCFU/g in Saudi Arabia, the author found that 34% of street foods were contaminated with *E. coli*, reinforcing that coliform contamination remains a persistent global challenge in informal food sectors despite geographic and cultural differences. Bacteria count on *Salmonella-Shigella* agar, though at relatively lower concentrations ranging

from 1.10 to 5.30 × 10^3 CFU/ml compared to total counts, represents a direct and serious public health threat due to the characteristically low infectous doses required for these pathogens to cause disease. Previous research by Edem, (2020) had a mean of 1.00 to 3.00 ×10^4CFU/g. Both organisms are well-established causative agents of gastroenteritis, bacillary dysentery, and in the case of *Salmonella typhi*, typhoid fever, which remains endemic in many Nigerian communities. Their presence in ready-to-eat foods indicates either post-cooking contamination through contact with contaminated surfaces or handlers, or alternatively, inadequate thermal processing that failed to eliminate these pathogens during cooking. (Kotloff *et al*., 1999; Crump *et al*., 2004)

The devastating 2018 cholera outbreak in Nigeria, which resulted in 43,996 reported cases and 836 deaths according to epidemiological investigations by Elimian *et al*. (2019), highlighted the critical role of contaminated food and water in facilitating disease transmission within communities. Subsequent analyses by Eneh *et al*. (2024) examining drivers of cholera outbreaks in Nigeria identified overcrowded markets, poor sanitation, and unsafe food and water practices as recurrent contributors, with informal food markets and street vending environments implicated as significant contributing factors even when water sources represented the primary transmission vehicle. These epidemiological patterns underscore that street-vended foods function not merely as isolated contamination events but as integral components of broader transmission networks linking environmental reservoirs, food chains, and human populations.

The isolation and comprehensive characterization of ten distinct bacterial species through systematic cultural, morphological, and biochemical approaches reveals substantial microbial diversity present in Benin City street foods. The Gram-negative organisms identified in this study *Escherichia coli*, *Salmonella* sp.[1], *Salmonella* sp.[2], *Shigella* sp., *Klebsiella* sp., *Pseudomonas aeruginosa*[1] and *Pseudomonas aeruginosa*[2] collectively represent well-

documented foodborne pathogens with established disease-causing potential and significant public health implications. Simultaneously, the Gram-positive isolates *Staphylococcus aureus*, *Staphylococcus* sp., and *Bacillus* sp. demonstrate the diverse contamination sources and pathogenic mechanisms operational in street food environments.

Escherichia coli functions simultaneously as both a hygiene indicator organism and a potential pathogen depending on strain characteristics and virulence determinant expression. Similarly, Petrin *et al*. (2023) performed whole genome characterization of *E coli* isolated from ready-to-eat products in Italy, confirming that food isolates frequently harbor both virulence determinants and resistance genes, thereby representing dual public health threats. Akinyemi and colleagues surveyed vended foods in Lagos, reporting broad bacterial contamination including *E coli*, *Salmonella* spp., and *Staphylococcus aureus* across many food types.

The identification of *Salmonella* species, represented by two distinct isolates in this study, is particularly concerning given the organism's specific association with typhoid fever and various forms of salmonellosis causing gastroenteritis and invasive disease. Typhoid fever remains endemic throughout Nigeria and broader sub-Saharan Africa, with Crump *et al*. (2004) estimating that developing countries bear the overwhelming majority of the global disease burden due to inadequate water and sanitation infrastructure combined with unsafe food handling practices. Contemporary investigations continue to document *Salmonella* prevalence in African street foods. Recent work examining street foods in various African contexts reinforces that *Salmonella* contamination persists as a recurrent challenge, with Campos *et al*. (2015) documenting non-typhoidal *Salmonella* in the pig production chain and emphasizing the organism's impact on human health when adequate food safety measures are absent.

Klebsiella species and *Pseudomonas aeruginosa* isolated from these street foods represent opportunistic pathogens that pose disproportionate risks to immunocompromised individuals, infants whose immune systems remain immature, and elderly consumers with declining immune function, as documented extensively by Podschun and Ullmann (1998). The presence of these organisms in food matrices reflects multiple contamination pathways including environmental sources, inadequate sanitation of food preparation surfaces, and possible biofilm formation on utensils and equipment that resist routine cleaning procedures. Both organisms are notorious for their intrinsic resistance to multiple antibiotic classes and demonstrated capacity to acquire additional resistance determinants through horizontal gene transfer mechanisms involving plasmids, integrons, and transposons, as detailed in the comprehensive review by Blair *et al*. (2015) examining mechanisms driving the global antimicrobial resistance crisis.

Staphylococcus aureus is frequently implicated in *staphylococcal* food poisoning through its production of preformed, heat-stable enterotoxins that cause rapid-onset symptoms including severe vomiting, abdominal cramping, and diarrhea typically within one to six hours after consuming contaminated food, as comprehensively reviewed by Argudín *et al*. (2010). This organism is most commonly transmitted to food products from human handlers through poor personal hygiene practices, with humans serving as the primary reservoir. The isolation of *S. aureus* from street foods by Adesetan *et al*. (2019) in their Nigerian survey revealed the presence of *mec*A genes indicative of methicillin resistance in several isolates, raising serious concerns about circulation of methicillin-resistant S. *aureus* (MRSA) strains within the food chain and their potential to cause infections that are extremely difficult to treat with available antibiotics. This finding aligns with growing global recognition that MRSA has transcended healthcare settings to establish reservoirs in food production systems and community environments.

Bacillus species represent spore-forming bacteria possessing the remarkable capability to survive standard cooking temperatures through formation of highly resistant endospores, subsequently germinating under favorable storage conditions to resume vegetative growth, as detailed by Stenfors Arnesen *et al*. (2008). Their isolation from cooked food samples suggests either survival of heat-resistant spores through the cooking process itself, or alternatively, post-cooking contamination through contact with environmental sources, contaminated surfaces, or handlers. Certain *Bacillus* species, particularly B. cereus, produce emetic toxins or diarrheal enterotoxins responsible for causing food poisoning, particularly problematic when foods are prepared in large batches, inadequately cooled, and subsequently held at ambient temperatures for extended periods that permit toxin accumulation to pathogenic levels.

The isolation of pathogens such as *Escherichia coli, Salmonella* sp., *Shigella* sp. and *Staphylococcus aereus* from the food samples directly corrobates the literature identifying these common contaminants in the informal food sector(Feglo and Sakyi, 2012; Alimi 2016). The distribution pattern, with *staphylococcus* sp. being the most frequently isolated (28.57%), underscores the critical role of human handling in food contamination, a factor repeated highlighted in studies focusing on vendor hygiene (Monney *et al*., 2014).

The pH measurements recorded across all food samples ranged from 4.55 ± 0.50 to 5.86 ± 0.05, positioning these foods within the slightly acidic to near-neutral range. While acidic conditions can theoretically inhibit growth of certain pH-sensitive pathogens, many enteric bacteria of clinical significance have evolved sophisticated acid tolerance mechanisms enabling survival and even limited growth at pH values below 5.0. For instance, *E coli* O157:H7 possesses a well-characterized acid tolerance response (ATR) system involving multiple genetic loci that facilitate bacterial survival through the harsh acidic environment of gastric juice (pH approximately 1.5-

3.5), subsequently enabling successful colonization of intestinal tissues, as demonstrated through extensive experimental work by Ryu and Beuchat (1999). Similarly, *Salmonella* species deploy inducible acid tolerance systems involving changes in membrane composition and expression of acid shock proteins that enhance survival under acidic stress conditions, mechanisms elucidated through molecular genetic studies by Foster (2004).

The relatively narrow pH range observed across different food samples in this study suggests that acidity alone provides insufficient protection against microbial contamination and pathogen survival. Multiple additional factors exert substantially greater influence on microbial survival and proliferation dynamics within food matrices, including initial microbial load at the point of contamination. Also, water activity levels affecting availability of free water for microbial metabolism, storage temperature regimens, intrinsic food composition including nutrient availability and presence of antimicrobial compounds, and presence of competing microflora that may inhibit pathogen growth through competition or antagonistic metabolite production.

Jay *et al*. (2005) reported that pH represents just one element within the "hurdle technology" concept of food preservation, wherein multiple antimicrobial factors operating simultaneously create cumulative inhibitory effects that exceed those achievable by any single factor alone. They noted that reliance on any single preservative factor whether pH, water activity, temperature, preservatives, or modified atmosphere rarely achieves adequate microbial control for food safety purposes without complementary measures. This principle appears validated by the current study's findings, where moderately acidic pH failed to prevent substantial microbial contamination across all sample types.

The moderately acidic nature of the examined foods likely reflects the common culinary use of tomatoes, peppers, and other naturally acidic ingredients in traditional Nigerian cuisine,

particularly in stews and sauces accompanying staple foods. However, this inherent acidity demonstrably failed to prevent contamination by acid-tolerant pathogens, thereby underscoring the critical importance of proper thermal processing to achieve pathogen reduction through heat inactivation, maintenance of strict hygienic handling practices throughout food preparation and service to prevent initial contamination or cross-contamination, and implementation of appropriate time-temperature controls during storage to prevent proliferation of surviving organisms.

The antimicrobial susceptibility testing conducted in this study revealed deeply concerning resistance patterns that closely mirror global trends documented in food-borne pathogen resistance surveillance systems. The universal resistance to amoxicillin observed across all bacterial isolates tested reflects decades of widespread use and systematic misuse of this broad-spectrum penicillin derivative in both clinical medicine and veterinary practice throughout Nigeria and similar developing country contexts. Laxminarayan *et al*. (2013) documented rising resistance prevalence to penicillin-class antibiotics across developing countries, attributing escalating trends to multiple contributing factors including unregulated over-the-counter availability of antibiotics without prescription requirements, patient non-compliance leading to incomplete treatment courses, widespread circulation of substandard or counterfeit antimicrobial drugs with inadequate active ingredient concentrations, and extensive non-therapeutic use in livestock production for growth promotion purposes prior to regulatory restrictions.

The resistance indices calculated for bacterial isolates in this study ranged from 0.2 observed in *E coli*, *Salmonella* sp.[2], *Pseudomonas aeruginosa*[1] to 0.5 documented in *Bacillus* sp., indicating that multidrug resistance phenotypes were present in 70% of isolates subjected to comprehensive antimicrobial susceptibility testing. According to the standardized definition proposed by

Magiorakos *et al*. (2012) in their influential international consensus paper establishing uniform terminology, multidrug resistance is operationally defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. Applying this internationally accepted criterion, the majority of isolates recovered in this investigation clearly qualify as multidrug-resistant organisms, presenting serious implications for empirical treatment of infections should these strains cause clinical disease in exposed consumers.

Recent investigations across multiple geographic contexts have documented escalating antimicrobial resistance in food-borne isolates, validating concerns raised by findings from the current study. Fusaro *et al*. (2024) characterized multidrug-resistant *E coli* in retailed chicken meat from different production systems, reporting that resistance to β-lactam antibiotics, tetracyclines, and fluoroquinolones was commonplace, with several isolates harboring extended-spectrum β-lactamase genes. Onohuean *et al*. (2025) conducted a comprehensive systematic review and meta-analysis of antimicrobial resistance patterns in food-borne pathogens from Nigerian street-vended foods, documenting resistance prevalence rates consistent with the current study and emphasizing that street foods serve as significant reservoirs for resistance gene circulation. Almashhadany *et al*. (2025) reviewing antimicrobial resistance patterns in food globally characterized the situation as a "growing global concern," with resistance mechanisms proliferating across food-borne bacteria through horizontal gene transfer and selection pressure from antibiotic misuse.

The relatively preserved activity demonstrated by fluoroquinolone antibiotics including ofloxacin (tarivid), pefloxacin, and levofloxacin represents a concerning finding when viewed within the broader context of increasing global reports documenting fluoroquinolone resistance emergence in food-borne pathogens. Hooper and Jacoby (2015) comprehensively described

chromosomal mutations occurring in genes encoding DNA gyrase (gyrA) and topoisomerase IV (parC) as the primary mechanisms conferring fluoroquinolone resistance in *Salmonella* and *E coli*, with accumulating mutations progressively elevating minimum inhibitory concentrations from susceptible through intermediate to fully resistant ranges. The intermediate susceptibility patterns observed for fluoroquinolones in multiple isolates from this study may represent a transitional stage in the evolution toward full high-level resistance, particularly concerning given continuing selective pressure through inappropriate antibiotic use in both human medicine and animal production systems. Akinyemi and colleagues' earlier Lagos survey documented resistance to fluoroquinolones in some *E coli*, *S. typhimurium*, and *Klebsiella* isolates, suggesting that resistance to this antibiotic class has been emerging progressively over recent decades.

Paterson and Bonomo (2005) emphasized in their landmark review that ESBL-producing Enterobacteriaceae have emerged as major threats to public health globally, substantially limiting therapeutic options for serious invasive infections and necessitating use of carbapenem antibiotics as last-resort agents, which themselves now face emerging resistance through carbapenemase production. The isolation of organisms exhibiting potential ESBL phenotypes from street foods indicates that these foods actively serve as environmental reservoirs for resistance determinants, facilitating dissemination of resistance genes throughout communities through multiple transmission pathways including direct consumption, environmental contamination, and gene transfer to commensal microbiota.

Several interconnected factors contribute to the high prevalence of antimicrobial resistance observed in Nigerian street foods, reflecting systemic challenges spanning agricultural production, pharmaceutical regulation, healthcare practice, and environmental management. First, widespread prophylactic and growth-promoting use of antibiotics in livestock and poultry

production creates intense selective pressure favoring emergence and maintenance of resistant bacterial strains, which subsequently enter the human food chain through contaminated meat products, as extensively documented by Wellington *et al*. (2013) in their comprehensive review of environmental dimensions of antibiotic resistance. Second, inadequate regulatory oversight and enforcement of antibiotic sales regulations allows over-the-counter purchasing of prescription antibiotics without medical consultation or proper diagnosis, leading to self-medication practices, inappropriate antibiotic selection for infections where antibiotics may not be indicated, inadequate dosing regimens that fail to achieve therapeutic concentrations, and premature treatment discontinuation when symptomatic improvement occurs but bacterial populations have not been eliminated, all of which promote resistance development, as highlighted by Ventola (2015) in her analysis of antibiotic resistance crisis drivers.

Third, environmental contamination with antibiotic residues originating from pharmaceutical manufacturing effluent, hospital wastewater discharge lacking adequate treatment, and agricultural runoff from livestock facilities creates selective environments in natural ecosystems including soil, water bodies, and food production environments that actively promote resistance gene evolution and horizontal transfer among environmental bacterial populations inhabiting these contaminated niches, mechanisms detailed by Martinez (2009) in influential work examining how pollution with antimicrobials drives resistance emergence outside clinical settings. Fourth, international food trade and global travel patterns facilitate geographic dissemination of resistant strains across national borders, creating interconnected resistance problems that transcend local contexts and require coordinated international responses.

The implications for public health are profound and multifaceted, extending beyond immediate treatment challenges to encompass broader societal impacts. Consumers regularly exposed to

multidrug-resistant organisms through consumption of contaminated street foods face substantially elevated risks of treatment failures requiring extended antibiotic courses or alternative agents, prolonged illness durations with extended periods of infectiousness and potential for transmission, increased hospitalization requirements consuming healthcare resources, higher medical costs for patients and healthcare systems, and elevated mortality risks particularly among vulnerable populations including young children, elderly individuals, pregnant women, and immunocompromised patients should clinical infection subsequently occur.

Beyond direct treatment challenges affecting individual patients, resistant bacteria ingested through contaminated food can establish themselves transiently or persistently as members of the intestinal microbiota following ingestion, where they may persist asymptomatically for extended periods measured in weeks to months while potentially transferring resistance genes to commensal flora through horizontal gene transfer mechanisms involving plasmids encoding resistance determinants, transposons capable of moving between replicons, and integrons capturing and expressing resistance gene cassettes, as comprehensively reviewed by Thomas and Nielsen (2005). This amplification effect through gene transfer among intestinal bacteria means that even transient dietary exposure to resistant food-associated organisms can generate lasting alterations in individual and community antimicrobial resistance profiles, with transferred resistance genes subsequently available within the resident microbiome for acquisition by pathogenic organisms causing future infections, thereby perpetuating and amplifying resistance problems across populations.

The phenotypic virulence screening performed in this study provided valuable functional insights into pathogenic capabilities possessed by isolated organisms extending beyond their simple taxonomic identification and presence in food matrices. These phenotypic assessments

complement genotypic approaches by demonstrating actual expression of virulence determinants under specific laboratory conditions, providing evidence that organisms not only carry virulence genes but can actively express functional virulence factors.

Biofilm formation capability, detected in 60% of tested isolates including *E coli*, *Pseudomonas aeruginosa*[1], *Pseudomonas aeruginosa*[2], *Salmonella* sp.[1], *Bacillus* sp. and *Stapylococcus aureus*, represents a critical bacterial adaptation that dramatically enhances environmental persistence characteristics, resistance to antimicrobial agents and sanitizing chemicals, protection against desiccation stress, and capacity for generating recurrent contamination events from persistent surface-associated bacterial populations, as extensively characterized by Costerton *et al*. (1999) in their pioneering work establishing the biofilm paradigm shifting how microbiology conceptualizes bacterial communities and chronic infections. Bacteria residing within biofilm structures become embedded in self-produced extracellular polymeric substance matrices composed primarily of polysaccharides but also incorporating proteins, extracellular DNA released from lysed cells, and lipids, which collectively provide robust protection against environmental stresses, antimicrobial agent penetration, immune system components, and mechanical removal through cleaning or washing procedures.

Within the specific context of street food vending environments, biofilm formation on cutting boards, knives, serving utensils, preparation surfaces, and even storage containers creates persistent contamination reservoirs that actively resist routine cleaning efforts and serve as continuous sources for food recontamination during subsequent food preparation cycles. Even when planktonic (free-floating) bacteria are successfully removed from contaminated surfaces through vigorous washing, residual biofilm structures and matrix components can persist in microscopic surface irregularities, cracks, scratches, or porous materials, serving as nucleation

sites facilitating rapid recolonization when environmental conditions become favorable again through moisture presence, nutrient availability from food residues, and appropriate temperatures.

Simões *et al*. (2010) demonstrated through systematic experimental studies comparing biofilm and planktonic bacterial susceptibilities that biofilm-associated bacteria exhibit antimicrobial resistance levels ranging from 10-fold to over 1,000-fold higher compared to genetically identical planktonic counterparts grown in suspension culture, with resistance enhancement primarily attributable to three major mechanisms: firstly, reduced penetration of antimicrobial agents through dense extracellular polymeric substance matrix material creating concentration gradients; secondly, presence of metabolically dormant or slowly-growing persister cells in biofilm depths exhibiting tolerance to antibiotics targeting active growth processes; and thirdly, expression of biofilm-specific resistance genes upregulated under sessile growth conditions but not expressed during planktonic growth. The demonstration of biofilm-forming capabilities in *E coli* and S. *aureus* isolates from street foods strongly suggests these organisms possess enhanced capacity to persist indefinitely in vending environments despite vendors' cleaning efforts, thereby maintaining continuous contamination risk throughout operational periods.

Recent investigations have reinforced the significance of biofilm formation in food safety contexts. Liu *et al*. (2024) examining biofilm formation by foodborne pathogens on medical devices emphasized that biofilm-associated bacteria on abiotic surfaces present particularly intractable contamination problems, while Singh *et al*. (2025) conducting a comprehensive review of biofilm formation in foodborne pathogens noted that understanding biofilm biology remains critical for developing effective intervention strategies targeting persistent contamination sources in food production and service environments.

The hemolytic activity patterns observed in this study provide direct phenotypic evidence of toxin production capabilities and tissue-damaging potential possessed by isolated organisms, functioning as indicators of virulence even when specific toxin identities have not been molecularly characterized. Beta-hemolysis, characterized by complete lysis of red blood cells producing clear zones surrounding bacterial colonies on blood agar plates, was demonstrated by both *Pseudomonas aeruginosa* variants, *E coli*, *Bacillus* sp., and *S. aureus* isolates. This phenotype indicates production of one or more hemolysins pore-forming cytolytic toxins that disrupt host cell membranes through formation of transmembrane pores or enzymatic degradation of membrane components—resulting in cell lysis, nutrient release exploitable by bacteria for growth, and direct tissue damage facilitating infection establishment and dissemination, as comprehensively reviewed by Welch (1991) in foundational work characterizing pore-forming toxins of gram-negative bacteria.

In *E coli*, α-hemolysin (*Hly*A) encoded by the *Hly*A gene represents a well-characterized virulence factor strongly associated with extraintestinal pathogenic strains including uropathogenic *E coli* (UPEC) causing urinary tract infections and neonatal meningitis-associated *E coli* (NMEC) strains, as well as some enterohemorrhagic *E coli* variants, as detailed through extensive molecular and epidemiological studies by Schmidt *et al*. (1995). Detection of hemolytic activity in *E coli* isolated from street foods suggests the recovered strain may possess significant pathogenic potential extending beyond simple intestinal colonization capability, potentially representing an extraintestinal pathogenic lineage that has contaminated food or a hybrid strain that has acquired hemolysin genes through horizontal transfer.

Similarly, *S. aureus* produces multiple distinct hemolysins including alpha-toxin (alpha-hemolysin), beta-toxin, gamma-toxin, delta-toxin, and additional leucocidins, which collectively

contribute to host tissue destruction, lysis of immune cells including neutrophils and macrophages, disruption of epithelial barriers, and evasion of host immune responses, mechanisms extensively characterized by Alonzo and Torres (2014) in their comprehensive review of *staphylococcal* toxins and virulence determinants. The demonstration of beta-hemolytic activity in *S. aureus* isolates from street foods confirms expression of functional toxins under laboratory conditions, supporting classification of these isolates as potentially virulent strains capable of causing disease if ingested in sufficient quantities, or if they establish infection following introduction through compromised epithelial barriers.

The *Salmonella* sp.[1] isolate exhibited alpha-hemolysis indicating partial red blood cell lysis producing greenish discoloration around colonies on blood agar. While *Salmonella* pathogenicity mechanisms depend primarily on sophisticated invasion systems encoded on *Salmonella* pathogenicity islands (SPI-1 through SPI-5) enabling entry into host cells and intracellular survival capabilities within membrane-bound vacuoles, rather than extensive extracellular toxin production characteristic of other enteric pathogens, hemolytic activity detected in this isolate may reflect acquisition of additional virulence determinants through horizontal gene transfer from other bacterial species sharing environmental niches or passage through animal hosts, potentially enhancing overall pathogenic potential through synergistic interactions between invasion systems and toxin-mediated tissue damage.

The organisms exhibiting gamma-hemolysis specifically *Klebsiella* sp., *Shigella* sp., *Salmonella* sp.[2], and an additional *Staphylococcus* sp. should emphatically not be dismissed as non-virulent or non-pathogenic based solely on absence of hemolytic activity in standard blood agar assays. These organisms possess well-documented alternative virulence mechanisms that do not manifest as hemolytic phenotypes but nonetheless enable significant disease-causing capability.

Klebsiella species produce thick polysaccharide capsules conferring resistance to phagocytic uptake and complement-mediated killing, enabling persistence in bloodstream and establishment of invasive infections; *Shigella* produces Shiga toxins that inhibit protein synthesis in host intestinal epithelial cells leading to cell death and dysentery; *Salmonella* utilizes type III secretion systems injecting effector proteins into host cells to trigger internalization and establish intracellular replication niches; and even coagulase-negative staphylococci can cause opportunistic infections particularly in immunocompromised hosts or when introduced via medical devices, diverse pathogenic strategies comprehensively reviewed by Pizarro-Cerdá and Cossart (2006) examining molecular mechanisms of bacterial virulence.

The complete absence of detectable gelatinase activity across all tested isolates indicates this particular proteolytic enzyme was not expressed at detectable levels under the specific laboratory culture conditions employed in this study, utilizing nutrient gelatin medium incubated at 37°C with refrigeration-based liquefaction assessment. However, this finding requires cautious interpretation avoiding overextension of conclusions, as virulence factor expression in bacteria is frequently regulated by complex environmental signal transduction systems responding to diverse environmental cues including temperature fluctuations, pH changes, osmolarity variations, nutrient availability or limitation, oxygen tension, cell density through quorum sensing mechanisms detecting accumulation of autoinducer molecules, and additional environmental signals that may substantially differ between standardized laboratory culture conditions optimized for bacterial growth and natural infection settings or actual food environments where organisms encounter different selective pressures, as discussed by Maurelli (2007) in his comprehensive review examining environmental regulation of virulence gene expression and noting that laboratory conditions may either fail to induce expression of virulence

factors requiring specific environmental triggers or alternatively may not provide conditions suitable for detecting certain enzymatic activities even when genes are expressed.

The documented co-occurrence of antimicrobial resistance phenotypes and multiple virulence characteristics within individual bacterial isolates including *E coli*, *S. aureus*, and *Pseudomonas aeruginosa* represents a particularly alarming finding with serious clinical implications that compound treatment challenges and elevate infection severity risks. Bacterial strains simultaneously exhibiting multidrug resistance limiting available therapeutic options and potentially requiring use of last-resort antibiotics or combination therapies and possessing capabilities for biofilm formation enhancing persistence and resistance, toxin production causing direct tissue damage, or both present compounded difficulties for healthcare providers managing infections. When such organisms cause clinical infections following ingestion through contaminated food or other transmission routes, they demonstrate enhanced capacity not only to evade antimicrobial therapy through diverse resistance mechanisms reducing drug efficacy but also to cause severe clinical manifestations through expression of virulence determinants promoting tissue invasion, immune evasion, and toxin-mediated pathology, thereby substantially elevating risks of treatment failure, prolonged illness duration, development of severe complications including systemic dissemination, and increased mortality particularly among vulnerable patient populations compared to infections caused by organisms lacking these combined threatening traits.

5.1 Conclusion

This study concludes that street-vended foods in Benin city are significantly contaminated with potentially pathogenic bacteria, including *Escherichia coli, Salmonella* sp., *Shigella* sp., *Bacillus* sp. and *Staphylococcus aereus*, posing a direct public health risk. The investigation confirmed

that these isolates possess key phenotypic virulence factors, such as biofilm-forming capabilities and hemolytic activity, indicating a high potential to cause disease. Most critically, these pathogens exhibited widespread multi-drug resistance to common antibiotics like amoxicillin, zinnacef and erythromycin, with some isolates demonstrating resistance indices as high as 0.5. This combination of high microbial loads, significant virulence and extensive antimicrobial resistance highlights that these foods are not only sources of infection but also potent vehicles for the dissemination of drug-resistant bacteria, underscoring an urgent need for improved hygiene practices and public health surveillance.