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Abstract

Indigenous fermented foods (IFFs) have a long history in Africa and are embedded in cultural norms and practices. Typically, these foods are produced at small or household scale using indigenous processing technologies. In addition, limited knowledge of good manufacturing and handling practices can lead to production under unhygienic conditions. This results in variations in the quality and safety attributes of IFFs, as spoilage and pathogenic bacteria can be introduced at any stage of the value chain. These foods have an important role in the African diet and can contribute to food security by increasing the availability of cheap, nutritious food and supporting livelihoods. However, the presence of foodborne pathogens and antibiotic-resistant bacteria in IFFs may constitute a health risk to consumers. Therefore, this review presents an overview of the microorganisms associated with IFFs from Africa, focusing on microbial food safety hazards. African indigenous fermented foods offer a vast genetic potential of undiscovered strains that possess valuable technical characteristics. However, IFFs may also serve as vehicles of pathogenic and antibiotic-resistant bacteria and genetic determinants. Significant research and data gaps exist regarding the microbiological safety of these food products, which warrant urgent attention. We propose practical solutions for improving the safety of African IFFs requiring action and collaboration from all stakeholders, including researchers, producers, governmental regulatory bodies, and consumers.

Keywords: Africa; antibiotic resistance; fermented foods; food safety; lactic acid bacteria; pathogens

1. Introduction

Indigenous fermented foods (IFFs) have a long history in Africa and are embedded in the cultural norms and practices. They make a valuable contribution to the continent's effort in achieving its sustainable development goals focused on food security, poverty alleviation and gender equality (Franz et al., 2014). Fermentation adds value, improving the organoleptic characteristics, variety, nutritional quality, digestibility, and safety of foods (FAO, 2010; Ganzle, 2020). As a low-cost technology, indigenous fermentations improves access to local, inexpensive, nutritious food. In addition, IFFs contribute to the livelihoods of many, especially women, through income generation via small-scale enterprise (Misihairabgwi & Cheikyoussef, 2017; Rolle & Satin, 2002).

Fermentation is a processing technique where desirable changes occur in a food product due to the metabolic activities of microorganisms (Caplice and Fitzgerald, 1999). There has been increasing interest from researchers to understand the diversity and technological properties of the microbial community in IFFs from Africa. This knowledge is required for producing local, well-characterised starter cultures to improve the quality and safety of IFF products (Akabanda et al., 2013; Anyogu et al., 2014; Bayili et al., 2019). Despite these efforts, IFF production in Africa largely remains a cottage-level technology, mainly reliant on spontaneous fermentation processes or backslopping, which may introduce spoilage or pathogenic organisms (Benkerroum and Tamime, 2004). Bacteria of public health interest have been isolated from IFFs, and some investigators have noted concerns about the safety of these foods (Ahaotu et al., 2013; Omemu et al., 2014; Samet-Bali et al., 2016; Walsh et al., 2017).

Data from the World Health Organisation (WHO) estimate that diarrhoeal diseases account for 70% of mortalities associated with foodborne disease in the African region (WHO, 2015). Also, antibiotic resistance (AR) has emerged as one of the utmost global public health concerns. Fermented food products have diverse microbial ecosystems, yet their ability to serve as vehicles for transferring AR microorganisms and genes remains unclear.

Reviews discussing IFFs from Africa have mainly focused on the microbial diversity of fermenting and technologically important bacteria (Achi and Ukwuru, 2013; Jans et al., 2017; Parkouda et al., 2009; Tamang et al., 2016). Recently, Nwaiwu et al. (2020) reviewed spoilage and pathogenic microbiota associated with indigenous fermented beverages but only concentrated on one country. Paudyal et al. (2017) analysed the prevalence of foodborne pathogens in foods from selected African countries; however, their focus was on raw and ready-to-eat foods and not IFFs specifically.

A comprehensive overview of the microbiological safety of IFFs from Africa is lacking. The purpose of this review is to summarise current knowledge on the microbiology of IFFs with a focus on pathogenic and AR bacteria. It also discusses approaches to improve the safety of these foods and highlights data gaps that could be explored in further research.

2. Indigenous African fermented food products

Fermentation has long been used as a preservation technology for extending the shelf life of various substrates. The characteristic flavour, texture and colour of IFFs due to the metabolic activities of fermenting microorganisms has guaranteed their widespread acceptability by consumers, thereby establishing a major role for IFFs in the African diet (Mokoena et al., 2016). Olasupo et al. (2010) classified IFFs from Africa into five major groups based on their raw materials. These include (1) fermented non-alcoholic cereals, (2) alcoholic beverages, (3) fermented animal proteins, (4) starchy root crops, and (5) fermented vegetable proteins.

Cereal grains comprising maize, sorghum, millet, and wheat are important staple crops in Africa, accounting for as much as 50% of the total daily calorific consumption (OECD/FAO, 2016). These cereals are common starting materials for lactic acid-fermented beverages and porridges known by different names such as *ogi* in Nigeria (Oguntoyinbo et al., 2011), *togwa* in Tanzania (Mugula et al., 2003), *koko* in Ghana (Lei and Jakobsen, 2004) and *hussuwa* in Sudan (Yousif et al., 2010).

Fermented non-alcoholic cereal-based products have an important role in the diet as complementary foods for infants or breakfast meals (Byakiya et al., 2019; Soro-Yao et al., 2014). The extensive use of cereal-based complementary foods makes them an attractive target in efforts

towards combating infant malnutrition in Africa. For example, *koko*, made from fermented corn dough, is the most commonly consumed complementary food in Ghana. However, it has been noted to be inadequate in meeting dietary protein and micronutrient needs (Suri et al., 2014). To improve the nutritional profile of *koko*, a legume-based supplement was developed recently, with preliminary studies suggesting good acceptability by consumers (Tano-Debrah et al., 2019).

Fermented alcoholic beverages are consumed across the continent. The majority of these are produced from cereals, e.g., sorghum, millet, and maize. These include *dolo, burukutu,* or *pito* in West Africa (Sawodogo-Lingani et al., 2007; Onyenekwe et al., 2015), *borde* in Ethiopia (Abegaz, 2007), and *sesotho* in South Africa (Cason et al., 2020). Fruit fermentation for alcohol production is uncommon. However, plantain and banana can be fermented to produce *agadagidi* (Sanni and Lonner, 1993). Palm wine is a popular alcoholic drink in many West African countries. Palm wine is obtained from the fermentation of sap obtained from palm tree species such as *Elaeis guineensis, Raphia hookeri*, *Borassus aethiopum,* and *Borassus akeassii*. The production of ethanol, lactic acid and acetic acids by yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) are the most significant activities contributing to the distinctive organoleptic characteristics and stability of the product (Amoa-Awua et al., 2007; Karamoko et al., 2012; Nwaiwu & Itumoh, 2017). The gradual accumulation of ethanol at the early stages of fermentation correlates with the increase in the AAB population, as AAB can use ethanol as a carbon source (Amoa-Awua et al., 2007). Organic acids produced by LAB & AAB contribute to the sour taste, aroma and colour, although AAB are often considered as spoilage organisms. The low pH and alcohol content control the growth of undesirable bacteria, including *Enterobacteriaceae* (Djeni et al., 2020; Ouoba et al., 2012).

Cassava (*Manihot esculenta*, Crantz) ranks third after rice and maize as a source of calories in tropical countries and is the most consumed starchy root crop in Africa. Total annual cassava consumption has tripled from 20 million tonnes in 1970 to just over 60 million tonnes in 2013 (FAOSTAT, 2020a; Szyniszweska et al., 2020). Fermentation practices involve either submerged fermentation for the production of *lafun* and *chikwangue* (Miambi et al, 2003; Padonou et al., 2009)

or solid-state to produce *attieke* and *garri* (Ahaotu et al., 2017; Djeni et al., 2015). Fermentation is essential for preventing the rapid post-harvest deterioration of cassava tubers. It is also an important processing technique for decreasing the amount of cyanogenic glucosides (CGs), which occur naturally in cassava. These CGs can be hydrolysed to produce hydrogen cyanide when the plant tissue is damaged, but it should be noted that the concentration of CGs can be cultivar specific (Abiodun et al., 2020). Cyanide can inhibit cellular respiration by binding to cytochrome oxidase in the electron transport chain, and high exposure to cyanide can lead to severe illness and, in some cases, death (Akintowa et al., 1994; Kimaryo et al., 2000).

Consequently, the WHO has recommended a safe limit of 10 mg cyanide/kg of cassava (FAO/WHO, 1995). Although the breakdown of cyanide has been attributed to the activity of endogenous enzymes, Obilie et al. (2004) observed that the highest loss of cyanogenic glucosides, from 72.4 mg cyanide/kg to below detectable limits, occurred during fermentation compared to other processing stages. These results are corroborated by Kivunde et al. (2000) during controlled fermentations using LAB as a starter where the total cyanide content reduced from 176.3 mg/kg in raw cassava to 8.2 mg/kg in *kivunde*. Their observation of higher cyanide content in spontaneously fermented and backslopped samples was attributed to β-glycosidase activity of the starter strains, suggesting an additional advantage of controlled fermentations in guaranteeing the safety of fermented cassava products. Another demerit of cassava is its low nutritional content, specifically protein, vitamins and minerals and studies on fermented cassava products biofortified with protein (Ahaotu et al., 2017) and Vitamin A (Abiodun et al., 2020) have recently been reported.

In Africa, milk has a long historical connection with pastoral communities, e.g., the Berbers, Fulani and Maasai ethnic groups in North, West and East Africa. Milk is a significant source of nutrients, and total milk production in Africa was 49 million tonnes in 2019 (FAOStat, 2020). Fermentation is an essential processing technology for extending the shelf life of milk, a highly perishable product. African fermented dairy products comprise of naturally fermented milks, e.g., *gariss* and *mabisi*; yoghurt-based products, e.g., *kindirmo* and *amasi*, and cheeses, e.g., *wara*, *jben* and *klilia*

(Abdelgadir et al., 2008; Mourad & Bettache, 2015; Moonga et al., 2020; Nyambane et al., 2014; Omemu et al., 2014; Osvik et al., 2013; Sudi et al., 2011) are widely consumed across Africa. Despite consumer preferences for fresh meat and seafood, the combination of a warm, tropical climate and variable access to refrigeration facilities, particularly in rural areas, means that meat and seafood products are usually available in processed form. Smoking, salting, and drying are common preservative strategies for meat and seafood products in Africa (El-Sheika et al., 2014). Compared to other parts of the world, meat and fish fermentation is not widespread across Africa. However, fermented meats, e.g., *sujuk* and *boubanita,* are consumed in different North African countries (Benkerroum, 2013). Fermented fish products serve as both flavouring agents and a source of protein (Anihouvi et al., 2007) and can be produced from a wide variety of fish species.

A significant amount of protein intake in the African diet comes from plant sources. The alkaline fermentation of proteinaceous oil seeds such as locust bean, melon, sesame, and castor oil have widespread use as flavour enhancers and meat substitutes (Ahaotu et al., 2013; Ezeokoli et al., 2016). These seeds are often inedible in their natural state as they contain anti-nutrients such as indigestible oligosaccharides and phytates, which are metabolised during fermentation (Parkouda et al., 2009). Plant leaves also serve as substrates for fermentation. Kawal and *ntoba mbodi* are produced from the fermentation of the leaves of *Cassia obtisufolia* and *Manihot esculenta,* respectively (Dirar, 1985; Vouidibio Mbozo et al., 2017).

3. Fermenting and technologically important microbiota of African indigenous fermented foods

Accurate identification and characterisation of the microbiota of IFFs is a critical first step for the selection of multi-functional starter cultures. Starter cultures are required for the controlled and large-scale production of IFFs with improved quality and safety attributes (Ahaotu et al., 2017; Edema and Sanni, 2008; Soro-Yao et al., 2014). The last four decades have seen an increase in research studies aimed at elucidating the microbial consortia present in IFFs from Africa. These studies have provided data on the vast diversity of microbial species, including lactic acid bacteria

(LAB), *Bacillus*, and yeasts that dominate the fermentation of cereals, legumes, dairy, and root crops (Table 1).

Lactic acid bacteria dominate the fermentation of cereals, dairy products, and starchy root tubers. Of the LAB, species belonging to *Lactobacillus*, *Lactococcus*, *Weissella*, *Leuconostoc, Streptococcus,* and *Enterococcus* are frequently isolated (Diaz et al., 2019; Oguntoyinbo et al., 2011).

Previously, the beneficial effects of fermented foods were focused mainly on preservation and desirable organoleptic characteristics. Nowadays, a better understanding of the microorganisms involved in fermentation processes has drawn more attention to the various health benefits associated with IFFs. Lactic acid bacteria are among the most studied microorganisms in this regard, given their interesting technological characteristics. During fermentation, LAB metabolise sugars to produce a wide range of organic acids, including lactic, acetic and propionic acid, which produce a low pH environment that is detrimental to the growth and survival of spoilage and pathogenic organisms (Caplice & Fitzgerald, 1999).

Other antimicrobial metabolites associated with LAB metabolism include ethanol, hydrogen peroxide, diacetyl, and bacteriocins showcasing the diversity of LAB in utilising different metabolic pathways and substrates (Oguntoyinbo and Narbard, 2015; Mokoena et al., 2016; Soro-Yao et al., 2014). As LAB species are important members of the gut microbiome, their contribution to human health as probiotics has been extensively studied. Positive actions have been reported to include restoration of gut microbiota after antimicrobial therapy, vitamin production, and stimulating the immune system (Markowiak and Slizewska, 2017). A role for IFFs from Africa as delivery vehicles for probiotics is receiving significant attention (Achi & Ukwuru, 2015; Mokoena et al., 2016). Recently, two cereal-based fermented foods, *obushera* and *kwete*, have been produced using probiotic strain *Lactobacillus rhamnosus* yoba (Mukisa et al., 2019; Wacoo et al., 2019). Although the probiotic strain is not of African origin, the inherent presence of LAB species in IFFs from Africa suggests that IFFs may be sources of novel probiotic strains and warrants further studies.

The presence of *Enterococcus* spp., notably *Enterococcus faecium* and *Enterococcus faecalis,* in food systems is complex due to their increasing medical relevance as aetiological agents of nosocomial infections and their ability to disseminate antibiotic resistance determinants, particularly vancomycin (Oladipo et al., 2013; Oguntoyinbo and Okesuo, 2013). However, *Enterococcus* spp. are usually observed in IFFs, particularly in fermented dairy products where they are considered as contaminants (Jans et al., 2017). Although not recognised on the Qualified Presumption of Safety list (EFSA Panel on Biological Hazards, 2021), the positive technological contributions of enterococci include the production of extracellular polysaccharides, which influence the texture of fermented dairy products, and flavour and aroma development (Jans et al., 2017; Obioha et al., 2021). Some strains also produce bacteriocins, and their consideration for probiotics is receiving more research attention (Hanchi et al., 2018).

S*accharomyces cerevisiae* is the most commonly isolated fungal species from African IFFs and has an essential role in the alcoholic fermentation of cereals and palm sap. Other dominant genera include *Candida*, *Pichia* and *Kluveromyces* (Table 1). In addition to fermenting the sugars available in the natural substrates to alcohol and carbon dioxide, fungi produce flavour and aroma compounds such as organic acids, esters and carbonyls and contribute to textural changes via pectinases and cellulases (Cason et al., 2020; Johansen, 2019). Yeasts may also contribute to organic acid production by LAB species present during the fermentation (Ferreira and Mendes-Faia, 2020). Fungi, therefore, play a significant role in the final organoleptic characteristics of alcoholic IFFs over and above alcohol production.

Bacillus spp. dominate the fermentation of protein-rich legumes and seeds. An important aspect of the production process of alkaline fermented condiments such as *iru*, *soumbala* and *bikalga* produced from these protein-rich substrates is the long cooking time, and this heating process may select for spore formers that are more heat resistant (Ouoba et al., 2007; Parkouda et al., 2009). In addition, the degradation of proteins during fermentation by *Bacillus* spp., most significantly, *B. subtilis*, *B. pumilus,* and *B. licheniformis,* leads to the accumulation of peptides and ammonia. This

leads to an increase in pH, which also favours the proliferation of *Bacillus* spp. (Ouoba et al., 2008). *Bacillus* spp. are also frequently isolated from fermented cassava products (Anyogu et al., 2014; Assanvo et al., 2017; Padonou et al., 2009). Their ability to produce enzymes that hydrolyse cassava tissue has been reported to be responsible for the textural changes that occur during fermentation (Amoa-Awua & Jakobsen, 1995).

Molecular based techniques including fingerprinting-based methods, e.g., repetitive element palindromic (rep)-, intergenic transcribed spacing (ITS)-PCR in combination with the sequencing of ribosomal RNA (16S and 26S) or other housekeeping genes, e.g. *rpoA, rpoB, gyrA* and *pheS* are now routinely used to identify and characterise microorganisms in IFFs (Oguntoyinbo and Okesuo, 2012; Owusu-Kwarteng et al., 2012; Tadesse et al., 2019). These have provided new insights compared to earlier studies, which relied only on phenotypic identification (Abegaz, 2007; Nyambane et al., 2014), which do not always provide sufficient information. For example, molecular studies on submerged cassava fermentation have highlighted the dominance of *Weissella confusa* (Anyogu et al., 2014; Padonou et al., 2009), a microorganism that had not been previously reported in studies relying on phenotypic methods (Omafuvbe et al., 2007; Oyewole & Odunfa, 1988). *Weissella* spp. are also now frequently identified in other IFFs, e.g. cereals (Angelov et al., 2017; Oguntoyinbo et al., 2011) and dairy products (Akabanda et al., 2013). The extensive species diversity revealed by the use of genotyping techniques has been reported for other IFFs (Achi, 2005; Aderigbigbe et al., 2011; Nwaiwu and Itumoh, 2017; Ouoba et al., 2012) and has also uncovered new microbial species (Ouoba et al., 2015, a, b).

More recently, culture-independent methods such as PCR-DGGE and next-generation sequencing techniques are used to investigate the metagenomics associated with IFF production (Cason et al., 2020; Djeni et al., 2020; Walsh et al., 2017). An advantage of this approach is that it provides detailed information about the microbial community associated with different fermentation stages without the need for isolation. This results in a less biased microbial profile compared to culture-dependent methods as IFFs may contain uncultivable species (Bigot et al., 2015). Accurate data of

the microbial community involved during IFF production eliminates chance isolates that can be recovered on agar from the screening process for potential starter cultures.

These more recent reports have confirmed that the microbes present in IFFs are more diverse than earlier reported. While investigating the microbial community of *soy-daddawa*, Ezeokoli et al. (2018) identified *Exiguobacterium* spp. for the first time. Diaz et al. (2019) observed *Zymomonas* spp. for the first time in fermented cereal, dairy, cassava and locust bean products. Although the contribution of these organisms to the fermentation process requires further study, their identification reveals new insights into IFFs. The factors that influence the composition of the microbial community has been noted to include fermentation conditions, pH changes, and geographical location (Houngbedji et al., 2018; Moonga et al., 2020). However, metagenomic studies have revealed that geographical location is not a consistent factor (Cason et al., 2020; Parker et al., 2018). Understanding the microbes involved in African IFF production will help in the design of starter cultures. However, culture-independent methods rely solely on DNA, so do not allow for the direct selection of microbial starters. Depending on the method used, DNA may be extracted from live and dead cells (Mukisa et al., 2012; Diaz et al., 2019). These limitations may be overcome by using both culture-dependent and independent methods (Adewunmi et al., 2012; Schoustra et al., 2013).

Despite these recent developments, there remain significant knowledge gaps concerning the microbiota of IFFs from Africa. Our review of the published research in this area highlights that further studies are required, particularly for alcoholic beverages, fermented meat and fish products (Gagaoua & Boudechicha, 2018; Djeni et al., 2020). These gaps limit the transition from household to large-scale controlled production of IFFs with consistent quality and safety attributes.

4. Microbial hazards in indigenous fermented foods

Globally, food safety remains a significant challenge. The World Health Organisation (WHO) estimates that as many as 1 in 10 people fall ill, and more than 120,000 children under 5 die each year after consuming unsafe food. Africa bears a high burden of the global incidence of foodborne

illness with an estimated annual morbidity of 90 million (WHO, 2015; WHO 2017b). Microbial hazards, including foodborne pathogens and their toxins, are primary aetiological agents of foodborne disease (FBD) and a growing public health issue.

Fermented foods are generally considered safe. Fermenting organisms, especially LAB, produce a range of antimicrobial compounds, e.g., organic acids, ethanol, bacteriocins and hydrogen peroxide, which are antagonistic to the growth and survival of foodborne pathogens (Adinsi et al., 2017; Devuyst and Vandamme, 1996; Mpofu et al., 2016). Cason et al. (2020) reported the decline of pathogenic and spoilage organisms during cereal fermentation for *sesotho* production. Similar observations were made by Karamoko et al. (2012), who noted a 4 – 7 log reduction in faecal coliform counts within a 24 h period in fermenting palm wine. When investigating the microbiological quality of milk products in Tanzania, Schoder et al. (2013) detected *Salmonella* and *Escherichia coli* in raw milk but not in fermented milk. In addition, Oguntoyinbo and Narbad (2015) isolated bacteriocin producing *Lactobacillus plantarum* strains from *kunu* and *ogi* that showed antimicrobial activity against *Salmonella enterica*.

However, indigenous practices for food production are often based on spontaneous fermentation, i.e., chance inoculation or the use of backslopping where utensils from a previous fermentation are reused (Caplice & Fitzgerald, 1999). Limited knowledge and utilisation of Hazard Analysis and Critical Control Points (HACCP) and good manufacturing processes (GMP) by farmers, food producers and handlers can lead to production and processing occurring under unhygienic conditions. These factors lead to variation in the microbial profile of IFFs, and consequently, the presence of spoilage and pathogenic bacteria in these foods cannot be ruled out (Oguntoyinbo, 2014; Olasupo et al., 2016).

Despite the lack of surveillance of foodborne infections in many African countries, several studies have investigated the prevalence of major foodborne pathogens in IFFs from Africa. Reports on microbiological hazards associated with IFFs from Africa are presented in Table 2. Microbes of public health significance, including *Bacillus cereus*, *Staphylococcus aureus,* and *Listeria monocytogenes,*

313 have been reported in IFFs from Africa. The introduction of pathogenic organisms can occur at
314 different stages of the value chain via raw materials, the processing environment, and food handlers.
315 An evaluation of the microbiological quality of water used for processing, fermenting broth, and the
316 fermented cassava product, *lafun*, identified microbial hazards such as coliforms, including
317 *Salmonella* spp. and *Staphylococcus* spp. (Lateef & Ojo, 2015). Potentially pathogenic bacteria have
318 been found in utensils used for the fermentation process (Gran et al., 2002; Jans et al., 2017);
319 however, only a few studies investigate the complete production chain to identify the source of
320 contamination (Ademola et al., 2018; Thorsen et al., 2015). Adedeji et al. (2017) reported
321 similarities in the microbial profiles of potentially pathogenic bacteria isolated from two fermented
322 condiments from the same producer at the retail level. It is important to note that these bacteria
323 were not present in the raw materials, suggesting unhygienic processing and handling. This
324 underscores the need for further research to identify contamination sources to support the
325 management of food safety hazards.
326 The microbiological safety of fermented vegetable proteins usually dominated by *Bacillus* spp. and
327 *Staphylococcus* spp. requires consideration, as acid production, a potent antimicrobial attribute in
328 lactic fermented IFFs, is not present (Ahaotu et al., 2013; Ouoba et al., 2019). *Bacillus cereus* and
329 *Staphylococcus aureus* can produce toxins in food during their growth and have been identified in
330 these products (Oranusi et al., 2015). *Bacillus* spp. are the dominant organisms involved in the
331 fermentation of oil bean seeds; however, the presence of *Bacillus cereus* is routinely reported
332 (Ahaotu et al., 2013; Ouoba et al., 2008b; Parkouda et al., 2009; Thorsen et al., 2015). Ahaotu et al.
333 (2013) isolated *Bacillus cereus* capable of producing enterotoxins in *ugba* under fermentation
334 conditions. A similar observation was made by Ouoba et al. (2008b) when investigating *B. cereus*
335 involved in locust bean fermentation for soumbala production. However, these are heat-labile toxins
336 that should be denatured with adequate cooking. Thorsen et al. (2015) detected the heat-stable,
337 emetic type toxin-producing *B. cereus* strains in fermented baobab seeds, which is of concern.

338 The metabolic activities of some microorganisms involved in the fermentation process provide
339 antagonistic conditions to the growth and survival of foodborne pathogens. However, inappropriate
340 handling and the use of unsanitary packaging material can introduce microbial hazards post-
341 processing (Adinsi et al., 2017; Mpofu et al., 2016; Schoder et al., 2013). The occurrence of
342 potentially pathogenic bacteria at the retail level across all food categories highlights the potential
343 risks to public health associated with IFFS (Odom et al., 2012; Owusu-Kwarteng et al., 2018). Of
344 particular concern is the isolation of pathogenic bacteria from cereal-based and dairy fermented
345 products, some of which are used as weaning or complementary foods (Adekoya et al., 2019; Samet-
346 Bali et al., 2016). The presence of these organisms in ready-to-eat products suggests that IFFs may
347 serve as vehicles of pathogenic bacteria. Therefore, the safety of these foods should not be taken for
348 granted or assumed. The use of next-generation sequencing methods has provided more insight in
349 identifying microbial hazards in IFFs, highlighting a role for metagenomic approaches as food safety
350 tools (Walsh et al., (2017).

351 **5. Antibiotic resistance of microbes from indigenous fermented foods from Africa**

352 The availability of antibiotics represented a landmark achievement in medicine and led to a
353 significant decrease in mortality and morbidity from infectious disease agents (Spellberg, 2014).
354 However, in recent times, an increasing number of pathogenic bacteria, including those that cause
355 foodborne diseases, have become resistant to treatment with the antibiotic drugs currently available
356 (WHO, 2017b). This scenario, termed antibiotic resistance (AR), has become one of the greatest
357 threats to global public health and food security (McEwen & Collingon, 2018). It has been estimated
358 that if left unchecked, the number of deaths attributed to AR each year could rise to10 million by
359 2050 (O'Neill, 2016).

360 Antibiotic resistance is increasingly recognised as a food safety issue. The consumption of food
361 contaminated with AR foodborne pathogens such as *Salmonella* could lead to treatment failure.
362 Commensal bacteria such as *Escherichia coli* and *Enterococcus* spp. in food may transfer AR genes to
363 human pathogens (WHO, 2017b). Drivers of AR include the overuse of antimicrobial drugs in human

364 and veterinary medicine. In addition to treatment, antimicrobials are used in agriculture and
365 aquaculture as prophylaxis and in some parts of the world as growth promoters (Nhung et al., 2017;
366 Van Boeckel et al., 2014). These factors lead to the spread of AR bacteria and AR genes (ARG) in the
367 environment.
368 A comprehensive evaluation of the safety of IFFs in Africa should consider their role as vehicles of
369 both pathogenic and AR bacteria. However, in Africa, the contribution of the food chain to the
370 overall burden of AR is mostly unknown as there are little to no surveillance systems for monitoring
371 antibiotic use in agriculture and food products (Founou et al., 2016; Oloso et al., 2018). For the most
372 part, antibiotic use remains unregulated in Africa (Oguntoyinbo & Okesuo, 2012; Van et al., 2020).
373 Reports of AR bacteria associated with some African IFFs are presented in Table 3. Some of these
374 studies have documented multiple drug-resistant (MDR)- bacteria in technologically relevant,
375 indicator and potentially pathogenic organisms. Phenotypic resistance to more than three classes of
376 antibiotics was observed in *Escherichia coli* 0157:H7 and *Shigella* spp. isolated from cheese products
377 available for retail sale in Egypt (Ahmed and Shimamoto, 2015 a,b). Fowoyo and Ogunbawo (2017)
378 isolated 255 coagulase-negative staphylococci (CoNS) from fermented dairy, cereal and oilseed
379 products in Nigeria. Of the total isolates, 27% exhibited MDR-phenotypes. Oguntoyinbo and Okesuo
380 (2012) observed MDR *Enterococcus* spp. in *wara*, a fermented cheese. Ouoba et al., (2019) also
381 identified MDR-*Staphylococcus* spp. in fermented vegetable products. However, other reports
382 showed AR to one or two classes or overall sensitivity to the antibiotics tested. Owusu-Kwarteng et
383 al., (2017) noted that *Bacillus cereus* isolated from fermented dairy products in Ghana only showed
384 resistance to beta-lactams. *Enterococcus* spp. in IF products in Tunisia were shown to be sensitive to
385 beta-lactams, aminoglycosides and macrolides (Rehaim et al., 2016).
386 Vancomycin is a critically important antibiotic, often used as a last resort treatment (WHO, 2019).
387 Vancomycin resistance has been noted in isolates from African IFFs (Awopetu et al., 2016;
388 Oguntoyinbo & Okesuo, 2012; Ouoba et al., 2008a). However, Rehaim et al., (2016) reported that
389 *Enterococcus* spp. isolated from Tunisian fermented food products were susceptible to vancomycin.

A similar observation was also noted by Owusu-Kwarteng et al., (2017, 2018) when investigating *B. cereus* and *L. monocytogenes* in fermented dairy products.

It is important to note that most bacterial species have intrinsic and induced resistance mechanisms to specific antibiotic drugs (Reygaert, 2018). From a food safety perspective, the ability of foodborne bacteria to transfer resistance traits via mobile genetic elements to other bacteria is a primary concern. There are some reports of AR genetic determinants associated with African IFFs. Ahmed and Shimamoto (2015a) screened shiga toxin-producing *Escherichia* coli for antibiotic-resistant genes (ARG) and identified beta-lactamase encoding genes in all (n=5) isolates. Two of these isolates possessed plasmid-mediated quinolone resistance genes. Conjugation experiments undertaken by Ouoba et al., (2019) showed that CoNS from fermented condiments produced transconjugants with increased resistance to erythromycin and tetracycline. Unfortunately, most investigations of AR in African IFFs only consider phenotypic resistance (Adimpong et al., 2012; Awopetu et al., 2016; Eruteya and Eze, 2017). For this review, we found only one study (Aka et al., 2020) where ARGs in IFFs from Africa were investigated using whole-genome sequencing.

The prevalence of AR phenotypes in bacteria isolates from retail level IFFs in Africa and the high levels of resistance observed in some strains provide some evidence that these foods may serve as a reservoir for AR and is of public health significance. There remain considerable research and data gaps in this area, underscoring the need for large scale and long-term surveillance studies coordinated at national and international levels.

6. Discussion & Recommendations

According to the United Nations, 50% of global population growth between now and 2050 is anticipated to occur in Africa (UN, 2020). However, Africa currently has the 2nd highest number of undernourished people globally, and about 20% of the population on the continent is already considered to be food insecure (FAO et al., 2020). The contribution of IFFs to combating food insecurity via increased food availability, improved nutrition and income generation is well established (Franz et al., 2014; Okafor, 1992; Rolle and Satin, 2002; Setta et al., 2020).

The global market for fermented products is predicted to surpass $20 billion by 2022 (Sivamaruthi et al., 2019). This demand is fuelled in part by the increasing popularity of some IFFs, such as *kombucha* and *kefir*, in the international market as consumers become more aware of their beneficial effects (Soni et al., 2014). Besides meeting domestic demand from an increasingly urban populace, African IFFs could also become a source of foreign revenue via exports for a growing diaspora community and beyond. This requires improving the value chains that deliver IFFs with consistent quality and safety attributes to local and international markets using modern, industrial processes, including well-characterised starter cultures.

More work remains to be done towards achieving large scale production of IFFs from Africa. However, there are some success stories. For example, the production of the widely consumed sorghum-based fermented beer *umqombothi* has been industrialised in South Africa. Cereal-based porridges *ogi* and *uji,* and the alcoholic beverage palm wine are also now produced commercially (Adebo, 2020; Nwaiwu & Itumoh, 2017). Significant advances have been made in identifying dominant strains in IFFs and characterising technological aspects which make them suitable for use in controlled fermentations (Aderigbigbe et al., 2011; Ahaotu et al., 2013; Aka et al., 2020; Houngbédji et al., 2018; Moodley et al., 2019; Oguntoyinbo & Narbad, 2015; Sawadogo-Lingani et al., 2008). However, transferring these technologies to producers presents some challenges, including the stability, activity, and viability of these cultures (Benkerroum & Tamime, 2004; Benkerroum, 2013; Rolle and Satin, 2002). Although several studies have identified and characterised potential starters for use in soumbala fermentation, most processors still utilise spontaneous fermentation processes (Ouoba et al., 2004; Compaore et al., 2020; Parkouda et al., 2009). To overcome some of these obstacles, an alternative approach used by some researchers is the utilisation of commercially available starter cultures, as has been reported for the production of *obushera* using *Lact. rhamnosus* yoba (Mukisa et al., 2019) and *pito* using *Lact. delbrueckii* and *Sacch. cerevisiae* (Djameh et al., 2019). However, this may not be a sustainable solution due to cost implications.

Selecting starter cultures from the autochthonous community is often recommended as these cultures are considered more adapted to the fermentation parameters of the IFF and may show wider metabolic diversity required for achieving desirable properties and functional characteristics of the product (Ashaolu, 2019; Casquete et al., 2011). However, there is a lack of data showing if autochtonous fermenting organisms possess unique biochemical properties compared to similar strains obtained from other sources, which could be further explored. There is also a need for more studies that evaluate the application of starter cultures as monocultures or in combination for IFF production outside the laboratory environment (Compaore et al., 2020; Kimaryo et al., 2000; Mukisa et al., 2016). Beyond technology transfer, more progress is required to improve raw materials, conduct laboratory, and pilot plant production before scaling up to industrial production (Okafor et al., 1992; Benkerroum, 2013).

Food fermentations are complex microbial ecosystems (De Filippis et al., 2017). The use of metagenomics in studying African IFFs has provided more insight into the microbiota and succession dynamics of fermenting microorganisms (Cason et al., 2020; Diaz et al., 2019). However, only a limited number of IFFs have been investigated using this approach, and future research efforts should be directed here. Metagenomic data can be combined with outputs from other 'omics' technologies such as metabolomics and transcriptomics to develop a more comprehensive understanding of the relationships between the microorganisms present in the food, their metabolic interactions, and what these contribute to the fermentation process (De Filippis et al., 2017; Kergourlay et al., 2015). This will allow for a more informed starter selection process for improved fermentation processes.

The prevalence of foodborne disease remains severe in many African countries. Factors contributing to this include food preparation with contaminated water, poor hygiene, inadequate storage facilities, food safety knowledge, and insufficient food safety legislation and implementation (Belli et al., 2013). Currently, IFFs are mainly marketed via the informal economy in open markets, street vending and household producer/seller, therefore outside the scope of official health regulatory

standards where these exist. The reviewed studies suggest that contamination of IFFs mainly occurs post-processing. Similar to our observations, a meta-analysis of the prevalence of foodborne pathogens in ready-to-eat food from seven African countries showed that *Enterobacteriaceae, Escherichia coli, Salmonella, Staphylococcus aureus, and Listeria monocytogenes* were the most frequently reported organisms (Paudyal et al. 2017).

Given the potential for the introduction of food safety hazards at each production stage, the design and implementation of quality assurance and management systems, including GMP and HACCP in commercial food production, is recommended or incorporated into legislation as an effective strategy for improving food safety (Kafetzopoulous, 2013; Ropkins & Beck, 2000). There are now several reports on the development of HACCP systems for IFF production (Asagbra et al., 1998; Fasoyiro et al., 2010; Lateef & Ojo, 2015; Oguntoyinbo, 2012;). Some studies have demonstrated the effective use of these approaches in improving the microbiological quality and safety attributes of some products such as *lafun* (Obadina et al., 2008) and *kenkey* (Amoa-Awua et al., 2007). However, there is a need for more data evaluating the application of HACCP systems in the commercial production of IFFs and on studies that report on producers and handlers' food safety knowledge, attitudes, and practices. Byakiya et al., (2019) noted that processors of *Obushera*, a fermented beverage widely consumed in Uganda, showed good food safety knowledge but observed poor hygienic practices. Amoa-Awua et al., (2007) reported significant improvements in the safety characteristics of kenkey after the implementation of a HACCP and GMP system. However, they noted that the level of formal education of processors was a significant hurdle when applying quality assurance systems in IFF production. After observing poor manufacturing and hygienic processes during the production of *ice kenkey* (Atter et al., 2015), researchers developed a simplified manual to train *ice kenkey* processors in GMP & HACCP principles. These studies highlight the need for more extension work, including training on basic food safety and hygiene, GMP, and HACCP, for food producers and handlers.

493 Assessing health risks to consumers from IAFFs requires more and better-quality data to underpin
494 quantitative exposure assessments. There is a scarcity of research studies that focus on the
495 microbiological safety of African IFFs. In some cases, the observation of pathogenic or indicator
496 bacteria are incidental, occurring where investigation of the fermented product's microbial
497 community is the main objective (Anyogu et al., 2014; Oranusi et al., 2015; Parkouda et al., 2010).
498 This means that beyond identification, important information such as microbial load, virulence
499 factors, or antimicrobial resistance determinants are not investigated and recorded.
500 Another constraint in the quality of data collected is study design. Some reports do not include the
501 number of samples collected to support estimating prevalence or utilised convenience sampling,
502 which may not be representative. Many reports rely on conventional techniques for identifying
503 foodborne pathogens, which may be misleading or do not provide sufficient information, e.g.,
504 species identification. While the presence of potential pathogens in a food product is a cause for
505 concern, health risks associated with consumption must consider any national, regional, or
506 international safety standards, such as the Codex Alimentarius. Except for a few exceptions (Byakika
507 et al., 2019; Kouame et al., 2013; Gran et al., 2002), these standards are often not referred to
508 indicate the microbiological quality of the food being studied.
509 Antibiotic resistance of microorganisms has not been extensively studied in African IFFs. A significant
510 AR data gap also exists in African clinical settings. In a systematic analysis, Tadesse et al., (2017)
511 reported that AR data was not available for as many as 40% of African countries. The analysis
512 concluded that resistance to commonly prescribed antibiotics was prevalent, and the quality of
513 microbiological data is of serious concern. In the absence of rigorous government surveillance and
514 regulation for IFF production in many parts of Africa, more studies will need to be carried out by the
515 scientific community to raise the necessary awareness required and monitor prevalence trends,
516 especially of acquired AR.
517 Many of the technologies required to investigate IFFs and produce the innovation needed in the
518 sector remain inaccessible to many researchers in low and middle-income countries (LMICs) due to

519 resource constraints in technical know-how and infrastructure. This limits opportunities to publish in
520 more impactful journals, reducing the visibility of the outputs obtained, despite their importance to
521 the scientific community. There is a need for more initiatives that support sustainable international
522 collaborative efforts that bring together scientists in LMICs and High-income countries to share
523 expertise and develop equitable capacity building research activities. An example of this is the
524 ENRECA/DANIDA project, "Capability Building for Research and Quality Assurance in Traditional Food
525 Processing in West Africa", which has supported several successful collaborative research efforts in
526 value-added processing of IFFs in some West African countries.
527 Research innovation must translate into improvements in IFF processing technology. This requires
528 significant investment in stakeholder management between policymakers, scientists, and producers.
529 Public sector funding could target knowledge transfer partnerships between research institutions
530 and small-medium- enterprise (SME) producers, which must benefit both partners. Research
531 projects can be designed in collaboration with SMEs, focusing on the real-world problems
532 encountered by producers (Moodley et al., 2019). This model can contribute towards the buy-in of
533 producers, required to drive change along the food processing value chain.
534 Weak enforcement of food safety regulations poses risks to public safety. Additionally, surveillance
535 systems that should provide accurate and reliable data on the burden of foodborne illness in IFFs are
536 often inadequate. This means that the number of illnesses or outbreaks associated with IFFs may go
537 unreported. In 2012, an outbreak of botulism in Canada was linked to an African fermented fish
538 product, fesikh, which led to a voluntary withdrawal from sale by the manufacturer (Walton et al.,
539 2014). Many public health agencies across Africa have limited access to the required infrastructure
540 to gather epidemiological evidence to support this type of timely action.
541 'One Health' is a transdisciplinary approach introduced by the WHO as a framework to be used by
542 relevant stakeholders in developing and implementing strategies to safeguard public health (WHO,
543 2017c). The African Center for Disease Control (Africa CDC) has recently published a 'One Health'
544 framework for managing zoonotic infections (Africa CDC, 2020). However, food safety and

antimicrobial resistance are also important priority areas for public health, requiring urgent attention in the African region. At a national level, policymakers also need to empower regulatory agencies with the required legal frameworks and infrastructure to develop and enforce food safety standards.

7. Conclusion

Indigenous fermented foods have great potential in combating food insecurity in Africa and harbour a vast genetic potential of valuable undiscovered strains. To achieve the goal of improving and scaling up fermentation technology, the use of advanced molecular biology tools, including whole-genome sequencing, is required to accurately identify the microbial community in IFFs, both beneficial and harmful. A comprehensive understanding of the microbial community of IFFs could identify biomarkers for assessing the quality and safety attributes of these foods, including technological characteristics, virulence factors and antibiotic resistance.

The presence of pathogenic & AR bacteria in ready to eat IFFs constitute a risk to public health. Harmful bacteria may enter the food chain via the raw material, inadequate fermentation to lower the pH sufficiently in lactic fermented foods or post-processing contamination. The production of antimicrobial compounds by fermenting organisms may be insufficient to eradicate pathogenic organisms in the final product, and they are not substitute for GMP. Hygiene improvement in handling raw food, increased surveillance, and uniform protocols for sampling and identification is suggested to help evolve a common approach to studies of indigenous foods.

Any strategies to guarantee IFFs free of microbial hazards require considerable investment and collaboration from relevant stakeholders – consumers, producers, industry, policymakers, and scientists.

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Author's contributions

Amarachukwu Anyogu: Conceptualisation, Data Collection, Formal Analysis, Writing - Original draft preparation, Project Administration. Ogueri Nwaiwu: Conceptualisation, Data Collection, Writing – Original draft preparation, Writing – Review and Editing, Supervision. Ayomide Olukorede: Data Collection, Writing – Original draft preparation. Writing – Review and Editing. Christian Anumudu: Data Collection, Writing – Original draft preparation. Helen Onyeaka: Data Collection, Writing – Review and editing. Esther Areo: Data Collection, Writing – Original draft preparation. Obadina Adewale: Writing – Review and editing, Supervision. All authors read and approved the final manuscript.

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