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Molecular and microbial identification of Microbiota of processed chicken products: Mini Review

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Chicken and its products, categorized as poultry meat, stand as a prominent source of animal protein, presenting a nutritious and cost-effective substitute for other protein options, such as red meat. The popularity of poultry meat and eggs in various countries stems from their affordability and health benefits. Despite the nutritional value and safety of fresh chicken, its perishable nature and susceptibility to microbial spoilage pose challenges. The entire process, from farm to fork, exposes chicken meat to potential contamination from diverse sources, impacting its quality and safety for human consumption. This review extensively examined both conventional microbial methods and contemporary, culture-independent techniques for identifying the microbiota in processed chicken products. Although there is an enhanced understanding of microbial communities in such products, the specific composition and behavior of this microbiota remain unclear, complicating efforts to manage their presence and activities. The review delved into culture-independent methods like 16S rRNA sequencing and Omic tools for microbiota identification.

Keywords: Microbiota, processed, products, chicken, Molecular

INTRODUCTION

Poultry, including chicken and chicken products, is a primary source of animal protein categorized as poultry meat. Gaining popularity as a healthy and cost-effective alternative to red meat, poultry meat, and eggs are increasingly becoming key components in diets globally, with approximately 137 million tons produced in 2020 to meet rising demand (FAO, 2021). Despite price increases, consumer demand remains high due to low-fat content, high protein levels, ease of production, environmental sustainability, and widespread social acceptance (FAO, 2019; Dourou et al. 2021; Heir et al. 2021). The production and consumption of poultry meat are expected to rise in the coming years, as chicken and poultry products are not strongly linked to major health effects (Dourouet al. 2021; Trijsburg et al. 2021).

However, despite the nutritional benefits, fresh chicken meat is highly perishable and susceptible to microbial contamination from various sources during handling from farm to table (Rouger et al. 2017; Odeyemi et al. 2020). Microbial contaminants compromise the quality and safety of chicken meat for human consumption, leading to economic losses and wastage (Nychaset al. 2008, 2016; Odeyemi et al. 2020). Chicken meat products undergo various processing methods for taste, safety, shelf-life, and consumer acceptability (Chmiel et al. 2018). However, these processed products are not sterile, and commensal and pathogenic microbes can survive processing, resulting in their presence in the final products (Dominguez and Schaffner, 2009; Zhang et al.2021).

Despite advancements in understanding microbial communities in processed chicken products, the specific microbiota composition remains poorly understood (Dourou et al. 2021). This review aims to evaluate both traditional microbial methods and modern, culture-independent approaches for identifying the microbiota in

processed chicken products.

Processed chicken products

Chicken and chicken products undergo physical or chemical modifications to enhance quality, safety, and economic value. Priced competitively, they face minimal cultural, religious, and nutritional opposition, fostering increased demand and production (Valceschini, 2006; Baéza, 2020). Growing concerns for animal rights prompt calls for improved conditions for chickens in meat and egg production. Processing facilitates convenience, making it easier for consumers to obtain freshly dressed chickens or packaged eggs. Ready-to-eat processed forms reduce preparation time, contributing to increased consumption in various settings such as fast-food outlets (Baéza, 2020). Whole chicken consumption has declined, with a rise in the popularity of cut-up sections and processed products, particularly in the US and France (USDA, 2011; La Volaille Française, 2018).

Advanced processing technology offers a diverse range of chicken products, including various forms of meat, whole or processed, and delivered in different packages. Despite this diversity, processed chicken meat products remain the most common focus (Baéza, 2020). Thus, processed chicken products fall into four categories based on processing methods and derived characteristics: breaded, deli meats, raw/marinated/cured, and cooked products (Baéza, 2020). The processing involves treating dressed chicken meat with various materials, gelling with milk and vegetable/animal proteins, and packing in natural or synthetic casings (Barbut, 2015; Baéza, 2020).

Regardless of the category, processing involves treating chicken meat with water, salts, spices, herbs, texturing compounds, and preservatives. Gelling agents from milk and proteins, along with complex sugars or hydrocolloid gums, are used to texture the products. Natural or synthetic covers are employed for packing, while the water content is reduced to increase acceptability and market value (Baéza, 2020). The processing significantly influences the characteristics of chicken products, affecting their nutritional composition. The level of salt, protein, fat, saturated fatty acids and carbohydrates in processed chicken products can vary, impacting daily nutritional intake (Albuquerque et al. 2016; Gibbs et al. 2013). Comparing raw and processed chicken meat reveals changes in chemical composition. Processing tends to increase salt and protein content while reducing fat. Collagen levels may remain stable, but total carbohydrate depends on supplements used during processing (Kayisoglu et al. 2003; Vazgecer *et al.* 2004).

Recipes and cooking methods also play a crucial role. Variations in recipes and cooking techniques can impact the sensory and nutritional value of chicken products, affecting microbial composition (Baéza, 2020). Additionally, the choice of cooking method influences the sensorial presentation and may generate potentially harmful chemicals, indirectly affecting microbial communities (Baéza, 2020; Krempa et al. 2019).

Microbiota of processed chicken products

The muscles of healthy, living chickens remain sterile, while external parts exposed to the environment, such as feathers, skin, lungs, and the gastrointestinal tract, harbor diverse microorganisms forming the chicken microbiota (Rouger et al. 2017; Shang et al. 2018; Carrasco et al. 2019). The host's biological activities play a crucial role in maintaining homeostasis, primarily through competitive exclusion, deterring hazardous pathogens, and reducing metabolic rates. The initial colonization of a bird's digestive tract starts spontaneously from hatching and may potentially begin earlier, mainly through microbes entering through eggshell pores (Roto et al. 2016; Lee et al. 2019). Intensive poultry production enforces strict hygiene to prevent pathogenic germs from colonizing the incubation setting (Khan et al. 2020).

Pathogens use various metabolic pathways to overcome the resident gut microbiota to establish a niche in the gut. AMPs, antimicrobial peptides; cdtA, cytolethal distending toxin subunit A; hybA, hydrogenase-2 electron transfer unit; frdA, fumarate reductase subunit; T4SS, type IV secretory system.

During processing, chicken products face contamination from various sources, with bacteria being the most abundant and diverse. Contaminants from air, liquids, and surfaces in the slaughterhouse, as well as from individuals involved in processing, contribute to bacterial contamination (Rougier et al. 2017). Although chickens are smaller, their carcasses remain vulnerable to contamination from the environment, with bacterial contaminants present on the body surface when freshly dressed (Luber, 2009). Subsequent processing steps, such as marination, reduce or eliminate surface contaminants, which by then have mostly migrated into the muscles (Warsow et al. 2008). As processing advances, the likelihood of environmental contamination decreases, but the risk from poor handling increases, resulting in higher bacterial loads in processed chicken products compared to fresh chicken (Álvarez-Astorga et al. 2002).

Processed chicken products have been implicated in various foodborne illnesses, including occurrences of Salmonella enteritidis linked to raw, refrigerated, and fried chicken items in Canada (Hobbs et al. 2017; Morton et al. 2019).

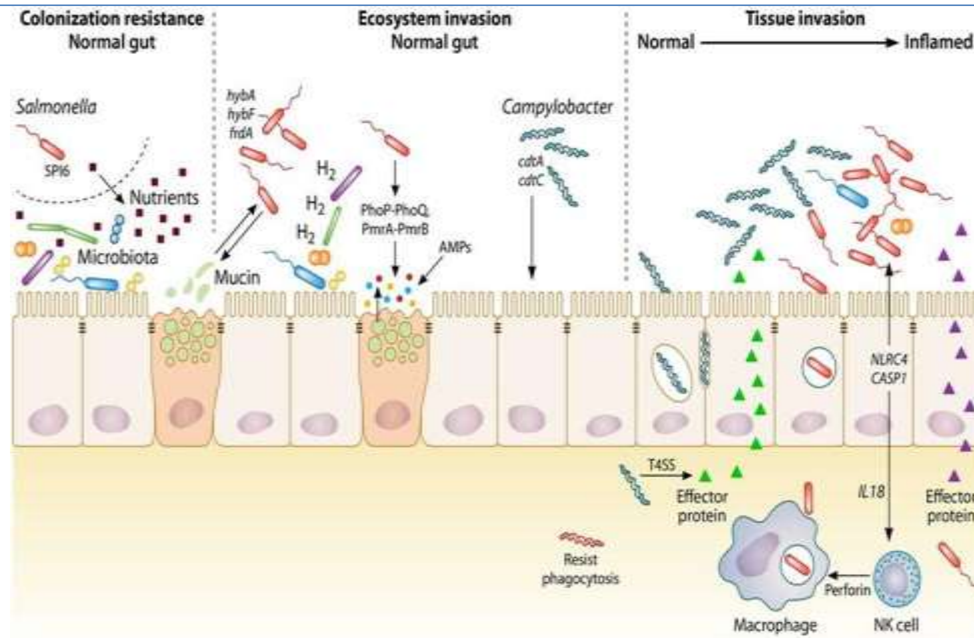

Figure 1: Generalized mechanisms of colonization by foodborne pathogens in the gut (Khan et al. 2020).

Studies have identified bacteria such as Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, and Enterococcus spp. in processed chicken products (Korkmaz et al. 2017; Vazgeceret al. 2004; Elmaliet al. 2005; Omurtag et al. 2012; Karada let al. 2013). Fungal species have also been recovered, with mycological analyses revealing the presence of Penicillium, Aspergillus, Cladosporium, Mucor, Fusarium, Rhizopus, Alternaria, and Candida spp. in chicken meat products (Ogu et al. 2017; Shaltout et al. 2014).

While standard microbial quality assurance processes aim to limit contamination, poor handling during storage poses challenges, and the presence of pathogenic microorganisms in processed chicken products represents a public health risk (Roccatoet al. 2015). The production of processed chicken with the potential presence of bacteria poses a high risk of food poisoning for consumers, particularly from notorious species like Campylobacter spp. and Salmonella spp., emphasizing the severity of illnesses, their impact on public health, and the risks within the processed chicken meat supply chain (Rouger et al. 2017).

Identification of microbiota of processed chicken products

Between 1998 and 2012, chicken emerged as the primary source of foodborne illnesses in the USA (Chai et al. 2016). The proliferation and heightened metabolic activity of numerous microbes, predominantly bacteria, contribute to the degradation of fresh meat quality and the onset of spoilage (Horváth et al. 2007b; Ercolini et al. 2009; Lorenzo et al. 2017). Notably, both fresh and spoiled meat contain significant yeast quantities, albeit in considerably lower numbers (Lucianezet al. 2010). The total aerobic bacterial count on chicken carcasses typically ranges from 102 to 106 CFU g-1, varying with factors such as the farm of origin, production processes, processing cleanliness, and external environment (Rougier, Tresse, and Zagorec, 2017). Gamma-proteobacteria, encompassing various taxa, dominates the bacterial composition of the natural spoilage microbiome in poultry flesh, with Moraxella, Shewanella, Pseudomonas, Aeromonas, and Acinetobacter being the most prevalent genera (Dourou et al. 2021).

Two primary methods for culturing, identifying, and characterizing microbial communities within a living system are culture-based (classical) and culture-independent (molecular) methods. The culture-based method involves cultivating the target organism in a selective medium under specific conditions, classifying bacteria based on phenotypical and biochemical characteristics. While the cultural approach is time-consuming and requires substantial laboratory effort, it remains a practical and cost-effective option for identifying and characterizing organisms in the chicken microbiota, especially those with unknown media requirements for growth (Apajalahti et al. 2004).

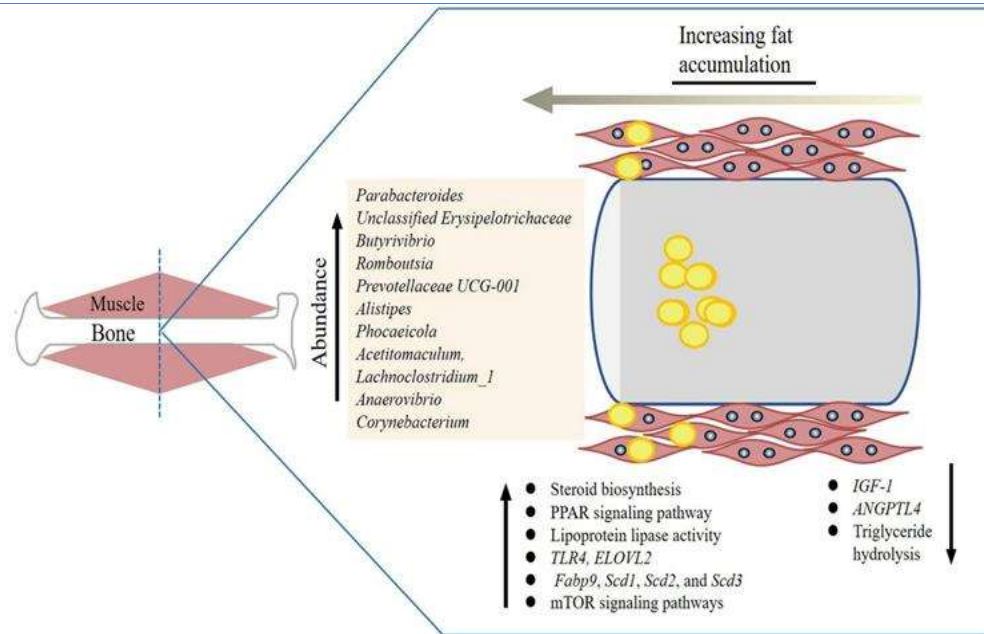

Figure 2: Gut microorganisms related to intramuscular fat accumulation (Chen et al. 2022). The yellow dots are lipid droplets and the bacterial genera related to intramuscular fat (IMF) content are listed. The genes and pathways are also listed.

Molecular Approaches

Accurate detection, characterization, and identification of pathogenic and food spoilage bacteria in processed and raw foods are essential for ensuring safe food production and protecting consumer health. PCR-based molecular biological methods present a promising avenue due to their precision, responsiveness, and significantly shorter processing times compared to traditional phenotypic and biochemical methods (Jasson et al. 2010). In a study by Belak et al. (2011), a multiplex PCR assay based on the co-amplification of the carA gene locus facilitated the simultaneous recognition of significant psychrotrophic meat-spoiling Pseudomonas species, including *P. lundensis, P. fragi, P. fluorescens,* and *P. putida*.

Broad-range PCR, utilizing universal primers targeting highly conservative loci like the 16S rDNA-encoding gene, allows for the amplification of species-specific sequences directly from affected patient tissues. This approach has unveiled new etiologic agents, such as *B. henselae* and *T. whipplei*, associated with bacillary angiomatosis and Whipple disease, respectively, showcasing the power of universal primers in identifying previously unknown pathogens (Relman et al. 1992; Houpikian & Raoult, 2002). The use of broad-range PCR enhances researchers' capacity to partially characterize organisms not traditionally grown and contributes to a better understanding of microbial diversity, evolution, and their potential impact on human health (Valones et al. 2009).

Although the 16S rRNA gene sequence is commonly used for prokaryotic molecular identification, its resolution is insufficient for distinguishing Pseudomonas species effectively (Srinivasan et al. 2015). The rpoB tree has demonstrated approximately three times the phylogenetic resolution of the 16S rRNA tree, providing more precise identification of Pseudomonas strains (Girard et al. 2020). Molecular identification and typing techniques for yeast species in fresh poultry flesh have proven to be more accurate than conventional phenotype-based procedures (Belak et al. 2011; Lopandic et al. 2006). Hence, the existing approach involves adopting a multiphase method for the accurate identification and characterization of the microbiota of chicken products. Unlike phenotypic methods, these molecular tools offer greater speed, reliability, and reproducibility. Moreover, they can discern distinctions among closely related species that may be phenotypically indistinguishable (Sharma et al. 2020).

Despite the benefits of broad-range PCR, concerns about microbial DNA contamination persist. Strict laboratory procedures and specialized reagents can mitigate contamination risks during the amplification process, addressing this challenge (Wernecke & Mullen, 2014). False-positive results may still occur even with meticulous technical measures, underscoring the need for caution. Additionally, examining non-sterile sites, such as chicken feces, poses limitations, which alternative techniques like family-restricted primers, in situ hybridization, or expression library screening can help overcome, delivering more tailored and specific findings (Liu et al. 2012; Young et al. 2020). Interpreting micro-heterogeneity

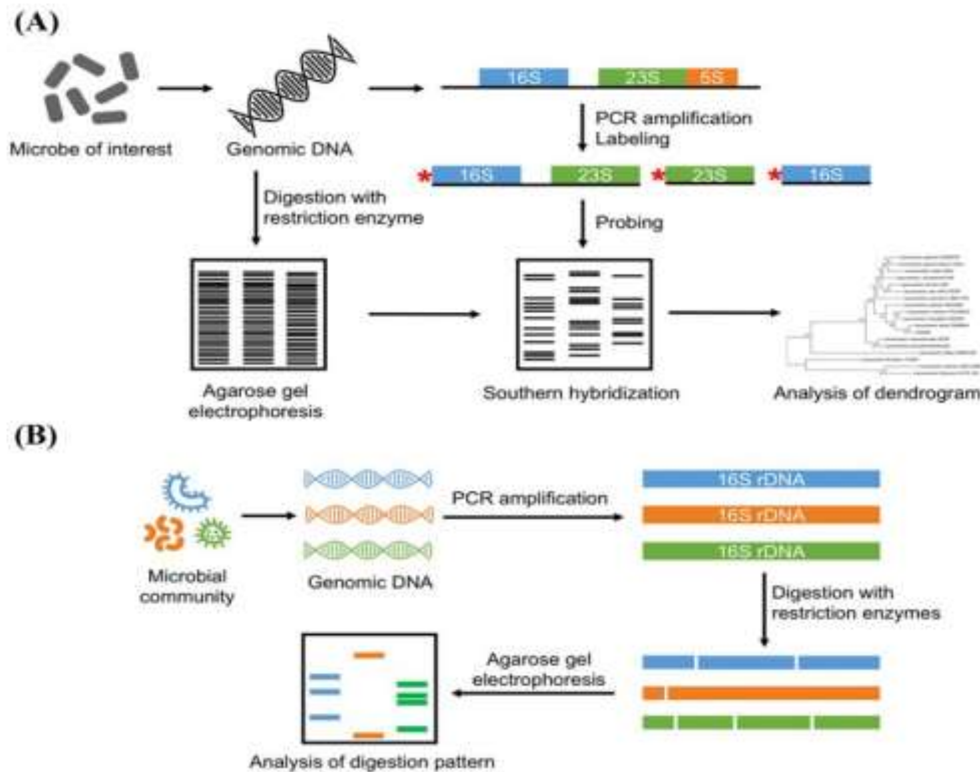

Figure 3: The schematic diagram for Ribotyping (A) and amplified ribosomal DNA restriction analysis (Sharma et al. 2020).

In microbial sequences directly from host tissues, particularly when used as the primary basis for microbial presence identification, presents another potential challenge (Almeida et al. 2010).

Traditional Approaches

Culture Condition

The field of microbiology has significantly progressed, primarily owing to advancements in culture media development. Culture media, composed of water and nutrients, serve as the fundamental basis for microbial growth, with additional growth factors tailored to each bacterium's specific needs (Bonnet et al. 2020). Koch's introduction of the first solid culture medium marked a pivotal moment in bacterial culture development, enabling the creation of colonies and the purification of bacterial clones (Ahern, 2018). Solid culture media, primarily utilizing agar as the gelling substance, have limitations, prompting the development of selective media that inhibit undesired bacterial groups, allowing for the isolation of desired microorganisms (Peterson and Kaur, 2018). Enhanced culture media and conditions tailored for challenging bacteria can result from a deeper understanding of the microbial microenvironment (Bonnet et al. 2020).

In the food industry, safety standards apply to the selection and use of protective food cultures, chosen for their ability to exploit bacterial competition and dominant processes to regulate and minimize foodborne pathogens and spoilage microorganisms (Bourdichon et al. 2021). Understanding microbial competition in complex environments enables the separation, classification, comprehensive categorization, and affirmation of cultures, ensuring added bio-preservative food cultures possess specific properties under circumstances (Bourdichon et al. 2021). The development of a new culture medium for bio-flocculant production, as studied by Mohammed and Degang (2017), highlighted the significant impact of culture conditions on microbe bio-flocculant production. Optimal conditions for *A. flavus* growth and bio-flocculant yield included pH 7, 150 rpm shaking, 35 °C temperature, and a 4% inoculum after 72 hours. However, there was a reduction in output and efficiency after 72 hours, attributed to the creation of enzymes breaking down bio-flocculants. Although bio-flocculants are generally considered safe, evaluating their toxicity is crucial before scaling up production due to the potential for fungi to produce toxins.

Selective Media

Selective media play a vital role in microbiological testing, allowing the separation of microorganisms for identification. The *Enterobacteriaceae* family, commonly used as an indicator for chicken and chicken product spoilage, helps achieve threshold sanitary proportions. Various selective media, such as Violet Red Bile Agar,

BAIRD-PARKER Agar, XLT4, Lactobacilli MRS Agar, and McConkey Agar, are frequently employed to isolate specific bacteria groups (Bonnet *et al.* 2020).

Gram Staining

Gram staining, a fundamental technique in microbiology, categorizes bacterial species into gram-positive and gram-negative groups based on their cell wall characteristics. Despite being reliable, Gram staining cannot categorize all bacteria with certainty. In the food industry, especially in meat-processing plants, Gram staining aids in detecting various microorganisms that may contaminate poultry meat (Wardhana *et al.* 2021).

Biochemical Identification

Biochemical identification, involving multiple biochemical tests and molecular methods, is crucial for identifying microbial flora in meat products, including antibiotic resistance and multi-resistance profiles. Techniques such as BOX, ERIC, (GTG)5, random amplified polymorphic DNA (RAPD), and API gallery tests are commonly used for genetic fingerprinting and microbial identification (Ashraf, 2018; Merieux, France; Franco-Duarte et al. 2019).

In summary, the evolution of culture media, selective media, and identification techniques has significantly contributed to microbiological advancements, ensuring safety and quality in various industries, particularly in food production and processing.

Characterization of microbiota of processed chicken products

Poultry, particularly chicken, and its products, stands out as a crucial animal protein source for low-income communities, recognized for its high protein content, low fat, and minimal religious constraints (Association of Poultry Processors and Poultry Trade, 2016; Tan et al. 2018). Its nutritional benefits have led to widespread consumption on a larger scale compared to other meat types (Belova, Smutka, and Rosochacka, 2012; Pandurevic et al. 2014).

The molecular characterization of bacteria often involves sequencing the 16S rRNA gene (Kim and Chun, 2014). This genetic method offers more precise bacterial identification than traditional phenotypic traits-based methods (Franco-Duarte et al. 2019). While 16S rRNA gene sequence analysis is highly accurate, its infrequent use outside of large facilities is attributed to technical and budgetary reasons (Johnson et al. 2019). It proves valuable in the routine identification of mycobacteria, enhancing the identification of rarely isolated or poorly described strains, and unveiling novel pathogens and non-cultured bacteria (Matsumoto and Sugano, 2013). The comparison of 16S rRNA gene sequences across bacterial phyla allows for classifying strains at various levels, providing deep taxonomic and evolutionary insights surpassing protein-encoding gene sequence comparisons for phylogenetic tree construction (Hassler et al. 2022).

Strength of methods

Traditional microbe identification techniques, encompassing physiological, morphological, biochemical, and chemical characterization, typically take a minimum of two to five days, with the possibility of extending to twelve days for molds (Franco-Duarte et al. 2019). However, these phenotypic methods often demand significant time and resources, and they may not consistently provide accurate identification at the species or strain level (Donelli, Vuotto, and Mastromarino, 2013; Dubourg, Laami, and Ruimi, 2018).

To expedite microbial identification, molecular biology techniques are increasingly employed, often complemented by various molecular fingerprinting methods (Adzitey, Huda, and Ali, 2013). The combination of these approaches, guided by multivariate methods, holds promise for faster and more accurate microbial characterization (Franco-Duarte et al. 2019). While the future of these methods looks promising, it is essential to carefully select appropriate approaches and understand their underlying mechanisms for precise estimation, categorization, and taxonomic classification of microorganisms (Pitt and Barer, 2012; Franco-Duarte et al. 2019).

Advancements in molecular technologies and sequence databases in the latter half of the 20th century significantly enhanced microbiology's capabilities and expanded the catalog of recognized bacterial communities (Franco-Duarte et al. 2019). The introduction of Polymerase Chain Reaction (PCR) in 1985 marked a pivotal moment in using genetics for microorganism identification, leading to the development and refinement of various techniques based on both culture-independent and culture-dependent approaches (Kadri, 2019). Omics tools, including proteomics, metagenomics, metabolomics, transcriptomics, and lipidomics, are now employed for comprehensive microbial characterization, offering high-throughput insights into the structure, function, and behavior of organisms (Klenk, 2019). These methods find applications across various domains, such as phylogeny, transcriptional profiling, microbial ecology, and functional genome analysis, with significant practical implications (Franco-Duarte et al. 2019).

Limitations of methods

As much as these methods have their advantages and are more frequently used now, they still have their limitations, and they will be discussed below.

Traditional immunoassays and culture procedures can be replaced with more rapid and accurate molecular diagnostic approaches. Nevertheless, despite their undeniable benefits, they have so far only partially

supplanted conventional approaches in analyses (Nichols, 2021). Numerous issues continue to prevent the widespread use of diagnostic tests that use the pathogen's nucleic acids rather than its phenotypic. The abundance of false positive and false negative outcomes is a significant contributing factor. A disease may be mistakenly identified due to DNA contamination in the environment, the lab, and even the tools used to prepare the reaction mix. In contrast to living cell pollutants, which can be easily cleaned off surfaces and lab equipment, DNA is more difficult to remove (Lauri and Mariani, 2009).

Additionally, the presence of inhibitors can result in erroneous negative results. It frequently happens that if the item being examined is a complex matrix, like cheese or salami, it may contain chemical substances capable of interfering with the activity of the enzymes. Thus, enzyme inhibition may result in a misleading negative test result. As a result, adding positive controls, such as the IPC for the TaqMan PCR, is essential to ensure the test's validity (Lauri and Mariani, 2009).

Recently, the potential to study the gut microbiota and its metabolic activity in poultry animals has increased thanks to the advent of novel omics technologies and platforms (Zampiga, 2018). The term "omics" refers to a collection of technologies used to define or measure a certain molecular level. Sadly, it is hard to identify a collection of compounds using a single method alone; as a result, numerous omics and innovations should be created and employed carefully in diverse settings to overcome each weakness in a particular technique (Dirong et al. 2021).

SUMMARY

Chicken and chicken products have rapidly become the dominant sources of animal protein, presenting an affordable and healthful alternative to red meat. Particularly popular among individuals with lower incomes, chicken meat and eggs are increasingly recognized as dietary staples. While fresh chicken flesh is nutritionally valuable, its high perishability poses challenges. The susceptibility of chicken to microbial cultivation is influenced by its physical and chemical characteristics. However, the processing journey from farm to table exposes it to various sources of microbial contamination, jeopardizing its quality and safety for human consumption.

This review aimed to assess both traditional microbial methods and modern, culture-independent techniques for identifying the microbiota in processed chicken products. Despite enhanced knowledge about microbial communities in these products, the specific microbiota remains poorly understood, complicating efforts to control their presence and activity. The review covers conventional methods like selective medium use, culture conditions, and gram staining for microbiota identification in chicken and processed products. Additionally, it explores advanced techniques such as 16S rRNA sequencing and Omic tools, shedding light on how these bacteria can be identified and managed to ensure safe consumption and mitigate the risk of food-borne illnesses.

CONCLUSIONS

The review underscores the superior performance of molecular techniques compared to traditional procedures in terms of both speed and high specificity. Literature suggests that molecular diagnostic tools exhibit greater sensitivity by amplifying and identifying target genetic material. Moreover, these methods are deemed more cost-effective in the long run as they can identify multiple diseases in a single test. The automation potential of molecular techniques enhances productivity and reduces human error. However, despite the advancements in molecular methods, the current systematic review notes that classical procedures continue to improve in sensitivity and specificity. As a result, these methodologies are considered complementary today, contributing to diagnostic and detection outcomes that are more reliable, standardized, and comprehensive. It is emphasized that while broad-range PCR is effective in taxonomy establishment, most newly identified infectious diseases are ultimately characterized after the culture and isolation of pathogenic agents.

Supplementary materials

Not applicable

Author contributions

Conceptualization, S.M.A. and D. N.; methodology, F.A. A.; validation, K. S. A.; formal analysis, A. H. A.; data curation, A. A. G.; writing-original draft preparation, S. M. A.; writing-review and editing, D. N. and A. M. S.; visualization, A. M. S.; supervision, B. O. A. and M. A. A.; project administration, S. M. A. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declared that the present study was performed in the absence of any conflict of interest.

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