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Molecular and Microbial Identification of Microbiota of Processed Chicken Products: Mini Review

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Abstract

Chicken and chicken products are a leading source of animal protein classified under poultry meat. They are a healthy and affordable alternative to other protein sources like red meat. This makes poultry meat and eggs a popular dietary component in most countries. Despite the relative safety and nutritional status of fresh chicken meat, it is a perishable product. Its physical and chemical properties make it ideal for microbial spoilage, while its handling from the farm to the fork makes it vulnerable to microbial contamination from multiple sources. The presence and activities of microbial contaminants in chicken meat compromise its quality and safety for human consumption. This review sought to evaluate the conventional microbial methods for identifying the microbiota of processed chicken products as well as the modern, culture-independent methods of microbiota identification. Despite an increased understanding of the presence and activity of microbial communities in processed chicken products, the microbiota that constitutes this community is not well understood, making their presence and activity difficult to control. Also, 16S rRNA sequence and Omic tools were reviewed as some culture-independent techniques for microbiota identification. Thus, the understanding of how bacteria in chicken and chicken products can be controlled and reduced using these methods to improve consumption of chicken and chicken products was systematically explored. The implementation of the outcome of this study would reduce the prevalence of foodborne illnesses in consumers.

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Introduction

Chicken and chicken products are a leading source of animal protein classified under poultry meat. As a healthy and affordable alternative to other protein sources such as red meat, poultry meat, and eggs are fast becoming popular dietary components, with about 137 million tons produced in 2020 to meet an ever-increasing global demand(FAO, 2021). Despite the steady increase in the prices of poultry meat over the years, consumer demand for poultry products remains high due to their low-fat content, high level of protein, ease of production, environmental sustainability, and wide 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 years ahead since the consumption of chicken and poultry products is not strongly linked with any major health effects (Dourou *et al.*, 2021; Trijsburg *et al.*, 2020).

Despite the relative safety and nutritional status of fresh chicken meat, it is a highly perishable product. Its physical and chemical properties make it ideal for microbial colonization, while its handling from the farm to the table makes it vulnerable to microbial contamination from multiple sources (Rouger *et al.*, 2017; Odeyemi *et al.*, 2020). The presence and activities of microbial contaminants in chicken meat compromise its quality and safety for human consumption (Nychas *et al.*, 2008; Odeyemi *et al.*, 2020), and with the attendant economic losses and wastage (Ishangulyyev*et al.*, 2019).

Chicken meat products are processed either by grilling, cooking, or via any of the several industrial processes to improve their taste, safety, shelf-life, and acceptability among consumers (Chmiel *et al.*, 2018). However, processed chicken products are not sterile. Commensal and pathogenic microbes have been shown to survive the chemical and physical processing of raw chicken meat (Dominguez and Schaffner, 2009), leading to their presence in processed products.

Despite an increased understanding of the presence and activity of microbial communities in processed chicken products, the microbiota that constitutes this community is not well understood(Dourou *et al.*, 2021), thereby making their presence and activity difficult to control. This review seeks to assess the traditional microbial methods for identifying the microbiota of processed chicken products, as well as the modern, culture-independent methods of microbiota identification.

Processed Chicken Products

Process chicken products are meat and egg products that have undergone physical or chemical modification to improve their quality, safety, and economic value. Chicken products are competitively priced and affordable, and there is little cultural, religious, and nutritional opposition to their use (Valceschini, 2006; Parrott & Walley, 2017). The features favor an increased demand for chicken products, spurring the production of chicken products, which is expected to rise even higher in the succeeding years (Baéza, 2020). Concerns about animal rights and welfare are, however, driving calls for better treatment and conditions for chickens raised for meat and eggs.

The processing of chicken products has also increased their consumption. It is more convenient for consumers to buy freshly dressed chickens or packaged eggs than to rear or dress the chickens by themselves. The availability of cut pieces and processed forms of chicken products that are ready-to-eat reduces the preparation time and makes the products appropriate for consumption in multiple places, such as fast-food outlets and restaurants (Baéza, 2020). As a result, the consumption of whole chickens has reduced in the last four decades (USDA-ERS, 2021). What is current, instead, is the increased consumption of cut-up sections and processed products from chicken, especially in the US (USDA, 2011) and France (La Volaille Française, 2018).

Thanks to improved processing technology, a wide variety of chicken products are now available on the market. The products of these technologies include different forms of chicken meat, processed as a whole or in parts, and delivered in a variety of packages and dimensions. Whole chicken eggs, egg yolks, and whites are processed, too, into liquid and powdered forms for use as food or in food manufacturing industries across the world (Lechevalier *et al.,* 2011; Roseland *et al.,* 2022). Despite the diversity of chicken products, the most common is derived from the meat component (Baéza, 2020). Therefore, processed chicken meat products will be the focus of this review.

There are four categories of processed chicken products depending on the processing method and characteristics of the derived products (Baéza, 2020). Breaded products are treated with brine before being breaded into crispy and crunchy end forms. Deli meats are cleared forms of chicken meat made into pâtés, ham, rillettes, sausages, terrines, and galantine. To create a single line of items, raw, marinated, or cured products are chopped into pieces and crammed together. The last category is cooked products made from steaming, frying, and roasting.

Regardless of the category of chicken product, the processing follows a common trajectory. Dressed chicken meat is treated with a variety of materials, including water, different assortments of salts, spices, aromatic herbs, texturing compounds, and preservatives (Barbut, 2015). The products are gelled with agents from milk and from both vegetable and animal proteins. Complex sugars or hydrocolloid gums may be used to texture the products (Sarteshnizi *et al.* 2015), while the final products are packed in natural casings or synthetic covers like cellulose and plastic (Baéza, 2020). Meanwhile, the initial water content of the products is reduced, while the product's natural content is maintained or amplified to increase their acceptability and market value.

The processing of chicken meat influences its characteristics. For instance, it is known that an adult can meet 49% of the daily requirement for salt with a serving of nuggets (Albuquerque *et al.* 2016), which is very rich in carbohydrates, fats,

saturated fatty acids, and salt (Gibbs *et al*., 2013). When this is adjusted with the nutritional or proximate composition of chicken meat, it will be seen that one is taking product rich in protein and products of supporting structures (De Shazo *et al*. 2013)

The influence of chicken processing on its chemical composition is more evident when the raw and processed forms of chicken meat are compared. Kayisoglu *et al*. (2003) found that processing caused an increase in the salt and protein content of chicken meat while reducing the fat content in the same product. Another study by Vazgecer *et al*. (2004) showed the level of collagen was maintained during processing, whereas the total level of carbohydrate in the product depended on the amount of carbohydrate supplement used during processing.

The kind of recipe used also matters. Poultry meat products are prepared through different methods with their corresponding set of recipes. The industry frequently modifies recipes and processes to boost output, improve nutritional and sensory value, and preserve products while also reducing prices. Studies have mostly examined cooking yield to increase output, looking at how independent kinds and amounts of fiber or starch affect the sensory and/or nutritional benefits (Baéza, 2020). In contrast to wheat bran, which had the opposite impact, egg white and cholesterol (5 to 15 g) added to nuggets diminished the product's solidity. (Pathera *et al*. 2017). However, the multiplicity of recipes also has the potential to derange the microbial composition of the products.

Aesthetics-wise, the cooking methods matter too. The choice of cooking method influences the sensorial presentation of the products and may generate chemicals that are harmful to users (Baéza, 2020). Since heating boosts proteins and mineral composition while reducing water content (Zweitering *et al*., 2021), the method adopted can indirectly affect the establishment and survival of microbial communities in the final product.

Microbiota of Processed Chicken Products

The muscles of healthy and living chickens are sterile. However, the parts of the chicken that are in constant exchange with the outside environment, such as the feathers, skin, lungs, and gastrointestinal tract, are colonized by a host of microorganisms that constitute chicken microbiota (Rouger *et al*., 2017) Shang *et al.,* 2018; Carrasco *et al.,* 2019). The influence and impact of the biological activities of the host are needed for homeostasis. This is accomplished primarily through the competitive exclusion of hazardous pathogens and bacteria which deters colonization and lowers the metabolic rate that birds would typically use to maintain their immune systems' readiness to combat these diseases.

The initial colonization of a bird's digestive tract is brought on by microbes that enter through the eggshell's pores and happen spontaneously from the moment of hatching and may potentially start earlier (Roto *et al.,* 2016; Lee *et al.,* 2019). To prevent pathogenic germs from colonizing the incubation setting and to ensure that freshly hatched birds receive their initial microbiome from an artificial environment on the farm rather than the natural maternal source, intensive poultry production requires strict hygiene standards. Nevertheless, throughout processing, different sources contaminate chicken products. Bacteria appear as the most abundant and diverse of these sources. Bacteria from the air, liquids, and surfaces in the slaughterhouse are prime contaminants, as are the bacteria from the individuals carrying out the processing

(Rouger *et al*., 2017).

Although chickens are easier to process due to their small size, their carcasses are still vulnerable to contamination from bacteria in the air and environment (Vihavainen *et al.,* 2007). Initially, when the chicken meat is freshly dressed, bacterial contaminants are present on the body surface (Luber, 2009). Subsequent processing, such as marination reduces or eliminates the surface contaminants, which by that time have mostly migrated into the muscles (Warsow *et al.,* 2008). At every step in the processing, the likelihood of contamination from environmental sources reduces, whereas the risk of contamination from poor handling increases (Álvarez-Astorga *et al.,* 2002,(Rouger *et al*., 2017). As a result, processed chicken products have higher bacterial loads than fresh chicken (Álvarez-Astorga *et al.,* 2002). These contaminants can thus persist in the final product until it reaches the final consumer.

Significantly, processed chicken products have been implicated in a range of foodborne illnesses. Studies of various human occurrences of *Salmonella enteritidis* illnesses linked to raw, refrigerated, fried chicken items in Canada have been reported (Hobbs *et al*., 2017; Morton *et al*., 2019). Other studies by Walker-York-Moore *et al.* (2017), Vazgecer *et al.* (2004), Kim *et al.* (2014), Abdou *et al.* (2012), Bennett *et al.* (2013) have indicated the presence of bacteria such as *Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Escherichia coli, Listeria monocytogenes, Enterococcus spp* and more in processed chicken products.

Fungal species have also been recovered from processed chicken meat products. For instance, mycological analysis of fresh and frozen chicken meat retailed in a Nigerian metropolis showed the presence of *Penicillium*, *Aspergillus, Cladosporium, Mucor Fusarium, Rhizopus*, *Alternaria*, and *Candida* spp. (Ogu *et al.,* 2017). When a similar analysis was done on packaged chicken meat products sold in Egypt (Ismail *et al.,* 2013), mold genera such as *Aspergillus Penicillium Geotrichum*, *Fusarium, Cladosporium Mucor Eupenicillium Scopulariopsis,* and *Acremonium* were recovered.

Although standard microbial quality assurance processes now include measures to limit microbial contamination and subsequent growth in chicken products. Their success, however, is often limited by poor handling during storage (Roccato *et al.,* 2015); therefore, given that some of these microorganisms are pathogenic, their presence in processed chicken products represents a public health risk.

The production of processed chicken with the actual or potential presence of bacteria puts consumers at a high risk of food poisoning, especially from notorious species like *Campylobacter spp.* and *Salmonella spp.* (European Food Safety Authority (EFSA), 2012). These pathogens deserve special mention due to the severity of illnesses that they cause, their potential impact on the public, and their risk of occurrence in the processed chicken meat supply chain (Rouger *et al*., 2017).

Identification of Microbiota of Processed Chicken Products

Between 1998 and 2012, chicken was identified as the primary source of foodborne illnesses in the USA (Chai *et al*., 2016). The growth and increase in metabolic activity of numerous microbes, predominantly bacteria, is responsible for

fresh meat's loss of quality as well as the advancement of rotting (Horváth *et al*., 2007b; Ercolini *et al*., 2009; Lorenzo *et al*., 2018). However, it has also been shown that both fresh and rotten meat contain significant amounts of yeasts, but in considerably lower numbers (Lucianez *et al*., 2010). The total aerobic bacterial count of chicken carcasses typically varies from 10^2 to 10^6 CFU g-1, but it also relies on the farm where the chickens were raised, the production process used, the processing cleanliness, and the outside environment (Rougher, Tresse, and Zagorec, 2017). *Gamma-proteobacteria* of various taxa are the most common bacteria that are linked to the natural rotting microbiome of poultry flesh. The most significant genera are *Moraxella, Shewanella, Pseudomonas, Aeromonas, and Acinetobacter* (Dourou *et al*., 2021).

The two main approaches for culturing, identifying, and characterizing microbial communities within a living system are the culture-based (classical) methods and culture-independent (molecular) methods. The microbial method works by growing the target organism in a selective medium under specific culture conditions, before classifying the bacteria based on their phenotypical and biochemical characteristics. The cultural approach is a tedious process. It requires a lot of laboratory time, and it is comprehensive enough to capture organisms whose media requirements for growth are unknown (Apajalahti *et al.,* 2004). However, it still presents a convenient and affordable option for the identification and characterization of organisms in the chicken microbiota.

Molecular Approaches

Accurate pathogenic and food spoilage bacterial detection, characterization, and identification in processed and raw foods are required to ensure the safe production of food and the protection of consumers' health. The use of PCR-based molecular biological methods for identifying spoilage and pathogenic microorganisms seems promising because of their high precision and adequate responsiveness, as well as the fact that their processing times are much shorter compared to phenotypic and biochemical processes (Jasson *et al*., 2010). According to a study by Belak*et al*. (2011), *P. lundensis, P. fragi, P. fluorescens,* as well as *P. putida* were thought to constitute the most significant psychrotrophic meat-spoiling *Pseudomonas* species, and a multiplex PCR assay based on the co-amplification of several *carA* gene loci was devised for the concurrent recognition of the species.

Species-specific sequences may be amplified directly from affected patient tissues and compared to a reference-sequence database to infer phylogenetic connections using universal primers that recognize extremely conservative loci, such as the 16S rDNA-encoding gene (Houpikian & Raoult, 2002). This broad-range PCR approach has increased researchers' capacity to partially characterize organisms that have never been classically grown. Thus, two inexplicable diseases have been linked to new etiologic agents: B. henselae in bacillary angiomatosis and, a year later, T. whipplei in Whipple disease patients (Relman *et al*., 1992; Houpikian & Raoult, 2002). These discoveries have highlighted the power of universal primers in uncovering previously unknown pathogens and have opened new avenues for research into the causes of various diseases (Boujelben *et al*., 2018). The use of broad-range PCR has allowed for a better understanding of the diversity and evolution of microorganisms, providing valuable insights into their ecological roles and potential impacts on human health (Valones *et al*., 2009).

Despite being commonly used in the molecular identification of prokaryotes, the 16S rRNA gene sequence, *Pseudomonas*, lacks sufficient sequence differences within this region to allow for the identification of species (Srinivasan *et al*., 2015). Previous studies showed that a substantial number of*Pseudomonas* strains could be more precisely identified because the phylogenetic resolution of the *rpoB* tree was about three times that of the 16S rRNA tree (Girard*et al*., 2020). In addition to the bacteria microbiota present in chicken and chicken products, fresh poultry flesh also contains species of yeast from the *Ascomycetes* or *Basidiomycetes* families, albeit these extremely fermentative species are rare (Lorenzo *et al*., 2018). The identification and characterization of yeasts using molecular identification and typing techniques rather than conventional phenotype-based procedures have proven to be far more accurate (Belak *et al*., 2011; Lopandic *et al*., 2006).

Several other issues are involved in the use of molecular diagnostic tools, including the issue of microbial DNA contamination (Houpikian & Raoult, 2002). However, outweighs the benefits of broad-range PCR. During the amplification procedure, microbial DNA contamination might take place and provide false-positive findings (Wernecke & Mullen, 2014). Strict laboratory procedures and the use of specialist reagents that reduce the danger of contamination can help to resolve this problem (Wernecke & Mullen, 2014). False-positive responses can happen even when meticulous technical measures are taken to reduce contamination of the PCR reaction (Borst *et al*., 2004). Another obvious limitation of broad-range PCR is the examination of non-sterile sites, such as chicken feces; however, the use of family-restricted primers, in situ hybridization with specific nucleic probes, or expression library screening with immune sera may help to overcome such limitations (Wernecke & Mullen, 2014). By concentrating on certain genetic markers or immunological responses, these alternative techniques can deliver more tailored and specific findings (Liu *et al*., 2012). However, when working with non-sterile materials such as chicken feces, the accuracy and sensitivity of the PCR study can be improved by employing family-restricted primers, in situ hybridization, or expression library screening (Young *et al*., 2020). Another possible concern arises when interpreting the microheterogeneity revealed in microbial sequences taken directly from host tissues, particularly when these sequences become the primary basis for identifying the presence of microbes (Almeida *et al*., 2010).

Traditional Approaches

Culture Condition

Microbiology has significantly advanced, largely due to the development and improvement of culture media. Water and nutrients are the basic building blocks of a culture medium into which additional growth factors that are particular to each bacterium and necessary for its development should be incorporated (Bonnet *et al*., 2020). The development of bacterial culture through the media used for their culture began with Koch's invention of the first solid culture medium, which allowed not only the creation of bacterial colonies but also the capacity to purify a bacterial clone (Ahen, n.d). The main gelling substance used in solid culture media is agar, but there are a number of limitations (Bonnet *et al*., 2020). The discovery of antimicrobials and their precise targets has served as motivation for the creation of selective media since

they can inhibit the growth of desired groups of bacteria. The elimination of undesirable bacteria from the microbiota and the selection of desired microorganisms are made possible by these inhibitory chemicals (Peterson and Kaur, 2018). Improved culture media and conditions that are best suited to some persistent bacteria that are difficult to isolate can be made possible by a better understanding of the microbial microenvironment (Bonnet *et al.*, 2020).

The same safety standards that apply to all food cultures employed in the food business must be met in the choice and use of protective food cultures (Lauland *et al.*, 2017). Food cultures are chosen for their capacity to take advantage of bacterial competitiveness and dominating processes to regulate and minimize foodborne pathogens and spoiling microorganisms (Bourdichon *et al.*, 2021). Utilizing the way microorganisms compete with one another in a complex environment, separation, classification, comprehensive categorization, and affirmation of cultures allows for the assurance that the added bio-preservative food culture possesses properties under a particular circumstance (Bourdichon *et al.*, 2021).

According to a study conducted by Mohammed and Degang (2019) on the development of a new culture medium for bio-flocculant production using chicken viscera, the culture conditions for the development of any microbe have a major effect on its bio-flocculant production or flocculation rate of the bio-flocculant. Five conditions (inoculum size, time, agitation speed, pH, and temperature) were taken into consideration at once to determine the best culture conditions for raising *A. flavus* and maximizing the bio-flocculant yield with viscera hydrolysate. Others were fixed, while the conditions of interest were varied to study a particular condition. The best culture conditions for *A. flavus* development and bio-flocculant yield were pH 7, 150 rpm shaking, 35 °C temperature, and 4% inoculum after 72 hours of incubation. The *A. flavus* growth profile's logarithmic and stationary phases showed parallel relationships between the mycelial weight and the bio-flocculant yield and efficiency. However, even with continuously increasing mycelial weight, there was a reduction in output and efficiency after 72 hours. This is partly caused by the creation of enzymes that break down bioflocculants. Even though bio-flocculants have generally been reported to be safe, it is important to evaluate the toxicity of bio-flocculants before scaling them up because fungi can produce toxins, and there is a lack of information on how safe bioflocculants made by microorganisms are.

Selective Medium

A selective medium facilitates the selection of one or more microorganism species. Only one microbe is allowed to grow on the medium, whereas all others are prohibited from doing so (Bonnet *et al.*, 2020). As a fair indicator of the entire bacterial load of meat and meat products, threshold sanitary proportions are achieved when the overall number of bacteria on fresh meat is between 10,000 (1.0×10^4) and 100,000 (1.0×10^5)/gram (Bersisa, 2019). Workable biologic standards typically include the amount of safe and hygienic microbes that can be used as a marker for specific hygienic dangers, in addition to the total plate count because the overall amount, however, does not allow for any inferences concerning the nature of the microbes, i.e., whether the pathogens are detrimental or inconsequential (Magar, 2021). It is possible to separate these microorganisms from the total population of bacteria using a selective bacterial culture medium that comprises additives that limit the development of all bacteria save the group of microorganisms that will be identified and employed as the indicator bacteria (Bonnet *et al.*, 2020).

The *Enterobacteriaceae* family of bacteria, which is a type of bacteria that causes chicken and chicken products to spoil, is the indicator bacteria that is most frequently utilized. The gut microflora includes the family *Enterobacteriaceae* (Rougher, Tresse, and Zagorec, 2017) and most importantly, this group includes dangerous food-poisoning bacteria like pathogenic *E. coli* and *Salmonella* (Bintsis, 2017). Large-scale contamination with dirt or even feces is possible if there are more *Enterobacteria* identified in food with all the repercussions it entails, especially the occurrence of bacteria that can cause foodborne illnesses (Todd, 2014). For the criterion of "excellent microbiological standard," the number of Enterobacteriaceae should not be more than 100 per centimeter (Heinz and Hautzinger, 2007; Zwietering, Ross, and Gorris, 2014). The Violet Red Bile (VRB) Agar, a selective culture medium used to identify *Enterobacteriaceae*, comprises crystal violet and bile salt to inhibit all other bacteria (Aryal, 2022). BAIRD-PARKER Agar for the isolation of *Staphylococcus aureus*, XLT4 for the isolation of *Salmonella*, Lactobacilli MRS Agar for the isolation of *Lactobacillus*, and Mc Conkey Agar for the isolation of molds are additional frequently utilized selective culture medium (Heinz and Hautzinger, 2007).

Gram Staining

In microbiology and bacteriology, Gram staining, also referred to as Gram's method, is a staining method used to categorize bacterial species into two main groups: gram-positive bacteria and gram-negative bacteria (Brukner, 2021). Bacteria are identified by gram staining by examining the physical and chemical features of their cell walls (Steward, 2022).

Gram-positive cells have a thick layer of peptidoglycan in their cell walls that helps to preserve the primary stain, crystal violet, whereas Gram-negative cells have a thinner layer that allows ethanol to be added to remove the crystal violet (Tripathi and Sapra, 2022). The initial stage in the investigation of bacteria is nearly always gram staining. Despite being a reliable test both in scientific and clinical contexts, this method cannot be used to categorize all bacteria with certainty (Ogawa *et al*., 2020). One of the most difficult issues facing the global food business is the contamination of chicken products, including raw broiler meat, by harmful microbes, particularly bacteria (Wardhana *et al*., 2021). During operations in meat-processing plants, poultry meat may become contaminated by various microorganisms (Rougher, Tresse, and Zagorec, 2017), which can be detected by Gram staining.

Biochemical Identification

Microorganisms are identified using biochemical tests (Ashraf, 2018). Combining two or more biochemical and molecular methods can also be used to identify different bacterial species (Peres *et al*., 2007; Mills, 2007). The conventional multiple biochemical tests such as API gallery 20E, 20 NE, and 50 CHL (Merieux, France) can be used to identify the microbial flora present in meat products as well as the bacteria's antibiotic resistance and multi-resistance profiles (González-Gutiérrez *et al*., 2020). BOX, ERIC, and (GTG)5 are examples of evolutionarily conserved repetitive sequences. Random amplified polymorphic DNA (RAPD) is another technique for creating genetic fingerprints (Vuyst *et al*., 2008).

For microbial diversity or variability and their ecological distribution, fingerprinting techniques have been applied. They are frequently used for biochemical identification and confirmation estimation because they are very practical and economical (Franco-Duarte *et al*., 2019). These are also regarded as useful resources for identifying and classifying a wide variety of Gram-positive and Gram-negative bacteria (El Sheikha, 2017). The methods produce DNA amplification with patterns unique to a particular bacterial species by amplifying variable sections of DNA surrounded by repetitive sequences (Braem *et al*., 2011).

Characterization of Microbiota of Processed Chicken Products

The best source of animal protein now available for low-income communities is poultry, especially chicken and its products, which is recognized to be a very good source of protein with low-fat content and little to no religious constraint (Castro *et al*. 2023; Tan *et al*., 2018). Due to these benefits, more people consume poultry meat on a wider scale than any other type of meat (Belova, Smutka, and Rosochacka, 2012; Pandurevic *et al*., 2014).

To characterize bacteria molecularly, the 16S rRNA gene is sequenced (Kim and Chun, 2014). A common genetic method is the comparison of the bacterial 16S rRNA gene sequence, which is more exact genetically than the traditional phenotypic traits-based bacterial identification methods (Franco-Duarte *et al*., 2019). Outside of large and reference facilities, 16S rRNA gene sequence analysis is not frequently employed despite its accuracy for a variety of technical and budgetary reasons (Johnson *et al*., 2019). It can be used routinely to identify mycobacteria, particularly those infrequently isolated, to better identify poorly described or phenotypically aberrant strains, and to identify novel pathogens and non-cultured bacteria (Matsumoto and Sugano, 2013). The classification of strains at various levels, including what we currently refer to as the species and subspecies level, is made possible by comparing the 16S rRNA gene sequences across all major phyla of bacteria (Johnson *et al*., 2019). The 16S rRNA gene appears to be a more reliable method for obtaining deep taxonomic and evolutionary relationships than protein-encoding gene sequence comparisons for obtaining phylogenetic trees (Hassler *et al*., 2022).

Strength of Methods

A minimum of two to five days, or possibly twelve in the case of molds, are thought to be required for microbe identification based on the conventional technique, which encompasses physiology, morphology, biochemical, and chemistry characterization (Franco-Duarte *et al*., 2019). Additionally, most phenotypic techniques used in microbiological laboratories require a lot of time and resources (Donelli, Vuotto, and Mastromarino, 2013). It’s crucial to keep in mind that phenotypic methods are not always successful at correctly identifying microorganisms at the species or, even more frequently, strain level (Dubourg, Laami, and Ruimi, 2018).

One way to reduce the time needed for microbial identification is to use molecular biology techniques, which should also be complemented by a variety of molecular fingerprinting techniques (Adzitey, Huda, and Ali, 2013). Each approach has advantages and disadvantages, and the most recent studies make use of a combination of multivariate methods (Franco-

Duarte *et al*., 2019). The future seems to hold a lot of promise for this implementation, but it is also crucial to select the right approaches and have a good grasp of the mechanisms underlying their action to acquire the precise estimation, categorization, and taxonomic classification of these microorganisms (Pitt and Barer, 2012; Franco-Duarte *et al*., 2019).

The development of molecular technologies and sequence databases in the latter half of the 20th century considerably enhanced the power of microbiology and the number of recognized bacterial communities (Franco-Duarte *et al*., 2019). The development of PCR (Polymerase Chain Reaction) in 1985 marked the beginning of the use of genetics as a method for identifying microorganisms. Since then, a variety of techniques have been created and refined (based on both culturally independent and dependent approaches) based on different principles (hybridization, 16S rRNA sequencing, and DNA sequencing) (Kadri, 2019). Additionally, Omics tools such as proteomics, metagenomics, metabolomics, transcriptomics, and lipidomics are employed for characterizing microorganisms (Breitwieser and Salzberg, 2017). These are intended to characterize and quantify pools of biological molecules in a collective high-throughput manner that corresponds to the architecture, functioning, and behaviors of an organism or organisms (Reiter & Klenk, 2018). These methods are currently employed for a variety of applications and have some significant practical ramifications (e.g., phylogeny, transcriptional profiling, microbial ecology, and functional genome analysis) (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.

1. 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 of 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).

2. 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

As far as poultry meat goes, chicken and chicken products are quickly overtaking all other sources of animal protein. They are a cheap and healthful substitute for other protein sources like red meat, and chicken meat and eggs are quickly gaining popularity as dietary staples since they are less expensive, especially for people with lower incomes. Fresh chicken flesh is a relatively healthy and nutritious commodity, but it is also a highly perishable one. Its physical and chemical characteristics make it the perfect material for microbial cultivation, but the processing it undergoes from the farm to the table leaves it open to microbial contamination from a variety of sources, compromising its quality and suitability for human consumption.

This review sought to evaluate the conventional microbial methods for identifying the microbiota of processed chicken products as well as the modern, culture-independent methods of microbiota identification. Despite an increased understanding of the presence and activity of microbial communities in processed chicken products, the microbiota that constitutes this community is not well understood, making their presence and activity difficult to control. Selective medium, culture conditions, and gram staining are a few of the conventional microbial techniques for identifying the microbiota of chicken and processed chicken products that are described in this review. 16S rRNA sequence and Omic tools are some culture-independent techniques for microbiota identification. This article went into greater detail about how these bacteria in chicken and chicken products can be found and controlled to ensure safe consumption and lower the risk of contracting foodborne illnesses.

Conclusion

The review makes it clear that molecular techniques outperform conventional procedures in terms of turnaround time and high specificity effects. Molecular diagnostic tools are far more sensitive since they amplify and identify target genetic material, according to the literature. Additionally, because molecular methods may identify several diseases in a single test, they have been found to be more economical in the long term. Finally, these techniques can be automated, which further improves productivity and decreases human error. However, according to the current systematic review, classical procedures have nevertheless never stopped improving in terms of sensitivity and specificity. Therefore, these methodologies are complementary today for diagnostic and detection output that is more trustworthy, standard, and complete. Most novel infectious illnesses are only eventually described after culture and isolation of the pathogenic agents since broad-range PCR is only useful in establishing the taxonomy.

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