Deciphering poultry microbial ecosystems by classical and modern tools

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A B S T R A C T

Food surveillance programs have traditionally relied on culture-dependent tools for the detection and enumeration of microbial groups along the food chain. While essential, these approaches provide a limited view of complex microbial ecosystems, often underestimating fastidious and viable but non-culturable microorganisms. In recent years, culture-independent tools, including sequencing and omics-based strategies, offer complementary insights into microbial diversity and function. Given the global consumption of poultry meat and its significance for food safety, spoilage, and antimicrobial resistance dissemination, a comprehensive characterization of poultry-associated microbial communities is essential. This review critically examines culture-dependent and culture-independent approaches to study the microbiome, resistome, virulome, and mobilome across the poultry production chain, comparing the type of information generated, their advantages and limitations. Culture-dependent methods enable quantification and isolation of viable strains, while culture-independent approaches reveal microbial diversity and functional genes related to antimicrobial resistance, virulence, and genetic mobility. Integrating both strategies strengthens surveillance, improves risk assessment, and supports targeted interventions throughout the poultry sector. This review also highlights key priorities for future research, including greater attention to post-slaughter processing environments, a more systematic investigation of the mobilome and virulome, and the integration of multi-omics, culturomics, and quasi-metagenomics to better link microbial diversity with functional activity and viability.

1. Introduction

Meat not only provides a large amount of protein (15–25 %) but also several vitamins [riboflavin (B2), niacin (B3), pantothenic acid (B5) and pyridoxine (B6)] and minerals (zinc, selenium, phosphorus and iron) (Williams, 2007). Due to its high nutritional value, meat constitutes a highly relevant component of the daily diet, and its demand has increased over the last 50 years. In 2023, 363.9 million tonnes of meat were produced worldwide (Food and Agriculture Organization [FAO], 2023), with a per capita consumption of 45 kg. In particular, the poultry sector showed a production of 142.7 million tonnes and a consumption of 14.9 kg per capita (OECD, 2021), being the most produced and consumed meat worldwide.

Due to its high nutritional content but also to its high water activity ($\geq$0.85) and moderate pH ($\geq$6.0), meat is a highly perishable food commodity susceptible to the growth of both spoilage and pathogenic microorganisms. Poultry meat quality is a complex and multivariate property, which is affected by multiple interacting factors including

genetics, feeding, husbandry, pre-slaughter handling, stunning and slaughter procedures, chilling, processing, and storage conditions (Petracci et al., 2010).

Although meat spoilage is a complex process involving microbiological, physical and chemical processes, the main cause of meat deterioration is the growth of spoilage microorganisms. This results in organoleptic alterations (off-odors, off-flavors and slime formation) and, consequently, in food waste and economic losses for producers, retailers and consumers. In addition, livestock animals are considered a reservoir of pathogenic microorganisms and have been strongly associated with food-borne outbreaks. In 2021, 21.7 % of reported strong-evidence food-borne outbreaks in the European Union (EU) were associated with meat and meat products, of which more than 25 % were specifically linked to poultry meat [European Food Safety Authority [EFSA] & European Centre for Disease Prevention and Control [ECDC] (2022)].

In this sense, determining the microbiota of meat and food processing environments (FPE) (including surfaces, equipment, utensils, walls, floors, ceilings, and air) along the food chain is very useful to identify the

sources of contamination and, consequently, to design measures to prevent the presence and growth of microorganisms. Culture-dependent techniques, i.e. those based on the growth, isolation and subsequent quantification and identification of microorganisms, have been extensively used to determine the food-associated microbiota. Nevertheless, culture-independent techniques, which include molecular methods, omics approaches combined with bioinformatics analysis and other non-culture-based strategies, are increasingly being used as they provide a more accurate and greater understanding of microbial communities (Jagadeesan et al., 2019).

On the other hand, the widespread use of antibiotics in primary production, whether for treating infectious diseases, for prophylactic purposes, or as growth promoters, has contributed to the emergence of antimicrobial-resistant (AMR) bacteria and, consequently, to the emergence of human infections difficult to treat (WHO, 2012). Although there are multiple pathways of AMR dissemination, the transmission of AMR bacteria via the food chain is often underrated and warrants significant attention. Bacteria associated with FPE may carry antimicrobial resistance genes (ARGs), which could be transferred from one cell to another by horizontal gene transfer (HGT) (Oniciuc et al., 2019), being particularly problematic when those ARGs are transferred to pathogenic bacteria which can contaminate food. Notably, multidrug resistant *Salmonella* spp. were recovered from 43.6 % of broiler carcasses and 39.4 % of turkey carcasses in the EU in 2021 (EFSA & ECDC, 2024). Mobile genetic elements (MGEs) such as plasmids, trasposons and integrons are the most frequent mechanism of horizontal transfer of ARGs (De Mesquita Souza Saraiva et al., 2022), so their presence in the food chain represents a significant public health concern. Beyond ARGs, bacteria present in FPEs may also harbour virulence factor genes (VFGs), which encode traits involved in adhesion, invasion, persistence and survival in several niches. The co-occurrence of ARGs and VFGs increases the risk that strains present in food and FPEs.

This review addresses a clear gap in the current literature by providing an integrated synthesis of both culture-dependent and culture-independent approaches used to study the microbiome (i.e., collection of microorganisms), resistome (i.e., collection of ARGs), virulome (i.e., collection of VFGs), and mobilome (i.e., collection of MGEs) within the poultry sector, which remains comparatively underrepresented in comparison to other meat types. Unlike previous works that have mainly focused on farm and slaughterhouse-level environments, this review compiles evidence across the entire poultry production chain, including also post-slaughter FPE that remain largely understudied. In addition, this work integrates studies addressing not only the microbiome and resistome, but also the virulome and mobilome, dimensions that have received considerably less attention to date. Finally, by bringing together studies employing both culture-dependent and culture-independent approaches, this review critically evaluates the type of information provided by each technique, their respective advantages and limitations, and their complementarity, thereby identifying current knowledge gaps and highlighting where additional or combined approaches are required to achieve a more comprehensive understanding.

Accordingly, we first revisit culture-dependent approaches, highlighting how isolation-based methods remain essential to link taxa with viability and phenotypes relevant for risk assessment and intervention design along the poultry chain. We then examine culture-independent strategies (metabarcoding and metagenomics) and discuss integrative workflows, including culturomics and quasi-metagenomics, to improve detection of rare taxa and to follow how genes spread across processing environments.

2. Literature search method

For the preparation of this narrative review, a targeted literature search was conducted using Web of Science, PubMed and Google Scholar databases. Combinations of search terms including *poultry,* *chicken meat*, *food processing environment, microbiome, resistome, virulome, mobilome, culturing, metabarcoding* and *metagenomics* were used to identify studies within the poultry sector applying culture-dependent and/or culture-independent methodologies to investigate microbial communities and associated genetic determinants.

Studies exclusively focused on the gastrointestinal tract of live chickens were excluded. Only studies addressing poultry carcasses, meat cuts, or environmental samples from poultry farms, slaughterhouses and food processing environments were included. For environmental studies, priority was given to samples collected from processing surfaces, equipment and facilities rather than faecal or soil material.

The search primarily covered peer-reviewed articles published in English between 2010 and 2025, although earlier articles were included when considered relevant. Additional relevant references were also identified through manual screening of the reference lists of selected studies.

3. Culture-dependent techniques

The characterization of microbial communities associated with food and FPE has traditionally relied on culture-dependent techniques, based on the isolation and growth of microorganisms in different culture media for subsequent investigation and enumeration (Fig. 1). Importantly, international standards and regulatory frameworks for food safety, such as ISO 7218:2024 "Microbiology of the food chain: General requirements and guidance for microbiological examinations" (International Organization for Standardization, 2024), are based on culture-dependent methods. Accordingly, this review first examines culture-dependent techniques, outlining their main applications, advantages, and limitations within the poultry production chain (Table 1). Table 2 summarizes studies that have used culture-dependent techniques to investigate microbial communities in the poultry sector.

3.1. Detection and isolation of pathogenic and spoilage microorganisms

The use of selective and/or differential growth media enabled the detection and isolation of specific microorganisms, including both pathogenic and spoilage ones. There are multiple examples of culture-dependent studies which employed these selective and differential growth media to detect the presence of specific microorganisms in food and FPE samples (Capita et al., 2001; Cerqueira-Cézar et al., 2025; Hill et al., 2017; Jeong et al., 2023). For instance, Hill et al. (2017) compared different enrichment media for the detection of *Campylobacter* spp., and Capita et al. (2001) compared different selective growth media for the detection of *Listeria* spp., including *Listeria monocytogenes*, both in poultry samples. The results of these studies enabled to optimize the detection of pathogenic microorganisms in poultry samples. Other pathogens commonly associated with poultry, like *Salmonella* spp.*, Escherichia coli*, or *Staphylococcus aureus*, can also be detected using molecular techniques, as discussed in Section 3.1.

3.2. Detection and isolation of AMR microorganisms

Beyond detecting specific microorganisms, these techniques have been used to detect AMR microorganisms. To assess AMR profiles, recovered isolates from growth media are tested against a panel of antibiotics, and their efficacy is measured using methods such as the Kirby-Bauer disk diffusion test or the minimum inhibitory concentration (MIC) determination via broth microdilution. An example is the study conducted by Wei et al. (2016), in which *Campylobacter* spp. isolates recovered from poultry meat were tested against various antibiotics using MIC determination. The authors reported that 91.5 % of the isolates were resistant to nalidixic acid and 83.0 % to ciprofloxacin. Another example is the study conducted by Cerqueira-Cézar et al. (2025), in which pathogens isolated from poultry meat were tested against various antibiotics using disk diffusion test. The authors reported
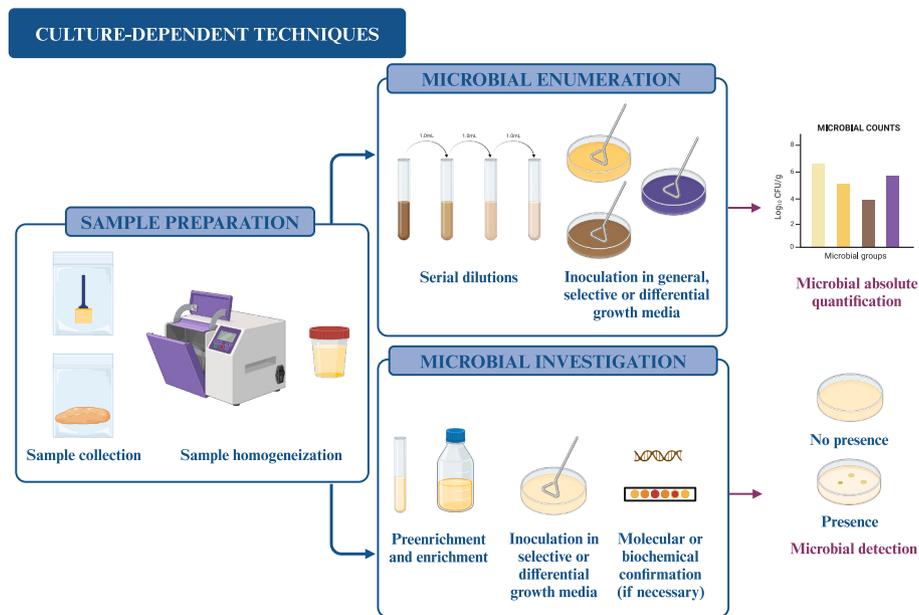
Fig. 1. Culture-dependent methodologies for microbial analysis. Samples are first collected and homogenized to ensure a representative distribution of microorganisms. Two main approaches can then be followed: (i) Microbial enumeration, which involves serial dilutions and inoculation on general, selective, or differential media to quantify microbial groups; and (ii) Microbial investigation, which includes pre-enrichment and enrichment steps, selective or differential plating, and when required, molecular or biochemical confirmation for accurate taxonomic identification.

Table 1
Comparative summary of the information provided, advantages, and limitations of culture-dependent and culture-independent (metabarcoding, and WMS) approaches that have been applied to poultry microbiome studies.

	Culture-dependent techniques	Culture-independent techniques	
		Metabarcoding	WMS
Information	Microbial detection Microbial isolation Microbial counts	Taxonomic profiling Relative quantification Microbial diversity	Taxonomic profiling Relative quantification Microbial diversity Functional profiling Taxonomic-functional association
Advantages	Absolute quantification Microbial isolation Capability of determining microbial viability Cheap	Comprehensive microbiome characterization Useful for samples with low DNA or high host DNA content	Comprehensive microbiome, resistome, virulome and mobilome characterization High taxonomic resolution (species level)
Disadvantages	Underestimation of microbial diversity Requirement of additional biochemical or molecular techniques for microbial identification (when necessary) Time-consuming Labor-intensive	Limited taxonomic resolution (genus level) Cannot determine microbial viability No absolute quantification Limited ability to detect rare or low-abundance taxa	Cannot determine microbial viability No absolute quantification Costly Requirement of high sequencing depth Requirement of advanced computational resources Limited ability to detect rare or low-abundance taxa

that 86.1 % of the *Salmonella* spp. isolates were resistant to at least one antibiotic tested, 95.1 % of *E. coli*, and 88.4 % of *Enterococcus* spp. Alternatively, AMR bacteria can be directly isolated using growth media containing specific concentrations of antibiotics. For instance, Aquilanti et al. (2007) isolated 123 AMR LAB from chicken meat by using a LAB specific growth medium supplemented with ampicillin, tetracycline and erythromycin, with most isolates recovered from medium containing tetracycline (51 of 123) or erythromycin (44 of 123).

3.3. Influence of production, processing and storage on microbial groups

There are different production, processing and storage factors influencing the microbial quality of poultry products. For example, rearing systems, defined as the combination of husbandry practices and environmental conditions under which animals are raised, can significantly influence the microbial quality of both animals and their products. In this sense, there are some culture-dependent studies focused on assessing the influence of the rearing system on the microbial quality of poultry products (Marmion et al., 2023; Voidarou et al., 2011). Voidarou et al. (2011) compared the microbial quality of broiler carcasses raised and slaughtered in free-range and conventional rearing systems. The higher levels of microbial contamination and a greater occurrence of pathogenic bacteria in free-range broiler carcasses reflected the poor hygienic conditions of the slaughtering process in free-range systems compared to conventional systems. Similarly, Marmion et al. (2023) concluded that the microbial load in final chicken cuts was not entirely influenced by the rearing system (free range or conventional) but can also be significantly affected by subsequent stages in the meat production process.

In this sense, there are several studies that employ culturing techniques to assess the influence of different slaughtering and post-slaughtering processing steps on the microbiota of poultry products (Chen, Fegan, Kocharunchitt, Bowman, & Duffy, 2020; Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al., 2020; Nieminen et al., 2012; Wang et al., 2020; H. Wang et al., 2019). By monitoring changes in microbial counts along the processing line, it becomes possible to assess the effectiveness of different stages in reducing microbial load. For instance, Chen, Fegan, Kocharunchitt, Bowman, and Duffy (2020) and

Table 2

Summary of the main applications of culture-dependent approaches for characterizing microbial communities in poultry meat and FPE, with referenced studies.

Reference	Sample types	Culture media/Incubation conditions/Target taxa	Main taxa	Main application and discovery
Aquilanti et al. (2007)	Chicken carcasses and raw poultry skewers	MRS supplemented with antibiotics/30 °C/48–72 h Anaerobiosis/AMR LAB	*Lactococcus garvieae* *Lactobacillus plantarum* *Lb. salivarius*	**Detection and isolation of AMR microorganisms** Lactobacilli and lactococci isolated from poultry samples harbored ARGs.
Blacha et al. (2014)	Turkey breast cuts packaged in vacuum or in different modified atmosphere mixtures and stored at 3 °C for 12 days	PCA/30 °C/72 h/TVC *Pseudomonas* Agar Base/30 °C/72 h/*Pseudomonas* spp.	TVC *Pseudomonas* spp.	**Influence of storage conditions on microbial groups** TVC varied with the atmosphere mixture, allowing the identification of MAP that extends poultry samples shelf-life
Capita et al. (2001)	Chicken carcasses	PALCAM/30 °C/48 h/*Listeria* spp. Modified Oxford/35 °C/24 h/*Listeria* spp.	*L. innocua* *L. monocytogenes* *L. welshimeri*	**Improving the detection and isolation of pathogens** PALCAM demonstrated greater sensitivity, selectiveness and differential capability for *Listeria* spp. (including *L. monocytogenes*) isolation from poultry samples.
Cerqueira-Cézar et al. (2025)	Chicken cuts	BPW + tetrathionate broth + Rappaport-Vassilidis Soya broth + XLD/37 °C/18–20 h + 37 °C/24 h + 41.5 °C/24 h + 37 °C/24 h/*Salmonella* spp. BPW + MacConkey agar/37 °C/18–24 h + 37 °C/18–24 h/*E. coli* BPW + BEA/37 °C/18–24 h + 37 °C/48 h/*Enterococcus* spp. Mueller-Hinton supplemented with antibiotics/37 °C/18–24 h/AMR *Salmonella* spp., AMR *E. coli* and AMR *Enterococcus* spp.	N/A	**Detection and isolation of pathogens** Detection and isolation of *Salmonella*, *E. coli* and *Enterococcus* from different chicken cuts. **Detection and isolation of AMR microorganisms** High prevalence of resistant *Salmonella*, *E.coli* and *Enterococcus* in poultry samples.
Chen, Fegan, Kocharunchitt, Bowman, and Duffy (2020)	Chicken carcasses collected at distinct slaughtering processing steps	PCA/TVC *E. coli*/Coliform Count Plate/*E. coli* mCCDA supplemented with antibiotics/42 °C/72 h under 5 % CO_2/*Campylobacter* spp.	N/A	**Influence of processing steps on microbial groups** Significant microbial reduction was achieved through slaughtering process, especially after scalding and chilling immersion, but *Campylobacter* spp. remained prevalent.
Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al. (2020)	Chicken carcasses collected at distinct slaughtering processing steps and at the beginning and at the end of the shelf-life Environmental samples (water and room and equipment surfaces) across the slaughtering processing line	PCA/TVC mCCDA/42 °C/72 h under 5 % CO_2/*Campylobacter* spp. *Pseudomonas* Agar Base supplemented with antibiotics/*Pseudomonas* spp.	N/A	**Influence of processing steps on microbial groups** Significant microbial reduction was achieved through slaughtering process, especially after scalding and chilling immersion, but *Campylobacter* spp. remained prevalent **Identification of contamination points** The presence of pathogens in chicken thighs depended on the poultry processor, indicating that the FPE had a strong influence on the microbiota of chicken thighs.
Chmiel et al. (2018)	Chicken breasts packaged under air and MAP (75 % O_2 and 25 % CO_2) conditions	PCA/30 °C/72 h/TVC MRS/30 °C/72 h/LAB KING B Agar with CFC supplement/22 °C/72 h/*Pseudomonas* spp.	*Pseudomonas* spp. TVC LAB	**Influence of storage conditions on microbial groups** MAP extended the shelf-life of chicken breast samples.
Dourou et al. (2021)	Chicken breast and thigh fillets stored under well-controlled and slightly abuse temperatures	PCA/25 °C/72 h/TVC *Pseudomonas* Agar Base with CFC supplement/25 °C/48 h/*Pseudomonas* spp. STAA/25 °C/48 h/*B. thermosphacta* MRS/30 °C/72 h/LAB	*Pseudomonas* spp., followed by *B. thermosphacta* and LAB	**Influence of storage conditions on microbial groups** Higher storage temperatures led to increased microbial loads, shortening the shelf-life of the chicken products
Hill et al. (2017)	Chicken carcasses	Different modified *Campylobacter* spp. enrichment media/42 °C/24 h Microaerophilia (5 % O_2/10 % CO_2/85 % N_2)/*Campylobacter jejuni*	N/A	**Improving the detection and isolation of pathogens** Modified Brucella enrichment media improved *C. jejuni* growth and recovery from poultry samples, offering a faster and cost-effective alternative.

Table 2 (*continued*)

Reference	Sample types	Culture media/Incubation conditions/ Target taxa	Main taxa	Main application and discovery
Jeong et al. (2023)	Chicken carcasses collected at distinct slaughtering processing steps Environmental samples from a slaughtering processing ling	*Listeria* enrichment broth + Fraser broth + Oxford agar/30 °C/24 h + 37 °C/48 h + 37 °C/48 h/*L. monocytogenes*	N/A	**Detection and isolation of pathogens** Detection and isolation of *L. monocytogenes* from chicken carcasses and different environmental samples from a slaughtering processing line **Identification of contamination point** Identification of chilling tank water as a contamination source of *L. monocytogenes* on chicken carcasses
Marmion et al. (2023)	Conventionally raised and free-range aerobically packaged chicken thighs collected from two different poultry processors and from a retail setting	PCA/37 °C for 48 h/Mesophiles PCA/4 °C for 240 h/Psychrotrophes VRBG/42 °C for 24 h/ Enterobacteriaceae TSB + Tryptone Bile X Glucuronide Agar/42 °C for 24 h + 42 °C for 24 h/ *E. coli* Bolton broth with horse blood + mCCDA/42 °C for 24 h + 37 °C for 48 h *Campylobacter* spp. Rappaport Vassiliadis Broth + XLD/ 37 °C for 24 h + 37 °C for 24 h/ *Salmonella* spp. MRS/30 °C for 72 h/LAB STAA/30 °C for 48 h/*B. thermosphacta* *Pseudomonas* Selective Agar with CFC supplement/37 °C for 48 h/ *Pseudomonas* spp.	Mesophiles followed by psychrotrophes, LAB, *B. thermosphacta, Pseudomonas* spp., and Enterobacteriaceae.	**Influence of rearing systems on microbial groups** Husbandry made little impact on the microbiota of chicken thighs. **Identification of contamination points** The presence of pathogens in chicken thighs depended on the poultry processor, indicating that the FPE had a strong influence on the microbiota of chicken thighs.
Meredith et al. (2014)	Skinless chicken fillets packaged under mixtures of CO_2 and N_2 and stored at 2 °C for 17 days.	PCA/30 °C/72 h/Mesophiles TVC PCA/6.5 °C/10 days/Psychrophiles TVC mCCDA/42 °C/48 h/ Microaerophilia (5 % O_2/10 % CO_2/85 % N_2)/*Campylobacter* spp./VRBG/ 37 °C/24 h/Enterobacteriaceae MRS/30 °C/72 h/LAB Cetrimide agar with CFC supplement/ 25 °C/48 h/*Pseudomonas* spp.	*Pseudomonas* spp. followed by LAB and Enterobacteriaceae (day 0)	**Influence of storage conditions on microbial groups** Higher concentrations of CO_2 resulted in a greater delay in microbial growth, extending the shelf-life of the chicken fillets. However, 50 % CO_2 was as effective as 90 % in controlling *Pseudomonas* spp., and 70 % CO_2 was equally effective as 90 % in inhibiting LAB.
Nieminen et al. (2012)	Marinated and unmarinated chicken strips stored under refrigeration and MAP	MRS/25 °C/LAB VRBG/25 °C/Enterobacteria STAA/25 °C/*B. thermosphacta*	LAB, followed by Enterobacteriaceae and *B. thermosphacta*	**Influence of processing steps on microbial groups** Marination inhibited the growth of Enterobacteriaceae and *B. thermosphacta*
Säde et al. (2013)	Boneless and skinless chicken fillets and turkey thighs packaged under mixtures of CO_2 and N_2 and stored at 2 °C until the use-by date	VRBG/30 °C/48 h/Enterobacteriaceae	N/A	**Influence of storage conditions on microbial groups** Enterobacteriaceae counts were >10^6 CFU/g in 34 % of the poultry samples, indicating that they may contribute to spoilage of MAP poultry.
Tsafrakidou et al. (2021)	MAP (30 % CO_2 70 % N_2) minced free-range chicken meat stored at 4 °C for 10 days	TSAYE/30 °C/72 h/Total mesophilic viable counts MRS/25 °C/72 h/LAB *Pseudomonas* Agar Base with CFC supplement/25 °C/48 h/*Pseudomonas* spp. VRBD/37 °C/24 h/Enterobacteria Baird-Parker agar base with egg yolk tellurite/37 °C/48 h/Staphylococci RBC/25^{0}C/5 days/Yeast	*Pseudomonas* spp., staphylococci and LAB (day 0); LAB, *Pseudomonas* spp. and staphylococci (day 10)	**Influence of storage conditions on microbial groups** MAP retarded the growth of LAB and suppressed the growth of the rest microbial groups.
Voidarou et al. (2011)	Organic chicken carcasses collected from rural households and conventional chicken carcasses from retail settings	PCA/35 °C for 48 h/Aerobic Plate Counts PCA/7 °C for 10 days/Psychrotrophic Counts Skim Milk Agar with 10 % Skim Milk/ 30 °C for 3 days/Proteolytic Bacterial Count VRBG/37 °C for 24 h/ Enterobacteriaceae VRBG/44.5 °C for 24 h/*E. coli* Baird-Parker/*S. aureus* *Salmonella* spp. *L. monocytogenes*	N/A	**Influence of rearing systems on microbial groups** The higher levels of microbial contamination and the greater occurrence of pathogenic bacteria in organic broiler carcasses reflected the poor hygienic conditions of the slaughtering process in organic production systems

Table 2 (*continued*)

Reference	Sample types	Culture media/Incubation conditions/Target taxa	Main taxa	Main application and discovery
		Campylobacter spp. *Clostridium perfringens*		
Wang et al. (2020)	Chicken carcasses	PCA/36 °C/48 h/Total aerobic mesophilic counts	N/A	**Influence of processing steps on microbial groups** The chilling method did not affect total aerobic mesophilic counts.
H. Wang et al. (2019)	Chicken carcasses Water samples from scalder and chilling tanks	Total aerobic mesophilic counts	N/A	**Influence of processing steps on microbial groups** Evisceration step increased the microbial load; while washing and chilling reduced the microbial load. Microbial load increased throughout the processing time. **Identification of contamination points** Identification of evisceration as a critical point of contamination.
Wei et al. (2016)	Retail chicken and duck meat	Bolton broth supplemented with antibiotics + mCCDA supplemented with antibiotics/37 °C/4 h + 42 °C/48 h Microaerophilia (5 % O_2/10 % CO_2/85 % N_2) + 42 °C/48 h/AMR *Campylobacter* spp.	*C. jejuni* *C. coli*	**Detection and isolation of AMR microorganisms** High prevalence of resistant and multidrug resistant *Campylobacter* spp. in poultry samples.

Abbreviations: MRS: De Man–Rogosa–Sharpe agar; mCCDA: modified charcoal cefoperazone deoxycholate agar; PCA: plate count agar; BPW: buffered peptone water; BEA: bile esculin azide agar; CFC supplement: cetrimide, fusidic acid, and cephalothin supplement; STAA: streptomycin–thallous acetate–actidione agar; VRBG: violet red bile glucose agar; TSB: tryptic soy broth; XLD: xylose lysine deoxycholate agar; TSAYE: tryptic soy agar with yeast extract; VRBD: violet red bile dextrose agar; RBC: Rose Bengal chloramphenicol agar; AMR: antimicrobial resistance; LAB: lactic acid bacteria; TVC: total viable counts; N/A: not applicable.

Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al. (2020) demonstrated that specific slaughtering steps (such as scalding, plucking, immersion chilling and air chilling) were effective in reducing overall microbial levels but not in completely eradicating *Campylobacter* spp. Similarly, Nieminen et al. (2012) enumerated poultry-associated microbiota (specifically LAB, Enterobacterales and *B. thermosphacta*) in marinated and unmarinated chicken strips stored under refrigeration and MAP throughout their shelf-life. LAB exhibited the higher counts in both sample types, exceeding 10^8 CFU/g at the end of storage (13 days from packaging), followed by Enterobacterales and *B. thermosphacta*. While LAB counts were similar between marinated and unmarinated strips, Enterobacterales and *B. thermosphacta* counts were higher in unmarinated samples at the end of the storage (13 days from packaging). These findings suggest that LAB predominates under MAP conditions and that the marination inhibits the growth of known poultry spoilage bacteria, specifically Enterobacterales and *B. thermosphacta*.

However, bacteria that spoil poultry products are usually unavoidable contaminants introduced with raw materials, and they may survive processing steps without being completely inactivated. Consequently, the microbial quality and shelf-life of these products are largely influenced by storage conditions (Møretrø & Langsrud, 2017). As a result, most research efforts focus on assessing how storage conditions affect the microbial quality and shelf-life of poultry products (Blacha et al., 2014; Chmiel et al., 2018; Dourou et al., 2021; Meredith et al., 2014; Säde et al., 2013; Tsafrakidou et al., 2021). For example, Dourou et al. (2021) evaluated the influence of different storage temperatures on the microbial quality of chicken breasts and thighs. *Pseudomonas* spp. stood out as the predominant microbial group, followed by *B. thermosphacta* and LAB, across all sample types and temperatures. While the microbial predominance remained consistent, higher storage temperatures led to increased microbial loads for all groups, shortening the shelf-life of the chicken products. Similarly, other investigations have assessed the impact of gas composition in MAP on the microbial quality and the shelf-life of chilled chicken samples (Chmiel et al., 2018; Meredith et al., 2014; Tsafrakidou et al., 2021). These studies confirmed that the shelf-life was extended when chicken samples were stored under MAP conditions compared to aerobic conditions. Meredith et al. (2014) tested

different gaseous mixtures with increasing concentrations of CO_2 balanced with N_2 and found that although higher concentrations of CO_2 resulted in a greater delay in microbial growth, 50 % CO_2 was as effective as 90 % in controlling *Pseudomonas* spp., and 70 % CO_2 was equally effective as 90 % in inhibiting LAB. Therefore, the use of culturing techniques enabled the identification of the most suitable packaging conditions to extend the shelf-life of poultry products.

3.4. Identification of contamination points

It is also worth noting that bacteria responsible for spoiling poultry products can also originate from FPE. In this sense, several studies have utilized culture-dependent techniques to analyze the specific microbial groups in both poultry samples and related FPE samples (specifically, surfaces from the slaughtering plant), aiming to identify potential sources of contamination. For example, H. Wang et al. (2019) reported a significant increase in total viable counts after evisceration, indicating that this step may serve as a critical point for contamination or cross-contamination. Similarly, Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al. (2020) employed culturing techniques to monitor microbial populations on poultry carcasses and associated FPE surfaces. They found high counts of *Pseudomonas* spp. on the walls of the air-chill room, while levels on carcasses were initially low after air chilling but increased during the shelf-life period, suggesting that airborne or environmental exposure from these surfaces can contribute to carcass contamination. Jeong et al. (2023) also identified the chilling water as a contamination point as most *L. monocytogenes* strains isolated from the final carcasses showed the same genetic cluster as the isolate in the chilling water.

3.5. Advantages and disadvantages

The main advantage of culturing techniques lies in their ability to selectively detect, quantify and isolate viable spoilage, pathogenic and AMR microorganisms in food and FPE (Table 1), which remains essential for regulatory compliance and risk assessment in the poultry sector. In particular, absolute counts of viable bacteria are critical for evaluating

process hygiene, the effectiveness of control measures in FPE, and product shelf-life.

Nevertheless, despite providing useful information and being cost-effective, culturing techniques are labor-intensive and time-consuming, often requiring several days to deliver results, which can delay decision-making in large-scale poultry production systems where rapid feedback is essential. Moreover, the inability of culturing techniques to detect fastidious microorganisms and those in a physiologically viable but non-culturable (VBNC) state (Forbes et al., 2017) results in underestimating bacterial diversity as well as neglecting the microbial interactions within complex communities. It is also noteworthy that the identification of cultured microorganisms necessitates the subsequent application of biochemical and/or molecular techniques (Table 1). Furthermore, culture-based monitoring typically focuses on a limited number of pathogenic microorganisms or ARGs based on their threat level (Allard et al., 2018), potentially overlooking emerging or non-target microorganisms that have acquired ARGs or VFGs through HGT. Therefore, it is important to consider culture-independent approaches that offer a more comprehensive understanding of poultry-associated microbiomes and resistomes.

4. Culture-independent techniques

Over recent years, culture-independent approaches have been developed to overcome all the limitations associated with culture-dependent techniques. This section will focus on culture-independent techniques applied in the poultry sector, covering molecular methods, first-generation sequencing, and next-generation sequencing (NGS).

4.1. Molecular methods

Molecular methods such as PCR, real-time quantitative PCR (qPCR) or denaturing gradient gel electrophoresis (DGGE) have been previously used in food microbial studies (Fig. 2). PCR has been utilized following isolation on specific growth media to confirm the presence of *Salmonella* spp. and *Campylobacter* spp. (Khan et al., 2018, 2019; Perez-Arnedo & Gonzalez-Fandos, 2019; Yang et al., 2020), recognized as the major foodborne pathogens in the poultry industry, as well as to detect other pathogenic microorganisms like *L. monocytogenes* (Panera-Martínez et al., 2022), *E. coli* (Rahman et al., 2020), and *S. aureus* (Abdalrahman et al., 2015). qPCR is based on traditional PCR and allows not only the amplification but also the quantification of a target sequences, making it a widely used tool for genotyping, gene quantification and targeted pathogen detection and quantification (Smith & Osborn, 2009). In the poultry sector, qPCR has been extensively applied for the rapid monitoring of key foodborne pathogens across the production chain. For example, Oakley et al. (2013) employed qPCR to detect and quantify the abundance of *Campylobacter* spp. and *Clostridium* spp. at various stages of poultry production from farm to fork, and Ito and Kishimoto (2023) developed an improved sampling and qPCR method without DNA extraction requirement for the quantitative detection of *Campylobacter* spp. in retail chicken. In addition, qPCR has been employed to quantify specific ARGs in broiler and turkey FPE (Yang et al., 2022), supporting targeted AMR surveillance. However, qPCR has some limitations such as the need of prior knowledge about the target sequence to design sequence-specific primers, limiting its capacity to detect emerging pathogens or novel ARGs; the requirement to perform calibration curves for each target gene to accurately measure the target copy number,
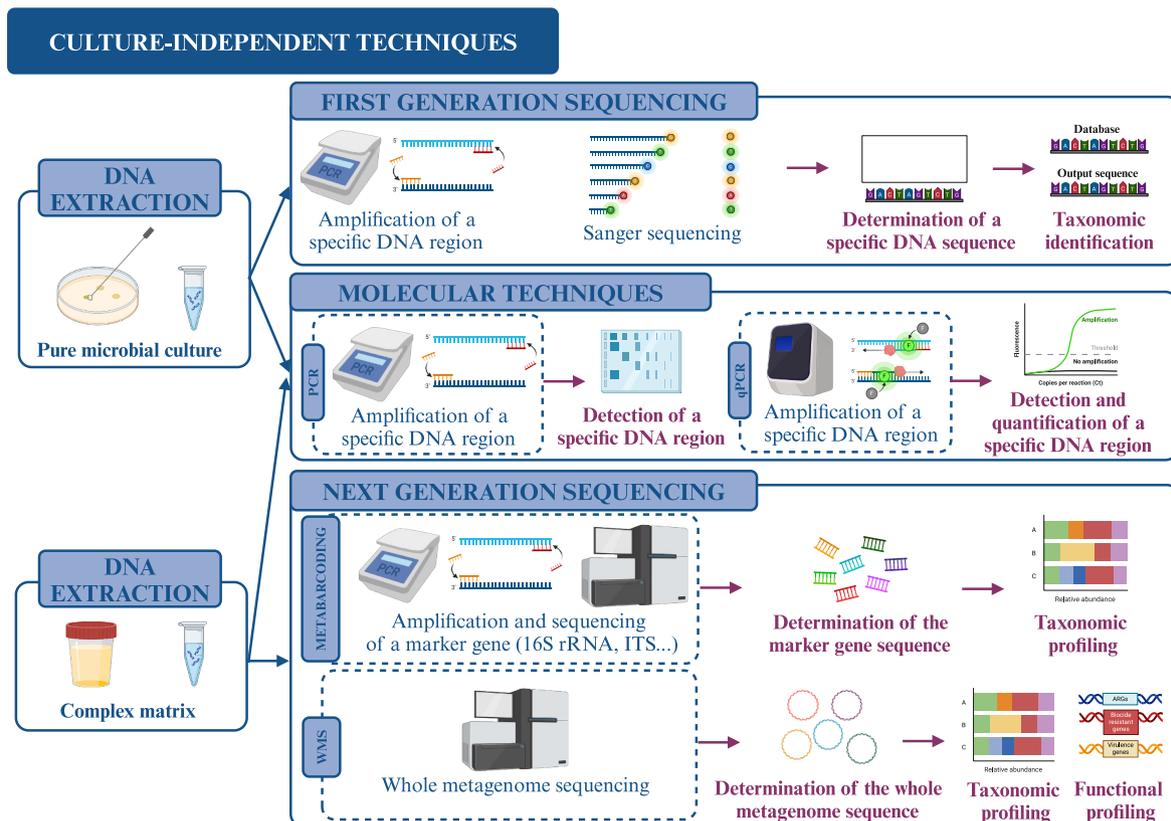

Fig. 2. Culture-independent methodologies for taxonomic identification and functional characterization. DNA is first extracted from either a pure microbial culture or directly from a complex matrix. Culture-independent approaches include: (i) first-generation sequencing (Sanger) for precise taxonomic identification of single microorganisms; (ii) molecular techniques (PCR, qPCR) for targeted detection and quantification of taxonomic or functional genes; (iii) metabarcoding for taxonomic profiling of microbial communities; and (iv) whole metagenome sequencing (WMS) for comprehensive taxonomic and functional characterization, including the resistome, virulome, and mobilome.

limiting the applicability of qPCR for large-scale or high-throughput monitoring; and the inability to distinguish whether the quantified DNA originates from live or dead cells when applied without prior cultivation, which may lead to an overestimation of microbiological risks (Smith & Osborn, 2009).

4.2. First-generation sequencing

First-generation sequencing, mainly represented by Sanger sequencing, is a DNA sequencing technology that enables the determination of a precise sequence of nucleotides by adding labelled deoxynucleotides during DNA amplification (Fig. 2).

There are some studies that have employed Sanger sequencing to taxonomically identify bacterial isolates from poultry samples. For example, this technique enabled the identification of LAB isolates from both chicken and FPE samples, thereby revealing that several FPE sources (cutting blades, conveyor belts, working tables and processing plant air) contributed to the contamination of chicken samples (Samapundo et al., 2019; Vihavainen et al., 2007).

Despite its limitations in terms of throughput and cost-efficiency, Sanger sequencing remains a valuable tool for applications requiring high accuracy, such as the sequencing of taxonomic marker genes or ARGs, where even minor errors may lead to misclassification or misinterpretation.

4.3. Next-generation sequencing

The arrival of NGS, such as Illumina, Ion Torrent, PacBio, or Oxford Nanopore, has significantly improved the characterization of microbiomes in complex matrices like food or FPE. NGS enables high-throughput sequencing, which provides deeper coverage by generating large volumes of data in a single run. This capability allows for the comprehensive sequencing of entire genomes or metagenomes, enabling the identification of both dominant and rare species and, consequently, leading to a more detailed and accurate understanding of microbial diversity compared to first-generation methods. These techniques are not only used for the identification of the microbiota present in a sample, but also for determining interactions and changes of the different microbial communities (Cao et al., 2017). Among NGS techniques, those intended for metabarcoding and WMS analysis are the prominent to characterize food-related microbiomes.

4.3.1. Metabarcoding, amplicon sequencing, or targeted sequencing

Metabarcoding, amplicon sequencing, or targeted sequencing taxonomically profiles microbial communities by extracting DNA, and amplifying and sequencing a taxonomic marker gene, most commonly 16 S rRNA for Archaea and Bacteria, and ITS for fungi when relevant (Jagadeesan et al., 2019) (Fig. 2). The obtained sequences are compared against reference databases to assign taxonomic identities and to estimate the relative abundance of taxa across samples (Cao et al., 2017).

A detailed overview of the main applications of metabarcoding in poultry and poultry FPE, as well as their advantages and limitations (Table 1), is provided in the next sections. Table 3 summarizes studies that have used metabarcoding to investigate microbial communities in the poultry sector.

4.3.1.1. Detection, identification and relative quantification of microbial taxa. Metabarcoding enables the identification of specific taxa that might be missed by culturing techniques, taxa that are in a physiologically viable but non-culturable state, and dead taxa that were initially present (Table 1). The identification of these taxa could offer valuable insights into the studies. An illustrative example is the study conducted by Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al. (2020), where metabarcoding results revealed that the presence of the genus *Anoxybacillus* occurred when scalding, defeathering, and plucking were

conducted in a single tunnel, rather than separately. Other examples include the investigations conducted by Dourou et al. (2021) and Lauritsen et al. (2019), where *Photobacterium* and *Vagococcus*, respectively, were identified as potential contributors to the spoilage microbiota of poultry samples through metabarcoding.

4.3.1.2. Influence of processing steps, storage conditions and different study factors on the microbiome. Most metabarcoding studies applied in the poultry sector focus on the slaughtering production line. Generally, poultry carcasses and environmental samples are collected at different stages of the slaughtering process to assess the impact of different processing steps, such as defeathering, evisceration, scalding or chilling, on poultry carcasses microbiota, and to identify putative sources of contamination throughout the processing line (Chen, Fegan, Kocharunchitt, Bowman, & Duffy, 2020; Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al., 2020; Wang et al., 2020; H. Wang et al., 2019). In comparison to culturing techniques, metabarcoding provides comprehensive information on the composition and diversity of the microbiota within the samples, offering a more complete view (Table 1). For example, in the study conducted by H. Wang et al. (2019), although culturing techniques demonstrated the effectiveness of the chilling step to reduce the overall microbial load, metabarcoding pinpointed the chilling step as a putative contamination point due to an increase in the microbial diversity of poultry carcasses after this procedure.

After the slaughtering process, only a limited number of studies have employed metabarcoding to investigate the microbial communities of both poultry products and their associated environmental samples within processing plants (Merino et al., 2025; Park et al., 2023). In fact, the vast majority of post-slaughter metabarcoding studies focus exclusively on the poultry product itself, often overlooking the surrounding FPE. These studies typically assess the influence of different storage conditions (Dourou et al., 2021; Lauritsen et al., 2019; Rouger et al., 2018) or different study factors such as rearing system (Lee et al., 2019), chicken part (Dourou et al., 2021), or seasonality (Kim et al., 2019; Merino et al., 2025; Song et al., 2021) on the microbiota of poultry products. Many of these studies also utilize a combined approach of culture-dependent and culture-independent techniques to tackle these questions. While culturing techniques only offer insights into differences in the abundance of certain microbial groups across different sample types, metabarcoding allows for the correlation of specific shifts in the composition and diversity of poultry microbiota with particular study factors, or even for the attribution of specific genera to these study factors. For example, culturing techniques did not reveal differences in the bacterial predominance among different chicken parts or among chicken samples stored at different temperatures, while metabarcoding demonstrated that the microbial composition of the chicken samples was determined by both chicken part and storage temperature (Dourou et al., 2021). Similarly, Lauritsen et al. (2019) showed that the influence of packaging conditions could only be detected using metabarcoding. Likewise, Merino et al. (2025) showed, using metabarcoding, that seasonal variations shape the microbiota of poultry products and food-contact surfaces, insights that were missed by culturing methods.

4.3.1.3. Establishment of associations between poultry products and FPE and identification of contamination sources. Comparing the microbiota of poultry carcasses with that of environmental samples from slaughterhouses (including scalding tank water, immersion chilling tank water, wall surfaces in the air-chill room, and air samples) enables the identification of contamination sources. Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al. (2020) demonstrated correlations between the microbiota found in the scalding tank water, on wall surfaces, and on poultry carcasses, while H. Wang et al. (2019) established a link between the microbiota of the chilling tank water and that of poultry carcasses. These findings suggest that these FPE samples may contribute to carcass contamination. This is relevant because, although the

Table 3

Summary of the main applications of metabarcoding for characterizing microbial communities in poultry meat and FPE, with referenced studies.

Reference	Sample types	Marker gene	Sequencing platform	Main application and discovery
Chen, Fegan, Kocharunchitt, Bowman, and Duffy (2020)	Chicken carcasses collected at distinct slaughtering processing steps	16 S rRNA (V4)	Illumina MiSeq	**Microbiome characterization** Firmicutes and Actinobacteria predominated in the first steps, while in the last steps there was a significant increase in Proteobacteria and Actinobacteria, and a significant decrease in Firmucutes. **Influence of processing steps on microbiome** Air chilling drastically changed the microbiome composition and diversity of chicken samples. After air chilling, *Pseudomonas*, *Paeniglutamicibacter*, *Chryseobacterium* and *Pseudarthrobacter* drastically increased. **Identification of contamination points** Identification of chilling step as a contamination/cross-contamination point.
Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al. (2020)	Chicken carcasses collected at distinct slaughtering processing steps and at the beginning and at the end of the shelf-life Environmental samples (water and room and equipment surfaces) across the slaughtering processing line	16 S rRNA (V4)	Illumina MiSeq	**Identification of taxa missed by culturing techniques** Detection of *Anoxybacillus* when scalding, defeathering and plucking were conducted in a single tunnel. **Microbiome characterization** Predominance of *Staphylococcus* on carcasses before scalding, and predominance of *Anoxybacillus* and *Gallibacterium* throughout the subsequent steps. Predominance of *Pseudomonas, Anoxybacillus* and *Lactobacillus* at the beginning of the shelf-life, and predominance of *Pseudomonas* at the end. Predominance of *Anoxybacillus* in environmental samples. **Influence of processing steps on the microbiome** Progressive decrease in the microbial diversity along the slaughtering processing line, more pronounced during storage. Scalding caused a significant decrease in *Staphylococcus* and a significant increase in *Anoxybacillus* and *Gallibacterium*. **Identification of contamination points** Identification of scalding tank water and wall surfaces as sources of contamination for chicken carcasses. Identification of the confined tunnel, which incorporates scalding, defeathering and plucking, as a distribution route for *Anoxybacillus*. Identification of chilling room wall as a distribution route for *Pseudomonas*.
Dourou et al. (2021)	Chicken breast and thigh fillets stored under well-controlled (0 °C) and slightly abuse temperatures (5 and 10 °C)	16 S rRNA (V2–4–8 and V3–7–9 regions)	Ion Torrent PGM	**Identification of taxa missed by culturing techniques** Detection of *Photobacterium* as potential contributors to the spoilage microbiota of poultry samples **Microbiome characterization** *Rubrivivax, Burkholderiales, Pseudomonas, Pelomonas, Flavobacterium,* and *Acinetobacter* predominated in fresh breasts; and *Pseudomonas* and *Acinetobacter* predominated in fresh thighs. **Influence of storage conditions on the microbiome** Decrease in microbial diversity during storage, being more pronounced at higher storage temperatures. Microbial composition was determined by storage temperature. *Photobacterium* was more associated with storage at 0 and 5 °C, while *Brochothrix* and LAB genera (*Carnobacterium, Vagococcus* and *Leuconostoc*) were more associated with storage at 5 and 10 °C. **Influence of other study factors on the microbiome** Poultry cut determines microbiome composition and diversity.
Kim et al. (2019)	Packaged chicken products from different companies collected in different seasons Unpackaged chicken carcasses from different butcher shops collected in different seasons	16 S rRNA (V1–V3)	Illumina MiSeq	**Microbiome characterization** Proteobacteria predominated in January samples and Firmicutes predominated in July samples. **Influence of other factors on the microbiome** Seasonality exerted a greater influence on microbiome composition than sample origin (region or company). *Moraxella, Chryseobacterium, Acinetobacter, Flavobacterium, Arthrobacter, Psychrobacter, Pseudomonas, Janithinobacterium,* and *Idobacter* were higher in January, whereas *Desemzia, Peptostreptococcus, Vagococcus, Serratia, Citrobacter, Lactococcus, Hafnia, Peptoniphilus,*

Table 3 (*continued*)

Reference	Sample types	Marker gene	Sequencing platform	Main application and discovery
				Carnobacterium, and *Streptococcus* were higher in July. Higher amount and lower microbial diversity in July.
Lauritsen et al. (2019)	Whole chicken meat packaged under 80 % CO_2 20 % CO_2 and under 50 % CO_2 50 % N_2 Environmental samples across the slaughtering processing line	16 S rRNA (V1–V3)	Illumina MiSeq	**Identification of taxa missed by culturing techniques** Detection of *Vagococcus* as a potential contributor to the spoilage microbiota of poultry samples **Microbiome characterization** *Lactobacillus, Bacillus, Chryseobacterium* and *Flavobacterium* comprised the initial microbiota, while *Brochothrix, Carnobacterium, Janthinobacterium* and *Vagococcus* dominated at the end of the shelf-life. **Influence of slaughtering and storage conditions on the microbiome** There was no microbiome in poultry samples when flocks are slaughtered and MAP packaged under the same conditions, so slaughtering and storage conditions strongly influenced the microbiome. **Identification of contamination points** Identification of the cooling condenser as a potential contamination point for *Janthinobacterium*.
Lee et al. (2019)	Poultry products from different producers that differ by cut (whole carcass, leg, breast and thigh) and product labeling (organic, free-range, no antibiotic, no label)	16 S rRNA (V4)	Illumina MiSeq	**Microbiome characterization** *Pseudomonas, Carnobacterium, Psichrobacter, Escherichia* and Lactobacillales comprised the core microbiome. **Influence of other factors on the microbiome** Poultry cut and producer exerted a greater influence on microbiome composition and diversity than product label (rearing system).
Merino et al. (2025)	Chicken thighs Chicken burger packages (70 % N_2 and 30 % CO_2). Food-contact surfaces across a chicken burger processing line sampled during production.	16 S rRNA (V3-V4)	Illumina NovaSeq X Plus	**Microbiome characterization** *Pseudomonas, Psychrobacter, Brochothrix, Acinetobacter, Carnobacterium* and *Lactobacillus* comprised the core microbiome. **Influence of other factors on the microbiome** Seasonality influenced the microbiome. Winter samples showed significantly higher abundance of *Acinetobacter, Brochothrix,* and *Lactococcus*, whereas summer samples exhibited a significantly higher abundance of *Pseudomonas, Psychrobacter* and *Enterococcus*.
Park et al. (2023)	Poultry samples (chicken abdomen, slaughtered carcasses, processed carcasses and final chicken products) collected across the poultry meat supply chain (from farm to market) Environmental samples collected across the poultry meat supply chain (from farm to market)	16 S rRNA (V4)	Illumina MiSeq	**Microbiome characterization** *Pseudomonas, Shewanella, Acinetobacter* and *Psychrobacter* comprised the core microbiome of the final products **Influence of processing steps on the microbiome** *Staphylococcus* drastically decreased after defeathering, while Enterobacteriaceae, *Acinetobacter* and *Pseudomonas* significantly increased after slaughter. **Identification of contamination points/routes** Identification of contamination routes throughout the meat supply chain, revealing that environmental samples from industry were the main contributors to the microbiota of final chicken products.
Rouger et al. (2018)	Chicken legs packaged under different modified atmosphere mixtures and stored at 4 °C	16 S rRNA (V1–V3)	Illumina MiSeq	**Microbiome characterization** *Brochothrix, Carnobacterium* and *Pseudomonas* comprised the core microbiome. **Influence of storage conditions on the microbiome** Microbiome composition was determined by atmosphere composition. *Pseudomonas* was not present or detected at very low levels at low O_2 concentration, while it was one of the predominant bacteria at high O_2 concentration.
Song et al. (2021)	Chicken carcasses Environmental samples from the slaughtering processing line (water and surfaces)	16 S rRNA (V3–V4)	Illumina MiSeq	**Microbiome characterization** Proteobacteria predominated in chicken carcasses and on contact surfaces, while Bacteroidetes and Firmicutes predominated in prechilling water. **Influence of seasonality on the microbiome** Seasonality exerts an influence on microbiome composition. *Psychrobacter* and *Acinetobacter* predominated in the warm season, while *Flavobacterium* and *Psychrobacter* predominated in the cold season. **Identification of contamination points** Identification of the chilling tank as a source of contamination of poultry carcasses.
Wang et al. (2020)	Chicken carcasses	16 S rRNA (V3–V4)	Illumina MiSeq	**Microbiome characterization** *Psychrobacter, Pseudomonas, Shewanella* and

Table 3 (*continued*)

Reference	Sample types	Marker gene	Sequencing platform	Main application and discovery
				Carnobacterium comprised the spoilage microbiome. **Influence of processing steps on the microbiome** The chilling method strongly influenced the microbiome. *Pseudomonas*, *Psychrobacter* and *Shewanella* were the spoilage bacteria in air-chilled carcasses, while *Psychrobacter*, *Pseudomonas* and *Carnobacterium* were the spoilage bacteria in water-chilled carcasses.
H. Wang et al. (2019)	Chicken carcasses Environmental samples (water samples from scalder and chilling tanks)	16 S rRNA (V3–V4)	Illumina MiSeq	**Microbiome characterization** Firmicutes and Proteobacteria predominated in chicken carcasses and in the water from the chilling tank, while Firmicutes and Deinococcus-Thermus predominated in the water from the scalder tank. **Influence of processing steps on the microbiome** Washing and chilling significantly reduced the abundance of *Escherichia-Shigella* and *Streptococcus*. **Identification of contamination points** Identification of the chilling step as a putative contamination point due to an increase in the microbial diversity of poultry samples after this step.

application of culturing techniques in these studies was able to identify some potential contamination sources, they cannot capture the full composition and diversity of microbial communities in the samples, missing certain information and possible contamination routes. An example is the identification of the confined tunnel system, which incorporates scalding, defeathering and plucking, as a distribution route of *Anoxybacillus* spp. between the FPE and poultry carcasses (Chen, Fegan, Kocharunchitt, Bowman, Duffy, et al., 2020).

Unfortunately, there are still limited metabarcoding studies that attempt to establish associations between poultry and environmental samples collected after the slaughtering process. In the study conducted by Merino et al. (2025), food-contact surfaces did not appear to act as a source of contamination, as no significant differences in microbial composition or diversity were observed between chicken thighs (used as raw material) and chicken burgers (final product). In contrast, in the study conducted by Park et al. (2023), some processing steps led to changes in the composition and diversity of the chicken samples' microbiomes. For instance, the defeathering step was found to significantly reduce the abundance of *S. aureus* in chicken samples, resulting in lower levels in slaughtered, processed, and market carcasses compared to live birds. Additionally, microbial contamination routes throughout the chicken meat supply chain could be identified, revealing that environmental samples from the industry processing plant were the primary microbial contributors to the final chicken products.

4.3.1.4. Advantages and disadvantages. Metabarcoding has been widely applied to describe poultry microbiota. In fact, the use of specific primers targeting microbial genetic markers (16 S rRNA, 18 S rRNA or ITS) during the amplification step makes this approach ideal for samples that are highly contaminated with non-microbial DNA (e.g. poultry tissue DNA). Moreover, since the amplification step generates numerous copies of the targeted fragment, it is particularly suitable for samples with low microbial density, such as surface samples from FPE (Knight et al., 2018) (Table 1). Metabarcoding is a simple, fast and cost-effective method which provides an overview of a microbial community (Knight et al., 2018), so it could be included to support routine microbial analysis (De Filippis et al., 2021). One of its main advantages is the availability of several bioinformatics pipelines free and easy to operate (QIIME2 (Bolyen et al., 2019), mothur (Schloss et al., 2009) or USEARCH (Edgar, 2010)), which eases the sequencing data analysis, reducing bioinformatics expertise requirements. Furthermore, there is a wide range of public databases for taxonomic classification based on 16 S and/or 18 S rRNA genes, such as Greengenes, RDP and Silva (Knight et al., 2018) (Table 1).

Nevertheless, this technique has some limitations that can impact its reliability in poultry studies (Table 1). First, the selection of specific marker genes and hypervariable regions may be a difficult task as it requires prior knowledge about the microbial communities present in a sample (Forbes et al., 2017). As mentioned above, the specific marker gene determines the microbial group to be studied (Archaea, Bacteria, Eucaryotes, Fungi); and different hypervariable regions provide different taxonomic information, with some domains being more suitable than others for specific microbial groups. Moreover, conditions during the amplification step, such as the choice of DNA polymerases and the number of PCR cycles, can influence the observed microbial composition and diversity (Wu et al., 2010). Therefore, it is strongly recommended to standardize amplification conditions across samples, particularly when the aim is to compare microbial composition and diversity. The amplification step may also introduce some biases related to primer affinity and operon copy number. The choice of primer set determines the taxonomic coverage, as not all primers hybridize equally well to the 16 S rRNA gene sequences of all microbial taxa, resulting in some bacterial groups being efficiently amplified while others are underrepresented or not amplified. Additionally, the variable number of 16 S rRNA gene copies among taxa can lead to overestimation of taxa with multiple copies after the amplification step. This leads to inaccurate estimation of some bacterial communities, affecting both the analysis of bacterial diversity and the estimation of microbial abundance (Kembel et al., 2012). Additionally, sequencing depth (number of sequencing reads obtained for a given sample) is a critical factor in metabarcoding studies, as it directly influences the ability to detect microbial taxa. The optimal sequencing depth depends largely on the complexity of the microbial community being studied. In fact, a sequencing depth sufficient to capture the full microbial diversity in one sample may be inadequate for another, particularly in communities with greater complexity. These disparities can complicate the comparison of microbial diversity across samples, particularly when some samples are not sequenced deeply enough to capture their complete taxonomic composition. A commonly employed strategy to address this issue is rarefaction, a normalization process in which all samples are subsampled to the same number of reads. However, while this facilitates fair comparisons, it also involves discarding a substantial amount of data, potentially reducing the sensitivity to detect low-abundant or rare taxa (Weiss et al., 2017). Another point to consider is that the taxonomic resolution is usually limited to genus level (Knight et al., 2018), as it has not enough variability to assign taxonomy beyond the genus level, preventing the identification of specific species and strains. Both the misestimation of bacterial communities and the limited taxonomic resolution could be

Table 4
Summary of the main applications of WMS for characterizing microbial communities in poultry meat and FPE, with referenced studies.

Reference	Sample types	Sequencing platform	Main application and discovery
Álvarez-Molina et al. (2023)	Food-contact and nonfood-contact surfaces from different meat (including poultry) processing plants (slaughter and processing plants)	Illumina NextSeq 500 platform	**High-resolution taxonomic profiling** Identification of the predominant species on food-contact surfaces (*Pseudomonas fragi*, *Psychrobacter* sp. P11F6 and *Staphylococcus equorum*). **Microbiome characterization** *Pseudomonas* and *Psychrobacter* predominated in slaughterhouses and processing plants; *Acinetobacter* was more abundant in slaughterhouses, and *Staphylococcus* and *Shewanella* in processing plants. Higher microbial richness in slaughterhouses than in processing plants. Greater microbial heterogeneity on food-contact surfaces than on non-food-contact surfaces. **Resistome characterization** ARGs conferring resistance to tetracyclines and aminoglycosides were predominant in slaughterhouses and biocide-resistant genes conferring resistance to QACs were predominant in processing plants. Higher abundance and diversity of ARGs in slaughterhouses than in processing plants. **Microbiome-resistome association** Identification of *Escherichia* as the main carrier of aminoglycoside ARGs, *Bacillus* as the main carrier of tetracycline ARGs, and *Staphylococcus* as the main biocide-resistant genes carrier. **Mobilome-resistome association** A substantial proportion of ARGs were located on MGEs. Aminoglycoside and tetracyclines ARGs were more frequently associated with MGEs than other ARGs.
De Cesare et al. (2022)	Chicken feces and carcasses from different rearing systems (conventional and antibiotic-free)	Illumina NextSeq500	**Microbiome characterization** *Acinetobacter*, *Pseudomonas*, *Psychrobacter* and *Propionibacterium* predominated. **Resistome characterization** ARGs conferring resistance to betalactams predominated. Higher ARGs abundance in carcasses than in feces. **Influence of study factors on microbiome and resistome** Rearing systems did not influence the microbiome composition or the abundance and composition of ARGs in chicken carcasses, but affected fecal samples, with higher ARG levels observed in conventional farms than in antibiotic-free farms.
Li et al. (2020)	Retail boneless and skinless chicken breasts of different brands (different processing establishments and different rearing systems) stored under vacuum or aerobically at 4 °C until their sell-by date.	Illumina MiSeq	**High-resolution taxonomic profiling** Detection of the species *S. enterica* in poultry samples, demonstrating even greater sensitivity than qPCR. **Microbiome characterization** *Pseudomonas* predominated in chicken samples stored aerobically. **Resistome characterization** Detection of low abundances of ARGs on chicken breasts. ARGs conferring resistance to aminoglycoside and betalactams predominated in chicken samples stored aerobically, while ARGs conferring resistance to betalactams, tetracyclines and aminoglycosides predominated in chicken samples stored under vacuum. **Influence of different factors on microbiome and resistome** Packaging type and processing environment, but no seasonality and rearing system, influenced microbiome composition and diversity. Packaging type but no rearing system influenced the abundance and composition of ARGs in chicken breasts.
Merino, Pagán, et al. (2024)	Chicken thighs Chicken burger packages (70 % N_2 and 30 % CO_2) stored at 4 °C, sampled on day 0 and after exceeding the sell-by date. Food-contact surfaces across a chicken burger processing line, sampled before and after C&D procedures	Illumina NovaSeq 6000	**Microbiome characterization** *Pseudomonas*, *Psychrobacter*, *Shewanella* and *Brochothrix* predominated in chicken samples at day 0, while *Latilactobacillus* and *Leuconostoc* predominated in expired chicken samples. *Hafnia* and *Acinetobacter* predominated in food-contact surfaces sampled after C&D procedures. **Resistome characterization** Low overall abundance of ARGs. Detection of ARGs and biocide-resistant genes on food-contact surfaces. **Microbiome-resistome association** Co-occurrence of *Pseudomonas* and biocide-resistant genes as well as LAB and lincosamides ARGs. Positive correlation between the main genera in clean surfaces (*Hafnia*, *Yersinia* and *Acinetobacter*) with several ARGs.

Table 4 (*continued*)

Reference	Sample types	Sequencing platform	Main application and discovery
Sequino et al. (2025)	Chicken carcasses at the beginning and at the end of a poultry processing line. Food-contact and non-food-contact surfaces sampled after C&D procedures across a poultry processing line. Chicken samples packaged aerobically, under MAP (30 % N_2 and 70 % CO_2) and under vacuum stored at 4 °C and 10 °C.	Illumina NovaSeq	**Microbiome characterization** *Brochothrix thermosphacta, Pseudomonas fragi* and *Psychrobacter immobilis* predominated in chicken carcasses and surfaces from the processing line. **Resistome characterization** High overall abundance of ARGs, mainly associated with resistance to aminoglycosides and betalactams. **Influence of different study factors on microbiome** Packaging and storage conditions determined the predominant microbiota. *Acinetobacter harbinensis* and different *Pseudomonas* species predominated in samples packaged aerobically, while *B. thermosphacta, Lc. gelidum* and *Ph. carnosum* predominated in samples packed in MAP. **Virulome characterization** Detection of a wide range of VFGs related to motility, adherence, and biofilm formation across the production line, with increased abundance in carcass processing areas. **Microbiome-resistome association** Identification of *Acinetobacter* as the main contributor of aminoglycoside and tetracycline ARGs, followed by *Staphylococcus* and *Bacillus*. **Mobilome-resistome association** ARGs conferring resistance to aminoglycosides, beta-lactams, tetracyclines and lincosamides were frequently associated with MGEs.
Vieira et al. (2023)	Retail frozen chicken carcasses from different rearing systems (conventional, antimicrobial-free and organic rearing systems)	Illumina MiSeq	**Microbiome characterization** *Pseudomonas, Acinetobacter* and *Enterobacter* predominated. **Resistome characterization** ARGs conferring resistance to betalactams, aminoglycosides and tetracyclines predominated. **Influence of study factors on microbiome and resistome** *Pseudomonas* was more abundant than *Acinetobacter* in samples from antimicrobial-free and organic rearing systems. ARGs abundance was lower in chicken from antimicrobial-free and ecological rearing systems.
Wang et al. (2017)	Yellow-feather broiler carcasses collected in a slaughtering processing line, packaged with penetrated-air packaging or MAP (80 % CO_2 20 % N_2) and stored at 4 °C for 4 and 8 days, respectively	Illumina HiSeq 4000	**High resolution taxonomic profiling** Identification of the species responsible for the spoilage of chicken carcasses. *Pseudomonas fragi,* CF149 and *fluorescens* dominated in air-permeable samples, and *Serratia liquefaciens, Lactobacillus raffinolactis, Aeromonas salmonicida,* and *Carnobacterium maltaromaticum* in MAP samples. **Microbiome characterization** *Pseudomonas, Arthrobacter* and *Janthinobacterium* predominated in samples packaged with penetrated-air packaging; while *Carnobacterium, Vagococcus, Enterococcus, Lactobacillus, Serratia* and *Hafnia* predominated in samples packaged with MAP **Influence of different study factors on microbiome** Packaging conditions strongly influenced microbiome composition and diversity. Air-permeable samples showed a higher microbial diversity than MAP samples; and air-permeable samples were dominated by *Pseudomonas*, whereas MAP samples were dominated by *Aeromonas, Lactococcus, Serratia* and *Shewanella*.

Abbreviations: ARGs: Antibiotic Resistant Genes; MGEs: Mobile Genetic Elements; QACs: Quaternary Ammonium Compounds; C&D: Cleaning & Disinfection; LAB: Lactic Acid Bacteria; MAP: Modified Atmosphere Packaging; VFGs: Virulence Factor Genes.

solved targeting different or even multiple marker genes. Targeting single-copy marker genes (e.g. *rpoB*, *gyrB*, *recA*) would avoid the over-estimation of some bacterial; and targeting marker genes with higher variability would improve specific identification (Van Reckem et al., 2020). However, the lack of complete databases in comparison to 16 S rRNA databases is a limiting factor for its application (D'Auria et al., 2006).

Finally, although metabarcoding is a high-throughput and cost-effective option for tracking community shifts in poultry meat and FPE, it offers little direct insights into functional determinants relevant to risk, such as VFGs or ARGs.

4.3.2. Whole metagenomic sequencing, shotgun sequencing or untargeted sequencing

Whole metagenomic sequencing (WMS), also called shotgun sequencing or untargeted sequencing, involves the extraction and sequencing of the total DNA content present in a sample without prior amplification of specific marker genes (Cao et al., 2017) (Fig. 2). Therefore, WMS provides comprehensive information on the entire repertoire of genes present in a sample, which can be employed to determine not only its microbiome and resistome, but also its virulome and mobilome by using specific databases (Jagadeesan et al., 2019).

The following sections present a detailed overview of the main applications of WMS in poultry and poultry FPE, as well as their advantages and limitations (Table 1). Table 4 summarizes studies that have used WMS to investigate microbial communities in the poultry sector.

4.3.2.1. Detection, identification and relative quantification of microbial taxa. Similar to metabarcoding, WMS also offers information regarding the composition and diversity of the microbial communities present in a

sample, as well as into the influence of different factors on microbial composition and diversity. For example, Li et al. (2020) and Wang et al. (2017) confirmed that packaging conditions shape the microbiome of chicken samples. Additionally, Li et al. (2020) also evaluated the influence of seasonality, rearing system, and processing plant, concluding that seasonality and rearing system did not result in differences in the microbiome.

However, differing from metabarcoding, the absence of an amplification step and the utilization of diverse marker genes lead to a less biased and higher-resolution taxonomic characterization, extending to the strain level (Yap et al., 2022). In this sense, the use of this technique enabled the identification of the bacterial species responsible for the spoilage of different chicken samples (Merino, Pagán, et al., 2024; Sequino et al., 2025; Wang et al., 2017), as well as the identification of the predominant bacterial species on food-contact surfaces from various meat processing plants (Merino, Pagán, et al., 2024; Sequino et al., 2025; Álvarez-Molina et al., 2023). Similarly, Li et al. (2020) employed WMS with sufficient sequencing depth to detect the pathogen *Salmonella enterica* in various chicken samples, demonstrating even greater sensitivity than qPCR. Overall, this comprehensive taxonomic profiling can be highly useful for assessing, and if necessary, for designing and implementing food preservation methods and cleaning and disinfection (C&D) procedures targeting these bacterial species in specific FPE.

4.3.2.2. Resistome characterization. In addition to providing valuable insights into the microbiome, shotgun sequencing also offers detailed information about the resistome. In fact, most WMS studies conducted in the poultry sector utilize this technique to evaluate the resistome of chicken meat and their environmental surroundings. Illustrative examples include those investigations aiming to correlate the antibiotic usage on farms with the abundance, composition and diversity of ARGs present in chicken meat samples (De Cesare et al., 2022; Li et al., 2020; Vieira et al., 2023). De Cesare et al. (2022) and Li et al. (2020) concluded that there were no differences in the abundance and composition of ARGs when comparing chicken meat samples from different rearing systems.

Although the vast majority of the studies applied in the poultry sector focus on chicken or farm environmental samples rather than on poultry FPE, there are two WMS studies have assessed the microbiome and resistome of surface samples from various slaughterhouses and industrial processing plants, including those involved in poultry product manufacturing (Merino, Pagán, et al., 2024; Álvarez-Molina et al., 2023). Álvarez-Molina et al. (2023) concluded that significant differences exist between slaughterhouses and meat processing plants in terms of resistome. Surface samples from slaughterhouses exhibited a higher abundance and diversity of ARGs compared to those from meat processing plants, indicating that live animals introduced a higher load of ARGs than carcasses, and/or that C&D protocols effectively eliminate a substantial proportion of ARG-harbouring microorganisms in slaughterhouses, thereby limiting the spread of AMR throughout the meat production chain. Additionally, the resistome composition varied between the two environments: ARGs conferring resistance to tetracyclines and aminoglycosides were predominant in slaughterhouses, while genes conferring resistance to quaternary ammonium compounds (QACs) were more prevalent in meat processing plants, which are likely more intensely sanitized. Similarly, Merino, Pagán, et al. (2024) also identified genes conferring resistance to QACs in surfaces from a poultry burger processing line.

Although resistome characterization has traditionally focused on ARGs, increasing attention is now being directed towards genes conferring resistance to biocides, chemical agents extensively used in the food industry for C&D procedures. This growing interest is largely due to the ability of bacteria carrying biocide-resistant genes to survive C&D treatments and persist in food processing environments, thereby acting as a continuous source of food contamination. Several studies have reported bacterial isolates from poultry products and FPE that

exhibit resistance to various biocides. For example, *Salmonella* spp. isolates resistant to specific biocides (chlorine and QACs) have been found on sanitized equipment in poultry processing plants where these biocides were regularly employed (Obe et al., 2021), and *L. monocytogenes* isolates from different surfaces in a turkey processing plant exhibited resistance to QACs (Mullapudi et al., 2008). Another reason for this increased focus is the potential association between biocide resistance and antibiotic resistance through co-resistance and cross-resistance mechanisms (Merchel Piovesan Pereira et al., 2021). Several studies have identified significant positive correlations between biocide use and reduced susceptibility to biocides and/or antibiotics. For instance, Xiao et al. (2021) not only observed a high prevalence of both antibiotic and chlorine-based biocide resistance but also significant positive correlations between resistance to a chlorine-based biocide and resistance to various antibiotics in *Salmonella* spp. isolates from poultry farms, slaughterhouses, and retail markets in China. Similarly, Wieland et al. (2017) observed significant positive correlations between resistance to a QAC-based biocide and resistance to various antibiotics in *E. coli* and enterococci isolates from different flocks from the same poultry farm. Other examples include studies by Alonso-Hernando et al. (2009) and Molina-González et al. (2014), which observed that *S. enterica* isolates from poultry products exhibited increased resistance to various antibiotics after exposure to increasing subinhibitory concentrations of chlorine-based biocides.

In response to these concerns, resistome databases such as CARD or ResFinder have expanded their scope to include genes associated with biocide resistance. In recent years, several WMS studies have confirmed the presence of biocide-resistant genes, particularly those conferring resistance to QACs, on surfaces within different poultry FPE (Merino, García-Castillo, et al., 2024; Álvarez-Molina et al., 2023).

4.3.2.3. Virulome characterization. As WMS provides information on the entire gene repertoire, this technique also enables the identification of VFGs, genes encoding products that allow microorganisms to adhere, invade, survive and persist in diverse niches. Similar to antibiotic resistance genes, which are classified based on the antibiotic families they confer resistance to, VFGs can also be categorized according to their functional roles. These categories include adherence, invasion, effector delivery system, motility, exotoxin, exoenzyme, immune modulation, biofilm, nutritional/metabolic factor, stress survival, post-translational modification, antimicrobial activity/competitive advantage, and regulation (Liu et al., 2021). The detection and characterization of VFGs in the food industry is particularly relevant, especially those related to motility (e.g. *fliP*, *flhA*, *fleQ*), which facilitate movement toward nutrient-rich niches; adherence, such as fimbria and pili components (e. g. *mrkD*, *pilT*, *ompA*), which mediate initial attachment to surfaces; and biofilm formation, such as *algD*, *algA* and *icaA*, which enable the production of the extracellular polymeric substance (EPS) matrix that stabilizes and protects microbial communities. Collectively, these traits enable microorganisms to colonize equipment surfaces, form biofilms, and resist C&D procedures, thereby increasing the risk of cross-contamination. In fact, biofilms are a major concern in poultry processing plants, where meat exudates may create favorable conditions for their formation. Compared to planktonic cells, the cells inside the biofilm (sessile cells) are more resistant to biocides and environmental stresses (López et al., 2010). This increased resistance is mainly attributed to several factors. First, the EPS matrix, which physically protects sessile cells and prevents or delays the penetration of antimicrobials into deeper layers (Mah & O'Toole, 2001). Second, cell differentiation within the biofilm (Ciofu et al., 2022), including metabolically active subpopulations that upregulate EPS synthesis (Friedman & Kolter, 2004) and efflux pump activity (Mah & O'Toole, 2001), and dormant subpopulations with reduced susceptibility to antimicrobial agents (Amato et al., 2014). Finally, the dissemination of both virulence and resistance genes among cells (Fux et al., 2005).

Although this information is highly valuable, to the best of our knowledge, only one study has employed WMS to investigate the presence and distribution patterns of VFGs along the poultry processing chain (Sequino et al., 2025). In this study, a wide range of VFGs were identified throughout the poultry production chain, with an increased abundance in the carcass processing area. Among them, there were VFGs associated with motility (*PA1464, PA1459, PA1458, fliP, fliN, fliI, fliG, flhA, flgI, flgH, fleQ, fleN*), adherence (*rpoN, pilT, pilJ, pilG, ompA, crc*) and biofilm formation (*mrkD, mrkC, mrkB, algW, algI, algD, algA, alg8*).

4.3.2.4. Microbiome-resistome association. While identifying the microbial community and the repertoire of ARGs present in a sample is essential, establishing correlations between them provides a deeper understanding of the ecological context and potential transmission pathways of AMR. Microbiome and resistome characterization can be performed by directly mapping metagenomic reads against reference databases containing known genomes or resistance genes based on sequence similarity. However, linking the microbiome and resistome requires an assembly process, in which sequencing reads are merged into longer contiguous sequences (contigs). These contigs allow not only for the identification of resistant genes, but also for the detection of other markers that serve as specific identifiers of particular taxa, thereby facilitating a more accurate assignment of resistant genes to their respective microbial hosts. For example, in the study carried out by Álvarez-Molina et al. (2023), the taxonomic mapping of ARG-carrying contigs pointed to specific bacterial genera, such as *Escherichia*, *Bacillus*, or *Staphylococcus*, as carriers of the most relevant resistance determinants. Most aminoglycoside ARGs were mapped to *Escherichia*, while *Bacillus* was primarily associated with tetracycline ARGs, and *Staphylococcus* to biocide resistance genes. In the study conducted by Sequino et al. (2025), most of the ARGs belonged to *Acinetobacter*, *Staphylococcus* and *Bacillus*. Specifically, *Acinetobacter* was the main contributor of aminoglycoside and tetracycline ARGs, followed by *Staphylococcus* and *Bacillus*. In this study, microbiome-virulome associations were also assessed, revealing that most VFGs were associated with various species within the *Pseudomonas* genus.

4.3.2.5. Mobilome-resistome association. Bacteria can acquire AMR not only through spontaneous mutations but also via HGT, a process by which ARGs are transferred from resistant to susceptible strains. HGT occurs through mechanisms such as transformation, transduction, or conjugation, with MGEs, including plasmids, transposons, and integrons, being the most frequent mechanisms of horizontal transfer of ARGs (De Mesquita Souza Saraiva et al., 2022). Given the potential for MGEs to mediate the spread of resistance genes across microbial populations, their presence in the food chain represents a significant public health concern.

The WMS assembly-based approach also enables the exploration of associations between the mobilome and the resistome, as it allows both the detection of resistance genes and the identification of specific genetic markers indicative of particular MGEs, thereby facilitating a more accurate assignment of these genes to their respective genetic contexts. In this sense, there are several studies which have employed WMS to study the presence of MGEs within poultry FPE, aiming to assess the potential mobilization of the resistome (Sequino et al., 2025; Álvarez-Molina et al., 2023). Álvarez-Molina et al. (2023) observed that about 63 % of all ARG reads were assigned to contigs classified as plasmid-associated, indicating that a substantial proportion of ARGs are located on MGE, which may facilitate their horizontal transfer among bacterial populations. Moreover, they observed that the diversity of ARGs conferring resistance to aminoglycosides and tetracyclines was higher in contigs associated with plasmids than ARGs conferring resistance to beta-lactams, suggesting that some ARGs have a higher risk of plasmid-mediated transfer than others. Sequino et al. (2025) observed that ARGs present in a poultry processing line were frequently associated to plasmids, specifically those conferring resistance to aminoglycosides, beta-lactams, tetracyclines and lincosamides, suggesting that they can be easily transferred along the food chain.

4.3.2.6. Advantages and disadvantages. WMS is being increasingly employed to obtain comprehensive information about microbial communities and AMR determinants, offering insights into the role of the poultry sector as a reservoir and/or disseminator of both relevant microorganisms and AMR. Therefore, integrating this technique into monitoring and food safety management programs should be considered. This perspective is reinforced by the recent Commission Implementing Regulation (EU) 2025/179 (European Commission, 2025), which establishes the mandatory use of whole genome sequencing for pathogenic microorganisms involved in foodborne outbreaks, underlining the importance of culture-independent approaches for food safety surveillance.

In addition to offering more comprehensive information on the entire repertoire of genes and providing higher taxonomic resolution, a significant advantage of WMS over other techniques is its ability to establish associations between resistance genes (resistome) and their taxonomic origin (microbiome) or genetic contexts (mobilome) when the assembly-based approach is feasible.

Nevertheless, although WMS provides valuable information and overcomes many of the biases associated with culturing techniques and metabarcoding, it faces distinct challenges. WMS is highly data-intensive and requires sufficient sequencing depth, coverage and read quality to enable reliable profiling of the microbiome, resistome, virulome and mobilome. These strict data requirements, together with the need for advanced computational power and bioinformatics expertise, increase analysis costs and limit the widespread use of WMS (Yap et al., 2022). In addition, one of the main challenges of WMS is that high concentrations of host DNA can reduce sequencing depth, leading to incomplete detection and characterization of microbial communities and ARGs, especially those present in low abundance (Yap et al., 2022). The detection limit in WMS is largely determined by the number of microbial reads recovered and the host-microbial DNA proportion. While most studies deplete host DNA reads during bioinformatic analysis, pre-analytical strategies are generally more effective. Traditional ones involve depleting host DNA or enriching microbial DNA using various chemical methods and commercially available kits (Marotz et al., 2018; Yap et al., 2020). Yap et al. (2020) compared different commercial kits and found that host depletion kits yielded a greater increase in microbial sequencing depth than microbial DNA enrichment kits. Marotz et al. (2018) demonstrated that osmotic lysis followed by a treatment with propidium monoazide was the most efficient method for removing host DNA compared to size filtration and commercially available host depletion kits. A particularly innovative solution is adaptive sequencing using Oxford Nanopore platforms, which can selectively enrich microbial DNA in real time by rejecting host-derived sequences as they enter the nanopores, thereby increasing the effective sequencing depth of microbial genomes and genes of interest without requiring additional chemical treatments. Similarly, low microbial loads in some samples, such as those from FPE, make it difficult to recover sufficient DNA content, thereby hindering comprehensive microbiome and resistome profiling (Álvarez-Molina et al., 2023).

Importantly, even when host DNA depletion or microbial DNA enrichment strategies are successfully applied, both metabarcoding and WMS remain limited in their ability to detect microorganisms present at very low abundance, including many clinically and food-relevant pathogens. Therefore, while integrating these techniques into monitoring and food safety management programs may be valuable, their use as a standalone approach cannot be recommended, and the combination of complementary methodologies is required. In this context, advanced culture-based approaches such as culturomics, as well as targeted culture enrichment prior to metagenomic sequencing (quasi-

metagenomics), can play a crucial role by increasing the relative abundance of low abundance or fastidious microorganisms, thereby enabling their detection and subsequent characterization through sequencing-based analyses. Finally, other common limitations of both metabarcoding and WMS are the inability to determine the absolute abundance of the identified taxa or ARGs, and the uncertainty regarding the viability or activity of these taxa or ARGs. Therefore, integrating these methods with complementary approaches is desirable and beneficial (Álvarez-Molina et al., 2023). For example, culturing techniques can quantify viable microorganisms, qPCR can provide absolute counts of specific taxa or ARGs, and metatranscriptomics reveals which genes are actively expressed under particular conditions.

5. Future research considerations

Despite the advances achieved through culture-dependent and culture-independent techniques, several areas remain underexplored, and future research should aim to further enhance our understanding of microbial ecosystems in the poultry sector.

The integration of multi-omics approaches, including metagenomics, metatranscriptomics and metaproteomics, can offer complementary insights by connecting microbial presence with functional activity. Metagenomics reveals the genetic potential of microbial communities, identifying ARGs, biocide-resistant genes and VFGs. However, without complementary approaches such as metatranscriptomics and metaproteomics, it is unclear which genes are actively expressed and translated into functional proteins. Metatranscriptomics indicates gene expression, while metaproteomics confirms the production of enzymes and other functional proteins. Combining these approaches is therefore crucial to bridge the gap between genetic potential and microbial function, enabling a more accurate understanding of spoilage, virulence, and AMR dissemination.

Despite the integration of culture-dependent and culture-independent approaches, some microorganisms remain undetected, including VBNC bacteria and species present at very low abundance. To overcome these limitations, culturomics has emerged as an innovative advancement in culture-based methodologies. By diversifying culture conditions and integrating identification tools such as MALDI-TOF MS or 16 S rRNA gene sequencing, culturomics can capture a broader spectrum of microbial diversity, including previously uncultured or rare taxa. Importantly, culturomics complements culture-independent approaches by confirming microbial viability and recovering low-abundance microorganisms, while information from multi-omics data can guide the design of targeted cultivation strategies and optimize media compositions, enhancing efficiency and reducing workload (Chen et al., 2024).

Similarly, quasi-metagenomics, which combines targeted culture enrichment with subsequent metagenomic sequencing, represents a promising strategy to focus analyses on viable microorganisms of interest, including foodborne pathogens, AMR bacteria, or microorganisms carrying MGEs. In addition to confirming microbial viability and recovering low-abundance microorganisms, this targeted enrichment approach is particularly valuable for mobilome studies, as it increases the likelihood of MGEs associated with viable cells, thereby improving the assessment of HGT potential in processing environments (Kocurek et al., 2023).

Despite the importance of understanding HGT events involving ARGs and VFGs in processing environments, studies investigating the mobilome in the poultry production chain remain comparatively limited, especially compared with microbiome and resistome research.

While many studies have focused on farms and slaughterhouses, food processing plants remain comparatively underexplored, particularly regarding surfaces and equipment where biofilms can form. These biofilms are critical sites for HGT, potentially spreading resistance genes and VFGs.

6. Conclusion

This review has provided an in-depth analysis and critical evaluation of the most relevant publications on the application of both culture-dependent and culture-independent techniques for characterizing the microbiome, resistome, virulome and mobilome in the poultry sector. Understanding the information each technique provides, along with their respective advantages and limitations, enables researchers and industry professionals to select the most appropriate method or combination of methods for specific study objectives. In fact, due to the different insights each approach offers, integrating both culture-dependent and culture-independent techniques seems to be the optimal strategy. Culture-dependent techniques allow the quantification of the absolute abundance of specific microbial groups of interest, while culture-independent techniques provide a more comprehensive understanding of the composition and diversity of the microbial communities and relevant genes, such as ARGs, biocide-resistant genes and VFGs. Integrating both approaches in microbiome and resistome studies could provide significant advantages: on the one hand culture-independent methods would facilitate the detection of microbiota harbouring ARGs, biocide-resistant genes and VFGs; while culture-dependent techniques would allow for the targeted isolation of these viable microorganisms, supporting their in-depth characterization and the development of specific control strategies to control these resilient strains.

Furthermore, this review underscores that future research should prioritize: (i) post-slaughter processing environments, where biofilms and equipment surfaces act as critical reservoirs for resistant and virulent microorganisms; (ii) a deeper exploration of the mobilome and virulome, which are considerably less studied than the microbiome and resistome; and (iii) the integration of multi-omics, culturomics, and quasi-metagenomics to link microbial presence with functional activity and viability. Therefore, this review not only synthesizes existing knowledge but also provides guidance for advancing poultry microbial ecology.

CRediT authorship contribution statement

Natalia Merino: Writing – original draft, Conceptualization. **Laura Espina:** Writing – review & editing, Conceptualization. **Daniel Berdejo:** Writing – review & editing, Conceptualization. **Rafael Pagán:** Writing – review & editing, Funding acquisition, Conceptualization. **Diego García-Gonzalo:** Writing – original draft, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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