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Review

Pseudomonas fluorescens and *P. aeruginosa* contaminations of poultry and poultry products: A review on food safety and quality

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Abstract

The presence of *Pseudomonas* spp. in food poses a health concern due to their ability to grow during cold storage. *Pseudomonas fluorescens* and *P. aeruginosa* are two important species that cause food spoilage and foodborne illness, respectively. *P. fluorescens* is responsible for food spoilage due to secretion of protease and lipase enzymes, which cause off-odours, off-flavours, and rancidity, even under refrigeration storage. *P. aeruginosa* is recognised as opportunistic pathogens that causes illness in infected individuals. *P. aeruginosa* harbours multiple virulence factors that enable it to be a successful pathogen to cause infection in humans. Both of these bacteria commonly contaminate poultry products which cause quality and safety issues. They are capable of forming biofilm in food processing environments, and exhibit multiple antibiotic resistances. The biofilm formation enables these bacteria to persist in the environments, and contaminate food if improper sanitation and handling happen. The contaminated food will have a shorter shelf life which leads to food wastage. Pathogenic *P. aeruginosa* that exhibits multiple antibiotic resistance will cause serious foodborne illness to infected individuals due to failure in clinical treatment. As such, controlling the growth of these bacteria in poultry is important which can be done through good hygiene practices, modified air packaging, biopreservatives, and low temperature storage. Detection of these bacteria in poultry will also help to ensure the quality and safety related to poultry. Selective agar plating is an important method to isolate *Pseudomonas* spp., which is important for further analysis. Molecular methods such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) are pivotal for rapid, robust, and specific detection of the targeted bacteria.

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Introduction

Taxonomy and characteristics

Pseudomonas spp. are ubiquitous in nature, and have become one of the most significant groups of known bacteria worldwide. The genus contains a total of 144 species, making it one of the bacterial genera with the largest number of species (Gomila *et al*., 2015), and incorporates species that have been isolated globally in various environments (Silby *et al*., 2011; Molina *et al*., 2013). All *Pseudomonas* spp. are Gram-negative rods with straight or long curves, and motile either by one polar flagellum or even more (Arslan *et al*., 2011; Kumar *et al*., 2019). They are non-spore forming, obligate aerobic, and oxidase-positive (Adams and Moss, 2008). Moreover, they are considered significant as psychotropic bacteria, and have been commonly isolated from foods, and they contributed to the spoilage in foods that have been stored at refrigeration temperatures due to their ability to grow at low temperature (Al-Rodhan and Nasear, 2016; Caldera *et al*., 2016). *Pseudomonas* spp. can grow optimally in a pH range from 6.5 to 8.0, and a temperature range from 2 to 35°C (Ercolini *et al*., 2010; Nowak *et al*., 2012). The majority of the isolates can thrive in low temperatures, and possess enzymes that may impact the overall quality of food products, particularly those stored under cold conditions (Caldera *et al*., 2016). Previous researchers reported that *Pseudomonas* spp. are highly active in producing enzymes especially proteases and lipases (Mhenni *et al*., 2023).

Among the 144 established species, most *Pseudomonas* spp. known to cause illness in humans are associated with opportunistic infection including *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. cepacia*,

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P. stutzeri, and *P. maltophilia* (Iglewski, 1996). *Pseudomonas* genus also includes most relevant specific agents of food spoilage (Mellor *et al*., 2011), important plant pathogens (Xin *et al*., 2018), and also opportunistic pathogens to animals and humans (Moradali *et al*., 2017). A recent study by Bloomfield *et al*. (2023) highlighted that *Pseudomonas* spp. are the predominant microbial genus found on retail foods such as seafood and foods from animal and plant origins.

Generally, *P. putida* is classified within the *fluorescent* group of opportunistic *Pseudomonas* species (Baykal *et al*., 2022). It has been noted that *P. putida* is less pathogenic than other *Pseudomonas* spp., and susceptible to numerous antimicrobial agents. As a result, infections caused by *P. putida* are uncommon in clinical settings (Cho and Lee, 2018). Several previous studies mentioned that although *P. fluorescens* infections are uncommon, the use of antibiotics may become ineffective. However, in rare cases, it can be found as an opportunistic pathogen, and lead to fatal diseases (Donnarumma *et al*. 2010; Naghmouchi *et al*., 2012), which is similar to *P. aeruginosa* as described by Austin and Austin (2016) and Duman *et al*. (2021).

Pseudomonas spp. contamination and their potential hazards towards food industry and human health

Spoilage refers to any alteration in a food product that makes it unsatisfactory to consumers based on its sensory characteristics (Gram *et al*., 2002). Besides physical damage, oxidation, and colour change, spoilage symptoms can also be attributed to the undesirable growth of microorganisms to unacceptable levels, as well as the activity of endogenous enzymes (Ercolini *et al*., 2010). Food spoilage caused by *Pseudomonas* spp. may happen in a number of ways. Most *Pseudomonas* spp. have been recognised as one of the crucial specific spoilage microorganisms due to their ability to excessively proliferate, which may accelerate the breakdown of nitrogenous compounds, and eventually contribute to product deterioration (Mhenni *et al*., 2023). Moreover, previous researchers highlighted that *Pseudomonas* spp. are capable of producing abnormal colours such as green fluorescent, yellow, red, and blue pigments in the contaminated foods or food products (Doulgeraki and Nychas, 2013; Mohareb *et al*., 2015). Fresh milk, fish, meat, and other items that are kept aerobically at low temperatures are common cold samples that include *Pseudomonas* spp. The development of *Pseudomonas* spp. is the cause of off-flavours, off-colours, and slime formation seen in meat and its derivatives (Stellato *et al*., 2017). Therefore, *Pseudomonas* spp. are frequently linked to poor quality food items (Molina *et al*., 2013).

P. fluorescens frequently contaminates a wide variety of foods, and exhibits a broad range of growth temperatures. Previous studies found that they tend to contaminate cooked and uncooked food products stored at refrigeration temperature. Chicken flesh that has been held in an aerobic environment deteriorates due to the production of *P. fluorescens* biosurfactants (Mellor *et al*., 2011). However, studies have shown that certain *P. fluorescens* strains were capable of surviving in 100% carbon dioxide-packed tofu (Stoops *et al*., 2012). *P. fluorescens* was also found in packed, ready-to-eat vegetables, according to an Italian study (Caldera and Franzetti, 2014). A number of factors, such as the raw vegetable quality, processing methods, packaging design, and storage temperature influence the microbial composition of the finished product. *P. fluorescens* is also crucially associated with the contamination in milk and dairy products. The post-pasteurisation contamination with *P. fluorescens* in high temperature, short time-pasteurised (HTST) milk remains a matter of concern, as it results in processor defects like reduced flavour acceptance level, altered coagulation texture, and the development of a fruity fermented milk characteristic (Reichler *et al*., 2018). *P. fluorescens* is commonly found as a contaminant in bulk milk tanks in Italy, demonstrating lipolytic, proteolytic, and lecithin activity (Decimo *et al*., 2014). *P. fluorescens* accelerates the breakdown of goat, cow, and buffalo milk mainly due to its proteolytic activity at different temperatures (Scatamburlo *et al*., 2015; Al-Rodhan and Nasear, 2016).

Andreani *et al*. (2015) revealed that mozzarella cheese was found with *P. fluorescens* that was associated with a particular spoilage occurrence. The iodine compound produced by *P. fluorescens* was determined as a substance responsible for the blue colour in this contaminated mozzarella cheese (Caputo *et al*., 2015). In a paper published by Belak *et al*. (2011), *Pseudomonas* spp. such as *P. fragi*, *P. lundensis*, *P. putida*, and *P. fluorescens* were recognised as significant psychrotrophic bacteria responsible for meat spoilage. Extensive research has focused on the proteases of these bacteria, demonstrating their involvement in producing

volatile organic compounds that contribute to the deterioration of meat quality (Ercolini *et al*., 2009).

Pseudomonas spp. are frequently dominant and persistent in foods and on food processing surfaces due to their ability to form biofilms, which increase their resistance to adverse conditions such as antimicrobial treatments (Moretro and Langsrud, 2017; Quintieri *et al*., 2019). Antibiotic-resistant *Pseudomonas* spp. have been reported to contaminate chicken and other meat products (Amos *et al*., 2015; Wong *et al*., 2015), as well as milk and other dairy products (Quintieri *et al*., 2019; Meng *et al*., 2020) and vegetables (Estepa *et al*., 2015), which suggested that cross-contamination might have occurred which caused organoleptic spoilage by producing unwanted volatile metabolites (Ercolini *et al*., 2010).

Pseudomonas spp., especially *P. fluorescens* and *P. aeruginosa*, are two of the most crucial bacteria causing spoilage of refrigerated food products because they actively secrete protease and lipase enzymes during pre-processing storage, even in chilled conditions or in high temperature treatments (Meliani and Bensoltane, 2015), and they have a strong ability to survive in the post-processing line, causing degradation in terms of appearance and sensory properties, hence reducing the shelf life and level of consumer acceptance of chilled and refrigerator dependent products. Therefore, it is crucial to understand the characteristics of these two important *Pseudomonas* spp. in food to improve quality, safety, and shelf life of poultry products.

Pseudomonas fluorescens

Characteristics and contamination in poultry

P. fluorescens is a predominant member of the *Pseudomonas* genus, and often discovered as spoilage species and crucial contaminant in various chilled food groups. This is due to its natural ability to produce heat stable hydrolytic enzymes although subjected to high temperature treatment and retain their enzymatic activities during cold storage (Quigley *et al*., 2013; Machado *et al*., 2017). *P. fluorescens* was found in chilled chicken drumstick samples, suggesting that this species could potentially cause the poultry industry's products to spoil (Can, 2022). This was consistent with the study by Morales *et al*. (2016) who found *P. fluorescens* in chicken breast meat, and also found that it exhibited both lipolytic and proteolytic activities.

P. fluorescens is also involved in nosocomial infections and spoilage of different types of food largely due to the presence of extracellular proteases, lipases, and also lecithinases that have heat stable characteristics, enabling this bacterium to survive well along thermal processing environments (Sillankorva *et al*., 2008; Rossignol *et al*., 2009). Previous researchers on fresh meat have shown that various strains within the same species can exhibit varying behaviours under identical food matrix and storage conditions. Consequently, distinct biotypes may exhibit distinct metabolic patterns that influence the activities linked to food spoilage, and ultimately dictate the likelihood of food spoilage (Ercolini *et al*., 2010; Casaburi *et al*., 2011; 2014).

Concerns about pathogenic microorganisms in meat or meat products have risen in recent years, even though there have been efforts to enhance the distribution of sanitary meat products (Bae *et al*., 2010). During processing, contamination commonly occurs, especially when meat comes into contact with equipment such as saws, belts, and grinders in slaughterhouses (Jay, 1992). It can also arise from food handlers' hands and knives, as well as from exposure to contaminated water. According to Stellato *et al*. (2017), *P. fluorescens* was discovered to be the most common species in settings related to the meat industry.

Enzymes produced and their roles in food spoilage

The release of fatty and amino acids due to lipase or protease enzymes produced by *P. fluorescens* causes rancidity, off-flavours, and off-odours before entering the later phase which is generally characterised by the development of extracellular slime and coloured growth (Martins *et al*., 2015). Triacylglycerol hydrolases, or lipases, catalyse the breakdown of fat molecules in foods, releasing unsaturated glycerol and fatty acids in the process (Andreani, 2016). It has been reported that rancid, free short-chain unsaturated fats contribute to unpleasant flavours, whereas bitter, frothy, or dirty flavours are associated with medium-chain unsaturated fats (Samaržija *et al*., 2012). According to a number of studies (Woods *et al*., 2001; Rajmohan *et al*., 2002), lipolytic activity is more prominent at refrigeration temperatures. The *AprX* gene has been identified as the unique protease linked to food degradation in a number of *Pseudomonas* spp. strains (Woods *et al*., 2001). Two genes responsible for the production of exoenzymes are the *AprX* and *LipM* genes as shown in Table 1.

Table 1. List of target genes of *P. fluorescens* detection and their functions.

Type of gene	Target gene	Function	Reference
Flagellin	*adnA*	- Biofilm formation related gene. - Flagellar synthesis.	Xu *et al*. (2017)
Alkaline metalloprotease	*aprX*	- Specific gene for enzyme protease secretion. - Causes degradation of extracellular protein to form grey colour and bitter flavour especially in milk.	Wong *et al*. (2023)
Flagellin	*fliC*	- Biofilm formation related gene.	Xu *et al*. (2017)
Lipase	*lipM*	- Specific gene for enzyme lipase secretion. - Hydrolyses fat to induce lipid deterioration, and produces bitter, foamy, and unclean flavour.	De Jonghe *et al*. (2011); Machado *et al*. (2017); Wong *et al*. (2023)

Wong *et al*. (2023) stated that as a psychrotrophic bacterium, *P. fluorescens* relies on low temperatures and extended storage periods to proliferate and significantly compromise the quality of food samples during refrigerated storage. This bacterium produces two key heat-resistant enzymes, protease and lipase, which withstand pasteurisation or UHT treatment, and maintain their functionality throughout refrigeration (de Oliveira *et al*., 2015; Martins *et al*., 2015). Basically, the enzymatic activity of spoilage bacteria is influenced by the initial psychotropic levels and the temperature at which food samples are stored prior to processing (Odeyemi *et al*., 2020). Previous researchers have mentioned that *P. fluorescens* will start to secrete protease and lipase to cause spoilage when the bacterial population has reached or exceeded approximately 10^6 CFU/mL or g. When the bacterial population is beyond 10^7 CFU/mL or g, the flavour deflection, especially in milk samples, is detected. Protease and lipase enzymes are typically synthesised during the early stationary growth phase or late logarithmic phase when cell density is high, where then a bitter and rancid smell is realised due to protein degradation and lipid breakdown (de Oliveira *et al*., 2015; Pazdzior, 2016).

Meng *et al*. (2017) highlighted that there was a strong correlation observed between extracellular peptidase activity on milk agar and the presence of *aprX*, as detected using PCR. This finding was in agreement with previous studies that reported and confirmed the role of *aprX* in the degradation of food products including milk (Dufour *et al*., 2008; Caldera *et al*., 2016). However, some isolates that are positive with the *aprX* gene exhibit no proteolytic activity across all storage temperatures, suggesting that *aprX* could be suppressed during the degradation of milk proteins. In the *Pseudomonas* genus, the *aprX* gene typically plays a role in nutrient utilisation by degrading extracellular proteins; thus, *Pseudomonas* spp. are commonly linked to the deterioration of milk and other dairy products (Dufour *et al*., 2008; Marchand *et al*., 2009; Zhang *et al*., 2009). Ercolini *et al*. (2010) added that compounds associated with protein breakdown were also found in non-proteolytic strains, probably originating from the breakdown of amino acids in meat or from enzymes naturally present in meat.

Biofilm formation

Pseudomonas spp. are frequently found bacteria in the food industry due to their capacity to produce biofilms, and adapt to a variety of environmental conditions. *Pseudomonas* spp. that produce biofilms are known to exist in food processing environments, and pose a threat to human health due to the biofilms production. These biofilms are encased in a self-produced exopolysaccharide matrix which consists of intricately structured microbial communities. Complexity, long production cycles, and nutrient availability in food processing lines that have large surface areas offer a perfect setting for the formation of biofilms (Yuan *et al*., 2021). When pathogenic bacteria are present in biofilms produced on surfaces that come into contact with food, they can become a persistent source of food contamination, resulting in both major sanitary issues and financial losses from food spoilage (Sofos and Geornaras, 2010). It is interesting to note that *Pseudomonas* spp. can generate biofilms that have the ability to capture and shield harmful microorganisms (Caraballo *et al*., 2020). Moreover, *Pseudomonas*

spp. are frequently found in multispecies biofilms containing pathogenic microorganisms (Quintieri *et al*., 2021). *P. fluorescens* is a strong biofilm-producing bacterium that poses a risk of contamination in poultry plants (Merino *et al*., 2024). Besides, it is also thought to enhance the growth and proliferation of other pathogens (Puga *et al*., 2018; Maggio *et al*., 2021).

Many foodborne microorganisms employ biofilm formation as a common survival strategy, distinct from their planktonic counterparts. Biofilms are recognised as a significant cause of bacterial cross-contamination in food processing areas (Maifreni *et al*., 2015). *Pseudomonas* species, especially *P. fluorescens*, are often involved in food spoilage, notably affecting dairy, poultry, and fresh produce. Ge *et al*. (2017) reported that the spoilage characteristics of *P. fluorescens* have been extensively studied across various products. *P. fluorescens* isolates from spoiled chicken meat have formed biofilm and showed an increasing attachment ability on stainless steel plates after 5 h of exposure (Wang *et al*. 2018). Dagang *et al*. (2016) indicated that *P. fluorescens* formed a strong biofilm by containing a high cell titre of 9.5 log/cm^2 cells, which was in agreement with another study that found out that the ability of *P. fluorescens* to form biofilms was greater than that of many other bacterial species, specifically foodborne pathogens, which frequently produced a cell number of less than 7 log/cm^2 cells during their biofilm formation (Yang *et al*., 2016).

According to Xu *et al*. (2017), 83.3% of 42 *P. fluorescens* strains had the *adnA* gene, and all of the strains with this gene were able to form biofilm under cultivation conditions of either 30 or 4°C. These conditions suggest that the *adnA* gene plays an important role in *P. fluorescens* biofilm formation (Table 1). Furthermore, it has been shown that the *AdnA* gene regulates both flagella-driven motility and surface adhesion in *P. fluorescens* (Mastropaolo *et al*., 2012). This may imply that the *adnA* gene's phylogenetic tree splits *P. fluorescens* into four groups based on their ability to adhere to biofilms. Therefore, the *adnA* gene is a useful target for both classifying *P. fluorescens* into distinct subgroups, and identifying *P. fluorescens* that is capable of generating biofilms (Xu *et al*., 2017).

In *P. fluorescens*, the *fliC* gene, which codes for flagella production, is controlled by *AdnA* (Table 1). The inactivation of the *fliC* gene prevents the mutant from forming biofilm, indicating the significance of the *fliC* gene for *P. fluorescens* biofilm formation (Mastropaolo *et al*., 2012). Redondo-Nieto *et al*. (2008) identified 15 *P. fluorescens* strains that had the *fliC* gene, and 12 of those also had the *adnA* gene. The *adnA* gene may be more crucial for *P. fluorescens* to create biofilm than the *fliC* gene. Indeed, the regulation mechanisms governing flagellar synthesis varied between strains, and beyond core genes, *P. fluorescens* exhibits quite complex flagellar synthesis regulation (Mastropaolo *et al*., 2012). According to Robleto *et al*. (2003), flagella are crucial but not required for *P. fluorescens* biofilm production; hence it is not surprising that there were strains with only the *adnA* gene that were also able to generate biofilm.

A number of earlier research studies reported no noticeable difference in the capacity to generate biofilms between strains carrying either one or both genes (*fliC* or *adnA*, or both). *P. fluorescens* ability to form biofilms depends on several factors which include nutrient availability (Aswathanarayan and Vittal, 2014), growing conditions (Rossi *et al*., 2016), and surface materials (Marchand *et al*., 2012). However, the temperature of incubation appeared to have a greater impact. *P. fluorescens* was more likely to develop biofilm at lower temperatures; but, at 30°C, it was more capable of forming biofilm with more adhesion.

Antibiotic resistance

Heir *et al*. (2021) highlighted that *P. fluorescens* strains isolated from chicken meat showed varying resistance rates towards antibiotics, where most of the isolates were resistant to aztreonam (72.6%), colistin (30.2%), imipenem (25.6%), and meropenem (12.6%); none of the isolates exhibited resistance to aminoglycosides such as amikacin, gentamicin, and tobramycin, whereas only 2.3% were resistant to the fluoroquinolone ciprofloxacin. The presence of resistance to one or more antibiotics and known resistance genes was observed in these *P. fluorescens* isolates from chicken meat, indicating there was significant variability in antibiotic resistance profiles.

Other than that, Shabana *et al*. (2022) revealed that all *P. fluorescens* isolate strains were resistant to piperacillin (100%), followed by ceftazidime (29.7%) and cefepime (25.8%). However, the strains were found to be highly sensitive to cefotaxime (74.2%), followed by ceftriaxone and levofloxacin (70.3% each). In addition, *P. fluorescens* showed substantial

resistance to benzylpenicillin, first and second generation cephalosporins, lipoglycopeptides, glycopeptides, fusidic acid, macrolides, streptogramins, lincosamides, rifampicin, and oxazolidinones (EUCAST, 2022).

Despite poultry industries using antibiotics as a control agent in the growth of spoilage microorganisms, aquatic products also showed huge resistance rate towards antibiotics. El-Sherifa *et al*. (2023) demonstrated that *P. fluorescens* harboured the highest rates of resistance to nalidixic acid (100%), streptomycin (100%), erythromycin (91.7%), penicillin G (79.2%), and cephalothin (75%). Thomassen *et al*. (2022) examined antibiotic resistance among *Pseudomonas* spp. isolates from a salmon-processing plant, where the majority of the isolates were *P. fluorescens* and highly resistant to ampicillin, amoxicillin, and cefotaxime.

The emergence of antibiotic resistance has led to the ineffectiveness of conventional antibiotics, and increased the failure rate for infection treatment, ultimately increasing the mortality rates associated with common infectious diseases (Sabeq *et al*., 2022). Thomassen *et al*. (2022) highlighted that *P. fluorescens* showed a multidrug resistance (MDR) pattern. El-Sherifa *et al*. (2023) reported that three out of 24 *P. fluorescens* isolates showed resistance to about 13 - 14 types of antibiotics belonging to seven antimicrobial classes, and five isolates showed resistance to 10 to 12 antibiotics belonging to six antimicrobial classes. Shabana *et al*. (2022) stated that *P. fluorescens* strains are considered as MDR microorganisms if they are resistant to more than two antibiotic classes. The wide growth of MDR microorganisms in the food chain poses a major food safety concern.

Pseudomonas aeruginosa

Characteristics and contamination in poultry

P. aeruginosa is a foodborne microorganism and opportunistic human pathogen extensively distributed in food and the environment (Gu *et al*., 2016). It is usually present in environmental sources, including soil and water. It can also be found on vegetables, fruits, and meat. Meat storage under aerobic conditions allows for *P. aeruginosa* growth and proliferation even in different temperatures (Neto *et al*., 2012). It can easily develop in milk, fish, meat, and dairy samples stored aerobically at low temperatures. The growth of the bacterium is responsible for off-flavours, pigmentation, slime, and malodour production in meat and derived products (Stellato *et al*., 2017).

Fresh meat available in the market has a higher chance of containing *P. aeruginosa* possibly due to the exposure during handling, idle time prior to processing and cleaning, or maintenance processes involving contaminated water. Numerous incidents of food product recalls, foodborne outbreaks, and economic losses due to food spoilage have been strongly linked to the occurrence of cross-contamination and recontamination. These incidences primarily involved inadequate hygiene practices, contaminated processing equipment, or insufficient storage conditions (Carrasco *et al*., 2012).

Pseudomonas infections in poultry industries are very crucial because they can rapidly spread among poultry flocks, leading to increased mortality across their different age groups (Shukla and Mishra, 2015). *P. aeruginosa* infections in chicken farming primarily occur *via* skin wounds, contamination of vaccines, egg dipping, and contamination of needles prior to injection. Chickens can often have skin injuries due to various reasons such as fighting and environmental hazards, where the wounds can be an entry point for *P. aeruginosa* into the chicken's body. Basically, in poultry industries, vaccination is a significant practice to prevent any infections, and if there is any lack of hygiene conditions during vaccination such as needles used for injections not being sterile or reuse of needles among the poultry flocks may lead to the contamination of microorganisms. Moreover, the diseases could spread from infected poultry flocks to vulnerable ones nearby due to inadequate hygiene practices, indicating chickens of any age can have high chances of being contaminated with *P. aeruginosa*, where young chicks are commonly the most prone to the infection (Saad *et al*., 2017).

Enzyme produced and role in food spoilage

Similar to *P. fluorescens*, *P. aeruginosa* is also a psychotropic bacterium that has the possibility to cause food deterioration during cold storage due to its ability to survive in low temperatures. Besides, it can compete with other types of spoilage microorganisms (Wong *et al*., 2023). *P. aeruginosa* has also been found in diverse food types including raw milk (Garedew *et al*., 2012) and raw vegetables (Ruiz-Roldan *et al*., 2021). Thus, the contamination of

P. aeruginosa food products has been identified as a potential source of nosocomial infections in human after consumption.

It is challenging to totally prevent the organoleptic deterioration of meat caused by microbiological consumption of meat components like sugars and free amino acids, and the generation of undesirable volatile metabolites, even in cold-stored meat (Goncalves *et al*., 2016). The preservation effect of low temperature may be diminished by psychrotrophic bacteria performing these activities at low temperatures (Ercolini *et al*., 2009). By altering their cytoplasmic membrane, and increasing the amounts of unsaturated fatty acids, these bacteria are able to survive at low temperatures. This keeps the membrane semifluid, which facilitates the transfer of nutrients and enzymes (Madigan and Martinko, 2006). Taking into consideration the quality of the meat and public health, one of the issues facing meat manufacturers is their capacity to develop at low temperatures.

Biofilm formation

P. aeruginosa is a crucial biofilm-forming species, and also a perspective microorganism for the biofilm investigations. Biofilm formation has been suggested as a bacterial survival strategy in adverse environments (Davies, 2003; Olsen, 2015; Moradali *et al*., 2017). Extracellular polymeric substances (EPSs) are a hydrated polymeric matrix that surrounds and supports living bacteria within biofilms. Environmental factors and bacterial species determine the physicochemical characteristics and composition of EPSs (Flemming and Wingender, 2010).

It has been well established that surfaces of equipment and utensils can facilitate the adhesion, growth, and proliferation of microorganisms, leading to the potential for spoilage and contamination by pathogenic bacteria like *P. fluorescens* and *P. aeruginosa*. These organisms are known for their involvement in adherence processes and the formation of biofilms.

P. aeruginosa is known to produce biofilms, which are regarded to be an important factor in the strain's pathogenicity and antibiotic resistance. A study published by Liang *et al*. (2023) showed this attribute of the bacterium, and revealed that almost 53% of the *P. aeruginosa* isolates showed MDR. According to Xu *et al*. (2021; 2022), biofilms are complex, interconnected communities of bacteria that can stick to the surfaces of industrial machinery, food, medical equipment, and plumbing in homes. The most crucial elements in the creation of the complex biofilm matrix, which shields bacteria from hostile surroundings, antimicrobial agents, and host immunological responses are EPSs, proteins, lipids, and eDNA (Li *et al*., 2023; Wen *et al*. 2023).

Antibiotic resistance

P. aeruginosa is expected to show resistance to ampicillin and amoxicillin, along with their combinations with lactamase inhibitors, ceftriaxone, cefotaxime, chloramphenicol, ertapenem, selected aminoglycosides such as kanamycin and neomycin, tetracycline, trimethoprim, and tigecycline (EUCAST, 2022). This was corroborated by several studies, including Rezaloo *et al*. (2022). *P. aeruginosa* isolated from meat products showed high resistance to ampicillin (89.65%), penicillin (86.20%), tetracycline (82.75%), cefoxitin (37.93%), gentamicin (34.48%), and clindamycin (31.03%). Besides, a study conducted by Poursina *et al*. (2023) also reported a similar pattern of antibiotic resistance rate, where *P. aeruginosa* obtained from raw meat samples had high resistance pattern to penicillin (87.23%), ampicillin (85.10%), tetracycline (85.10%), gentamicin (65.95%), and trimethoprim (57.44%).

The inherent resistance of *P. aeruginosa* towards chloramphenicol, tetracyclines, and trimethoprim can be due to the presence of MDR on the cell surface, and active efflux systems (Langendonk *et al*., 2021). Benie *et al*. (2019) discovered that *P. aeruginosa* isolates from raw food samples were resistant to aztreonam, ticarcillin, and ciprofloxacin. Bhuiya *et al*. (2018) reported that a total of 100% of *P. aeruginosa* isolated from frozen meat and chicken nuggets showed resistance to ampicillin, penicillin, cefixime, and cefpodoxime, and 30% of isolates were not susceptible to cefotaxime.

Algammal *et al*. (2023) highlighted that the primary factors contributing to the emergence of MDR strains are the extensive utilisation of antibiotics in poultry farming and processing sectors, as well as the ability of *P. aeruginosa* to acquire resistance genes from other highly resistant bacteria. Moreover, antimicrobial resistance in *P. aeruginosa* primarily arises from acquired and inherent mechanisms, characterised by the outer membrane's low permeability, and the presence of resistance

genes. Consequently, accurate implementation of susceptibility testing and thorough investigation into the presence of MDR pathogens are essential for selecting the most optimal antibiotics (Makharita *et al*., 2020; Langaee *et al*., 2000). The presence of MDR *P. aeruginosa* strains highlights the importance of consistently performing sensitivity tests, and promoting limited antibiotic usage in both the poultry farming industry and the healthcare sector (Algammal *et al*., 2023).

Virulence genes

P. aeruginosa is indeed notable for possessing a variety of virulence factors that contribute to its pathogenicity as shown in Table 2. These include both cell-mediated and secreted virulence determinants (Algammal *et al*., 2023). Cell-mediated virulence determinants basically involve structures and mechanisms that directly interact with host cells or tissues. These determinants in *P. aeruginosa* may include lipopolysaccharide (LPS), pili, and flagella that facilitate adherence to host cells, and movement within host tissues (Mesquita *et al*., 2013). Besides, secreted virulence determinants may refer to toxicity and enzymatic reactions produced by the microorganisms, and secreted into the desired environment or directly to the host tissues such as exotoxins (*ExoS*, *ExoU*, *ExoT*, and *ExoY*), elastase, and protease enzymes that degrade host tissues and proteins, aiming for immunology evasion (Fazeli and Momtaz, 2014; Jurado-Martín *et al*., 2021).

Table 2. List of target genes of *P. aeruginosa* detection and their functions.

Type of gene	Target gene	Function	Reference
Alginate	*algD*	- Biofilm formation related gene.	Mohamed *et al*. (2022)
	algU	- Biofilm formation related gene.	Mohamed *et al*. (2022)
Exoenzyme	*exoS*	- Plays bifunctional toxin activity that disrupts cell to cell adhesion.	Horna and Ruiz (2021)
		- The longest *P. aeruginosa* effector. - Has relevant cytotoxic power, and is considered the main driver of the cytotoxic phenotype. - Exhibits lysophospholipase, and acts as a lipase targeting neutral lipids. - Considered as exclusive gene cause human acute infection.	Horna and Ruiz (2021)
Elastase	*lasA*	- Protease production marker gene.	Aslantas *et al*. (2022)
	lasB	- Major virulence factor of protease cleavage activity. - Affects biofilm formation. - Disrupts epithelial junctions.	Behzadi *et al*. (2021)
Phenazine operon	*phzH*	- Secretes the precursor proteins responsible for intracellular oxidative effects.	Higgins *et al*. (2018)
	phzM		
	phzS		
Phospholipase	*PlcH*	- Haemolytic gene.	Aslantas *et al*. (2022)
	plcN	- Non-haemolytic gene.	Aslantas *et al*. (2022)
Other protein (Exotoxin A)	*toxA*	- Pathogenic related gene. - Responsible for the synthesis of exotoxin A. - Key virulence determinant of *P. aeruginosa*.	Liang *et al*. (2023); Ertugrul *et al*. (2017); Algammal *et al*. (2023)
Other protein (Lipoprotein)	*oprL*	- Species specific identification gene. - A reliable indicator of *P. aeruginosa* infection.	Ghazaei (2023); Wessel *et al*. (2013)

Another types of virulence factors of *P. aeruginosa* responsible for the pathogenesis of diseases and related infections include phenazine operon genes (*phzH*, *phzM*, and *phzS*) that secrete the precursor proteins to encode three phenazine compounds responsible for the intracellular oxidative effects (Higgins *et al*., 2018). Furthermore, the elastase gene (*lasA* and *lasB*), haemolytic and non-haemolytic phospholipase C (*plcH* and *plcN*), and alginate-encoded genes (*algD* and *algU*) are also other essential virulence factors of *P. aeruginosa* (Rocha *et al*., 2019; Veetilvalappil *et al*., 2022). *P. aeruginosa* strains containing these genes can easily attach to epithelial cells, and cause invasion which results in inflammation and injury (Moissenet and Khedher, 2011).

The *exoU* gene can rapidly destroy the membrane of host cells, and consequently leads to severe lung injury, proinflammatory response, sepsis, and mortality (Jurado-Martin *et al*., 2021). The most abundant protease elastase B (*lasB*) is recognised as a major virulence factor due to its protein cleavage activity that could interfere with bacterial clearance, disrupt epithelial junctions, and affect biofilm formation (Behzadi *et al*., 2021).

Control methods

Microorganisms or their by-products are utilised to eradicate unwanted microorganisms in food, thereby enhancing food safety and prolonging shelf life. Several studies have documented the use of microorganisms like bacteria, fungi, actinomycetes, and algae, along with their antimicrobial metabolites as biopreservatives (Marrez and Sultan, 2016; Sultan *et al*., 2016; Marrez *et al*., 2017). Certain bacterial strains have the capability to generate antagonistic substances that serve as antimicrobial and biocontrol agents targeting microorganisms in food. In this regard, *Pseudomonas* spp. have been highlighted as one of the predominant species employed in the biocontrol of foodborne pathogenic bacteria and fungi producing mycotoxins (Sabry *et al*., 2016).

Marrez *et al*. (2017) stated that the major control methods to eliminate the foodborne bacteria are cooking and chilling. Many cases of foodborne infections can be minimised by preventing cross-contamination. This involves following good manufacturing practices (GMP) and good hygienic practices (GHP), such as storing raw and cooked foods separately, and consistently washing hands before and after handling raw ingredients. These essential hygiene measures should be observed throughout food production, storage, transportation, and preparation to reduce the proliferation and transmission of harmful pathogens (Malhotra *et al*., 2015). There are diverse approaches for preserving food, including traditional techniques like drying, heating, freezing, fermentation, and salting, as well as contemporary methods such as biopreservatives and active antimicrobial packaging systems (Sung *et al*., 2013). The activity of removing water from food during drying will be able to minimise the growth of microorganisms including *Pseudomonas* spp. since they normally require moisture to grow and reproduce. Besides, cooking the food such as chicken meat at an adequate temperature can kill *Pseudomonas* spp. by denaturing their proteins and disrupting their cellular structure. Since *Pseudomonas* spp. can survive in freezing conditions, lowering the temperature below their optimal growth range may become a crucial way to inhibit their growth and thereby extend the shelf life of frozen meat and other foods. To date, various biopreservatives have been widely established worldwide. For example, the use of organic acid such as acetic acid in vinegar can create an acidic environment that inhibits the growth of *Pseudomonas* spp. and other spoilage organisms. Furthermore, modified atmosphere packaging that involves replacing the atmosphere around a food product with low oxygen levels and high carbon dioxide levels can reduce the chances for the growth of aerobic microorganisms like *Pseudomonas* spp. (Darwesh *et al*., 2018).

Using synthetic preservatives is a common method to prevent food spoilage, and inhibit the growth of pathogenic microorganisms. The common synthetic preservatives include sulphites that are used to prevent the browning of processed meat, and nitrites that are primarily used as colour preservatives in cured meats. However, these chemicals raise high concerns due to human health, and can contribute to microorganisms developing resistance (Liu *et al*., 2021).

Detection methods

Culture-based method

The oldest approach for determining whether contaminated foods contain foodborne pathogens is the culture-based method. It is a progressive process

of cultural enrichment that includes strain typing, confirmation, and selective and differential plating (Kim and Kim, 2021). It can be separated into two groups: pre-enrichment, which restores damaged cells to their original states, raises the target pathogen concentration in food samples, and rehydrates cells from dehydrated foods in selective enrichment that increases the concentration of a certain pathogen in food samples (Dwivedi and Jaykus, 2011). The culture condition is influenced by the atmosphere, temperature, incubation period, and different nutrients in the media (Lagier *et al*., 2015). However, using a differential medium to identify the targeted pathogenic bacteria, foodborne pathogens can be detected in a selective and distinctive manner using culture-based methods that restrict the growth of unneeded microbes.

Similar to most other strains of *Pseudomonas*, *P. fluorescens* complex grows best in a rich, peptide-containing medium with an energy source of 0.1 to 1.0% (w/v) (Moore *et al*., 2006). Due to increased production of *P. fluorescens* siderophores, the natural fluorescence produced by this bacterium can be detected using selective media that are weak in iron. The selective pigment-enhancing media include King's A and B media (Taguett *et al*., 2015), Pseudosel agar medium, and *Pseudomonas* agar F medium. In addition, these media include other substances like cetrimide, magnesium, and potassium that help the *P. fluorescens* grow more selectively. Specifically, cetrimide assists in preventing the growth of non-*Pseudomonas* microbial flora, and permits *P. aeruginosa* to produce sufficient amounts of pigment (Milligan *et al*., 2023).

According to Algammal *et al*. (2023), the recovered *P. aeruginosa* isolates' colonies on cetrimide agar were big, irregular, smelled like fruit, and spread a yellowish-green fluorescent pigment. Furthermore, on McConkey agar, the isolated isolates showed smooth, pale (non-lactose fermenter), and flat colonies. Biochemical assays for oxidase, mannitol fermentation, gelatine hydrolysis, catalase, citrate utilisation, and nitrate reduction revealed that the *P. aeruginosa* isolates under test were positive. Additionally, the isolates of *P. aeruginosa* that were recovered were negative for the Voges-Proskauer, urease, indole, methyl red, and hydrogen sulphate (H_2S) generation tests.

Romalho *et al*. (2002) evaluated the survival of *P. aeruginosa* isolates on *Pseudomonas* agar base with the addition of several types of supplements such as Cefsulodin-Irgasan-Novobiocin (CN), cetrimide, nalidixic acid, and ferrous sulphate ($FeSO_4$). While the addition of $FeSO_4$ did not speed up *P. aeruginosa*'s recovery, it yielded colonies a distinctive dark brown colour that made them easily distinguishable from other species that could grow at 42°C.

Molecular method

Polymerase chain reaction (PCR) assay

Currently, the conventional culture method remains the standard approach for detecting *Pseudomonas* spp. in foods despite being time-consuming, and labour- and cost-intensive (Zhou *et al*., 2020; Chon *et al*., 2021). Isolating and identifying P. *aeruginosa* using conventional procedures takes a long time, especially when there are a lot of samples (Gharieb *et al*., 2022). Furthermore, *P. aeruginosa* is identified using the conventional culture method based on the green pigment that the strain produces. This approach will result in incorrect assessments during the actual inspection. For example, certain *P. aeruginosa* strains are not pigment producers, which causes inspections to be overlooked. Another scenario is when *P. fluorescens* and *P. aeruginosa* produce the same pigment, rendering differentiation impossible and leading to false positives (Schroth *et al*., 2018; Junaid *et al*., 2021). Researchers have been working for a while to develop a quick and accurate way to identify *P. aeruginosa*, but each approach has advantages and disadvantages (Tang *et al*., 2017).

As earlier mentioned, using traditional microbiology methods in the detection of *P. aeruginosa* is extensively time- and labour-consuming, where in most circumstances the finished goods would possibly be moved out from the factory before releasing results of total *P. aeruginosa* colony count. This condition eventually reduces the chance of appropriate and successful product release systems as written in pre-requisite program by that food manufacturing company. Thus, in order to monitor the contamination status and provide a scientific foundation for the prevention and control of foodborne *P. aeruginosa*, it is important to create quick, accurate, straightforward, and efficient diagnostic techniques or tools for the detection of *P. aeruginosa* in food (Wang *et al*., 2022). Despite traditional detection methods for *Pseudomonas* spp., molecular techniques are increasingly favoured for diagnosing *Pseudomonas* down to the species level, owing to their simplicity, speed, and reliability (Bej

et al., 1991). A common PCR assay has been widely utilised as a specific and rapid method for the detection of *P. aeruginosa* in a variety of foods and processing areas due to its high specificity, huge sensitivity, less time consuming, and simple operation (Wang *et al*., 2022).

In the beginning of PCR application, the specific target gene *oprL* has been used precisely as a marker for the detection of *P. aeruginosa* in various food samples (Wang *et al*., 2022). However, with the increasing of new strains globally, some of the earlier target genes are unable to detect new threats. Thus, it is necessary to allow researchers to detect specifically *P. aeruginosa* using other variety of virulence genes. This was in agreement with studies conducted by Taee *et al*. (2014), Heidari *et al*. (2018), Khademi *et al*. (2021), and Wang *et al*. (2022) which identified and differentiated *P. aeruginosa* from other *Pseudomonas* spp. using several target genes including the *algD* forward and reverse sequences.

Several researchers agreed that PCR with gel electrophoresis evaluation are identification methods with consistency, able to provide a detailed outcome of specific bacterial communities, and determine the contamination sources (Aslam *et al*., 2003; Carraro *et al*., 2011). Hummel and Unger (1998) developed the first PCR technique that used the exotoxin A gene to precisely identify *P. aeruginosa*. They also evaluated the method's suitability for quickly identifying *P. aeruginosa* in patients on mechanical ventilation. The findings demonstrated that the exotoxin A gene-based PCR method had superior sensitivity because it identified 57 positive samples out of 364 total samples, while the traditional culture method only found 36 positive samples. As *P. aeruginosa* PCR assays continue to advance, more and more specific genes, including *gyrB*, *algD*, and *oprL* are being identified. While specificity is crucial for the success of traditional PCR, it is also the primary reason for PCR detection failures. As a result, numerous studies have looked into the gene specificity of various *P. aeruginosa* strains. De Vos *et al*. (1997), for instance, looked at the *oprL* gene's specificity in *P. aeruginosa* detection.

Isothermal amplification (LAMP) assay

The drawbacks of traditional PCR techniques, which need thermocycling for amplification, have been addressed by isothermal amplification techniques. Rapid nucleic acid molecule amplification at a constant temperature is made possible by isothermal amplification, which offers a higher sensitivity and requires less equipment (Dhama *et al*., 2014). Based on several recent studies, *P. aeruginosa* can be found using the loop-mediated isothermal amplification (LAMP) approach. Goto *et al*. (2010) created a LAMP assay that selectively targeted the *oprL* gene of *P. aeruginosa*. It was based on a hydroxynaphthol blue (HNB) colorimetric assay. The outcomes demonstrated that compared to traditional PCR, the LAMP assay had > 10-fold higher sensitivity and 100% specificity for the serogroup. Furthermore, the assay only took two hours to complete from DNA extraction to detection.

Conclusion

The challenges posed by *P. fluorescens and P. aeruginosa* in poultry and poultry products are significant owing to their great adaptability, psychrophilic nature, growth potential, and multidrug resistance. Rapid growth of *P. fluorescens* at refrigeration temperature that produces exoenzymes protease and lipase significantly changes the physicochemical properties of poultry products. The persistence of spoilage microorganisms in cold storage conditions, facilitated by enzymatic activity and biofilm formation, emphasises the importance of food hygiene standards and effective protocols throughout food storage and handling. A high number of *P. aeruginosa* poses risk of human infection should the poultry meat be undercooked, leaving sufficient numbers to cause disease. Therefore, effective control and detection methods are important to ensure poultry product safety for consumers.

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