



## Importance of *Pseudomonas aeruginosa* in Food Safety and Public Health

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### ABSTRACT

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*Pseudomonas aeruginosa* (*P. aeruginosa*), the most pathogenic species among the pseudomonas species, is a bacterium that causes opportunistic infections resulting in significant damage to host tissues. *P. aeruginosa*, which is resistant to antibiotics, also causes fatal infection in human and animals. Infections caused by *P. aeruginosa* are difficult to treat due to its rapid proliferation in the environment and its ability to form biofilms that confer resistance to antibiotics. One of the main virulence factors of *P. aeruginosa* is its direct damage to host tissues, which disrupts the host's defense mechanisms. *P. aeruginosa* is a food-borne pathogen often detected in various food groups such as meat, milk, fruit, vegetables, and water. In recent years, there has been a noticeable rise in food-borne contamination with *P. aeruginosa*. New measures are urgently needed in the treatment of patients with infections due to this agent, since *P. aeruginosa* can develop resistance to most antibacterials. In this review, general information about *P. aeruginosa*, which has gained importance for public health, will be given.

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## Introduction

*Pseudomonas aeruginosa* is a Gram-negative bacterium that can cause various infections in human and animals (Pang et al., 2019). *P. aeruginosa* is an ubiquitous bacterium that can multiply in a wide variety of environments and foods. The most important feature that enables *P. aeruginosa* to be successful as an opportunistic pathogen is its wide metabolic diversity (Sadikot et al., 2005). Its rapid proliferation in the environment, wide range of phenotypes and genotypes, and strong virulence effect makes it important among opportunistic pathogenic microorganisms (LaBauve and Wargo, 2012). It is a well-known microorganism that causes persistent chronic infections and develops robust biofilms (Lee and Yoon, 2017).

*P. aeruginosa* is a pathogenic microorganism that can be found on the surface of foods such as fruits and vegetables, vegetation, water, soil and hospitals and causes many diseases (Brady, 2009). *P. aeruginosa* causes important health problems due to its presence in hospital environments. It is also considered a bacterium of great medical importance due to its adaptability to different environments and also its ability to cause chronic infections in immunocompromised individuals (Spiers et al., 2000). It can be easily recognized among other *Pseudomonas* species with its typical colony morphology, pigments and grape-like odor (Maçin, 2014). These

pigments are named “*aeruginosa*” because they form a blue or greenish color at pH (Siriken and Öz, 2017). Molecular methods are used for identification, apart from traditional methods such as sowing on media (Paul and Sinha, 2017).

The pathogenicity of *P. aeruginosa* is due to its virulence factors and antibiotic resistance in its genome (Moradali et al., 2017). These virulence factors are; lipopolysaccharides, alginate, flagella, pili, exotoxin A, pyoverdine, pyocyanin, phospholipase C, Quorum sensing and rhamnolipid (Jurado-Martin et al., 2021). *P. aeruginosa* is a common pathogen in hospitals and especially in intensive care units due to its ability to acquire resistance mechanisms against many antibiotic classes and antiseptics and to survive in humid environments (Santajit and Indrawattana 2016). *P. aeruginosa* has acquired resistance to antibiotics used to destroy environmental bacteria. In the treatment of infectious diseases, treatment of infections caused by *P. aeruginosa* is becoming increasingly difficult, especially thanks to the resistance it has developed against multiple antibiotic classes (Jeukens et al., 2019). Therefore, it is necessary to develop new antimicrobials to prevent the antibiotic-resistant pathogen *P. aeruginosa* (Xu et al., 2019). This review will focus on *P. aeruginosa*, which has gained importance in terms of public health.

### Classification

*Pseudomonadaceae* family consists of 5 genera: *Azotobacter*, *Mesophilobacter*, *Oblitimonas*, *Permianibacter* and *Pseudomonas*. The genus *Pseudomonas* also includes different subspecies. These subspecies are classified according to the 16s ribosomal nucleic acid (rRNA) gene sequence and pigments. These; *P. aeruginosa*, *P. alcaligenes*, *P. fluorescens*, *P. fragi*, *P. mendocina*, *P. oleovorans*, *P. pseudoalcaligenes*, *P. putida*, *P. stutzeri*, *P. pseudomallei*, *P. mallei*, *P. solanacearum*, *P. marginalis*, *P. cepacia* and *P. syringae* (Charles et al., 2006). The most important animal pathogens are *P. aeruginosa*, *P. pseudomallei* and *P. mallei*. The most important plant pathogens are *P. solanacearum*, *P. syringae* and *P. marginalis* (Bilgehan, 2000). It was defined by Schroeter in 1872 with the special name *Aeruginosa* because the color of the colonies in certain environments turns to coppery rust or copper color and then turns green. Schroeter added it to the genus *Bacterium* and named it *Bacterium aeruginosum*. Later, Migula redefined the species and transferred it to the genus *Pseudomonas*. The scientific classification made by Migula is shown in Table 1. (Migula, 1894; Palleroni, 2010).

Table 1. Scientific classification of *Pseudomonas* and some species (Migula, 1894).

Scientific Classification		Some Species
Domain	Bacteria	<i>P. aeruginosa</i>
Phylum	Proteobacteria	<i>P. gelidicola</i>
Class	Gamma proteobacteria	<i>P. fluorescens</i>
Order	Pseudomonadales	<i>P. fragi</i>
Family	<i>Pseudomonadaceae</i>	<i>P. putida</i>
Genus	<i>Pseudomonas</i>	<i>P. syringae</i>

### Morphology and General Characteristics of *Pseudomonas aeruginosa*

*P. aeruginosa* has a Gram-negative, motile, non-spore forming, rod-shaped, monoflagella structure. *P. aeruginosa*, which is positive for catalase, oxidase, and citrate in biochemical tests, cannot ferment glucose and lactose. It is easily detectable on agar because it produces water-soluble pigments such as pyoverdine, a yellow-green fluorescent pigment, and pyocyanin, a blue-green pigment (Peix et al., 2019). These pigments are called “*aeruginosa*” because they form a blue or green-like color when the pH becomes alkaline (King and Philips, 1978).

*Pseudomonas* spp. it is a bacterium that can grow in aerobic and humid environments. Unlike other species in this genus, *P. aeruginosa* can also live in anaerobic conditions due to its ability to use nitrate (NO<sub>3</sub>). In addition, they are known as an opportunistic pathogen that causes significant problems in the food industry, pharmaceutical industry and hospital environments due to their resistance to wide temperature ranges (20-42°C), high salt concentrations, many antibiotics and environmental conditions for their multiplication, as well as being able to reproduce even in distilled water. (Siriken and Öz, 2017; Özdemir et al., 2009). Optimal growing temperature is 37°C and it has been observed that they can reproduce between 20°C and 42°C. In addition, their ability to grow at 42°C makes it easy to distinguish this bacterium from many other

*Pseudomonas* species (Wu et al., 2015). They can provide the minimum nutritional conditions necessary for the reproduction of *Pseudomonas* by using a wide variety of environmental resources. *P. aeruginosa* generally requires only acetic acid salt acetate (CH<sub>3</sub>COO) and ammonia as a carbon and nitrogen source (Abreu et al., 2014).

### Virulence Factors of *Pseudomonas*

*P. aeruginosa*, being an opportunistic pathogen, has the ability to cause both acute and chronic infections. Its pathogenic profile is due to the large and variable virulence factors and antibiotic resistance markers that reside in the genome of *P. aeruginosa* (Moradali et al., 2017). The enormous adaptability of *P. aeruginosa* greatly facilitates its capacity to cause chronic infections. The variability and flexibility of the pathogenicity of *P. aeruginosa* is shaped by the fact that it has a large number of virulence factors and rapidly adapts to stress factors (Jurado-Martín et al., 2021). The pathogenicity of *P. aeruginosa* consists of virulence factors that act both intracellularly and extracellularly (Balasubramanian et al., 2013). These virulence factors cause *P. aeruginosa* to proliferate, survive, and cause disease, especially without being affected by the immune responses formed in the host. It causes inflammation of the patient's lung tissues and serious tissue damage, especially during lung infections (Sadikot et al., 2005). The virulence factors of *P. aeruginosa* are, respectively, lipopolysaccharides, alginate, flagella, pili, exotoxin A, pyoverdine, pyocyanin, phospholipase C, Quorum sensing, and rhamnolipid (Jurado-Martín et al., 2021).

#### Lipopolysaccharide

Lipopolysaccharide (LPS) in the bacterial membrane is an important virulence factor in Gram-negative pathogenic bacteria. This LPS structure in the cell membrane consists of three different structures: lipid A, core region, and O-antigen or O-polysaccharide (Maldonado et al., 2016). Besides acting as a physical barrier, the LPS structure interacts with the host receptors and causes tissue damage with its endotoxic activity (King et al., 2009). Bacteria serotyping is performed with the O-polysaccharide or O-antigen structure in the LPS structure. These chains are protective against complement lysis and also show resistance to antimicrobial proteins. Lipid A structure in this structure activates many inflammatory precursor cells and causes adhesion by binding to asialo GM1, a ganglioside receptor (Salysers et al., 1994).

#### Alginate

It produces alginate with a mucoid structure so that the infection caused by the pathogen *P. aeruginosa* in the host can turn into a chronic one (Cross et al., 2020). Alginate is in an exopolysaccharide structure and is also responsible for biofilm formation and stability. Alginate, also called mucoid exopolysaccharide, is the main component of the most studied *P. aeruginosa* biofilms (Ghafoor et al., 2011). Alginate production plays a role in the adhesion of the bacteria by fixing *P. aeruginosa* to epithelial cells. This situation is mostly observed in respiratory tract infections (Salysers et al., 1994). In addition, alginate weakens the host's response to bacteria and protects against phagocytosis, but also reduces the effect of antibiotics used (Wozniak et al., 2003).

### Flagella (Whip)

*P. aeruginosa* has a polar flagella and thus, besides gaining movement, it provides chemotaxis by directing the movements of the bacteria according to the chemicals in the environment. These polar flagella, which play an important role in adhesion to the cellular surface, help the formation of the first biofilm with its ability to attach flagels (Haiko and Westerlund-Wikström, 2013; Fooladi et al., 2013). The flagella of *P. aeruginosa* are approximately 25 nanometers (nm) in diameter and consist of 20 different parts in total. This structure consists of a filament made of polarized flagellin, a cap protein, hook and hook attachment proteins, and a series of basal bodies. Flagella are connected to the cell membrane by basal trunk pathways and take a long spiral shape (Karaderi and Kahraman, 2017). Although *P. aeruginosa* swarms on solid surfaces, flagella are primarily responsible for swimming through corkscrew rotation in aqueous or low-viscosity environments, and generate a force that moves the bacteria forward (Sampedro et al., 2015).

### Pili (Fimbriae)

*P. aeruginosa* has hair-like appendages that enable it to adhere to surfaces and move. These extensions are called pili (fimbriae) and are short surface structures. Pili helps *P. aeruginosa* spread rapidly by colonizing the respiratory tract (Kipnis et al., 2006; Jacobsen et al., 2020). Bacteria have 4 different pili, namely Type I, Type II, Type III and Type IV. Type IV pili in bacteria are 5-8 nm in diameter and are responsible for adherence, biofilm formation, motility and adhesion. *P. aeruginosa* uses Type IV pili to provide motility, adhesion, and colonization and thus initiates the infection (Leighton et al., 2015; Burrows, 2012).

### Protein Secretion Systems

Bacteria release the toxins and enzymes they produce into the outer environment with 8 different protein secretion systems (Types I, II, III, IV, V, VI, VII and IX) (Pena et al., 2019). Type I and Type V are the simplest secretory pathways and are responsible for releasing enzymes such as proteases into the external environment. (Zhao et al., 2019). Type II, Type III, Type IV, and Type VI are more complex systems and release a wide variety of exoproteins. Type III and Type IV protein secretion systems also increase the virulence of bacteria by injecting exoproteins directly into the cytoplasm of the target cell (Bleves et al., 2010; Sana et al., 2016; Pena et al., 2019).

The most important secretion system is the Type III secretion system, which is used to disable and destroy the host's immune system (Anantharajah et al., 2016). Thanks to the type III protein secretion system, the formation of a bridge between two cells is provided and effector proteins are transmitted to the cytoplasm of the eukaryotic cell. Type III protein secretion system also secretes S, T, Y and U exoenzymes (Kipnis et al., 2006). *P. aeruginosa* uses all protein secretion systems effectively and thus attacks the host and causes chronic infections various toxins and hydrolytic enzymes (Pena et al., 2019). Table 2 shows that exoenzymes secreted by the Type III protein secretion system in *P. aeruginosa* and their functions.

### Exotoksin A

Exotoxin A is an adenosine diphosphate-ribosyl transferase, which is secreted into the extracellular space through the Type II protein secretion system and has a toxic effect on body tissues (Lederberg, 2000). Exotoxin A is an important virulence factor for *P. aeruginosa* (Javanmardi et al., 2019). The toxin of interest is subdivided into three structurally prominent domains and one minor subdomain. The N-terminal domain, which is composed of antiparallel threads, is responsible for attachment to host cells. The middle domain, which consists of six  $\alpha$ -helices, has membrane translocation activity. The third part, called the C-terminal domain, is the toxic part. There is a small Ib subdomain located between the mid-domain and the C-terminal domain and does not affect on toxin activity (Michalska and Wolf, 2015).

Exotoxin A causes death in experimental animals even in very small doses. Bacteria release exotoxin A into the extracellular space via the type II secretion system. Exotoxin A shows its effect by inhibiting protein synthesis in cells. Exotoxin A secreted during infection plays an important role in the formation of tissue damage by suppressing the host response. In addition, it is known that exotoxin A has an immunosuppressive effect on lymphocytes (Lederberg, 2000).

### Pyoverdine

Pyoverdine is a virulence agent that ensures the transport of iron in bacteria and ensures proliferation. In addition to binding the iron required for the metabolism of *P. aeruginosa*, important virulence factors such as Exotoxin A and endoprotease are involved in the regulation of secretion (Song et al., 2010).

Table 2. Type III secretion system toxins and their functions

Secretion system	Functions
Exoenzyme S	This toxin causes cellular apoptosis. It causes tissue damage, especially in lung infections, leading to the spread of bacteria (Nicas ve Iglewski, 1985).
Exoenzyme T	It is a toxin that inhibits the uptake of <i>P. aeruginosa</i> by macrophages (Shaver and Hauser, 2004).
Exoenzyme Y	It is the second most common exotoxin of <i>P. aeruginosa</i> and inhibits the production of proinflammatory cytokines from the macrophage and epithelial cells of the host (Javanmardi et al., 2019; He et al., 2017).
Exoenzyme U	This toxin, which can be found in <i>P. aeruginosa</i> , is among the potent cytotoxins such as phospholipase and destroys various cells (Mitov et al., 2010).

### **Pyocyanin**

Pyocyanin, belonging to the class of tricyclic phenazine compounds, is a zwitterion that contains a phenol group and exhibits weak acidic properties. In recent years, it has attracted attention as an important virulence factor produced by *P. aeruginosa*. Its low molecular weight and zwitterionic properties enable the toxin to easily cross the cell membrane (Hall et al., 2016; Fothergill et al., 2007; Lau et al., 2004; Reszka et al., 2004).

The virulence factors enhancing the effect of pyocyanin on *P. aeruginosa* is stated below.

- It allows the toxin to easily penetrate the cell membrane (Hall et al., 2016; Zeng et al., 2020).
- It triggers oxidative stress by increasing the amount of intracellular peroxides (Hall et al., 2016; Zeng et al., 2020).
- It causes cell lysis by damaging various enzymes and DNA of the target cell (Hall et al., 2016; Zeng et al., 2020).
- Pyocyanin causes apoptosis of neutrophils by increasing mitochondrial Reactive Oxygen Species (ROS) formation in neutrophils (Managò et al., 2015).
- It contributes to lung colonization of *P. aeruginosa* by disrupting of epithelial tissue and increased mucous secretion in the respiratory tract (Hall et al., 2016).
- It alters the host immune response by increasing the production of interleukin-8 (IL-8) from macrophages and neutrophils (Hall et al., 2016).
- In addition to inhibiting catalase activity, it also reduces transcription of genes encoding catalase (O'Malley et al., 2003).
- It is a redox active secondary metabolite responsible for the blue-greenish color of *P. aeruginosa* colonies in culture (Gellatly and Hancock, 2013).

### **Rhamnolipid**

Discovered in 1949, rhamnolipids are amphipathic extracellular secondary metabolites (Jarvis and Johnson, 1949; Alfiniyah et al., 2019). In addition, thanks to the rhamnolipid secreted by *P. aeruginosa*, the surfactant structure in the lung tissue deteriorates. Tight junctions in the respiratory epithelium are disrupted as a result of the subsequent decrease in transepithelial electrical resistance. It contributes to the formation of the biofilm layer by increasing the release of LPSs to the cell surface at low or normal concentrations of rhamnolipids. However, as a result of the overproduction of rhamnolipids, biofilm formation is prevented (Zulianello et al., 2006; Nickzad and Déziel, 2014; Köhler et al., 2010).

### **Phospholipase C**

Phospholipase C is an enzyme that hydrolyzes the ester bond between phosphoric acid and glycerol in glycerophospholipids and is a heat-stable thermolabile hemolysin (Khalifa et al., 2011). Phospholipase C is secreted from the Type 2 secretion system and breaks down phospholipids in the membrane of the target cell and sphingomyelin, which has hemolytic activity (Kipnis et al., 2006).

### **Quorum Sensing**

Quorum sensing system is known as a communication mechanism. Bacteria determine their density by measuring the signals generated by the related system and other

bacteria (Venturi, 2006; Chu et al., 2015). Such communication between cells plays an important role in the formation of biofilms and the initiation of infection (De Kievit, 2009; Davies, 2003). The induction of stimulating signal proteins bound to the membrane of bacterial cells allows the establishment of bridges between bacterial cells and then the formation of bacterial colonies on the surface (Hall-Stoodley et al., 2004; Şahin and Kaleli, 2018).

### **The Epidemiology of *Pseudomonas Aeruginosa***

*P. aeruginosa* is a pathogenic microorganism found on the surface of foods such as fruits and vegetables. It settles in the gastrointestinal tract after food consumption. *P. aeruginosa*, which can colonize in the small intestines, can also cause temporary colonization in the large intestine. This microorganism has a high ability to adapt to different environmental conditions. The reason for this is that it can multiply in different environments with minimum nutritional requirements (Brady, 2009).

Epidemiological studies are concerned with the possibilities, sources and mechanisms of transmission of *P. aeruginosa* to patients in the hospital setting. Environments where bacteria are found include solutions, creams, faucets, sink drains, incubators, personnel, and inhalation and resuscitation equipment (Lowbury et al., 1970). *P. aeruginosa* is reported at a rate of 13.2–22.6% in intensive care units and is responsible for 11–13.8% of all nosocomial infections (Weinstein et al., 2005; Kim et al., 2000).

*P. aeruginosa* can be found on the skin, nasal mucosa, throat and normal flora of healthy people (Shannon and French, 2004). There are three stages in the development of the infection. These; colonization, invasion and systemic spread (Bergagne, 2004). During infection, the degree of infection depends on the host's defense mechanism and bacterial virulence factors. In this way, it is determined whether there will be systemic spread at the colonization stage or not. *P. aeruginosa* is an important pathogen in patients with both primary and acquired immunodeficiencies (Lee et al., 2006). In addition, it is an important cause of bacteremia in patients with acute leukemia and is responsible for 14–21% of bacteremia attacks in these patients (Chatzinikolaou et al., 2000). *P. aeruginosa* plays an important role in patients with Cystic Fibrosis, where chronic and recurrent infections of the respiratory tract are common (Burns et al., 2001).

### **Antibiotic Resistance and Formation of Biofilm in *Pseudomonas aeruginosa***

#### **Antibiotic Resistance**

Antibiotic resistance is the ability of an organism to resist the action of an antimicrobial agent to which it was previously sensitive (Bagge et al., 2004). Thanks to the existing resistance to many antibiotics and antiseptics, *P. aeruginosa* is among the most common pathogens seen in hospitals and intensive care units (Santajit and Indrawattana 2016).

One of the most notable distinctions of *P. aeruginosa* from other bacteria is its exceptionally low cell membrane permeability. For example, *Escherichia coli* shows intrinsic resistance to antimicrobial agents such as  $\beta$ -lactam and penem group antibiotics because it is 1/100 times more

selective than the outer membrane permeability (Martin-Loeches et al., 2013). In addition to the intrinsic resistance mechanism, it also creates resistance through some mechanisms such as a flow system that expels the antibiotic from the bacterial cell and the production of antibiotic inactivating enzymes (Poole, 2005). In many studies, it has been observed that *P. aeruginosa* strains develop antibiotic resistance during antibacterial treatment and thus remain viable (Rello et al., 2006; Dietz et al., 1997; Pachori et al., 2019).

#### Biofilm Formation

Biofilm formation is an integral part of the microbial life cycle in nature. In the food processing process, the transmission route of bacteria primarily occurs through non-compliance with hygiene rules and cross-contamination of raw or undercooked foods. Foodborne pathogens form biofilms as a survival strategy in a variety of adverse environments. In this way, they cause recurrent contamination and food-borne infections and intoxications in the food business (Bai et al., 2021). A biofilm is a community of microbes that typically live on surfaces and are contained within an extracellular matrix. *P. aeruginosa* is a microorganism famous for developing robust biofilms that are highly resistant to antibiotics, disinfectants, and host defenses, disrupting bacterial clearance (Lee and Yoon, 2017). *P. aeruginosa* is an opportunistic microorganism that can attach to food or food contact surfaces and form a biofilm. It is difficult to eradicate because the growth of the agent occurs in a biofilm and it is resistant to antibiotics and disinfectants (Sriken and Öz, 2017). Studies have reported that biofilm formation occurs in five stages. These stages include:

- In the first stage of biofilm formation, bacteria control whether oxygen, osmolality, nutrient concentrations and temperature factors are at appropriate levels among environmental factors. They are then reversibly attached to the surface using extensions such as pili and flagella (Garrett et al., 2008).
- After the first contact on the surface, in the second stage, bacteria form stronger irreversible bonds thanks to components such as exopolysaccharide matrix (Branda et al., 2005).
- After microorganisms become stable by attaching to a biotic or abiotic surface, they initiate a proliferation and division process initiated by certain chemical signals in extracellular polymeric Extracellular Polymeric Substance (EPS) materials. This process then leads to the formation of micro-colonies (Costerton et al., 1999).
- At this stage, cells communicate with each other with the help of auto-inducing signals, increase the microbial cell density and begin to form three-dimensional structures (Parsek and Singh, 2003).
- At this stage, the microbial cells in the biofilm undergo rapid proliferation and spread to transform from the dormant sessile form to the motile form. Thus, the separated microbial cells allow the biofilm layer to spread to the environment (Donlan and Costerton, 2002; Jamal et al., 2018; Olivares et al., 2020).

The biofilm layer formed when *P. aeruginosa* becomes infected is somewhat difficult to treat. Because the antibiotic-tolerant cells in the biofilm layer are not affected

by the treatment or can re-proliferate in the biofilm by reducing its effect (Akiyama et al., 2017). In the food sector, polymicrobial biofilms are formed on the surfaces by *P. aeruginosa*. Thus, it helps the persistence of many foodborne pathogens and raises concerns in terms of food safety and public health (Bai et al., 2021).

#### Isolation and Identification of *Pseudomonas aeruginosa*

Various methods have been developed to isolate *P. aeruginosa* (Lambe and Stewart, 1972). The most important media used in classical cultivation methods are *Pseudomonas* CN (Cetrimide) Agar and *Pseudomonas* CFC (Cephaloridine, Fucidin, and Cetrimide) Agar. These media are left to incubate for 24-48 hours at a temperature of 30-37 °C, where *P. aeruginosa* ideally grows. After the incubation, the pyocyanins formed by the suspicious colonies are examined in ultraviolet light, and the reddish brown colonies are treated as suspicious and biochemical tests are performed. Although blue-green colonies are accepted as *P. aeruginosa*, they should be subjected to biochemical tests (Kristiansen, 1983).

Biochemical analyses used in the isolation and identification of *P. aeruginosa* are performed on 5% blood agar, beta hemolysis, catalase, oxidase and motility tests, methyl red, Voges-Proskauer, Indole, H<sub>2</sub>S, xylose, gram staining and citrate tests (Kleeberger and Busse, 1975; Paul and Sinha, 2017). The biochemical test results of *P. aeruginosa* are shown in Table 3. (Ezemba et al., 2022).

Table 3. Identification of *P. aeruginosa* isolates (Ezemba et al., 2022).

Biochemical Tests	<i>Pseudomonas aeruginosa</i>
Catalase test	+
Oxidase test	+
Motility test	+
Citrate test	+
Methyl Red test	-
Gram staining	-
Xylose test	-
Indole test	-
Hydrogen Sulfide (H <sub>2</sub> S) test	-
Voges-Proskauer	-

In addition to microbiological cultivation methods, *P. aeruginosa* in foods is also confirmed by molecular methods. Polymerase Chain Reaction (PCR) based molecular techniques are used to detect specific genes of *P. aeruginosa*. *16S rDNA*, *16S-23S rDNA ITS*, *ETA*, *fliC*, *algD*, *oprI*, *oprL*, *toxA*, *gyrB* and *ecfX*, *lasB*, *phzM*, *toxA*, *ExoU* and *ExoS* gene regions are generally searched for the detection and identification of *P. aeruginosa*. The genomic DNA of *P. aeruginosa* is extracted from bacterial colonies by the set buffer method. After performing the necessary PCR procedures, the 16S rRNA gene sequence of *P. aeruginosa* is compared with those in the NCBI/Eztaxon Ribosomal Database Project (RPD) and EMBL nucleotide sequence databases using the BLAST (blastn) program (Wei et al., 2020). There are also molecular typing methods to identify *P. aeruginosa* transmission sources and routes. These; Pulsed-field gel electrophoresis (PFGE) and

variable-number tandem repeat (VNTR) are current molecular epidemiological typing systems such as amplified fragment length polymorphism analyses (Eckmanns et al., 2008; Turton et al., 2010). Using both Roche 454 and Illumina, which are new generation sequencing systems, the whole genome sequence of the *P. aeruginosa* isolate can be determined (Snyder et al., 2013).

### Presence of *Pseudomonas aeruginosa* in Foods

*P. aeruginosa* can be found as a spoilage factor in various foods such as meat, milk, vegetables and fruits, especially in water (Gram et al., 2002). *P. aeruginosa* causes major problems for the food industry (Collins et al., 1989).

### Detection of *P. aeruginosa* in meats

*P. aeruginosa* is the most common psychrotrophic organism that causes spoilage in aerobically stored foods with high water content and natural pH, especially beef and poultry (Gram et al., 2002). *P. aeruginosa* limits the shelf life of chicken meat in cold storage by creating slipperiness and unpleasant odor on the surface (Lopez et al., 2015). *P. aeruginosa*, which multiplies easily at refrigerator temperature, causes significant economic losses by causing deterioration of beef in cold storage (Liao et al., 2019).

In a study on beef and poultry, 86 *Pseudomonas* spp. isolates were obtained. As a result of the biochemical tests performed for the identification of these strains isolated from beef, 3 (3.49%) of them were found to be *P. aeruginosa*. *Pseudomonas* spp. obtained from chicken meat. It was stated that none of the isolates were *P. aeruginosa* (Akan and Gürbüz, 2016).

In another study, 110 *Pseudomonas* spp. isolated from pork and beef spoiled under aerobic conditions. 13 of the species were identified as *P. aeruginosa* (Shaw and Latty, 1982).

Elbehiry et al. (2022) stated that only 3 of the 69 *Pseudomonas* species they detected in a total of 320 chicken meats were *P. aeruginosa*.

In a study conducted in the Alborz province of Iran, 29 (7.83%) of 370 samples from raw, frozen and imported beef were found to be contaminated with *P. aeruginosa* (Rezaloo et al., 2022).

In the study of Poursina et al. (2022), 350 meat samples were taken, 175 of which were beef and 175 were sheep. *P. aeruginosa* was detected in 13 of the beef samples (7.42%) and in 10 of the sheep meat samples (5.71%). In total, *P. aeruginosa* was found in 23 of 350 meat samples (6.57%).

### Detection of *P. aeruginosa* in milk and dairy products

*P. aeruginosa* contamination causes significant problems in the dairy industry (Dhanashekar et al., 2012). *P. aeruginosa* is among the most frequently isolated bacteria from surfaces in the food industry in general. Biofilm layers caused by *P. aeruginosa* may form on the inner surface of milk cooling tanks and pipelines before heat treatment in the enterprise (Marchand et al., 2012). When *P. aeruginosa* is mixed with milk, it multiplies very quickly and causes changes in the color, smell, structure and consistency of milk (Şen and Halkman, 2006). Enzymes such as esterase secreted by *P. aeruginosa* in cold stored milk become active again after pasteurization because they are heat resistant. Therefore, they can cause

bitterness by causing the breakdown of triglycerides in products such as milk, cheese, cream and butter (Cousins and Bramley, 1983).

In a study, 45 *Pseudomonas* spp. isolates were obtained from raw milk. It was determined that only 4 (8.88%) of the isolates were *P. aeruginosa* (Jooste et al., 1986).

In another study, 11 (22.9%) of the *Pseudomonas* isolates obtained from 48 milk samples were found to be *P. aeruginosa* (Uraz and Çıtak, 1998).

Arslan et al. (2011) reported that 32 *Pseudomonas* spp. isolates were obtained. of these isolates, 15% were *P. pseudoalcaligenes*, 5% *P. alcaligenes*, 0.7% *P. fluorescens* biovar V, 0.7% *P. pseudoalcaligenes* subspecies citrulli and 1.4% was also identified as *P. aeruginosa*.

Okuno et al. (2021) reported that conducted in Rio de Janeiro, Brazil, the presence of *P. aeruginosa* was investigated in 33 traditional Minas cheeses. As a result, *P. aeruginosa* was detected in 4 (12.1%) of the samples.

### Detection of *P. aeruginosa* in water sources

*P. aeruginosa* is a pathogen that grows in marine habitats and marshes (Garvey et al., 2018).

In a study, 7904 water samples obtained from various water sources were examined for the presence of *P. aeruginosa*. As a result, *P. aeruginosa* was detected in 524 (6.6%) of the examined water samples. Of the samples, 243 are from hot pool water, 51 of them (21%), 40 of them are tap water, 3 of them (8%), 5811 of them are from jacuzzis, 432 of them (7.4%) are from swimming pools, 270 of them are from swimming pools and 5 of them (2%) are from swimming pools, 67 of them are bottled natural mineral waters, 2 of them (3%) and 1234 of them are port irrigation hydrates, and 24 of them (2%) were found to be contaminated with *P. aeruginosa* (Caskey et al., 2018).

It was observed that 19 (7.6%) of 251 water samples obtained from fountains in common areas in Brazil were contaminated with *P. aeruginosa* (Anversa et al., 2019).

As a result of a study conducted with samples taken from wastewater treatment plants, 40 *Pseudomonas* spp. isolates were obtained. It was determined that 22 (55%) of the 40 isolates identified were *P. aeruginosa* (Keloğlu et al., 2019).

Xu et al. (2019) reported that conducted in different water samples, *P. aeruginosa* was detected in 3% of drinking water, 9% of tap water, 18.8% of bottled water and 90% of sewage water.

In a study conducted in India, the microbiological quality of the waters in the water tanks in 32 different villages was investigated. According to the results obtained, it was determined that 17 samples (53.1%) contained *P. aeruginosa* (Rizvi and Mohammed-Aslam, 2019).

In a study conducted in China, 314 drinking water and 133 mineral water samples were collected from 23 cities. *P. aeruginosa* was found positive in 77 (24.5%) of the obtained drinking water samples and in 18 (13.5%) of the mineral water samples (Wei et al., 2020).

### Detection of *P. aeruginosa* in vegetables

*P. aeruginosa* is a ubiquitous bacterium. It is commonly found in foods. In vegetables (Hardalo and Edberg, 1997), *P. aeruginosa* is frequently seen especially in tomato, eggplant, spinach, celery, onion, carrot, lettuce and cucumber (Shooter et al., 1971; Xu et al., 2019).

In a study using molecular methods, 26 untreated vegetable samples consisting of chard, lettuce, green beans, potatoes, zucchini, cucumbers and onions were examined for *P. aeruginosa*. As a result, *P. aeruginosa* was found in 18% of the samples (Ruiz-Roldán et al., 2021).

In a study in which 38 raw vegetable samples consumed in hospital meals consumed by inpatients in the oncology department of a University Hospital were examined for *P. aeruginosa*, it was found in 19% of the vegetable samples. Among the vegetable samples, lettuce, chicory and watercress were the most active (Correa et al., 1991).

## Conclusion

*P. aeruginosa*, is a very flexible and changeable microorganism that adapts to various living conditions. This microorganism becomes important in food businesses as it can easily survive in foods such as meat and meat products, milk and dairy products, water, fruits and vegetables. Besides being an opportunistic pathogen, it is among the best-known biofilm-forming bacteria. It is very easy to stick as a result of contact with the surface of the food. Therefore, it is a microorganism that is included in the scope of food safety among *Pseudomonas* species. *P. aeruginosa* causes difficult-to-treat infections with high incidence and various virulence factors. In recent years, the effectiveness of antimicrobials in the treatment of *P. aeruginosa* infections has gradually decreased. Infections are difficult to eradicate due to high levels of antibiotic resistance and their growth in biofilms. Therefore, unnecessary use of antibiotics in this bacterium should be abandoned and different treatment methods should be developed. Personnel working in food establishments should be made aware, and surfaces (floor, wall, counter, etc.) and cracks that may form biofilm should be cleaned and renewed well by paying attention to Hazard Analysis and Critical Control Point (HACCP) and Good Manufacturing Practices (GMP) practices.

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