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Research Article

Prevalence, Antimicrobial Resistance, and Molecular Description of *Pseudomonas aeruginosa* Isolated from Meat and Meat Products

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Resistant and virulent *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria are measured as the major cause of food spoilage and food-borne diseases. This survey assesses the prevalence, antibiotic resistance properties, and virulence factors distribution in *P. aeruginosa* bacteria isolated from meat and meat products. A total of 370 raw, frozen, and imported bovine meat samples and diverse types of meat product samples were collected from Alborz province, Iran. *P. aeruginosa* bacteria were identified by culture. Disk diffusion was used to assess the antibiotic resistance of bacteria. Furthermore, the PCR was used to assess the virulence and antibiotic resistance genes. Twenty nine out of 370 (7.83%) samples were contaminated with *P. aeruginosa*. Imported frozen bovine meat (20%) harbored the highest distribution, while sausage (2%) harbored the lowest. High resistance rates were observed toward ampicillin (89.65%), penicillin (86.20%), tetracycline (82.75%), cefoxitin (37.93%), gentamicin (34.48%), and clindamycin (31.03%). The most commonly detected antibiotic resistance genes were *bla*DHA (93.10%), *bla*CTX-M (83.65%), and *bla*SHV (48.27%). *Bla*DHA (93.10%), *bla*CTX-M (83.65%), and *bla*SHV (48.27%) were the most frequently detected resistance genes. The most commonly detected virulence genes were *exoS* (75.86%), *lasA* (68.96%), *exoU* (58.62%), *lasB* (51.72%), *plcH* (48.27%), and *algD* (44.82%). Meat and meat product samples may be sources of *P. aeruginosa,* which show an important threat to their consumption. Nevertheless, additional inquiries are obligatory to find supplementary epidemiological properties of *P. aeruginosa* in meat and meat product samples.

1. Introduction

Meat is one of the most important sources of protein in the world. Additionally, red meat is a rich source of fatty acids, vitamins, proteins, and minerals. Red meat also can be converted into a wide range of meat products, such as burgers, kebabs, sausages, and salami. Reports indicate that meat products are a large part of the world's diet [1, 2]. However, there are many reports of food-borne illnesses due to consuming contaminated meat and meat products, which increases the importance of hygiene and inspection of these products[3–6].

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic Gram-negative bacterium responsible for hospital infections, particularly bacteremia, urinary and respiratory tract infections (UTIs and RTIs, respectively), soft and hard tissues, and gastrointestinal and systemic infections [7]. It is also responsible for severe burn and wound infections in immunosuppressed patients [8]. The bacterium may be involved in food spoilage characterized by off-flavors, pigment secretion, and slime and malodor production [9]. Food spoilage caused by this bacterium is a thoughtful global issue, particularly in developing countries, owing to insufficient processing and refrigeration technologies.

There is a growing sign of the occurrence of food-borne infections caused by *P. aeruginosa* [10]. Diverse virulence factors are responsible for the occurrence of infections caused by this bacterium. Phenazine operons (*phzH*, *phzM*,

and *phzS*) secrete the precursor proteins that encode three phenazine compounds [11]. These compounds are responsible for increasing intracellular oxidative effects [11]. Exoenzymes (*exoS*, *exoT*, *exoU*, and *exoY*), alkaline protease (*apr*), toxin A (*toxA*), elastase gene A and B (*lasA* and *lasB*), alginate-encoded genes (*algD* and *algU*), hemolytic and nonhemolytic phospholipase C (*plcH* and *plcN*), pilus genes (*pilA* and *pilB*), and pyoverdine encoded gene (*pvd*) are other *P. aeruginosa* virulence factors [12, 13]. The role of these factors is often adhesions and attachments, inflammatory reactions, and ultimately invasion of the host cell.

Another critical aspect of *P. aeruginosa*-related infections is antibiotic resistance. *P. aeruginosa* progresses resistance to varied types of antibiotic agents [14]. In 2017, multidrug-resistant *P. aeruginosa* was responsible for about 32,500 infections and 2,700 deaths in hospitalized patients in the United States [15]. Resistant strains cause more complicated diseases for an extended period, which causes a lot of economic losses due to treatment and control. *P. aeruginosa* reveals resistance to varied types of antimicrobial agents, predominantly β-lactams, aminoglycosides, tetracyclines, quinolones, and macrolides [16]. Throughout the last years, extended-spectrum beta-lactamase (ESBLs) types (encoding resistance to all beta-lactams, except carbapenems) have arisen in the hospital and community setting among *P. aeruginosa* strains on plasmids commonly harboring other genes encoding resistance to non-beta-lactam antibiotics. The most important and clinically relevant genes encoding ESBL enzymes in *P. aeruginosa* are *bla*SHV, *bla*TEM, *bla*DHA, *bla*OXA, *bla*VEB, and *bla*CTX-M genes [17, 18]. Additionally, quinolone (nalidixic acid, ciprofloxacin, ofloxacin, etc.) resistance has been increasingly reported in *P. aeruginosa* clinical isolates. This resistance is mainly associated to point mutations in *gyrA* (encoding *GyrA* subunit of DNA gyrase) and *parC* (encoding *parC* subunit of topoisomerase IV) [19].

Rendering the boost portion of meat consumption in Iran and also *P. aeruginosa's* importance as a food spoilage agent, the present survey was performed to assess the prevalence as well as phenotypic and genotypic patterns of antibiotic resistance and virulence factors distribution in *P. aeruginosa* bacteria isolated from diverse kinds of meat and meat products.

2. Materials and Methods

2.1. Ethics. This research was confirmed by the Ethics Committee of the College of Veterinary Medicine, Islamic Azad University.

2.2. Study Design. The targeted population of the present study was fresh meat, frozen meat, imported frozen meat, hamburgers, kebabs, sausages, and salami samples presented in the winter of 2020 in Alborz province, Iran. Samples were collected from different sales centers in Alborz province. The sampling unit for each sample was 100 g of meat or meat products.

2.3. Sampling Procedure. A total of 370 dissimilar kinds of meat and meat product samples, such as fresh bovine meat ($n = 60$), frozen bovine meat ($n = 60$), imported frozen bovine meat ($n = 50$), burger ($n = 50$), kebab ($n = 50$), sausage ($n = 50$), and salami ($n = 50$), were randomly collected from the sale centers of the Alborz province, Iran. The samples (100 g) were transferred to Food Hygiene Research Center using cool boxes.

2.4. Bacterial Isolation and Identification. Twenty-five grams of the collected meat and meat product samples were put in sterile stomacher bags containing 225 ml peptone water (Oxoid, Basingstoke, UK). The bags underwent maceration within the stomacher (Seward 400 circulator, 4 min at 260 beats per min). A total of 100 μl homogenate samples were placed on CN selective agar (Oxoid SR 102E, UK) supplemented with *Pseudomonas* agar base (Oxoid, UK). Media were incubated at 37°C for 24 h in aerobic conditions. Microscopic morphology, Gram-staining, oxidase, urease, and catalase activities, starch and casein hydrolysis, methyl red and Voges Proskauer (MR-VP) and gelatin liquefaction and indole and citrate utilization tests were applied to identify that *P. aeruginosa* was identified by API 20NE strips (BioMerieuxVitek, Inc., MO, USA) system. The polymerase chain reaction (PCR) was used to identify *P. aeruginosa* isolates based on the detection of *rpoB* household genes [20].

2.5. Antimicrobial Susceptibility Testing. A simple disk diffusion method (Kirby Bauer) was used to investigate the pattern of antibiotic resistance. *P. aeruginosa* isolates were cultured overnight in Tryptone Soya broth (TSB, Merck, Germany) medium and incubated in aerobic conditions at 37°C for 24 h. After the preparation of 0.5 McFarland dilution, isolates were cultured on the Mueller–Hinton agar (Merck, Germany) in the presence of diverse antibiotic discs. The following antibiotics were used (μg/disk): tetracycline (30), chloramphenicol (30), sulfamethoxazole (25), gentamicin (10), ciprofloxacin (5), trimethoprim (5), ampicillin (10), penicillin (10), cefoxitin (10); clindamycin (2), imipenem (10), and aztreonam (30) (Oxoid, UK). Cultures were incubated at 37°C for 24 h. To assess the resistance pattern, the diameter of the growth inhibition halo was measured. In this experiment, *P. aeruginosa* ATCC 1014 was used as a positive control. Findings were interpreted according to the Antimicrobial Susceptibility Testing European Committee (EUCAST, 2018) [21] and CLSI criteria (2016 and 2018) [22–24].

2.6. Detection of Virulence and Antibiotic Resistance Genes. *P. aeruginosa* isolates were subcultured on TSB media (48 h at 37°C). Isolate's DNA was extracted using a DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). Guidelines of the kit instruction were applied. Quality (using electrophoresis on a 2% agarose gel) and quantity (using a spectrophotometer (A260/A280)) of extracted DNA were measured [25]. Table 1 indicates the

Table 1: PCR conditions [26–30].

Molecular characters	Targeted genes	Primer sequence (5′-3′)	PCR product (bp)	PCR programs	PCR volume (50 μL)
Virulence factors	*algD*	F: AAG GCG GAA ATG CCA TCT CC R: AGG GAA GTT CCG GGC GTT TG	275	1 cycle: 2 min at 95°C	
	algU	F: CGC GAA CCG CAC CAT CGC TC R: GCC GCA CGT CAC GAG C	410	30 cycles: 30 s at 94°C 30 s at 58°C 60 s at 72°C 1 cycle: 7 min at 72°C	
	*exoT**	F: CAA TCA TCT CAG CAG AAC CC R: TGT CGT AGA GGA TCT CCT G	1159	1 cycle: 5 min at 96°C	
	lasA	F: GCA GCA CAA AAG ATC CC R: GAA ATG CAG GTG CGG TC	1075	30 cycles: 30 s at 94°C	
	pvdA	F: GAC TCA GGC AAC TGC AAC R: TTC AGG TGC TGG TAC AGG	1281	30 s at 47–63 °C 1 min at 72°C 1 cycle: 3 min at 72°C	
	lasB	F: ACA CAA TAC ATA TCA ACT TCG C R: AGT GTG TTT AGA ATG GTG ATC	284	1 cycle:	
	toxA	F: ATG TGC AGY ACC AGT AAR GT R: TGG GTR AAR TAR GTS ACC AGA	270	3 min at 94°C 30 cycles:	
	plcH	F: CAC ACG GAA GGT TAA TTC TGA R: CGG TTA RAC GGC TGA ACC TG	608	30 s at 94°C 60 s at 55°C	
	plcN	F: CGA CTT CCA TTT CCC GAT GC R: GGA CTC TGC AAC AAA TAC GC	481	90 s at 72°C 1 cycle:	
	exoS	F: GTG TGC TTT ATG CCA TGA G R: GGT TTC CTT TTC CAG GTC	444	5 min at 72°C	
	exoY	F: TAT CGA CGG TCA TCG TCA GGT R: TTG ATG CAC TCG ACC AGC AAG	1035		
	exoU	F: GAT TCC ATC ACA GGC TCG R: CTA GCA ATG GCA CTA ATC G	3308		
	apr	F: TGT CCA GCA ATT CTC TTG C R: CGT TTT CCA CGG TGA CC	1017	1 cycle: 5 min at 96°C	
	phzM	F: ATG GAG AGC GGG ATC GAC AG R: ATG CGG GTT TCC ATC GGC AG	875	30 cycles: 30 s at 94°C	
	phzS	F: TCG CCA TGA CCG ATA CGC TC R: ACA ACC TGA GCC AGC CTT CC	1752	30 s at 47–63 °C 1 min at 72°C	
	phzH	F: GCC AAG GTT TGT TGT CGG R: CGC ATT GAC GAT ATG GAA C	1036	1 cycle: 3 min at 72°C	
	pilA	F: ACA GCA TCC AAC TGA GCG R: TTG ACT TCC TCC AGG CTG	1675		
	pilB	F: TCG AAC TGA TGA TCG TGG R: CTT TCG GAG TGA ACA TCG	408		dNTP: 200 μM PCR buffer (10X): 5 μL Primer F: 0.5 μM Primer R: 0.5 μM Mgcl$_2$: 1.5 mM DNA: 2.5 μL Taq DNA polymerase: 1.25 U
Antibiotic resistance genes	*bla*TEM[a]	F: ATG AGT ATT CAA CAT TTC CG R: CTG ACA GTT ACC AAT GCT TA	747	1 cycle: 5 min at 96°C 35 cycles: 60 s at 96°C 60 s at 58°C 60 s at 72°C 1 cycle: 10 min at 72°C	
	*bla*SHV[b]	F: GGT TAT GCG TTA TAT TCG CC R: TTA GCG TTG CCA GTG CTC	867	1 cycle: 5 min at 96°C 35 cycles:	
	*bla*OXA[c]	F: ACA CAA TAC ATA TCA ACT TCG C R: AGT GTG TTT AGA ATG GTG ATC	885	60 s at 96°C 60 s at 60°C 60 s at 72°C 1 cycle: 10 min at 72°C	
	*bla*CTX-M[d]	F: ATG TGC AGY ACC AGT AAR GT R: TGG GTR AAR TAR GTS ACC AGA	593	1 cycle: 7 min at 94°C 35 cycles: 50 s at 94°C 40 s at 50 °C 60 s: At 72°C 1 cycle: 5 min at 72°C	
	*bla*DHA[e]	F: CAC ACG GAA GGT TAA TTC TGA R: CGG TTA RAC GGC TGA ACC TG	970	1 cycle: 5 min at 94°C 35 cycles: 30 s at 94°C 45 s at 50°C 60 s at 72°C 1 cycle: 8 min at 72°C	
	*bla*VEB[f]	F: CGA CTT CCA TTT CCC GAT GC R: GGA CTC TGC AAC AAA TAC GC	1014	1 cycle: 5 min at 96°C 30 cycles: 60 s at 96°C 60 s at 55°C 2 min at 72°C 1 cycle: 10 min at 72°C	
	gyrA	F: GTG TGC TTT ATG CCA TGA G R: GGT TTC CTT TTC CAG GTC	287	1 cycle: 14 min at 95°C	
	parC	F: CAT CGT CTA CGC CAT GAG R: AGC AGC ACC TCG GAA TAG	267	35 cycles: 45 s at 95°C 45 s at 51°C 60 s at 71°C 1 cycle: 7 min at 71°C	

*Annealing temperatures of *exoT*, *exoY*, *exoU*, *apr*, *phzM*, *phzS*, *phzH*, *lasA*, *lasB*, *pvdA*, *pilA*, and *pilB* virulence factors were 54, 61.8, 61.6, 51, 54, 63, 51, 47, 57, 59, 59, and 54°C, respectively. [a]Temoneira: the name of the first patient providing the first sample. [b]Sulfhydryl reagent variable. [c]Active on oxacillin. [d]Active on cefotaxime, first isolated at Munich. [e]Discovered at Dhahran, Saudi Arabia. [f]Vietnam extended-spectrum β-lactamase.

Table 2: *P. aeruginosa* prevalence amongst the examined meat and meat product samples.

Sample types		N. collected	N. positive for *P. aeruginosa* (%)
Fresh bovine meat		60	3 (5.00)
Frozen bovine meat		60	8 (13.33)
Imported frozen bovine meat		50	10 (20.00)
Meat products	Burger	50	3 (6.00)
	Kebab	50	4 (8.00)
	Sausage	50	1 (2.00)
	Salami	50	0
	Total	200	8 (4.00)
Total		370	29 (7.83)

Table 3: *P. aeruginosa* antibiotic resistance pattern.

Samples (N. *P. aeruginosa*)		N. isolates resist to each antibiotic agent (%)											
		T	C	S	G	CIP	T	A	*P*	Cef	CLN	Im	AZ
Fresh bovine meat (3)		2 (66.66)	0	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	3 (100)	2 (66.66)	1 (33.33)	1 (33.33)	0	1 (33.33)
Frozen bovine meat (8)		7 (87.50)	0	2 (25)	3 (37.50)	2 (25)	1 (12.50)	8 (100)	8 (100)	2 (25)	2 (25)	1 (12.50)	2 (25)
Imported frozen bovine meat (10)		10 (100)	2 (20)	3 (30)	7 (70)	5 (50)	3 (30)	10 (100)	10 (100)	5 (50)	4 (40)	3 (30)	3 (30)
Meat products	Burger (3)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)	0	2 (66.66)	2 (66.66)	1 (33.33)	0	1 (33.33)	0
	Kebab (4)	2 (50)	1 (25)	1 (25)	1 (25)	1 (25)	2 (66.66)	2 (50)	2 (50)	1 (25)	1 (25)	1 (25)	1 (25)
	Sausage (1)	1 (100)	1 (100)	0	1 (100)	0	0	1 (100)	1 (100)	1 (100)	1 (100)	0	0
	Total (8)	5 (62.50)	3 (37.50)	2 (25)	4 (50)	2 (25)	2 (12.50)	5 (62.50)	5 (62.50)	3 (37.50)	2 (12.50)	2 (12.50)	1 (12.50)
Total (29)		24 (82.75)	5 (17.24)	8 (27.58)	15 (51.72)	10 (34.48)	7 (24.13)	26 (89.65)	25 (86.20)	11 (37.93)	9 (31.03)	6 (20.68)	7 (24.13)

*T: tetracycline (30 μg/disk); C: chloramphenicol (30 μg/disk); S: sulfamethoxazole (25 μg/disk); G: gentamicin (10 μg/disk); CIP: ciprofloxacin (5 μg/disk); T: trimethoprim (5 μg/disk); A: ampicillin (10 μg/disk); P: penicillin (10 μg/disk); Cef: cefoxitin (10 μg/disk); CLN: clindamycin (2 μg/disk); Im: imipenem (10 μg/disk); AZ: aztreonam (30 μg/disk).

PCR ingredients and circumstances applied for the virulence and antibiotic resistance genes identification [26–29].

PCR was done using a thermocycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany). For electrophoresis, 2.5% agarose gel containing ethidium bromide (0.1%, 0.4 μg/ml) at a constant level of 120 V/208 mA was used. Visualization was done by UVI doc device (Grade GB004, Jencons PLC, London, UK).

2.7. Data Analysis. SPSS software (21.0 version, Chicago, IL, USA) was used for analysis. For data assessment, Chi-square and Fisher's exact two-tailed tests were used. P value <0.05 was determined as levels of significance.

3. Results

3.1. P. aeruginosa Prevalence. The *P. aeruginosa* prevalence in different examined meat and meat product samples is shown in Table 2. Twenty nine of 370 (7.83%) meat and meat product samples were contaminated with *P. aeruginosa*. Imported frozen bovine meat (20.00%) harbored the highest prevalence of bacteria, while sausage (2.00%) harbored the lowest. Data analysis revealed significant differences between the types of samples and the prevalence of *P. aeruginosa* ($P < 0.05$). Statistically, significant differences were observed for the *P. aeruginosa* prevalence between fresh and frozen bovine meat samples ($P < 0.05$), fresh and imported bovine meat samples ($P < 0.05$), and meat and meat product samples ($P < 0.05$). The applied method failed to detect any *P. aeruginosa* in sausage samples.

3.2. P. aeruginosa Antibiotic Resistance Pattern. *P. aeruginosa* antibiotic resistance pattern is shown in Table 3. *P. aeruginosa* bacteria showed the highest resistance rate toward ampicillin (89.65%), penicillin (86.20%), and tetracycline (82.75%) antibiotic agents. The resistance rates of examined bacteria toward cefoxitin, gentamicin, clindamycin, and sulfamethoxazole were 37.93%, 34.48%, 31.03%, and 27.58%, respectively. The lowest resistance rate was observed against chloramphenicol (17.24%) and imipenem (20.68%). *P. aeruginosa* isolates of imported frozen bovine meat samples harbored the highest resistance against examined antibiotic agents ($P < 0.05$). Data analysis revealed significant differences between the types of samples and the

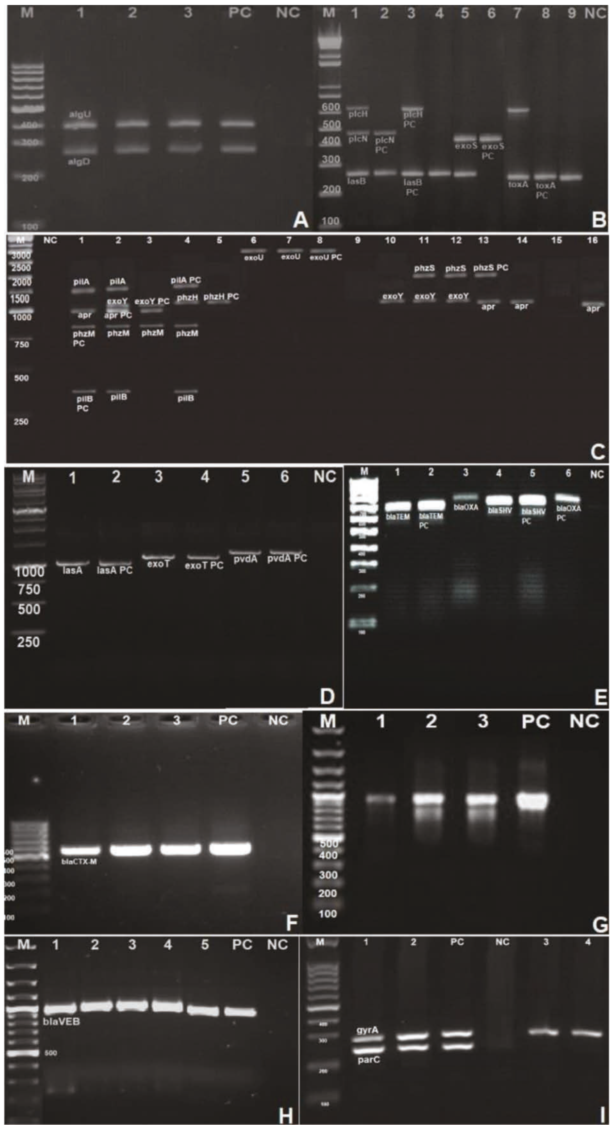

Figure 1: (a) Gel electrophoresis of PCR products for the detection of *algD* and *algU* virulence factors. M 100 bp ladder, 1–3: positive samples for *alg*D (275 bp) and *alg*U (410 bp) virulence factors, PC: positive control, and NC: negative control. (b) Gel electrophoresis of PCR products for the detection of *lasB*, *toxA*, *plcH*, *plcN*, and *exoS* virulence factors. M 100 bp ladder, 1–9: positive samples for virulence factors and their positive controls, and NC: negative control. (c) Gel electrophoresis of PCR products for the detection of *pilA*, *pilB*, *phzM*, *apr*, *phzH, phzS, exoU*, and *exoY* virulence factors. M 1000 bp ladder, 1–8 and 10–14 and 16: positive samples for virulence factors and their positive controls, NC: negative control, and 9 and 15: negative samples for virulence factors. (d) Gel electrophoresis of PCR products for the detection of *lasA*, *exoT*, and *pvdA* virulence factors. M 1000 bp ladder, 1–6: positive samples for virulence factors and their positive controls, NC: negative control. (e) Gel electrophoresis of PCR products for the detection of *bla*TEM, *bla*OXA, and *bla*SHV antibiotic resistance genes. M 100 bp ladder, 1–6: positive samples for virulence factors and their positive controls, NC: negative control. (f) Gel electrophoresis of PCR products for the detection of the *bla*CTX-M antibiotic resistance gene. M 100 bp ladder, 1–3: positive samples, PC: positive control, and NC: negative control. (g) Gel electrophoresis of PCR products for the detection of *bla*DHA antibiotic resistance gene. M 100 bp ladder, 1–3: positive samples, PC: positive control, and NC: negative control. (h) Gel electrophoresis of PCR products for the detection of *bla*VEB antibiotic resistance gene. M 100 bp ladder, 1–5: positive samples, PC: positive control, and NC: negative control. (i) Gel electrophoresis of PCR products for the detection of *gyrA* and *parC* antibiotic resistance gene. M 100 bp ladder, 1–4: positive samples, PC: positive control, and NC: negative control.

resistance rate of bacteria against antibiotic agents ($P < 0.05$).

3.3. P. aeruginosa Antibiotic Resistance Genes. Gel electrophoresis of the PCR products of virulence factors and antibiotic resistance genes is presented in Figure 1. The distribution of *P. aeruginosa* antibiotic resistance genes is shown in Table 4. According to obtained data, *bla*DHA (93.10%), *bla*CTX-M (83.65%), and *bla*SHV (48.27%) were the most frequently detected antibiotic resistance genes amongst the *P. aeruginosa* isolates. Findings showed that *bla*TEM (20.68%) and *gyrA* (24.13%) had lower frequencies

Table 4: *P. aeruginosa* antibiotic resistance genes.

Samples (N. *P. aeruginosa*)		N. isolates harbored each antibiotic resistance gene (%)							
		*bla*TEM	*bla*SHV	*bla*OXA	*bla*CTX-M	*bla*DHA	*bla*VEB	*gyrA*	*parC*
Fresh bovine meat (3)		1 (33.33)	1 (33.33)	1 (33.33)	2 (66.66)	2 (66.66)	1 (33.33)	1 (33.33)	1 (33.33)
Frozen bovine meat (8)		1 (12.50)	2 (25)	2 (25)	7 (87.50)	8 (100)	3 (37.50)	2 (25)	1 (12.50)
Imported frozen bovine meat (10)		3 (30)	7 (70)	4 (40)	10 (100)	10 (100)	3 (30)	2 (20)	2 (20)
Meat products	Burger (3)	1 (33.33)	1 (33.33)	1 (33.33)	2 (66.66)	3 (100)	1 (33.33)	1 (33.33)	0
	Kebab (4)	1 (25)	2 (50)	2 (50)	4 (100)	3 (75)	2 (50)	1 (25)	1 (25)
	Sausage (1)	0	1 (100)	0	1 (100)	1 (100)	0	0	0
	Total (8)	2 (12.50)	4 (50)	3 (37.50)	7 (87.50)	7 (87.50)	3 (37.50)	2 (12.50)	1 (12.50)
Total (29)		6 (20.68)	14 (48.27)	10 (34.48)	26 (89.65)	27 (93.10)	10 (34.48)	7 (24.13)	5 (17.24)

amongst the examined antibiotic resistance genes. The distribution of *bla*OXA and *bla*VEB was 34.48% and 34.48%, respectively. *P. aeruginosa* isolates of imported frozen bovine meat samples harbored a higher distribution of antibiotic resistance genes ($P < 0.05$). Data analysis revealed significant differences between the types of samples and the distribution of antibiotic-resistance genes ($P < 0.05$).

3.4. P. aeruginosa Virulence Factors. The distribution of *P. aeruginosa* virulence factors amongst the examined meat and meat product samples is shown in Table 5. Findings showed that *exoS* (75.86%), *lasA* (68.96%), *exoU* (58.62%), *lasB* (51.72%), *plcH* (48.27%), and *algD* (44.82%) were the most frequently detected virulence factors. Distribution of *pilA*, *phzM*, *toxA*, and *exoT* virulence factors was 24.13%, 20.68%, 17.24%, and 17.24%, respectively. *PhzH* (3.44%), *plcN* (6.89%), *pvdA* (6.89%), and *pilB* (6.89%) had the lower distribution among examined virulence factors. Statistically, significant differences were observed amid the distribution of *algD* and *algU* ($P < 0.05$), *plcH* and *plcN* ($P < 0.05$), *exoS* and *exoT* ($P < 0.05$), *exoU* and *exoT* ($P < 0.05$), *exoS* and *exoY* ($P < 0.05$), *exoU* and *exoY* ($P < 0.05$), *phzM* and *phzH* ($P < 0.05$), *lasA* and *lasB* ($P < 0.05$), and *pilA* and *pilB* ($P < 0.05$). *P. aeruginosa* isolates of imported frozen bovine meat samples harbored a higher distribution of virulence factors ($P < 0.05$). Data analysis revealed significant differences between the types of samples and the distribution of virulence factors ($P < 0.05$).

4. Discussion

An emerging food bacterium, *P. aeruginosa* is a frequent opportunistic human pathogen that is extensively found in the environment; it plays an essential portion in the spoilage of different foods [30]. Thus, it is essential to determine its prevalence and other epidemiological properties.

In the present study, 7.83% of meat and meat product samples were contaminated with *P. aeruginosa*. A higher prevalence rate of *P. aeruginosa* was reported in imported frozen bovine meat (20.00%) and frozen bovine meat (13.33%). Considering the psychrophilic nature of the bacterium, it is logical that the frozen samples had higher bacterial contamination. The high contamination rate of imported meat samples may be due to the importation of low-quality meat or their long storage period in customs in harmful conditions. Improper application of heat chain and cooking time and contamination in samples of meat products after production can be possible reasons for *P. aeruginosa* in these samples.

Sheir et al. [31] described that *P. aeruginosa* was detected in 4.00% of frozen burger samples. They revealed that unnecessary handling, malpractices, poor hygienic quality of raw materials, particularly spices, and unsanitary practices during production and storage were the main reasons for *P. aeruginosa* in frozen meat samples. Benie et al. [32] reported that the *P. aeruginosa* prevalence amongst smoked fish, fresh fish, and bovine meat samples collected from West Africa was 23.57%, 37.69% 53.04%, respectively. Up to now, the highest *P. aeruginosa* prevalence among food samples has been reported by Benie et al. [33]. They disclosed that the *Pseudomonas* species prevalence was higher in beef (97.90%), followed by fresh fish (87.80%), and smoked fish (63.10%). *P. aeruginosa* prevalence amongst the chicken meat [34], camel meat [35], retail meat [36], frozen imported meat [37], and sausage [38] samples was 46.70%, 80.00%, 3.00%, 6.67%, and 8.33%, respectively. Highly distribution of *P. aeruginosa* in examined samples may be due to high *P. aeruginosa* adaptation to a different environment, ambient temperatures (4 to 42°C), and low water activities (72%–97%) [39]. Thus, it is crucial to consider this bacterium in meat and meat products.

In addition to the high prevalence of bacteria in the studied meat and meat product samples, *P. aeruginosa* was also highly resistant to most of the evaluated antibiotics, especially ampicillin, penicillin, tetracycline, cefoxitin, gentamicin, clindamycin, and sulfamethoxazole. The high prevalence of antibiotic resistance amongst examined bacteria was also assisted by the high distribution of antibiotic resistance genes, especially *bla*DHA, *bla*CTX-M, and *bla*SHV. Similarly, a high prevalence of resistance toward such antibiotic agents was reported by Meng et al. (China) [40], Khan et al. (Australia) [41], Yayan et al. (Germany) [42], and Khan and Faiz (Saudi Arabia) [43]. In a survey conducted by Benie et al. [32], *P. aeruginosa* isolates of meat samples revealed the highest resistance rate toward kanamycin (100%), aztreonam (97.60%), and ciprofloxacin (35.40%). Allydice-Francis and Brown [44] stated that the *P. aeruginosa* isolates of vegetable samples harbored a high resistance rate toward ceftazidime (79.00%), ciprofloxacin

Table 5: *P. aeruginosa* virulence factors.

Samples (N. *P. aeruginosa*)		N. isolates resist each virulence factor (%)																	
		algD	*algU*	*toxA*	*plcH*	*plcN*	*exoS*	*exoT*	*exoY*	*exoU*	*apr*	*phzM*	*phzS*	*phzH*	*lasA*	*lasB*	*pvdA*	*pilA*	*pilB*
Fresh bovine meat (3)		1 (33.33)	0	1 (33.33)	2 (66.66)	0	2 (66.66)	1 (33.33)	0	2 (66.66)	0	0	0	0	2 (66.66)	2 (66.66)	0	1 (33.33)	0
Frozen bovine meat (8)		3 (37.50)	1 (12.50)	1 (12.50)	3 (37.50)	0	6 (75)	1 (12.50)	0	4 (50)	1 (12.50)	1 (12.50)	1 (12.50)	0	5 (62.50)	3 (37.50)	0	1 (12.50)	0
Imported frozen bovine meat (10)		5 (50)	2 (20)	2 (20)	6 (60)	2 (20)	8 (80)	2 (20)	2 (20)	7 (70)	2 (20)	3 (30)	1 (10)	1 (10)	8 (80)	6 (60)	1 (12.50)	4 (40)	1 (12.50)
Meat products	Burger (3)	1 (33.33)	0	0	1 (33.33)	0	2 (66.66)	1 (33.33)	0	1 (33.33)	0	1 (33.33)	0	0	2 (66.66)	1 (33.33)	0	0	1 (33.33)
	Kebab (4)	2 (50)	1 (25)	1 (25)	1 (25)	0	3 (75)	0	1 (25)	2 (50)	1 (25)	1 (25)	1 (25)	0	2 (50)	2 (50)	1 (25)	1 (25)	0
	Sausage (1)	1 (100)	0	0	1 (100)	0	1 (100)	0	0	1 (100)	0	0	0	0	1 (100)	1 (100)	0	0	0
	Total (8)	4 (50)	1 (12.50)	1 (12.50)	3 (37.50)	0	5 (62.50)	1 (12.50)	1 (12.50)	4 (50)	1 (12.50)	2 (25)	1 (12.50)	0	5 (62.50)	4 (50)	1 (12.50)	1 (12.50)	1 (12.50)
Total (29)		13 (44.82)	4 (13.79)	5 (17.24)	14 (48.27)	2 (6.89)	22 (75.86)	5 (17.24)	3 (10.34)	17 (58.62)	4 (13.79)	6 (20.68)	3 (10.34)	1 (3.44)	20 (68.96)	15 (51.72)	2 (6.89)	7 (24.13)	2 (6.89)

(93.00%), gentamicin (97.00%), and imipenem (100%) antibiotic agents. Odumosu et al. [45] mentioned that the *P. aeruginosa* isolates of ready-to-eat vegetable samples collected from Nigeria were resistant against carbenicillin (63.00%), amikacin (1.90%), and ceftazidime (83.30%). *P. aeruginosa* strains isolated from imported frozen meat samples harbored a higher distribution of antibiotic resistance. One possible explanation for this finding is the importation of low-quality meat samples from other countries to Iran. Additionally, the addition of antibiotic resistant *P. aeruginosa* strains from other foodstuffs into imported meat samples and during storage are other probable reasons. Chloramphenicol is a prohibited antibiotic agent. Resistance against this antibiotic may show its unauthorized prescription in veterinary. In this survey, resistance toward chloramphenicol was obtained in 20% of *P. aeruginosa* strains isolated from imported frozen bovine meat, 33.33% of burger-based isolates, 25% of kebab-based isolates, and finally all (100%) of strains isolated from sausage samples. This finding may indirectly show the application of poultry products in the production of sausage, burger, and kebab samples.

Irregular and irrational administration of antibiotics in veterinary medicine is the probable reason for antibiotic resistance in bacteria. Additionally, it could be transferred by plasmids and transposons. In the food systems, ESBL-producing *P. aeruginosa* occurrence is emerging [46]. Our findings revealed that the genes that encode resistance toward all beta-lactams (*bla*ShV, *bla*DHA, *bla*VEB, *bla*CTX, *bla*TEM, and *bla*OXA), and quinolones (*gyrA* and *parC*) were predominant amongst isolated bacteria. In keeping with this, as we did not perform the ESBL production test, we cannot exactly mention whether the isolated strains are ESBL producers or not. In another Iranian survey, Bahrami et al. [47] described that *bla*CTX-M, *bla*SHV, *bla*TEM, and *bla*OXA were the most frequent antibiotic resistance genes detected in 23.95%, 23.08%, 57.29%, and 12.5% of *P. aeruginosa* strains, respectively. A survey on camel meat [48] showed that the total distribution of ESBL-producing *P. aeruginosa* was 45%, and *bla*CTX-M (38.00%), *bla*SHV (33.30%), and *bla*TEM (28.50%) were the most commonly detected antibiotic resistance genes. Extended-spectrum b-lactamases are encoded by *bla* plasmid-mediated genes, particularly *bla*CTX-M, *bla*SHV, *bla*TEM, *bla*VEB, and *bla*OXA, which have extended the substratum specificity toward cefotaxime, ceftazidime, and ceftriaxone [49]. Hosu et al. [50] stated that *bla*SHV, *bla*TEM, and *bla*CTX-M antibiotic resistance genes amongst the *P. aeruginosa* isolates of nonclinical samples were distributed 93.30%, 40.00%, and 20.00%, respectively.

Another critical aspect of the *P. aeruginosa* presence in examined samples was the high distribution of virulence factors, particularly *exoS*, *lasA*, *exoU*, *lasB*, *plcH*, *algD*, *pilA*, *phzM*, *toxA*, and *exoT*. These genes are mainly responsible for the adhesion and invasion of bacteria into the host cells. In keeping with this, consuming food containing virulent *P. aeruginosa* strains may cause severe food-borne infection. *ToxA*, *aprA*, *plcH*, and *las* virulence factors were predominant in the *P. aeruginosa* strains isolated from camel meat [35]. Benie et al. [32] recognized that the distribution of *exoU*, *pilB*, *plcH*, *algD*, *exoS*, and *lasB* virulence factors in the *P. aeruginosa* strains isolated from bovine meat samples were 4.10%, 42.60%, 72.10%, 74.50%, 96.70%, and 96.70%, respectively.

The high *lasA* and *B* genes distribution specifies that these protease enzymes can be significant for the *P. aeruginosa* pathogenesis cutting collagen and elastin [51]. It also can destruct the junctions amid the epithelial cells [52]. *LasA* and *lasB* genes increase IL-8 production and decrease the innate immune response, immunoglobulins, and complement compounds [51, 52]. The high *algD* distribution caused alginate overproduction. Its high distribution maybe shows that isolated strains of meat and meat product samples are involved in biofilm formation as alginates have been extensively regarded as the main biofilm matrix exopolysaccharides [53]. It can protect the bacteria from antibiotics, decrease phagocytosis and polymorphonuclear chemotaxis, and inhibit complement activation [53]. *ExoS* and *plcH* virulence genes were also detected in 75.86% and 48.25% of *P. aeruginosa* isolates. Thus, the isolates can secrete hemolytic exoenzymes and phospholipase C, which can facilitate the occurrence of some kinds of human clinical infections [54]. The *exoS* was responsible for bacterial dissemination and tissue destruction [54]. The *pilA*, *pilB*, and *exoU* genes were detected in 24.13%, 6.89%, and 58.62% of *P. aeruginosa* isolates. Both *pil* genes are responsible for pili formation and further motilities [55]. The *ExoU* gene is cytotoxic, and its distribution is a marker of invasive *P. aeruginosa* [56]. It seems that *P. aeruginosa* isolates of meat and meat product samples in this survey can cause severe gastrointestinal disorders owing to the high distribution of virulence factors. However, some additional works should be performed to approve it.

5. Conclusion

In assumption, the high *P. aeruginosa* prevalence in samples predominantly imported frozen bovine meat and frozen bovine meat samples with a high distribution of virulence factors and antibiotic resistance was reported in this study. A high prevalence of resistance to ampicillin, penicillin, tetracycline cefoxitin, sulfamethoxazole gentamicin, and clindamycin antibiotic agents and the presence of *Bla*DHA, *bla*CTX-M, *bla*SHV, *Bla*TEM, and *gyrA* antibiotic resistance genes were reported in the present survey. These findings may show the high antibiotic resistance of *P. aeruginosa* and the potential role of meat and meat products in its transmission to the human population. Some strains harbored different virulence factors, particularly *exoS*, *lasA*, *exoU*, *lasB*, *plcH*, *algD*, *pilA*, *phzM*, *toxA*, and *exoT*. These genes are mainly involved in the adhesion and invasion to host cells. It seems that the consumption of contaminated meat and meat products with virulent *P. aeruginosa* may cause severe food-borne diseases. Additional investigations are suggested to illuminate additional epidemiological aspects of antibiotic resistant and virulent *P. aeruginosa* in raw meat, frozen meat, imported frozen meat, and meat product samples.

Data Availability

The data used to support the findings of this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Authors' Contributions

Zohreh Mashak and Manizhe Rezaloo were involved in the conceptualization, designing, and support of the study and carried out molecular examinations and statistical analysis. Abbasali Motalebi supported the study and carried out the disk diffusion. Amirali Anvar carried out the bacterial isolation and identification. The authors read and approved the final manuscript.

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