Hindawi
Journal of Food Quality
Volume 2022, Article ID 8990912, 8 pages
https://doi.org/10.1155/2022/8990912

Research Article

The Prevalence Rate, Pattern of Antibiotic Resistance, and Frequency of Virulence Factors of *Pseudomonas aeruginosa* Strains Isolated from Fish in Iran

Gholam Reza Shahrokhi, Ebrahim Rahimi, and Amir Shakerian

Department of Food Hygiene, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Correspondence should be addressed to Ebrahim Rahimi; ebrahimrahimi55@yahoo.com

Received 18 January 2022; Revised 25 March 2022; Accepted 6 April 2022; Published 25 April 2022

Academic Editor: Slim Smaoui

Copyright © 2022 Gholam Reza Shahrokhi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Pseudomonas aeruginosa (*P. aeruginosa*) is a pathogenic bacterium and one of the seafood's most common spoilage microorganisms. In this study, 470 fish samples collected randomly were evaluated for the presence of *P. aeruginosa*, antibiotic resistance, and frequency of virulence factors. Isolation of *P. aeruginosa* from fish samples was performed on cetrimide agar after an initial enrichment. Representative colonies were selected, and biochemical tests were conducted. An antibiotic resistance test was performed using the disk diffusion method. DNA was extracted, and antibiotic resistance genes, as well as virulence genes, were detected using PCR. Fresh fish showed the highest prevalence of *P. aeruginosa* (5%). No positive samples contaminated with *P. aeruginosa* were isolated from frozen fish samples. From smoked, salted, and dried fish, two samples (2.85–4%) were contaminated with *P. aeruginosa*. The antibiotic resistance against meropenem, imipenem, carbapenem, erythromycin, gentamicin, chloramphenicol, and enrofloxacin was 0%. The lowest antibiotic resistance pattern was observed in fresh fish, and the highest was observed in smoked, salted, and dried fish. Respectively, bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} were the most abundant genes encoding antibiotic resistance. The most virulence genes were *algD, algU, lasB, toxA, exoS, exoT*, and *apr*. This study suggests that raw seafood could be a source of antibiotic-resistant *P. aeruginosa* and helps to spread resistance genes through the food chain. It seems that cross-contamination in the fishing, transportation, and supply of seafood can cause increased contamination with *Pseudomonas aeruginosa* in these products. Therefore, the hygienic principles can effectively reduce contamination by *P. aeruginosa*. Also, the prophylactic use of antibiotics in these products should be controlled.

1. Introduction

With the increasing consumption of fish and various types of seafood in Iran, the microbial quality of these products must be controlled. Fish is rich in protein, omega 3, omega 6, vitamins, and minerals. The presence of omega-3 fatty acids, including alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, has an influential role in consumer health and prevents some common diseases such as cardiovascular disease, Alzheimer's, stroke, asthma, cancer, and diabetes [1].

The Food and Agriculture Organization estimates the world consumption of fish as about 17.7 kg, and in some developed countries, its consumption has increased to 26 kg (Western Europe) and 80–90 kg (Japan). The FAO has announced the fish consumption in Iran as 7.7 kg [2].

Fish contains 80% water, 18% protein, 1% lipids, and 1% carbohydrates. Fish proteins contain large amounts of nonprotein nitrogen compounds (NPNs) and peptides. The pH of fish is generally higher than 6. In general, fish and seafood are more susceptible to microbial spoilage than other meat due to a lower pH and moisture content. The microbial spoilage may cause discoloration, off-odor, off-flavor, and slime formation that makes it undesirable for consumption. The types of spoilage microorganisms in fish and seafood will depend on the type of product, muscle, and storage conditions [3].

Pseudomonas aeruginosa is one bacterium that can easily cause contamination and spoilage of fish meat. This organism is a Gram-negative, aerobic, oxidase-positive, and catalase-positive bacillus, without spores. They are found in various environments, have simple nutritional requirements, and survive under a wide range of temperatures. *Pseudomonas aeruginosa* with high pathogenicity has been causing the most common infections over 30 years [4]. This bacterium can be transmitted to humans by consuming contaminated meat products and causes gastrointestinal infections [5].

Pseudomonas aeruginosa is more critical than other spoilage bacteria due to its high resistance to a wide range of antibiotics. It has a wide range of virulence factors that can cause severe and aggressive infections in humans and animals. The genes encoding antibiotic resistance can be easily transmitted to humans through consuming contaminated seafood causing severe antibiotic resistance [6].

This Gram-negative bacterium is one of the factors limiting the shelf-life of fish and seafood and must be considered an important microbiological problem in seafood [7]. Therefore, the present study aimed to investigate its prevalence in fish and types of fish products in Iran and the pattern of antibiotic resistance and virulence genes.

2. Materials and Methods

2.1. Sampling. A total of 470 fish samples, including 200 fresh fish, 70 smoked fish, 70 salted fish, 50 dried fish, and 80 frozen fish samples, were collected randomly and under aseptic conditions from Isfahan, Ilam, Bushehr, and Mazandaran provinces (Iran) during six months. Samples were immediately sent to the laboratory.

2.2. Isolation of Pseudomonas aeruginosa from Fish Samples. To isolate *Pseudomonas aeruginosa* from samples, 25 g of the sample was homogenized with 225 ml of peptone water. After initial enrichment, the bacteria were cultured in cetrimide agar (PCA) and incubated at 37°C for 24 hours. The colonies were selected based on color and odor (green-blue pigment with a specific odor). To confirm *Pseudomonas aeruginosa* species, biochemical tests such as fermentation of lactose, citrate, indole, oxidase, DNase, and hemolysis in a blood agar medium were performed. Colonies containing lactose-negative, citrate-positive, indole-negative, oxidase-positive, DNase-negative, and hemolytic bacteria were selected as *Pseudomonas aeruginosa*. They were cultured in the brain heart infusion (BHI) medium at 37°C for 24 hours.

2.3. The Antibiotic Resistance Pattern of Pseudomonas aeruginosa Strains. The antibiotic resistance of *Pseudomonas aeruginosa* isolated from fish samples was tested using the disk diffusion method (Kirby–Bauer) according to the Clinical and Laboratory Institute (CLSI) instructions of 2017. *Pseudomonas aeruginosa* isolates were cultured overnight in a BHI medium. They were concentrated in Müller–Hinton agar, and after incubation at 37°C for 24 hours, bacterial susceptibility or resistance to the antibiotics was determined by the growth inhibition zone. The *Pseudomonas aeruginosa* standard strain (ATCC 10145) was used in this experiment as a control.

The antibiotic discs include tetracycline (30 μg/disc), chloramphenicol (30 μg/disc), imipenem and carbapenem (30 μg/disc), sulfamethoxazole (25 μg/disc), gentamicin (10 μg/disc), enrofloxacin (5 mg/disc), cephalothin (30 μg/disc), ciprofloxacin (5 μg/disc), trimethoprim (5 μg/disc), ampicillin (10 units/disc), penicillin (10 μg/disc), and erythromycin (15 μg/disc).

2.4. DNA Extraction. DNA was extracted from the overnight culture of bacteria in the brain heart infusion medium (Merck, Germany) using the DNA Purification Genomic Kit (Fermentas Germany). The DNA samples were then placed at −20°C until polymerase chain reaction (PCR).

2.5. Molecular Detection of Virulence Factors and Antibiotic Resistance Genes. PCR technique was used to detect virulence (*algD, algU, lasB, toxA, plcH, plcN, exoS, exoT, exoY, exoU, apr, phzII, phzM, phzS, phzI, phzH, lasA, pvdA, pilA*, and *pilB*) and antibiotic resistance genes (bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{CTX\text{-}M}$, bla_{DHA}, and bla_{VEB}). The list of utilized primers and the conditions for the reactions are given in Table 1.

The gene amplification process was done in 25 μl of a mixture including 1 unit of Taq DNA Polymerase (Fermentas, Lithuania), 200 μmol dNTP (Fermentas, Lithuania), 2.5 μl of 10x buffer solution (Fermentas, Lithuania), 1 μmol of manganese chloride (Fermentas, Lithuania) 10 picomoles of each primer, and 3 μl of template DNA, and nuclease-free water was added to 25 μl. The thermal program includes the initial denaturation at a temperature of 94°C for 6 minutes, denaturation stage at a temperature of 94°C for 60 seconds, annealing stage at a temperature of 55°C for 1 minute, extension stage at a temperature of 72°C for 5.5 minutes, and final extension stage at a temperature of 72°C for 5 minutes.

2.6. PCR Product Electrophoresis. The distilled water was used as a negative control, and *Pseudomonas aeruginosa* strain ATCC 10145 was used as a positive control. PCR products were electrophoresed on 2% agarose gel. The gels were stained with Sybergreen (Fermentase, Germany), and DNA strips were evaluated using UV light.

2.7. Statistical Analysis. The percentage of contamination in different sources and products has been calculated. The differences between groups were analyzed with SPSS statistical software (version 18) using the K^2 method. The significant level was determined at $p < 0.05$.

3. Results and Discussion

3.1. Isolation of Pseudomonas aeruginosa from Samples. In this study, a total of 470 fish samples and fish products were evaluated for the presence of *Pseudomonas aeruginosa*. The results of contaminated samples based on the culture

Table 1: List of primers used to detect virulence factors in *Pseudomonas aeruginosa* strains isolated from fish and seafood [8].

Virulence genes	Sequence (5′–3′)	Size of the product (bp)
algD	F: AAGGCGGAAATGCCATCTCC R: AGGGAAGTTCCGGGCGTTTG	275
algU	F: CGCGAACCGCACCATCGCTC R: GCCGCACGTCACGAGC	410
lasB	F: ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	284
toxA	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	270
plcH	F: CACACGGAAGGTTAATTCTGA R: CGGTTARACGGCTGAACCTG	608
plcN	F: CGACTTCCATTTCCCGATGC R: GGACTCTGCAACAAATACGC	481
exoS	F: GTGTGCTTTATGCCATGAG R: GGTTTCCTTTTCCAGGTC	444
exoT	F: CATCGTCTACGCCATGAG R: AGCAGCACCTCGGAATAG	1159
exoY	F: GGAATGAACGAAGCGTTCTCCGAC R: TGGCGTCGACGAACACCTCG	1035
exoU	F: CTGCGCGGGTCTATGTGCC R: GATGCTGGACGGGTCGAG	3308
Apr	F: GCACGTGGTCATCCTGATGC R: TCCGTAGGCGTCGACGTAC	1017
phzII	F: TCCGTTATCGCAACCAGCCCTACG R: TCGCTGTCGAGCAGGTCGAAC	1036
phzM	F: CGTCGTGTTCAAGCAGATGGTGCTG R: CCGAACCGCTTCACCAGGC	875
phzS	F: CAATCATCTCAGCAGAACCC R: TGTCGTAGAGGATCTCCTG	1752
phzI	F: TATCGACGGTCATCGTCAGGT R: TTGATGCACTCGACCAGCAAG	392
phzH	F: GATTCCATCACAGGCTCG R: CTAGCAATGGCACTAATCG	1752
lasA	F: TGTCCAGCAATTCTCTTGC R: CGTTTTCCACGGTGACC	1075
pvdA	F: GCCAAGGTTTGTTGTCGG R: CGCATTGACGATATGGAAC	1281
pilA	F: ATGGAGAGCGGGATCGACAG R: ATGCGGGTTTCCATCGGCAG	1675
pilB	F: TCGCCATGACCGATACGCTC R: ACAACCTGAGCCAGCCTTCC	408

and molecular method (PCR) are summarized in Table 2. As the results show, the prevalence rate in samples was reported at 14.7%. The fresh fish showed the highest prevalence rate of *Pseudomonas aeruginosa*. No positive samples with *Pseudomonas aeruginosa* were isolated from frozen fish samples. The smoked (2.85%), salted (2.85%), and dried fish (4%) samples were found to be contaminated by *Pseudomonas aeruginosa*. No significant difference was observed between the samples ($p > 0.05$).

Pseudomonas aeruginosa count in fish samples is significant because this bacterium is often considered an indicator of food quality and may cause food-borne illnesses [9, 10].

The present study showed the highest and lowest prevalence of *Pseudomonas aeruginosa* in fresh and processed fish. Preservation processes such as smoking, salting, and drying may have played an essential role in reducing the bacterial population in these products.

Algammal et al. [11] isolated *Pseudomonas aeruginosa* at a prevalence of 31.57% from 90 fish samples in Egypt. Benie et al. [5] reported the prevalence of *Pseudomonas aeruginosa* in beef, fresh fish, and smoked fish at 47.8%, 33.1%, and 20%, respectively. Benie et al. [12] isolated 153 multidrug-resistant strains of *Pseudomonas aeruginosa* from beef (93%), fresh fish (36%), and smoked fish (24%). Abd-El-Maogoud et al. [13] isolated *Pseudomonas aeruginosa* at a prevalence of 65% from frozen mackerel (33%), frozen saurus (30%), and tilapia samples (23%). The highest amount of *Pseudomonas aeruginosa* was seen in cold tilapia. Abd El-Aziz [14] reported that all fish samples collected from Assiut (Egypt) were contaminated with *Pseudomonas aeruginosa*. Duman et al. [15] isolated 90 strains of *Pseudomonas aeruginosa* from fish farms in Turkey, classified them into 12 species, and reported seven new species. Abd-El-Maogoud et al. [13] reported the highest prevalence of *Pseudomonas aeruginosa* in mackerel, followed by the frozen fish samples. Fewer

Table 2: The prevalence of *Pseudomonas aeruginosa* in types of fish.

Sample	*N.* samples collected	Bacteriological methods *N.* samples positive (%)	Molecular detection *N.* samples positive (%)
Fresh fish	200	10 (5)	10 (5)
Smoked fish	70	2 (2.85)	2 (2.85)
Salted fish	70	2 (2.85)	2 (2.85)
Dried fish	50	2 (4)	2 (4)
Frozen fish	80	0 (0)	0 (0)
Total	470	16 (14.7)	16 (14.7)

No significant difference was observed between samples ($p > 0.05$).

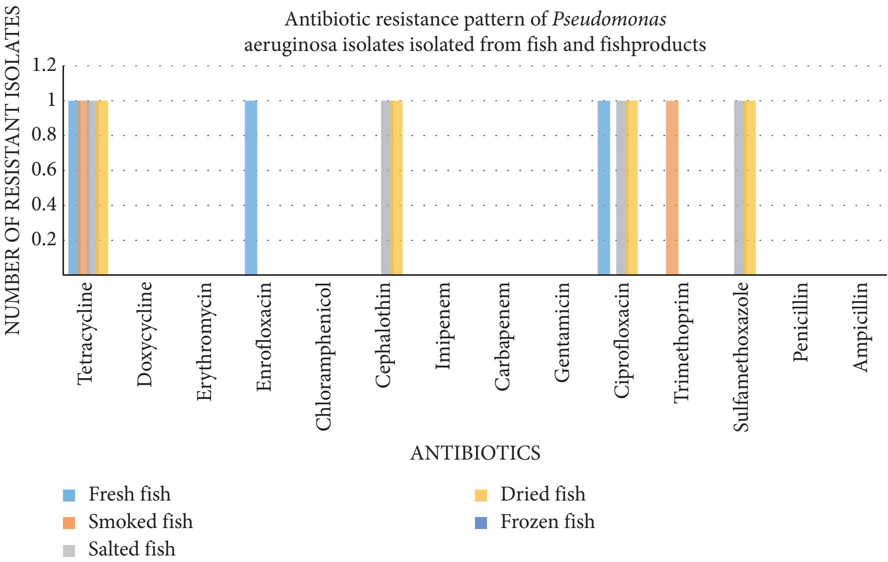

Figure 1: The number of resistant isolates of antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates isolated from fish and fish products.

prevalence rates were reported by Benie et al. [16], Salem et al. [17], and Ibrahim et al. [18].

Our results confirmed the contamination of fish samples with *Pseudomonas aeruginosa*. The results may be due to differences in hygienic principles during fishing, handling, freezing, storage, and thawing of fish, as Salem et al. reported [17].

3.2. The Antibiotic Resistance Pattern of Pseudomonas aeruginosa Strains. Figures 1–4 summarize the antibiotic resistance of *Pseudomonas aeruginosa* strains isolated from fish and fish products. The results show that the prevalence of antibiotic resistance against meropenem, imipenem, carbapenem, erythromycin, gentamicin, chloramphenicol, and enrofloxacin was reported to be 0%. The prevalence of antibiotic resistance against antibiotics used in frozen fish was 0%. The highest antibiotic resistance was observed in smoked, salted, and dried fish samples. *Pseudomonas aeruginosa* was resistant to tetracycline, cefotaxime, chloramphenicol, imipenem, carbapenem, and penicillin antibiotics. Four semisensitive samples were reported against doxycycline, gentamicin, penicillin, and ampicillin antibiotics.

Pseudomonas aeruginosa strains isolated in Egypt showed multidrug resistance (MDR) to amoxicillin, cefotaxime, tetracycline, and gentamicin [11]. Carol et al. [19] observed that *Pseudomonas aeruginosa* had high resistance to amoxicillin, moderate resistance to ampicillin, ceftazidime, nitrofurantoin, and gentamicin, and sensitivity to tobramycin, cefotaxime, ethylene, ciprofloxacin, and amikacin.

Benie et al. [5] examined the prevalence of *Pseudomonas aeruginosa* in beef, fresh fish, and smoked fish. They found that *Pseudomonas aeruginosa* strains were mainly resistant to cefepime, imipenem, ceftazidime, ciprofloxacin, and piperacillin. The resistance of *Pseudomonas aeruginosa* to cephalosporins (ceftazidime and cefepime) may be due to chromosomal mechanisms (disruption of the OprD purine) and an association of resistance mechanisms such as extended-spectrum beta-lactamase (ESBL).

In the study by Benie et al. [12], *Pseudomonas aeruginosa* isolates were resistant to aztreonam, ticarcillin, and ciprofloxacin. Aman et al. [20] found resistance of *Pseudomonas aeruginosa* to cefepime (34%) and ceftazidime (37%).

Imipenem resistance is due to the reduction of purine permeability, according to Bricha et al. [21]. It is related to the enzymatic mechanism with carbapenemases, which causes a high level of resistance to all beta-lactamases.

Comparison of the results obtained in the present study with other studies shows that antibiotic resistance differs

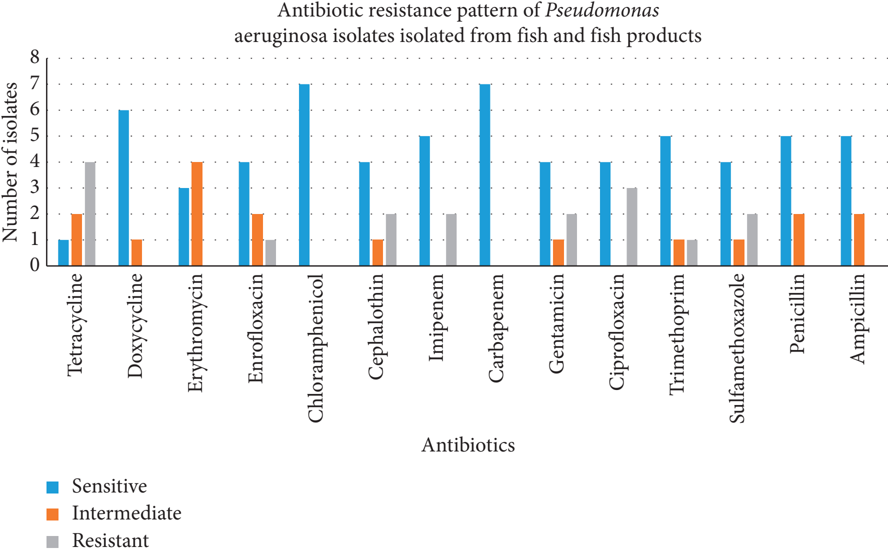

Figure 2: The number of isolates of antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates isolated from fish and fish products..

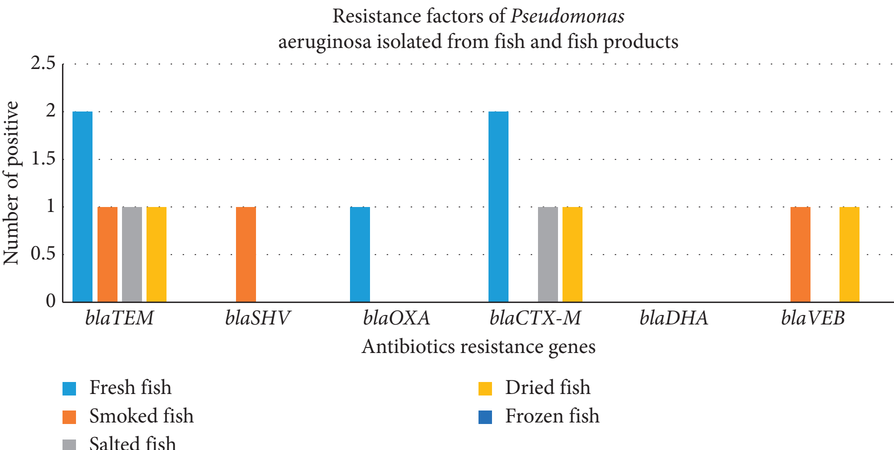

Figure 3: Resistance factors of *Pseudomonas aeruginosa* isolated from fish and fish products.

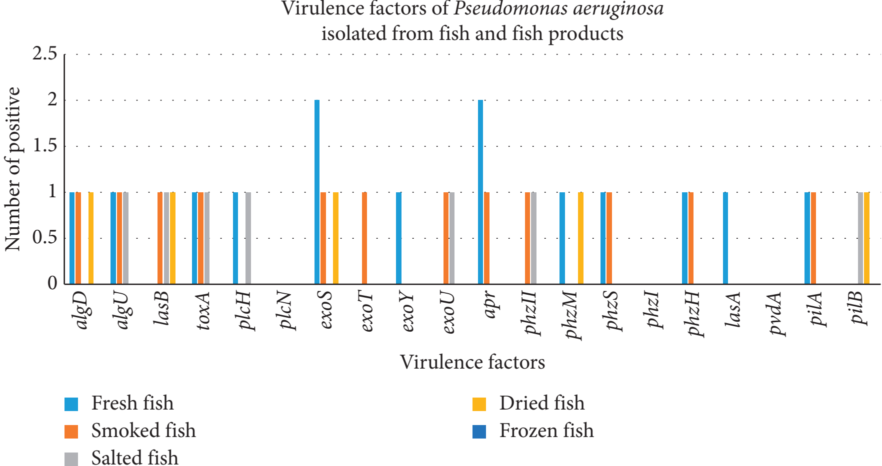

Figure 4: Virulence factors of *Pseudomonas aeruginosa* isolated from fish and fish products.

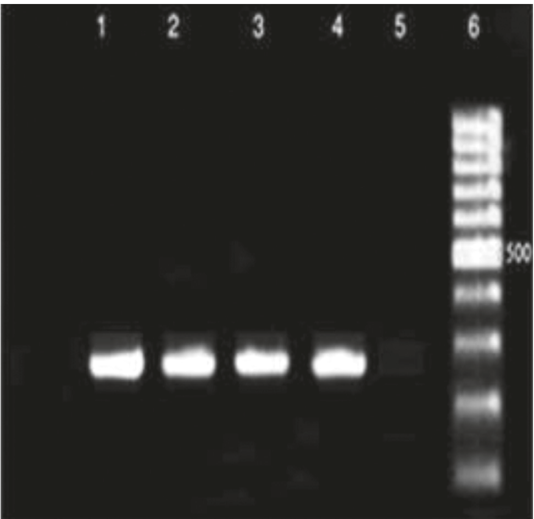

Figure 5: PCR product electrophoresis of 275 bp *algD* gene (lanes 1–3: positive samples, lane 4: positive control, lane 5: negative control, and lane 6: marker 100 bp).

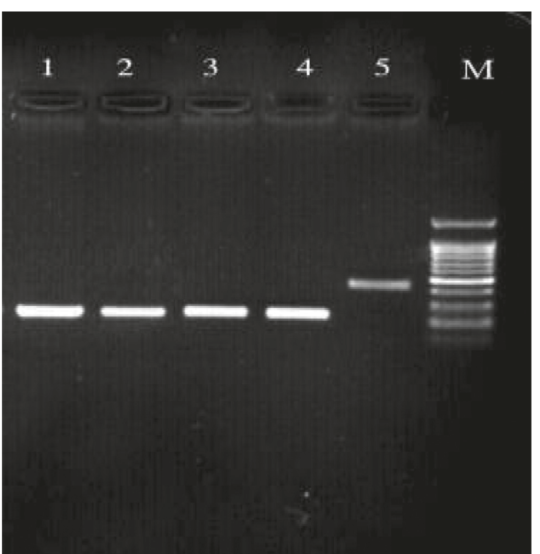

Figure 6: PCR product electrophoresis of 284 bp *lasB* gene and 444 bp *exoS* gene (lanes 1–3: positive samples 286 bp, lane 4 positive control, lane 284: *lasB* gene, lane 5: positive sample of 444 bp *exoS*, and lane M: marker 100 bp).

across countries. The antibiotic resistance of *Pseudomonas aeruginosa* seems to be constantly changing, and it is necessary to be attending to this change in different countries.

3.3. The Virulence and Antibiotic Resistance Genes. Figure 1–4 summarize the antibiotic resistance and virulence genes of *Pseudomonas aeruginosa* isolated from fish samples. As can be seen, the most abundant genes encoding antibiotic resistance in *Pseudomonas aeruginosa* strains were bla_{TEM}, $bla_{CTX\text{-}M}$, and bla_{SHV} from smoked, salted, and dried fish samples, respectively. The most abundant virulence genes

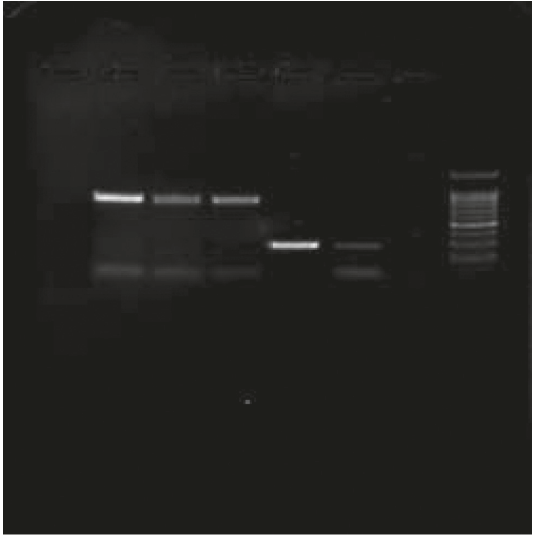

Figure 7: PCR product electrophoresis of 270 bp *toxA* gene and 875 bp *phzM* (from left, lanes 1–3: positive samples of 875 bp *phzM* gene, lanes 4 and 5: positive sample of 270 bp *toxA* gene, lane 6: negative controls, and lane 6: marker 100 bp).

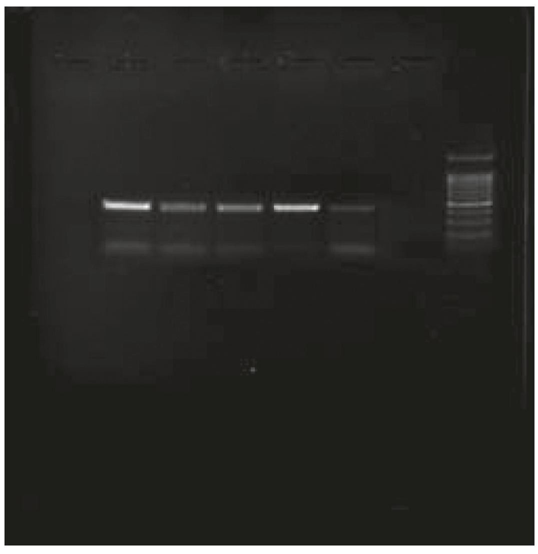

Figure 8: PCR product electrophoresis of 410 bp *algU* gene (from left, lanes 1–4: positive samples, lane 4: positive control, lane 5: negative control, and lane 6: marker 100 bp).

detected in *Pseudomonas aeruginosa* were *algD*, *algU*, *lasB*, *toxA*, *exoS*, *exoT*, and *apr*.

These findings are consistent with those of other researchers [11, 12]. Nonadherent virulence factors play an essential role in bacterial survival under specific conditions such as human blood, iron deficiency, and intestinal diseases. However, these nonadherent virulence factors are not essential for the pathogenesis of food-borne illness.

Algammal et al. [11] reported the bla_{CTXM}, bla_{TEM}, and *tetA* genes as the significant antibiotic resistance genes in *Pseudomonas aeruginosa* strains isolated from fish, which induces a pattern of resistance to cefotaxime, amoxicillin,

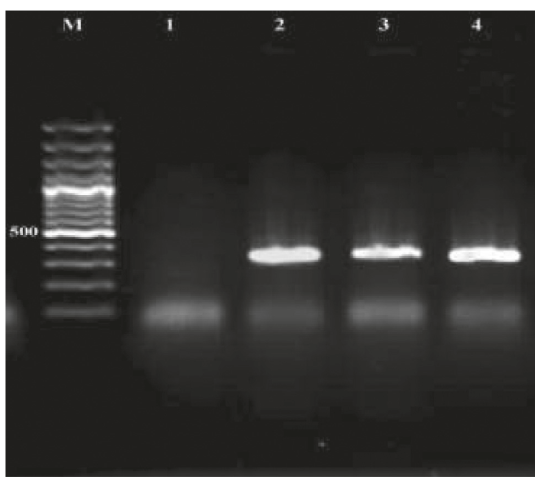

FIGURE 9: PCR product electrophoresis of 392 bp *phzI* gene (from left, lane M: marker 100 bp, lane 1: negative control, and lanes 2–4: positive samples).

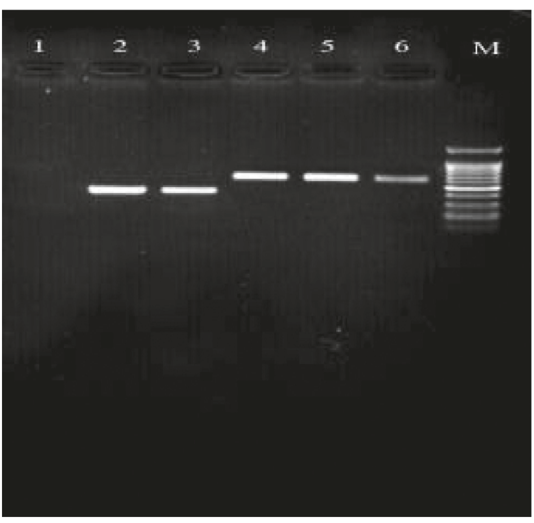

FIGURE 10: PCR product electrophoresis of 593 bp $bla_{CTX\text{-}M}$ gene and 642 bp bla_{VEB} gene (from left, lane 1: negative control, lanes 2 and 3: positive samples of 593 $bla_{CTX\text{-}M}$ bp, lanes 4 and 5: positive samples of 642 bp bla_{VEB} gene, lane 6: positive control, and lane M: marker 100 bp).

and tetracycline. This highlights multidrug resistance (MDR) of *Pseudomonas aeruginosa* strains in public health. Benie et al. [12] identified bla_{SHV}, bla_{TEM}, bla_{PER}, bla_{VEB}, bla_{IMP}, and bla_{VIM} genes in *Pseudomonas aeruginosa* isolated from beef, fresh fish, and smoked fish.

Our findings differed from other studies mentioned above, which could be due to the geographical conditions and the level of health and improper use of some antibiotics in the livestock.

3.4. PCR Product Electrophoresis. Figures 5–10 show electrophoresis profiles of the genes detected in this study.

4. Conclusion

The results of this study confirmed the prevalence of *Pseudomonas aeruginosa* in fish and seafood, which is a severe warning to prevent the further spread of this microorganism. Due to the abundance of *Pseudomonas aeruginosa* in meat products, especially fish, much attention should be paid to the health and quality of meat products. Fish play an essential role in human nutrition. They are a suitable food environment for the growth of bacteria such as *Pseudomonas aeruginosa*, so farms should be completely healthy.

Fish should be caught by public health standards and kept out of direct and indirect contact with external sources of contamination at all stages of fishing, collection, handling, and transportation. Therefore, with the lack of hygienic and cooling systems, *Pseudomonas aeruginosa* in the fish will increase. The hygienic principles must be applied to reduce contamination with *Pseudomonas aeruginosa*.

Data Availability

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

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors thank M. Momeni Shahraki and his laboratory staff for their support throughout this study.

References

[1] M. Ćirković, B. Jovanović, and S. Maletin, *Ribarstvo-biologija-tehnologijaekologija-ekonomija*, Univerzitet u Novom Sadu, Poljoprivredni fakultet Novi Sad, 2002.

[2] FAO, "Food and agricultural organization," 2010, https://www.fao.org.

[3] L. Gram and P. Dalgaard, "Fish spoilage bacteria-problems and solutions," *Current Opinion in Biotechnology*, vol. 13, no. 3, pp. 262–266, 2002.

[4] P. R. Murray, K. S. Rosenthal, and M. A. Pfaller, *Medical Microbiology*pp. 425–432, Mosby, Maryland Heights, MO, USA, 7th edition, 2012.

[5] C. K. D. Benie, G. Nathalie, D. Adjéhi et al., "Prevalence and antibiotic resistance of *Pseudomonas aeruginosa* isolated from bovine meat, fresh fish and smoked fish," *Archives of Clinical Microbiology*, vol. 8, p. 3, 2017.

[6] N. Gorgani, S. Ahlbrand, A. Patterson, and N. Pourmand, "Detection of point mutations associated with antibiotic resistance in *Pseudomonas aeruginosa*," *International Journal of Antimicrobial Agents*, vol. 34, no. 5, pp. 414–418, 2009.

[7] A. Ardura, A. Linde, and E. Garcia-Vazquez, "Genetic detection of Pseudomonas spp. in commercial Amazonian fish,"

International Journal of Environmental Research and Public Health, vol. 10, no. 9, pp. 3954–3966, 2013.
[8] S. Finnan, J. P. Morrissey, F. O'Gara, and E. F. Boyd, "Genome diversity of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients and the hospital environment," *Journal of Clinical Microbiology*, vol. 42, no. 12, pp. 5783–5792, 2004.
[9] M. Begum, A. Abu Ahmed, M. Das, and S. Parveen, "A comparative microbiological assessment of five types of selected fishes collected from two different markets," *Advances in Biological Research*, vol. 4, pp. 259–265, 2010.
[10] J. M. Jay, M. J. Loessner, and D. A. Golden, *Modern Food Microbiology*, Springer Science, Berlin, German, 7th edition, 2005.
[11] A. M. Algammal, M. Mabrok, E. Sivaramasamy et al., "Emerging MDR-Pseudomonas aeruginosa in fish commonly harbor oprL and toxA virulence genes and blaTEM, blaCTX-M, and tetA antibiotic-resistance genes," *Scientific Reports*, vol. 10, no. 1, p. 15961, 2020.
[12] C. K. F. Benie, D. K. N'zebo, N. A. N'gbesso-Kouadio et al., "High prevalence of multidrug resistant *Pseudomonas aeruginosa* strains recovered from bovine meat, fresh and smoked fish in côte d'Ivoire," *Microbiology and Nature*, vol. 1, pp. 44–54, 2019.
[13] H. Abd-El-Maogoud, A. B. Edris, A. Mahmoud, and M. Maky, "Occurrence and characterization of *Pseudomonas* species isolated from fish marketed in sohag governorate, Egypt," *SVU-International Journal of Veterinary Sciences*, vol. 4, no. 2, pp. 76–84, 2021.
[14] D. Abd El-Aziz, "Detection of *Pseudomonas* spp. in chicken and fish sold in markets of assiut city, Egypt," *Journal of Food Quality and Hazards Control*, vol. 2, pp. 86–89, 2015.
[15] M. Duman, M. Mulet, S. Altun et al., "The diversity of Pseudomonas species isolated from fish farms in Turkey," *Aquaculture*, vol. 535, 2021.
[16] C. Benie, A. Dadie, N. Guessennd et al., "Prevalence and diversity of *Pseudomonas* spp. isolated from beef, fresh and smoked fish in Abidjan, Côte d'Ivoire," *Journal of Food and Dairy Technology*, vol. 4, no. 4, pp. 52–61, 2016.
[17] A. Salem, I. Osman, and S. Shehata, "Assessment of Psychrotrophic Bacteria in frozen fish with special reference to *Pseudomonas* Species," *Benha Veterinary Medical Journal*, vol. 34, no. 2, pp. 140–148, 2018.
[18] H. Ibrahim, R. Amin, N. Shawkey, and H. Mohammed, "Bacteriological evaluation of some fresh and frozen fish," *Benha Veterinary Medical Journal*, vol. 31, no. 1, pp. 24–29, 2016.
[19] G. Carol, K. Immaculate Jeyasanta, A. Elizabeth Mani, and J. Patterson, "Prevalence of *Pseudomonas* sp. in fin fishes and their antibiotic susceptibility," *Journal of Pure and Applied Microbiology*, vol. 7, no. 1, pp. 677–681, 2013.
[20] U. Aman, D. Rabia, A. Ghadir, and A. Safia, "Prevalence of antimicrobial resistant *Pseudomonas aeruginosa* in fresh water spring contaminated with domestic sewage," *Journal of Biological and Food Science Research*, vol. 1, pp. 19–22, 2012.
[21] S. Bricha, K. Ounine, S. Oulkheir, N. E. E. L. Haloui, and B. Attarassi, "Virulence factors and epidemiology related to *Pseudomonas aeruginosa*," *Tunisian Journal of Infectious Diseases*, vol. 2, pp. 7–14, 2009.