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Identification of Multidrug-Resistant *Aeromonas salmonicida* from Imported Seafood in Mosul City Shops via Microarray Technology

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ABSTRACT

The widespread emergence of multidrug-resistant (MDR) pathogens in imported seafood is an increasing public health concern. The present study aimed to detect *Aeromonas salmonicida* (*A. salmonicida*) in seafood samples and to assess the antibiotic resistance gene profiles of the isolated bacteria using conventional polymerase chain reaction (PCR) and DNA microarray analysis. Twenty samples consisting of shrimp, mussels, oysters, and salmon (five of each) were collected from shops in Mosul city, Iraq. The biochemical characteristics of the strains were determined using the VITEK 2 compact system, and their identities were confirmed by *16S rRNA* gene sequencing, which showed ≥99% identity to database sequences. Nine samples tested positive for *A. salmonicida*, with a prevalence of 45 percentage, with shrimp and salmon each accounting for three isolates (60%), mussels for two (40%), and oysters for one (20%). Microarray testing identified seven isolates as MDR, whereas two strains had no resistance genes and were antibiotic-sensitive. The current results revealed the absence of genes encoding extended-spectrum β-lactamases (ESBLs), including *bla*SHV, *bla*CTX-M, *bla*FOX, *bla*PER, *bla*VEB, and *bla*CMY, in all MDR strains. Tetracycline resistance was observed in 28.6% (2/7) of MDR isolates, with the *tetA* gene present, whereas the *tetB* gene was completely absent from all MDR isolates. Concerning sulfonamide resistance, all MDR isolates were found to carry sul1, whereas none carried sul2. The *aphA* and *aadA1* genes were detected in all seven MDR strains, while the *aac(6')-Ib* gene was found in only three of the seven MDR isolates (42.9%). Additionally, each of these strains carried *intI1* and *qacEΔ1* genes. The widespread occurrence of MDR *A. salmonicida* carrying diverse resistance genes in imported seafood presented a significant public health risk.

Keywords: *Aeromonas salmonicida*, Multidrug-resistant, Microarray, Public health, Seafood

INTRODUCTION

Seafood is a crucial component of human nutrition because it provides high-quality protein, minerals, and essential fatty acids that support healthy metabolism and cardiovascular health (Jayasekara et al., 2020). Global seafood consumption is rising steadily as more people recognize its health benefits. Consequently, several countries, including Iraq, are importing large quantities of frozen and chilled fish products (Al-Busaidi et al., 2016). However, globalization of the seafood supply chain has enabled the cross-border spread of microbial contaminants, including antibiotic-resistant bacteria, which are important for clinical and veterinary health. In recent decades, different non-cholera pathogenic *Vibrio* species have attracted increasing attention from public health authorities (Muzembo et al., 2024). *Aeromonas* species, particularly *Aeromonas salmonicida* (*A. salmonicida*), are significant because they thrive in aquatic environments, cause fish infections, and may harbor multidrug-resistant (MDR) genes that might be transmitted to humans through seafood consumption (Tao et al., 2026).

Aeromonas salmonicida was previously identified as the causative agent of furunculosis (Elbaiomy et al., 2025). *Aeromonas salmonicida* is a Gram-negative, facultatively anaerobic, psychrophilic bacterium that lives in freshwater and seawater. Several species of *Aeromonas* are recognized as causative agents of human infections. Although primarily identified as pathogens in fish, numerous species have been associated with gastroenteritis, wound infections, septicemia, and illnesses affecting the skin and soft tissues (Ali Eltabey, 2023).

The emergence of MDR in *A. salmonicida* is potentially linked to the widespread use of antimicrobial agents in aquaculture, including tetracycline, fluoroquinolones, sulfonamides, β-lactams, and aminoglycosides, as well as to environmental contamination from wastewater and other aquatic pollutants (Santos and Ramos, 2018).

Aeromonas salmonicida can acquire resistance genes via mobile genetic elements such as plasmids, integrons, and transposons under the selective pressure of antimicrobial agents. These mechanisms can promote MDR and the spread of resistant strains through imported fish products (Mutuku et al., 2022).

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The increasing consumption of seafood in Iraq, particularly in Mosul, has been observed over the past decade, linked to evolving consumption trends and the availability of imported frozen fish from regional and international markets. There is significant demand for monitoring; however, microbiological screening of imported seafood is rarely conducted. The current gaps highlighted the need for advanced, rapid molecular tools capable of detecting antibiotic resistance markers and distinguishing resistant strains from susceptible strains with high accuracy (Butz et al., 2019). Bacteriological techniques such as culture, biochemical identification, and the disk diffusion assay (Barker and Kehoe 1995) provide valuable information, but they are time-consuming, often taking several days to complete. Additionally, bacteriological techniques generally lack the sensitivity necessary to characterize complex resistance profiles (Vasala et al., 2022). Molecular techniques such as polymerase chain reaction (PCR) and real-time PCR (qPCR) have improved detection capabilities. However, these methods are limited to assessing only a select number of genetic markers per reaction (Butz et al., 2019). In contrast, the DNA microarray technique offers a rapid, high-volume screening system that enables concurrent screening of hundreds of virulence factors, species-specific sequences, and antibiotic resistance genes in a single assay (Peterson et al., 2010). The DNA microarray technique is considered more efficient and faster at identifying MDR pathogens, supporting regulatory compliance and food safety decisions (Ali Eltabey, 2023). The use of microarray technology to identify *A. salmonicida* in imported seafood sold in Mosul City, Iraq, is a significant concern, given the lack of local data on the prevalence of resistant aquatic pathogens in the retail environment. Fish products imported from countries with high antibiotic use in aquaculture may carry resistance genes, contributing to the spread of MDR organisms worldwide. Studying *A. salmonicida* in seafood products is essential for assessing potential transmission pathways (Cabello et al., 2016). Therefore, the present study aimed to identify *A. salmonicida* in seafood samples and to evaluate antibiotic resistance genes in the isolated bacteria using conventional PCR and DNA microarray analysis.

MATERIAL AND METHODS

Ethical approval

The Scientific Committee of the College of Veterinary Medicine at the University of Mosul, Iraq, approved this study under reference number VET.MED/2026/0173 on January 5, 2026. All procedures were conducted in strict accordance with the institution's animal ethics guidelines.

Sampling

Twenty samples of imported seafood were collected randomly from retail shops and batches in Mosul City, Iraq, for the present investigation. The sampling consisted of four species, including shrimp (n = 5), mussels (n = 5), oysters (n = 5), and salmon (n = 5). The selected samples were representative of the different seafood commonly consumed by the local population. Furthermore, the sampling was practically feasible, as supported by preliminary studies on microbial contamination, dosage, and antimicrobial resistance in seafood products. Although a formal power calculation was not conducted, the sample size was adequate to acquire multiple bacterial isolates for subsequent molecular characterization. Each sample was transported in a sterile, insulated container at 4°C to the laboratory. All processing steps were initiated within four hours of collection to prevent any fluctuations in bacterial proliferation (Odour-Odote, 2020).

Preparation and isolation of *Aeromonas* spp.

About 10 g of each seafood sample was collected using an aseptic technique and placed in a sterile stomacher bag containing 90 mL of phosphate-buffered saline (PBS) for two minutes of homogenization (Bolivar et al., 2016). Subsequently, the homogenates were kept at 37°C for 18 to 24 hours to facilitate pre-enrichment. *Aeromonas salmonicida* is psychrophilic; therefore, the incubation temperature of 37°C might have favored the proliferation of mesophilic contaminants or induced stress on the target organism (Boziaris et al., 20214). Then, a loopful of the enriched suspension was streaked onto the selective medium (Chromogenic agar). Colonies exhibiting characteristics typical of *Aeromonas*, such as smoothness, convexity, and a greyish to translucent appearance, were subcultured onto tryptic soy agar (TSA) to obtain pure cultures for subsequent analysis (Sarder, 2017).

VITEK 2 compact system

The automated VITEK 2 compact system (bioMérieux, France) was used to identify *A. salmonicida* isolates in accordance with the manufacturer's instructions. Initially, presumptive *Aeromonas* isolates were subcultured onto TSA and incubated at 37°C for 24 hours to obtain pure, fresh culture colonies (Alghabshi et al., 2018). A standardized bacterial suspension was made by transferring a well-isolated colony into sterile 0.85% saline solution. The turbidity of the solution was then adjusted to a range of 0.50 to 0.63 McFarland, using the DensiCHEK™ turbidity meter. The prepared inoculum was used to inoculate the VITEK 2 Compact system, specifically the Gram-negative identification card, which incorporates multiple biochemical substrate panels for identifying Gram-negative bacteria. The Gram-negative cards evaluated several biochemical reactions, such as carbohydrate utilization, enzyme activities, decarboxylation, and fermentation. The biochemical reaction group demonstrated predominantly positive responses, whereas the other groups exhibited negative responses (Table 1). Enzymatic and assimilation tests, including ornithine

decarboxylase (ODC), citrate utilization (CIT), gamma-glutamyl transferase (GGT), and β-galactosidase, further reinforced species-level identification (Table 1). The identification of isolates was performed by comparing their biochemical profiles with the system's database. Isolates having a confidence level of ≥ 95% was confirmed as *A. salmonicida* (Nakasone et al., 2007).

Table 1. Biochemical reaction profile of *Aeromonas salmonicida* using the VITEK 2 compact Gram-negative identification system

No.	Biochemical test	Reaction	No.	Biochemical test	Reaction	No.	Biochemical test	Reaction	No.	Biochemical test	Reaction	No.	Biochemical test	Reaction
1	APPA	Negative (-)	**11**	OGT	Positive (+)	**21**	PLE	Negative (-)	**31**	ILATk	Positive (+)	**41**	CIT	Positive (+)
2	ADO	Positive (+)	**12**	dXYL	Positive (+)	**22**	TYR	Positive (+)	**32**	AGLU	Negative (-)	**42**	BGLU	Positive (+)
3	PyrA	Positive (+)	**13**	IGLU	Positive (+)	**23**	URE	Positive (+)	**33**	SUCT	Positive (+)	**43**	O129R (vibriostatic)	Resistant (+)
4	LARL	Positive (+)	**14**	dMAL	Positive (+)	**24**	dSOR	Positive (+)	**34**	NAGA	Positive (+)	**44**	GGAA	Positive (+)
5	dCEL	Negative (-)	**15**	dMAN	Positive (+)	**25**	SAC	Positive (+)	**35**	AGAL	Positive (+)	**45**	MLTa	Negative (-)
6	BGAL	Positive (+)	**16**	dMNE	Positive (+)	**26**	dTAG	Negative (-)	**36**	PHOS	Positive (+)	**46**	ELLM	Positive (+)
7	H2S	Negative (-)	**17**	IXYL	Negative (-)	**27**	CIT	Positive (+)	**37**	GlyA	Positive (+)	**47**	ILATa	Negative (-)
8	BNAG	Positive (+)	**18**	BAlap	Negative (-)	**28**	CMT	Positive (+)	**38**	ODC	Positive (+)			
9	AGLTp	Negative (-)	**19**	ProA	Positive (+)	**29**	MNT	Negative (-)	**39**	LDC	Positive (+)			
10	dGLU	Positive (+)	**20**	LIP	Negative (-)	**30**	5KG	Negative (-)	**40**	IHISa	Negative (-)			

Positive (+) and negative (−) reactions represented substrate utilization and enzymatic activities used for species-level identification.

Molecular identification

The molecular identification of *A. salmonicida* isolates was performed by amplifying and sequencing the *16S rRNA* gene (Zhu et al., 2025). Genomic DNA from each isolate, obtained from a pure overnight culture, was extracted employing a commercial bacterial DNA extraction kit (Bangalore Genei, India) in accordance with the manufacturer's protocols. Universal bacterial forward and reverse primers were designed using conserved *16S rRNA* sequences, targeting the conserved regions on both sides of the gene. The *16S rRNA* (~600 base pairs) was amplified using PCR (Table 2). A 25 µL PCR reaction mixture was prepared, comprising 5 µL of template DNA, 5 µL of each forward and reverse primer at 10 pM, 1 µL of PCR master mix, and 12.5 µL of nuclease-free water. The PCR amplification was conducted for 35 cycles, starting with an initial denaturation step at 95°C, followed by 30 cycles consisting of denaturation at 95°C, annealing at 65°C, and extension at 72°C. The PCR process concluded with a final extension step (Kumar 2022). The PCR products were confirmed by gel electrophoresis (1%) at 120 volts on a 400 mm gel, and then purified for Sanger sequencing. The sequences were further analyzed using the BLAST tool of the National Center for Biotechnology Information (NCBI) GenBank (NCBI, USA). The species-level identification of the isolates was confirmed through molecular methods, exhibiting over 99% sequence similarity (Wojnarowski et al., 2024).

Table 2. Primer sequences used for PCR amplification of the *16S rRNA* gene in *Aeromonas salmonicida*

Primers	Oligonucleotide sequence (5′–3′)	Reference
Forward	AGAGTTTGATCMTG CTCAG	Khalaf et al. (2025)
Reverse	TACGGYTACCTTGTTACGACTT	Khalaf et al. (2025)

Microarray technique

Aeromonas salmonicida has been examined for antibiotic resistance genes utilizing a DNA microarray platform. In the present study, 41 gene-specific oligonucleotide probes were designed using reference sequences from the NCBI GenBank database. These probes were resistant to β-lactams, tetracyclines, fluoroquinolones, aminoglycosides, sulphonamides, phenicols, and polymyxins, all of which were classified as MDR factors according to the British Pharmacopeia (Lough, 2025). The probes target the *intI1*, *qacEΔ1*, *acrB*, *tolC*, *marA*, and *soxS* genes, which were involved in the resistance strain. To confirm probe specificity, an in silico BLASTn search was conducted, and no significant matches to non-target sequences were observed. Positive-control strains containing each target gene and negative-control strains lacking these genes were used to experimentally validate probe performance, along with no-template controls. Genomic DNA from different bacterial isolates, labeled with Cy5 and Cy3 fluorescent dyes, was hybridized to the array under optimized conditions, then washed post-hybridization to remove nonspecific binding. Microbial Analysis Software was utilized to scan slides and analyze fluorescence. Three housekeeping genes (*rpoB*, *gyrB*, and *recA*) were assessed for controls. The detected resistance profiles are shown in Table 3.

Table 3. Oligonucleotide probes used in the DNA microarray for the detection of antibiotic and multidrug resistance genes in *Aeromonas salmonicida*

No.	Gene symbol	Antibiotic	Antibiotic group	Resistance mechanism	Oligonucleotide probe sequence (5′–3′)
1	*bla*TEM	Ampicillin	β-Lactams	β-lactamase production	ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTGATGCGTCT
2	*bla*SHV	Amoxicillin	β-Lactams	β-lactamase production	TTGACCGCTGTTGTTAGCGTTGCTGTTATCGCTCATGGTGTTGTTAT
3	*bla*CTX-M	Cefotaxime	ESBL	Extended-spectrum β-lactamase	GCTGATGAAGGTCGCTGATACCGCTGATGATGCGTCTGCTGTTAT
4	*cphA*	Imipenem	Carbapenems	Metallo-β-lactamase	CGCTTATCGGCTGTTACGATGCTGATGACGTTGTTACCGATGCT
5	*bla*FOX	Cefoxitin	AmpC β-lactams	Cephamycinase	GCTGTTGATCGCTGATGATGACGTTGCTGATGTTGTTACGCT
6	*bla*OXA	Oxacillin	β-Lactams	Oxacillinase	ATGTTGATCGCTGATGTTGCTGACGTTGATGATGCGTTGCT
7	*tetA*	Tetracycline	Tetracyclines	Efflux pump	CGCTGATGTTGATGATGCTGTTGCTGACGTTGCTGATGTTGTT
8	*tetB*	Tetracycline	Tetracyclines	Efflux pump	TTGATGCTGTTGACGTTGATGATGCTGTTGCTGATGTTGAT
9	*tetE*	Tetracycline	Tetracyclines	Efflux pump	GATGCTGTTGACGTTGCTGATGTTGATGATGCTGTTGCT
10	*tetD*	Doxycycline	Tetracyclines	Efflux pump	GCTGATGTTGTTGACGTTGCTGATGATGCTGTTGATGCT
11	*gyrA*	Ciprofloxacin	Fluoroquinolone	Target site mutation	GCTGATGACGTTGCTGTTGATGCTGATGTTGATGCTGTT
12	*parC*	Nalidixic acid	Quinolones	Target site mutation	GTTGATGCTGATGTTGCTGTTGATGACGTTGCTGAT
13	*qnrA*	Ciprofloxacin	Fluoroquinolone	Target protection	GCTGTTGATGCTGATGTTGACGTTGCTGATGTTGTT
14	*qnrB*	Ciprofloxacin	Fluoroquinolone	Target protection	TTGCTGATGTTGACGTTGATGCTGTTGATGCTGAT
15	*qnrS*	Ciprofloxacin	Fluoroquinolone	Target protection	GATGTTGCTGATGACGTTGATGCTGTTGATGCT
16	*aac(6')-Ib*	Gentamicin	Aminoglycoside	Acetyltransferase	GCTGATGTTGCTGACGTTGATGCTGTTGATGCTGTT
17	*aphA*	Kanamycin	Aminoglycoside	Phosphotransferase	GTTGATGCTGATGTTGCTGATGACGTTGCTGTT
18	*aadA1*	Streptomycin	Aminoglycoside	Adenyltransferase	GCTGATGTTGACGTTGCTGATGTTGATGCTGTT
19	*armA*	Amikacin	Aminoglycoside	*16S rRNA* methylation	TTGCTGATGTTGATGCTGACGTTGCTGATGTT
20	*sul1*	Sulfamethoxazole	Sulfonamides	Folate pathway inhibition	GATGCTGTTGATGACGTTGCTGATGTTGCTGTT
21	*sul2*	Sulfadiazine	Sulfonamides	Folate pathway inhibition	TTGCTGATGTTGACGTTGCTGATGTTGATGCT
22	*dfrA*	Trimethoprim	Diaminopyrimidines	DHFR modification	GCTGATGTTGCTGACGTTGATGCTGTTGAT
23	*floR*	Florfenicol	Phenicols	Efflux pump	GTTGATGCTGATGTTGCTGACGTTGATGCT
24	*cat*	Chloramphenicol	Phenicols	Acetyltransferase	GCTGATGTTGACGTTGCTGATGCTGTT
25	*mcr-1*	Colistin	Polymyxins	Lipid A modification	GTTGCTGATGTTGACGTTGATGCTGATGTT
26	*arnT*	Polymyxin B	Polymyxins	LPS modification	GCTGATGTTGCTGACGTTGATGCTGTT
27	*intI1*	—	Integrons	Gene cassette integration	GATGCTGTTGATGACGTTGCTGATGTT
28	*qacEΔ1*	Disinfectants	QAC resistance	Efflux pump	TTGCTGATGTTGACGTTGATGCT
29	*acrB*	Multiple	MDR	RND efflux pump	GCTGATGTTGACGTTGCTGATGTT
30	*tolC*	Multiple	MDR	Efflux channel	GTTGCTGATGTTGACGTTGAT
31	*marA*	Multiple	MDR	Transcriptional regulator	GCTGATGTTGCTGACGTTGAT
32	*soxS*	Multiple	MDR	Stress response regulator	TTGCTGATGTTGACGTTGCT
33	*strA*	Streptomycin	Aminoglycoside	Phosphotransferase	GCTGATGTTGACGTTGCT
34	*strB*	Streptomycin	Aminoglycoside	Phosphotransferase	GTTGCTGATGTTGAC
35	*ant(3")-Ia*	Gentamicin	Aminoglycoside	Nucleotidyltransferase	GCTGATGTTGCTGAC
36	*bla*CMY	Cephalosporins	AmpC β-lactams	β-lactamase	GTTGCTGATGTTGAC
37	*bla*PER	Ceftazidime	ESBL	β-lactamase	GCTGATGTTGAC
38	*bla*VEB	Cephalosporins	ESBL	β-lactamase	GTTGCTGATGTT
39	*rpoB*	—	Control	Housekeeping gene	GCTGATGTTGACGTTGCT
40	*gyrB*	—	Control	Housekeeping gene	GTTGCTGATGTTGAC
41	*recA*	—	Control	DNA repair protein	GCTGATGTTGAC

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RESULTS

A total of nine different strains were successfully isolated. Three strains were isolated from shrimp, and three strains were isolated from salmon fish, two strains from mussels, and one strain from oysters. All isolates grew successfully on the selective agar. *Aeromonas salmonicida* isolates were distinguished phenotypically by colony morphology, such as shape, size, and growth features, rather than by the green coloration of the colonies (Figure 1). All observations confirmed the presence of *A. salmonicida* isolates in different types of imported seafood, with colony morphology on selective agar used as a preliminary identification step, followed by confirmatory biochemical and molecular characterization (Table 4).

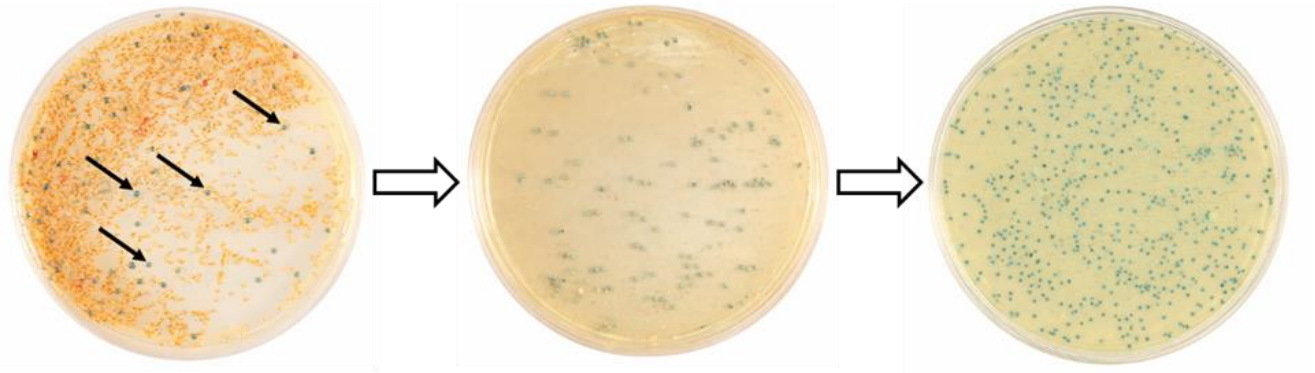

Figure 1. Isolation and identification of *Aeromonas salmonicida* from imported seafood in Mosul city, Iraq. Selective plating was used to isolate presumptive colonies (marked by arrows), which were then sub-cultured to reduce background flora. The last re-streak has produced well-isolated, shiny green colonies characteristic of *Aeromonas salmonicida*, aiding identification.

Table 4. Distribution of *Aeromonas salmonicida* isolates obtained from imported seafood samples in Mosul city, Iraq

Seafood Type	Samples (number)	Isolates (number)	Isolation rate (%)
Shrimp	5	3	60%
Mussels	5	2	40%
Oysters	5	1	20%
Salmon fish	5	3	60%
Total	20	9	45%

VITEK 2 compact system identification

The VITEK 2 compact system was used to further identify nine presumptive *A. salmonicida* isolates recovered on selective agar. Biochemical analysis revealed a similar reaction pattern across all isolates. The isolates exhibited positive reactions in different key enzymatic and metabolic assays, including pyrrolidonyl arylamidase (PyrA), glycine arylamidase (GlyA), ODC, lysine decarboxylase (LysA), D-mannose utilization/fermentation (dMNE), tyrosine arylamidase (TyrA), sucrose fermentation/utilization (SAC), D-glucose utilization/acid production (dGLU), oxidative–fermentative test for glucose metabolism (OFF), and alanine arylamidase (AlaA). Isolates revealed no significant differences; thus, nine strains were phenotypically homogeneous. According to the identification results obtained with the VITEK 2 compact system, all green colonies isolated from selective agar were confirmed as *A. salmonicida*. Selective culturing and automated biochemical identification were accurate methods for detecting *A. salmonicida*, as indicated by biochemical cassette results from imported seafood (Figure 2).

Figure 2. Identification card of the VITEK 2 compact system used for biochemical profiling of the bacterial isolates. The cassette contains a range of micro-wells containing dehydrated substrates that generate colorimetric signals upon inoculation and incubation, enabling automated identification of *Aeromonas salmonicida* in the VITEK 2 compact system.

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Molecular identification

All nine *A. salmonicida* isolates successfully underwent PCR amplification of the *16S rRNA* gene. The distinct bands of DNA were clearly visible in all nine lanes of the agarose gel electrophoresis (Figure 3). The size of each DNA band was approximately 600 base pairs in comparison with the 1 kb DNA ladder (Lane M). No nonspecific bands or primer-dimer formation was observed, indicating high primer specificity and optimal PCR conditions. The current results demonstrated that genomic DNA was successfully isolated and that the PCR assay employed was dependable. Amplification of the *16S rRNA* gene confirmed the molecular identification of the bacterial isolates as *A. salmonicida*. These findings suggested that *16S rRNA*-based PCR was an effective method for identifying *Aeromonas* species in bacterial isolates.

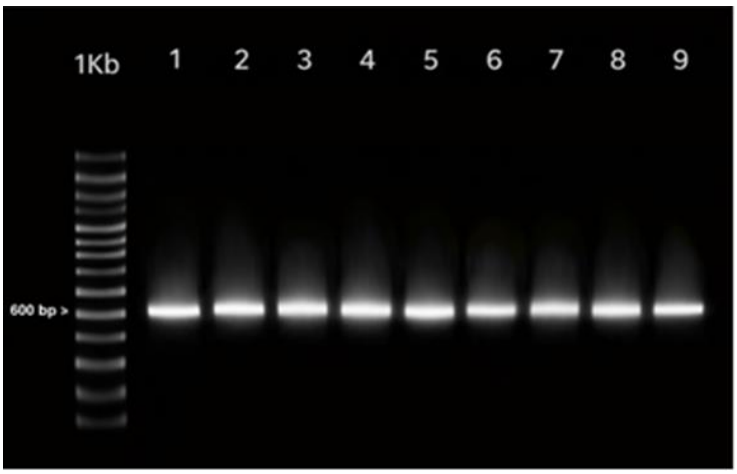

Figure 3. Gel electrophoresis results of nine *Aeromonas salmonicida* isolates obtained from imported seafood samples in Mosul city, Iraq. Lane M: 1 kb DNA ladder. Lanes 1-9: DNA fragments with a size of approximately 600 bp, indicative of *Aeromonas salmonicida*

Phylogenetic tree relationships of *Aeromonas salmonicida* strains

The evolutionary relationships among *A. salmonicida* strains based on *16S rRNA* gene sequences are illustrated in Figure 4. A phylogenetic tree was determined to be closely related to the *A. salmonicida* ATCC 33658 strain and the *A. salmonicida* strain NCIMB 1102. The CECT 894 strain demonstrated an earlier divergence, establishing a separate lineage distinct from the ATCC and the NCIMB strains. These strains formed a cluster with strong bootstrap support (85%), indicating close relationships and suggesting a common ancestry with high confidence. Based on isolate analysis, the wild-type *A. salmonicida* genome from NCBI was identified as the most distantly related among the four strains. The scale bar represented 0.00200 substitutions per nucleotide site, with shorter branch lengths indicating greater sequence similarity and closer phylogenetic relatedness.

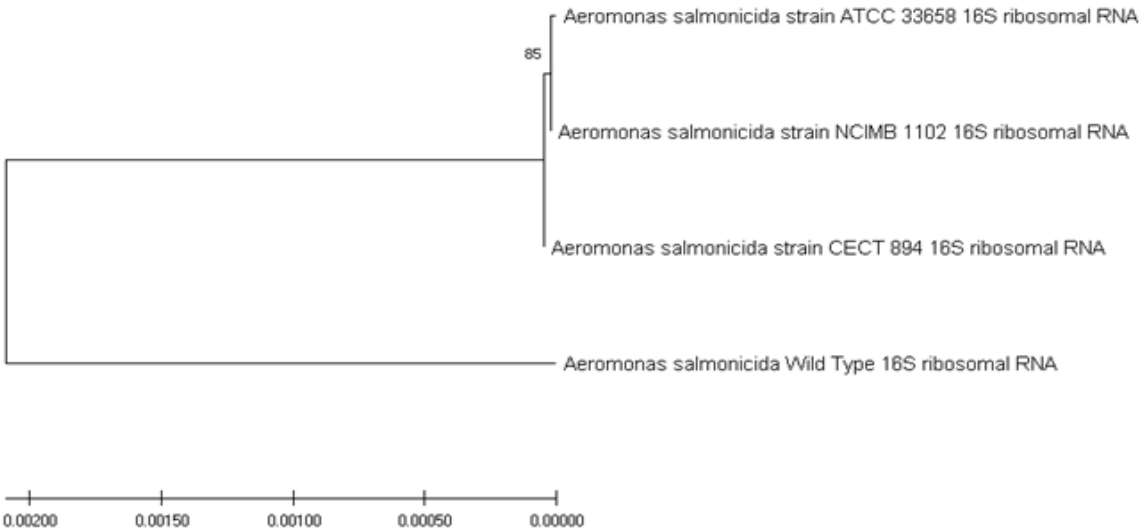

Figure 4. Phylogenetic tree of the *Aeromonas salmonicida* strains based on sequences of the *16S rRNA* gene. The tree was constructed by the Neighbor-Joining method, and bootstrap values (based on 1000 replications) are shown beside the branches.

Microarray detection of antibiotic resistance genes

Microarray analysis revealed significant variation in the distribution of antibiotic resistance genes among the nine strains of *A. salmonicida* isolated from imported seafood. All seven strains (samples 1 to 7) were identified as MDR, exhibiting resistance against common antibiotics. In contrast, samples 8 and 9 exhibited no antibiotic resistance genes (0 out of 38 screened) and were classified as antibiotic-sensitive isolates.

All seven MDR isolates were positive for *bla*TEM, *cphA*, and *bla*OXA(I), demonstrating broad-spectrum β-lactamase activity across the entire group of MDR. None of the MDR isolates carried genes coding for extended-spectrum β-lactamases (ESBLs) such as *bla*SHV, *bla*CTX-M, *bla*FOX, *bla*PER, *bla*VEB, or *bla*CMY. Sensitive isolates had no resistance genes detected (samples 8 and 9). Importantly, all the isolates, including the MDR strains, were devoid of any ESBL-associated genes, suggesting that the resistance patterns resulting from these mechanisms are unlikely in the present case. The consistent presence of *bla*OXA(I) in all MDR isolates indicated that this oxacillinase plays a specific role in β-lactam resistance. In contrast, non-MDR strains exhibited multiple *bla*OXA gene sequences (Figure 5).

All the MDR strains (1-70) carried the *tetE* genes, detected in all samples. In contrast, *tetA* was detected in only two, specifically in samples 1 and 4 (28.6%), which had the highest number of resistance genes. The current finding indicated that all MDR isolates lacked the *tetB* and *tetD* genes. The present investigations identified the sulfonamide resistance gene *sul1* in all seven MDR isolates. However, *sul2* was absent in all of the MDR strains. Even though sul2 was part of the screened targets onto the micro-array panel, it was not detected in any isolate. Significantly, the sensitive strains (samples 8 and 9) showed the absence of resistance genes since sul2 and other resistance genes were not found in strains. All MDR strains exhibited resistance to trimethoprim, which was attributed to the presence of the *dfrA* gene (Figure 6).

The MDR isolates exhibited a broad distribution of aminoglycoside resistance genes. Comprehensive gene profile analysis demonstrated that all seven MDR strains contained the *aphA* and *aadA1* genes. Additionally, three of the seven strains (42.9%), specifically samples 1, 4, and 5, possessed the *aac(6')-Ib* gene. Moreover, the *armA* gene, associated with high-level aminoglycoside resistance, was absent from all MDR isolates. The *floR* gene, conferring resistance to phenicol, was identified in all seven MDR isolates, whereas the cat gene was not detected in any MDR isolate (Table 5).

The present results confirmed that *gyrA* plays a crucial role in fluoroquinolone resistance within the MDR group. In comparison, *parC* was identified in three of seven strains (42.9%), specifically in samples 1, 4, and 5. Additionally, *qnrA*, *qnrB*, and *qnrS* genes were co-detected in these samples 1, 4, and 5, indicating a combined resistance mechanism. The presence of *intI1* and *qacEΔ1* genes, associated with integrons, was noted in all seven strains. *AcrB* and *TolC* components of the efflux pump were found in all MDR strains, suggesting that efflux-mediated resistance was a widespread mechanism across MDR strains.

The MDR isolates harbored more resistance genes than the sensitive isolates. The average number of resistance genes in MDR isolates (16.43 ± 3.05) was higher than in sensitive isolates (0.00 ± 0.00). According to the present results, microarray-based profiling can distinguish between resistant and sensitive isolates and characterize the distribution of antibiotic resistance genes (Figure 7).

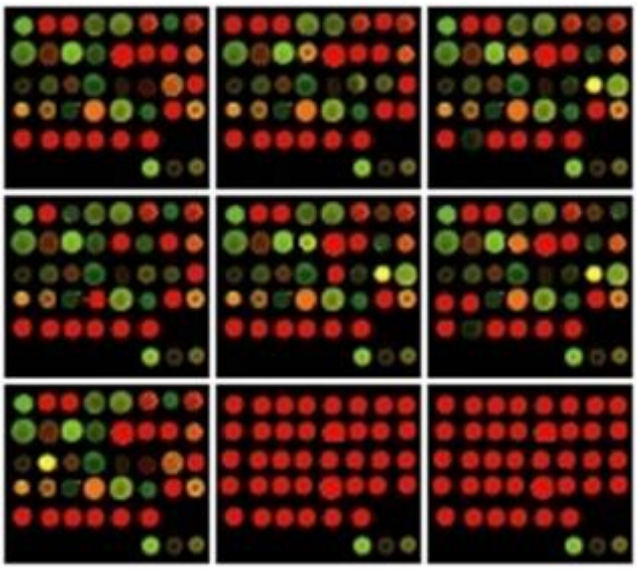

Figure 5. Antibiotic resistance genes' hybridization patterns of *Aeromonas salmonicida* strains in a DNA microarray chip. Green spots indicated the gene is present, red spots indicated the gene was absent. Samples 1 to 7 were multidrug-resistant, and samples 8 and 9 contained no resistance genes. Internal controls were housekeeping genes.

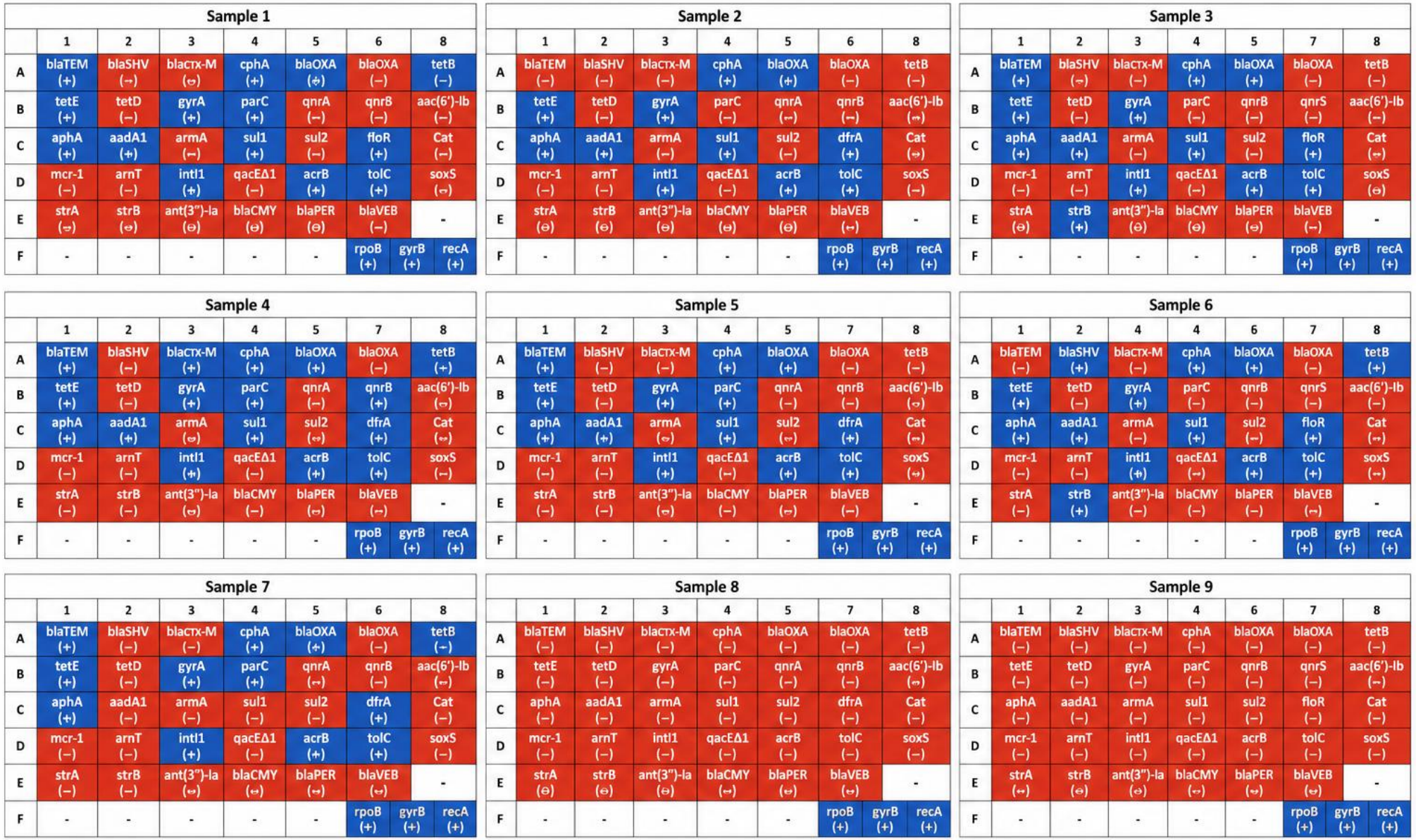

Figure 6. Microarray detection of antibiotic resistance and multidrug resistance genes in nine *Aeromonas salmonicida* strains isolated from imported seafood in Mosul city, Iraq. The layout shows the distribution of gene probes in a 6 × 8 array. Rows A-F and columns 1-8 represent positional coordinates used to identify individual probe locations

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Table 5. The microarray analysis results based on antibiotic resistance probes

Sample ID	Status	Resistance genes (n)	Susceptible loci (n)	Resistance genes detected
1	MDR	20	18	*bla*TEM, *cphA*, *bla*OXA(I), *tetA, tetE, gyrA, parC, qnrA, qnrB, qnrS, aac(6')-Ib, aphA, aadA1, sul1, dfrA, floR, intI1, qacEΔ1, acrB, tolC*
2	MDR	14	24	*bla*TEM, *cphA*, *bla*OXA(I), *tetE, gyrA, aphA, aadA1, sul1, dfrA, floR, intI1, qacEΔ1, acrB, tolC*
3	MDR	14	24	*bla*TEM, *cphA*, *bla*OXA(I), *tetE, gyrA, aphA, aadA1, sul1, dfrA, floR, intI1, qacEΔ1, acrB, tolC*
4	MDR	20	18	*bla*TEM, *cphA*, *bla*OXA(I), *tetA, tetE, gyrA, parC, qnrA, qnrB, qnrS, aac(6')-Ib, aphA, aadA1, sul1, dfrA, floR, intI1, qacEΔ1, acrB, tolC*
5	MDR	19	19	*bla*TEM, *cphA*, *bla*OXA(I), *tetE, gyrA, parC, qnrA, qnrB, qnrS, aac(6')-Ib, aphA, aadA1, sul1, dfrA, floR, intI1, qacEΔ1, acrB, tolC*
6	MDR	14	24	*blaTEM*, *cphA*, *bla*OXA(I), *tetE, gyrA, aphA, aadA1, sul1, dfrA, floR, intI1, qacEΔ1, acrB, tolC*
7	MDR	14	24	*bla*TEM, *cphA*, *bla*OXA(I), *tetE, gyrA, aphA, aadA1, sul1, dfrA, floR, intI1, qacEΔ1, acrB, tolC*
8	Sensitive	0	38	None detected
9	Sensitive	0	38	None detected

MDR: Multidrug-resistant. Resistance Genes (n): The number of resistance determinants detected per isolate. Susceptible Loci (n): The number of screened panel targets returning no detectable resistance gene (total panel = 38 targets). The two susceptible isolates (Samples 8 and 9) had no detectable resistance genes (n = 0).

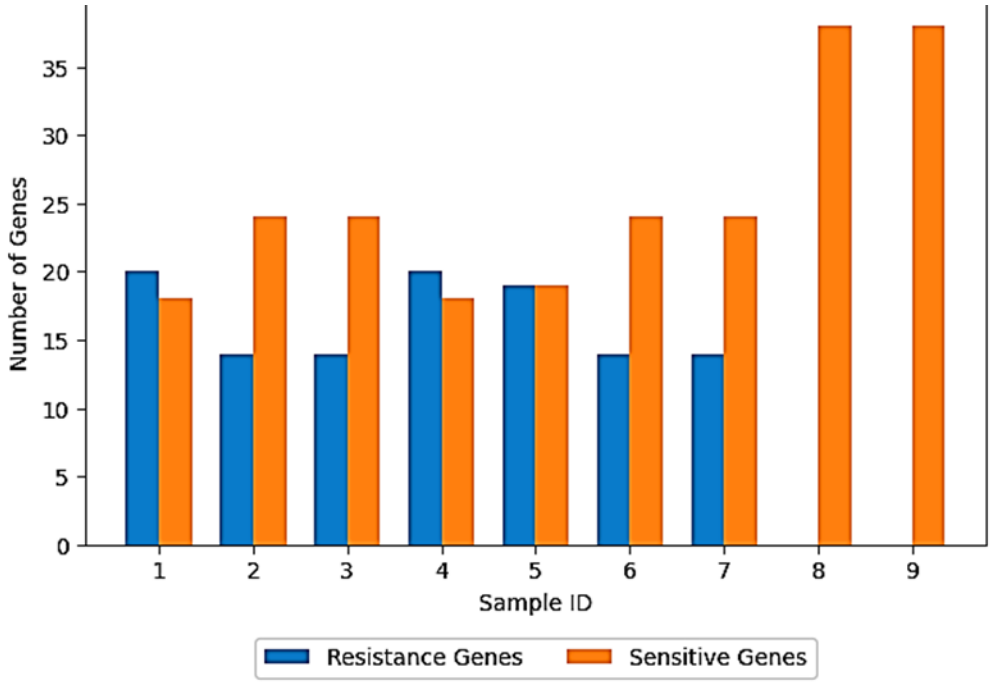

Figure 7. Distribution of resistance and sensitive gene across nine *Aeromonas salmonicida* strains isolated from imported seafood in Mosul city, Iraq, via the microarray technique

DISCUSSION

Antibiotic-resistant aquatic pathogens pose a major public health threat to humans due to the increasing international seafood trade (Odeyemi et al., 2023). Similar to the present study, Tate et al. (2022) identified *A. salmonicida* in retail seafood at a relatively high prevalence of 45%, and assessed its potential impact on food safety and public health. The detection rate for *A. salmonicida* in imported seafood, ranging from 30% to 50%, was similar across Asia and Europe, likely due to their shared origins and characteristics (Pradhan et al., 2023). The high detection levels might be attributed to extensive use of antibiotics in aquaculture, insufficient monitoring of imported products, and virulence factors (Bondad-Reantaso et al., 2023). Although the current study did not provide experimental confirmation, Lerfall et al. (2024) indicated that MDR isolates can survive refrigerated and frozen storage, potentially enabling their persistence in the seafood supply chain and increasing consumer exposure. Notably, shrimp and salmon had the highest detection rates for *A. salmonicida*, at 60% each, with mussels at 40% and oysters at 20%. These differences might be attributed to differences in farming practices, environmental exposure, and post-harvest handling (Blosser, 2022). The isolation rates in oysters and mussels might be due to their harvest from natural or semi-natural environments (Blosser, 2022). Intensive aquaculture systems are employed to cultivate shrimp and salmon, which frequently use antibiotics for prophylaxis and growth promotion (Getahun et al., 2025). Selective culture media facilitated preliminary identification, and the VITEK 2 compact system biochemically verified the isolates as *A. salmonicida*. Homogeneous biochemical profiles with a confidence level of ≥ 95% across all isolates supported the validity of this method (Feregrino et al., 2025). The VITEK 2 compact system is well-documented for its rapid and reliable identification of *Aeromonas* species from food and environmental samples, corroborating the previous observations of Kitagawa et al. (2022).

During the present study, molecular confirmation using the *16S rRNA* gene identified nine isolates as *Aeromonas* species. However, phenotypic identification with the VITEK 2 compact system consistently classified all strains as *A. salmonicida*. Phylogenetic analysis revealed that the isolates clustered closely with reference strains from different geographical origins (Long et al., 2023). According to the DNA microarray analysis in the present study, 77.8% of isolates carried multiple antibiotic resistance genes, whereas only 22.2% were entirely susceptible (Connor et al., 2023). The presence of MDR bacteria in retail seafood poses a major health threat to consumers, particularly immunocompromised individuals, and indicates a growing public health concern due to antimicrobial resistance (Sharma et al., 2023).

Although β-lactam resistance was the most frequently detected phenotype among the isolates, no ESBL genes were identified in any of the MDR strains. However, the presence of *bla*TEM, *cphA*, and *bla*OXA was concerning because these enzymes confered resistance to critically important antibiotics used in both human and veterinary medicine. Consistent with the present findings, Long et al. (2023) reported detecting *Aeromonas* isolates from aquaculture

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environments, emphasizing the widespread presence of β-lactam resistance, likely due to the extensive use of these antibiotics in fish farming.

Among the tetracycline resistance genes, *tetE* was identified in all isolates, *tetA* in 28.6% of isolates, and *tetB* was not observed. The widespread presence of the *tetE* gene indicated extensive exposure to tetracycline and the spread of tetracycline resistance genes in the aquaculture environment. The most commonly used antibiotic on fish farms is tetracycline due to its low cost and broad-spectrum activity (Amangelsin et al., 2023). The frequent administration of these antibiotics has driven the development of resistant strains, reflected in the high detection rates of efflux pump resistance genes (Vilela et al., 2022).

The sulfonamide resistance gene *sul1* was detected in 100% of MDR isolates, whereas *sul2* was absent in all strains, consistent with the established association between sulfonamide resistance and integrons (Bhat et al., 2023). The detection of sulfonamide- and β-lactam-resistance genes in the same strains indicated the concurrent accumulation of diverse resistance determinants, raising notable epidemiological concerns (Elbaiomy et al., 2025).

Aminoglycoside resistance genes were identified in 100% of MDR isolates; the *aphA* and *aadA1* genes were universally present, while *aac(6')-Ib* was detected in 42.9% (3/7) of strains. These genes encode enzymes that modify and deactivate aminoglycosides. These genes are predominantly carried on integrons and plasmids (Ali et al., 2024). These resistance genes indicated that *A. salmonicida* was developing MDR, potentially reducing the effectiveness of treatments in human infections.

Integrons are essential for the acquisition, expression, and dissemination of resistance gene cassettes. In the present study, all seven MDR isolates (77.8% of the nine total isolates) contained integron-associated genes (*intI1* and *qacEΔ1*). The detection of *A. salmonicida* underscored the potential for horizontal gene transfer within the *Aeromonas* genus and to other enteric pathogens through consumption of contaminated seafood (Pessoa et al., 2022), thereby exacerbating the public health concern associated with MDR bacteria in food. The overall prevalence of MDR strains distinctly indicated deficiencies in the surveillance and regulation of imported seafood.

The present findings indicated that marine seafood was a major reservoir of MDR *A. salmonicida*. The current study employed DNA microarray technology, which was advantageous for comprehensive resistance profiling and enabled the detection of multiple resistance factors within a single assay (Yamin et al., 2023). The high prevalence of MDR *A. salmonicida* in imported fish sold in Mosul city, Iraq, posed a notable public health risk. The current findings highlight the urgent necessity to enhance regulatory policies on antibiotic use in aquaculture, as indicated by Ljubojević Pelić et al. (2024).

CONCLUSION

The current study indicated that imported seafood in Mosul City, Iraq, was a potential reservoir for MDR *A. salmonicida*. *Aeromonas salmonicida* was detected in 45% of seafood samples, predominantly in shrimp and salmon, with the highest contamination levels. Molecular and phenotypic investigations indicated that the majority of isolates (77.8%) harbored genes conferring multiple antibiotic resistance, mainly for beta-lactams, tetracyclines, sulphonamides, and aminoglycosides. Genes associated with integrons have been identified, indicating the potential of horizontal transfer of *A. salmonicida* within the food chain. The current results indicated a public health threat associated with imported seafood sold in Mosul, Iraq. The primary constraint of the present study was its limited sample size, which might not adequately represent the prevalence of MDR *A. salmonicida* in imported seafood. Future studies should focus on improved regulation and consistent monitoring of imported seafood in markets. Furthermore, it is essential to ensure enhanced stewardship of antibiotic use in aquaculture to mitigate the dissemination of antimicrobial resistance.

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Authors' contributions

Ayman Albanna, Aseel A. H. Al-Layla, and Sahar Lukman Al-Saleem equally contributed to the study's design, data collection, analysis, and manuscript preparation. All authors collaborated in a distinct revision of the manuscript's final edition. All authors have read and approved the final edition of the manuscript.

Availability of data and materials

The data that support the present study are available within the article. The corresponding author can provide additional datasets upon reasonable request.

Competing interests

The authors declared no conflict of interest.

Ethical considerations

All procedures were carried out following institutional standards and data confidentiality. The authors confirmed that the study is original, not under consideration elsewhere, complies with data integrity and anti-plagiarism standards, and that no artificial intelligence tools were used in the preparation of the manuscript.

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