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Prevalence and Antibiogram of Multidrug-Resistant *Aeromonas* spp. Isolated from Clinical and Environmental Sources in Duhok City/ Iraq

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ABSTRACT

The genus *Aeromonas* comprises opportunistic pathogens of increasing clinical and environmental concern, mainly in aquatic and healthcare-related environments. This study was aimed at ascertaining the occurrence and antimicrobial resistance of *Aeromonas* bacteria from different environmental and clinical samples obtained from Duhok City, Kurdistan Region, Iraq. A total of 387 samples (180 environmental and 207 clinical) were analyzed using phenotypic, biochemical, automated Vitek 2 compact system, and molecular methods, utilising the PCR amplification of the *GCAT* gene, to confirm the presence of *Aeromonas* bacteria. Environmental samples had a higher rate of isolation (38/180; 21.1%) than clinical samples (3/207; 1.45%). In general, out of 51 phenotypically presumptive isolates, 41 (80.39%) were confirmed by PCR. The cumulative rate of multidrug resistance MDR was 97.56%. The environmental isolates recorded an MDR rate of 97.37%, and 100% within the clinical isolates.

The highest resistance was exhibited by tested antimicrobials against imipenem (95.12% total; 94.74% environmental; 100% clinical), followed by amikacin (90.24% total; 92.11% environmental; 66.67% clinical) and tetracycline (85.37% total; 86.84% environmental; 66.67% clinical). Moderate resistance rates were detected for trimethoprim/sulfamethoxazole, meropenem and aztreonam.

On the other hand, the lowest resistance rates were against ciprofloxacin (39.02%; environmental: 34.21%, clinical: 100%) and chloramphenicol (26.83%; environmental: 26.32%, clinical: 33.33%). This study reveals the environmental and clinical prevalence and multidrug resistance of *Aeromonas* bacteria, especially in hospital-associated microhabitats, and their significance to public health. The results suggest environmental surveillance on a regular basis, increased infection control, and more restrictive antibiotic policies in the region.

Keywords: *Aeromonas*, environmental reservoirs, multidrug resistance, Duhok.

INTRODUCTION

The genus *Aeromonas*, first described by Sanarelli in 1891, has undergone tremendous taxonomic and nomenclatural advancement in the last century. Initially placed under the family Vibrionaceae, *Aeromonas* is now part of the family Aeromonadaceae, class Gammaproteobacteria. *Aeromonas* includes a range of Gram-negative, facultative anaerobic rod-shaped bacteria that mainly thrive in aquatic systems (Janda and Abbott, 2010). They can be found in all sorts of water, from salty to fresh, wastewater, and even drinking water (Fernández-Bravo and Figueras, 2020).

Infections triggered by *Aeromonas* are not reported very often, but studies in different areas show that the rates can vary. This might be due to differences in public health systems, weather conditions, environmental factors, and socioeconomic conditions (Li *et al*., 2022; Sadeghi *et al*., 2023). While some places can maintain stable incidence rates, factors like seasonal flooding, exposure to recreational waters, climate change, rising antibiotic resistance, and growing populations can change the patterns (Habeeb *et al*., 2014; Assafi *et al*., 2022; Sinclair *et al*., 2022). Warmer temperatures have been linked to higher rates of antibiotic resistance, making it harder to treat infections caused by *Aeromonas*. This resistance can spread more quickly in places with weak public health systems (Grilo *et al*., 2021).

The pathogenicity of *Aeromonas* is usually considered multifactorial because there are different antibiotic resistance genes and virulence determinants reported, and metabolic adaptation can be associated with the survival of such bacteria in other habitats (de Melo *et al*., 2019). The bacteria are established pathogens in a broad range of aquatic animals and are consistently implicated in disease outbreaks in aquaculture systems. In humans, there is an increasing hospitalization prevalence for aerobic infections ranging from gastroenteritis to systemic and life-threatening infections (Bartie and Desbois, 2024). Gastrointestinal disease is commonly linked to the consumption of contaminated water or food, particularly in populations with inadequate hygienic amenities (Pessoa *et al*., 2022). *Aeromonas* spp. are multidrug resistant when isolated from clinical and environmental habitats (Nhinh *et al*., 2021). They have been suggested to be ecological markers of water pollution since they harbor antibiotic-resistance genes acquired from, for example, wastewater effluents (Conte *et al*., 2021).

The 237 bp *GCAT* gene, which encodes the glycerophospholipid-cholesterol acyltransferase, is commonly used as a molecular probe for the identification of bacterial members in the genus *Aeromonas*. Initially, it was identified in *A. salmonicida* as a virulence-related toxin, then it was discovered in all *Aeromonas* species as a genus-specific probe (Buckley *et al*., 1982; Chacón *et al*., 2003). Compared to phenotypic differentiation often compromised by biochemically overlapping features with those of other genera in research, the *GCAT* PCR is more specific and diagnostically precise. The assay proved effective in accurately identifying *Aeromonas* isolates, particularly in distinguishing them from morphologically or biochemically related species. The assay's broad applicability aligns with previous findings, having been validated in environmental and clinical samples (Puthucheary *et al*., 2012; Latif-Eugenín *et al*., 2016), supporting its use in both culture-dependent and -independent systems. Although some isolates may not be detected, likely due to *GCAT* locus variability or PCR inhibitors, the gene's other advantages of being highly specific, having a rapid turnaround, and being economical render it an effective frontline genus-level screen for *Aeromonas*. Its application to routine diagnostics is especially handy in resource-limited environments, and performance on this score justifies its continued utilization as a foundation of molecular identification procedures (Wilson, 1997).

Therefore, the present study aimed to determine the prevalence and antimicrobial resistance profile of *Aeromonas* bacteria isolated from diverse environmental and clinical sources in Duhok City, Kurdistan Region, Iraq, using a combination of phenotypic methods and GCAT gene-based PCR for molecular identification.

MATERIALS AND METHODS

Sample collection

Between October 2024 and January 2025, 387 samples were collected, consisting of 180 environmental and 207 clinical samples from Duhok City. Environmental samples were gathered from such sources as tap water, spring water bottles, sinkholes, sewer drains, and surfaces of hospital equipment. The clinical samples were collected from patients exhibiting gastrointestinal symptoms and consisted of stool, urine, and blood – specimens suspected of harboring *Aeromonas* due to clinical presentation.

Water samples were aseptically collected with sterile 0.5-liter bottles and processed immediately. Ten milliliters of each water sample were inoculated into 90 mL alkaline peptone water with ampicillin (APW-A; 10 mg/L), incubated at 37 °C for 24 hours, and sub-cultured on M–*Aeromonas* Selective Agar (Himedia®, India) and then incubated at 37 °C for 24 hours (Didugu *et al*., 2015). Surface swab samples were similarly enriched in APW-A before subculturing.

Clinical specimens (stool, urine, and blood) were collected using sterile swabs or containers, enriched in APW-A, and incubated at 37°C for 24 hours. Following enrichment, samples were sub-cultured on M–*Aeromonas* Selective Agar and incubated under the same conditions (El-Hossary *et al*., 2023).

Morphological identification

Gram staining was initially carried out to observe the morphological characteristics of the bacterial cells. This was followed by phenotypic characterization of the bacterial isolates using certain biochemical tests. These included lactose fermentation on MacConkey agar and beta-hemolytic activity on blood agar after a 24-hour incubation at 37°C (Srivastava *et al*., 2023; Mzula *et al*., 2019).

Since *Aeromonas* bacteria are known to possess catalase and oxidase enzymes, the catalase and oxidase reactivity of the isolates were assessed (Mailafia *et al*., 2021; Mahmood *et al*., 2024). For further confirmation, the presumptive Gram-negative *Aeromonas* isolates were analyzed using the Vitek 2 compact system (bioMérieux, France), which identifies bacterial species based on biochemical profiles derived from colorimetric and fluorometric readings (Du *et al*., 2021).

Molecular identification

For subsequent sample analysis, genomic DNA was extracted from overnight bacterial cultures using the Genomic DNA Extraction Kit (HIMEDIA®, India) according to the manufacturer's regulations. The quality and concentration of the DNA were determined using a NanoDrop spectrophotometer (Thermo Scientific, USA) based on the 260/280 absorbance ratio. Pure DNA produced 260/280 values in the range of 1.8-2.0.

The PCR of the *GCAT* gene, using primers *GCAT*-F (5'-CTCCTGGAATCCCAAGTATCAG-3') and *GCAT*-R (5'-GGCAGGTTGAACAGCAGTATCT-3'), was used to verify the identity of *Aeromonas* isolates (Latif-Eugenín *et al*., 2016). The PCR thermal cycler (Applied Biosystems, USA) was used to run the reactions, and the PCR reaction mixtures (20 µL total volume) included 10 µL 2X PCR Master Mix, 1 µL of each primer (100 pmol/µL), 2 µL DNA template (90–100 ng), and 6 µL nuclease-free water. Cycling parameters comprised an initial denaturation at 95°C for 3 minutes and 30 cycles comprising denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, with a terminal extension at 72°C for 3 minutes.

Amplicons were resolved by 2.0% agarose gel electrophoresis prepared with 1X TBE buffer and stained with Safe Gel Stain Dye (AddBio, Korea). Electrophoresis was carried out after loading the PCR products and the 100 bp DNA ladder molecular weight marker (Genesand, India) in the wells at 45 V for 15 minutes, followed by 80 V for 30 minutes. The DNA bands were subsequently visualized under UV transillumination.

Antimicrobial susceptibility testing

The antibiotic susceptibility of the isolates was measured using Kirby-Bauer disc diffusion on Mueller-Hinton agar. The isolates were suspended in sterile saline and adjusted to 0.5 MacFarland turbidity, after which a sterile swab was used to apply the bacterial inoculum evenly across the top of the agar plate. In the current study, nine selected antimicrobial agents (Bioanalyse, Turkey), representing eight distinct antimicrobial classes, were used for this test in accordance with CLSI (2024) guidelines. The names of these antibiotics, along with their respective disc contents, are detailed in Table 1. From then on, antibiotic discs were positioned on the agar and incubated for 24 h at 37°C. The diameter of inhibition zones was then individually measured and interpreted as per CLSI guidelines (Igbinosa and Okoh, 2012; CLSI, 2024).

Table (1): Key Antimicrobial Agents Employed in the Current Study

Antimicrobial Class	Antimicrobial Agent	Abb.	Disc cont. (µg/ml)
Penicillin and β-Lactam/ β-Lactamase Inhibitor Combinations	Piperacillin-Tazobactam	TPZ	100/10
Aminoglycosides	Amikacin	AK	30
Carbapenems	Imipenem	IPM	10
	Meropenem	MEM	10
Monobactams	Aztreonam	ATM	30
Tetracyclines	Tetracycline	TE	30
Fluoroquinolones	Ciprofloxacin	CIP	5
Phenicols	Chloramphenicol	C	30
Folate Pathway Inhibitors	Trimethoprim/Sulfamethoxazole	STX	23.75

Statistical analysis

Statistical analysis was conducted on the data through employing the chi-square test with SPSS software version 23 and Microsoft Excel (2021). A p-value of less than 0.05 was taken to be statistically significant, providing a basis for significant results interpretation.

Ethical approval

The study was conducted with the approval of the Ethics Committee at the Ministry of Health, Duhok Directorate General of Health, Kurdistan Region of Iraq (Approval ID: 30102024-9-30). All participants provided informed written consent and agreed to adhere to the study's procedures.

RESULTS AND DISCUSSION

Isolation and phenotypic identification of *Aeromonas* species

After selective culturing and phenotypic testing, presumptive *Aeromonas* isolates were retrieved from clinical specimens and environmental samples, with the confirmed organisms in some processed specimens being presumptive *Aeromonas* isolates post-pre-enrichment in alkaline peptone water (APW), which has been established to enhance the growth of *Aeromonas* while inhibiting the growth of interfering organisms (Didugu *et al*., 2015). Subculturing onto M-*Aeromonas* Selective Agar (Himedia®, India), with ampicillin supplementation, yielded colonies with *Aeromonas* species characteristics: smooth, convex, faintly mucoid, and yellow-pigmented, due to acid-pigment production during dextrin fermentation Fig. (1A) (Parker and Shaw, 2011).

The *Aeromonas* spp. were differentiated from other Gram-negative bacteria on the MacConkey and blood agar by further differentiation of the isolates, as the non-lactose-fermenting isolates produced colorless colonies in the MacConkey agar, in line with the non-fermentative characteristics of *Aeromonas* spp. Fig. (1B) (Janda and Abbott, 2010; Srivastava *et al*., 2023). β-hemolysis was observed on blood agar due to lysis of red blood cells by the bacteria, supporting *Aeromonas* identification and possible pathogenicity through hemolysin-mediated cytotoxic effects Fig. (1C) (Soltan Dallal et al., 2016; Mzula *et al*., 2019). Biochemical tests showed that the suspected isolates were catalase- and oxidase-positive, and the Gram staining revealed Gram-negative bacilli morphology (Parker and Shaw, 2011; Mailafia *et al*., 2021; Mahmood *et al*., 2024). To accurately depict taxonomically, isolates showing phenotypic characteristics consistent with *Aeromonas* were identified by an automated identification using the Vitek 2 Compact system that unequivocally confirmed their belonging to the *Aeromonas* genus (Du *et al.*, 2021).

Fig. 1: Phenotypic Characterization of *Aeromonas* Isolates on Selective and Differential Media
(A) Growth of distinctive yellow colonies on *Aeromonas* selective agar, indicating selective isolation.
(B) Non-lactose fermenting colonies on MacConkey agar suggest presumptive identification.
(C) Beta-hemolysis on blood agar demonstrates hemolytic activity for further differentiation.

Targeted molecular detection of *Aeromonas* using specific marker

A total of 51 *Aeromonas* isolates, identified based on their phenotypic characteristics, were put through a genus-specific PCR test that focused on the *GCAT* gene. The results from the electrophoresis analysis showed that 41 of these isolates, corresponding to 80.39%, successfully amplified the 237 bp product, confirming their classification as *Aeromonas* spp. Nevertheless, the other 10 isolates, making up 19.61%, did not produce any amplification and were thus excluded from the study.

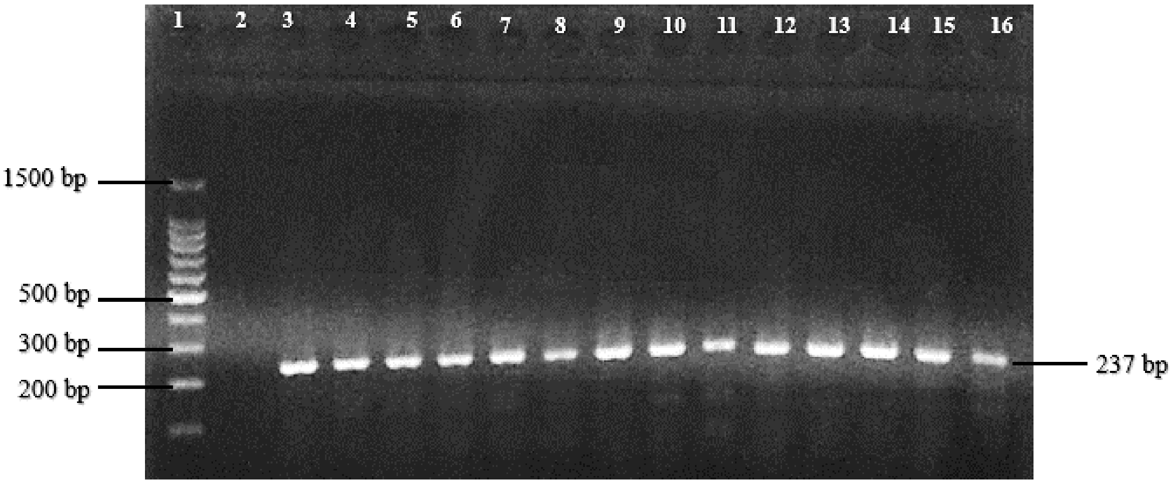

Fig. 2: Agarose gel electrophoresis of PCR amplicons targeting the *GCAT* gene (237 bp) in *Aeromonas* isolates on a 2% agarose gel. Lane 1: 100 bp DNA ladder (molecular weight marker); Lane 2: negative control, showing no nonspecific amplification; Lanes 3–16: positive amplification of the *GCAT* gene in *Aeromonas* isolates, indicated by distinct bands at 237 bp.

Prevalence of *Aeromonas* species in environmental and clinical samples

A total of 111 environmental samples from sinkholes across various locations were screened, yielding 29 presumptive *Aeromonas* isolates. PCR confirmation validated 28 isolates, indicating a notably high prevalence among suspected samples. The greatest incidence was observed at Azadi Teaching Hospital, where 12 of 13 presumptive isolates were confirmed positive. Similarly, all six isolates from residential towers tested positive. Additional confirmed isolates were recovered from Burn and Plastic Surgery Hospital (3/3), Kidney and Diseases Transplantation Center (1/1), a public eatery (1/1), a private American educational facility (2/2), and a private clinical laboratory (3/3).

Conversely, no *Aeromonas* isolates were detected in sinkhole samples from Heevi Pediatric Teaching Hospital, University of Duhok's Colleges of Nursing and Dentistry, and a premium fitness center.

Extended environmental sampling of lentic water swabs, septic waste system, and sewage systems revealed further positives. Two PCR-confirmed isolates were obtained from lentic water samples; four positive isolates were gained from septic waste system from the Kidney and Diseases Transplantation Center; samples from Azadi Teaching Hospital and Heevi Pediatric Teaching Hospital were, however, negative. Confirmed isolates were found in septic waste system from residential towers (1/1), Kidney and Diseases Transplantation Center (1/1), as well as Burn and Plastic Surgery Hospital (2/4), whereas septic waste system isolates from Azadi Teaching Hospital and Heevi Pediatric Teaching Hospital were negative. Samples taken from sewage conveyance systems that tested positive for isolates included residential towers (2/5) and the University of Duhok - College of Nursing (1/2). A sample of effluent taken from a thoracic device from the Azadi Teaching Hospital that was tested was also confirmed positive for *Aeromonas*. No *Aeromonas* bacteria were isolated from other environmental sources such as oxygen humidifiers, syringe blood residues, hemodialysis water supplies, environmental dust, dialysate filtrate water, damp soil swabs, catheter blood residues, pacemaker fluid concentrates, hose nozzle effluents, hemodialysis apparatus basins, device containers, shower enclosure glass perimeters, intensive care unit sinks, spring bottled water, or tap water.

From 207 clinical specimens tested, 10 presumptive *Aeromonas* isolates were recovered from diarrheal and mucoid stool samples (9 from diarrheal stool samples at Heevi Pediatric Teaching Hospital and one from a mucoid stool specimen at the Central Public Health Laboratory), and 3 of these were confirmed by PCR. Conversely, 41 diarrheal stool samples from the Central Public Health Laboratory and Azadi Teaching Hospital, 15 mucoid stool samples from Heevi Pediatric Teaching Hospital and Azadi Teaching Hospital, and 22 normal stool samples from Heevi Pediatric Teaching Hospital and the General Health Directorate yielded no *Aeromonas* isolates. Furthermore, 16 blood samples from Azadi and Heevi Pediatric Teaching Hospitals, as well as 24 urine samples from Azadi Teaching Hospital, Kidney and Diseases Transplantation Center, and Heevi Pediatric Teaching Hospital, did not yield *Aeromonas* isolates. This low prevalence of *Aeromonas* in clinical specimens compared with environmental samples is reflected by the fact that all confirmed isolates were found only in diarrheal and mucoid stool specimens.

Table (2): Prevalence of *Aeromonas* Isolates in Diverse Environmental Sources

Source of Isolation	Total Samples	PCR-Confirmed Positives: n (%)	p-Value
Sinkhole	111	28 (25.23%)	0.1266
Lentic Water Swab	2	2 (100%)	0.2163
Septic Waste System	10	4 (40%)	0.26811
Sewage Conveyance System	7	3 (42.86%)	0.3342
Thoracic Device Effluent	3	1 (33.33%)	0.8491
Other sources*	47	0 (0%)	-
Total	180	38 (21.11%)	0.4019

*Other sources investigated in this study included oxygen humidifiers, syringe blood residues, hemodialysis water supplies, environmental dust samples, dialysate filtrate water, damp soil swabs, catheter blood residues, pacemaker fluid concentrates, hose nozzle effluents, hemodialysis apparatus basins, device containers, shower enclosure glass surfaces, intensive care unit sinks, spring bottled water, and tap water.

Table (3): Prevalence of *Aeromonas* Isolates Across Clinical Specimen Types

Sample Type	**Total Samples**	**PCR-Confirmed Positives: n (%)**
Diarrheal Stool	98	2 (2.04%)
Mucoid Stool	47	1 (2.13%)
Normal stool	22	0 (0%)
Blood	16	0 (0%)
Urine	24	0 (0%)
Total	207	3 (1.45%)

Antimicrobial susceptibility profile of *Aeromonas* isolates

The conducted antimicrobial susceptibility testing on confirmed *Aeromonas* isolates demonstrated their widespread resistance against multiple antibiotics. The resistance levels of imipenem stood at 95.12% as the highest, along with amikacin at 90.24% and tetracycline at 85.37%. The isolates showed moderate resistance to meropenem at 41.46% and aztreonam at 41.46%, and ciprofloxacin resistance was observed in 39.02% of samples. Of the tested antibiotics, chloramphenicol demonstrated the loftiest susceptibility rate (51.22%), followed by trimethoprim-sulfamethoxazole (43.90%). Resistance to piperacillin-tazobactam was especially high (70.73%), emphasizing a challenge in β-lactam therapy. These results indicate a multidrug-resistant profile in *Aeromonas* spp. and punctuate the need for watchful antibiotic stewardship and localized resistance monitoring.

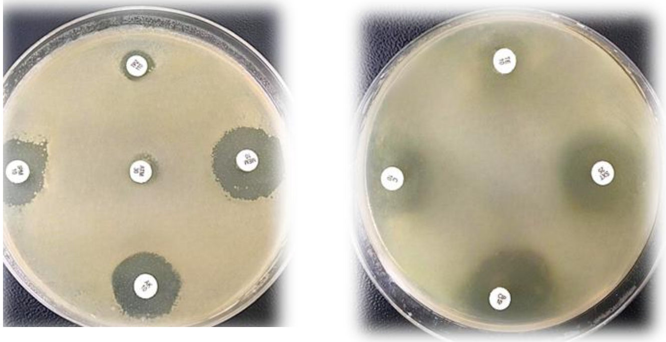

Fig. 3: Antimicrobial Susceptibility Patterns of *Aeromonas* Isolates on Mueller-Hinton Agar.

Table 4. Antimicrobial Susceptibility Profile of the Confirmed *Aeromonas* Isolates

Antibiotics	**Sensitive: n (%)**	**Resistant: n (%)**
TPZ	3 (7.32%)	29 (70.73%)
AK	2 (4.88%)	37 (90.24%)
IPM	1 (2.44%)	39 (95.12%)
MEM	12 (29.27%)	17 (41.46%)
ATM	17 (41.46%)	17 (41.46%)
TE	1 (2.44%)	35 (85.37%)
CIP	17 (41.46%)	16 (39.02%)
C	21 (51.22%)	11 (26.83%)
STX	18 (43.90%)	19 (46.34%)

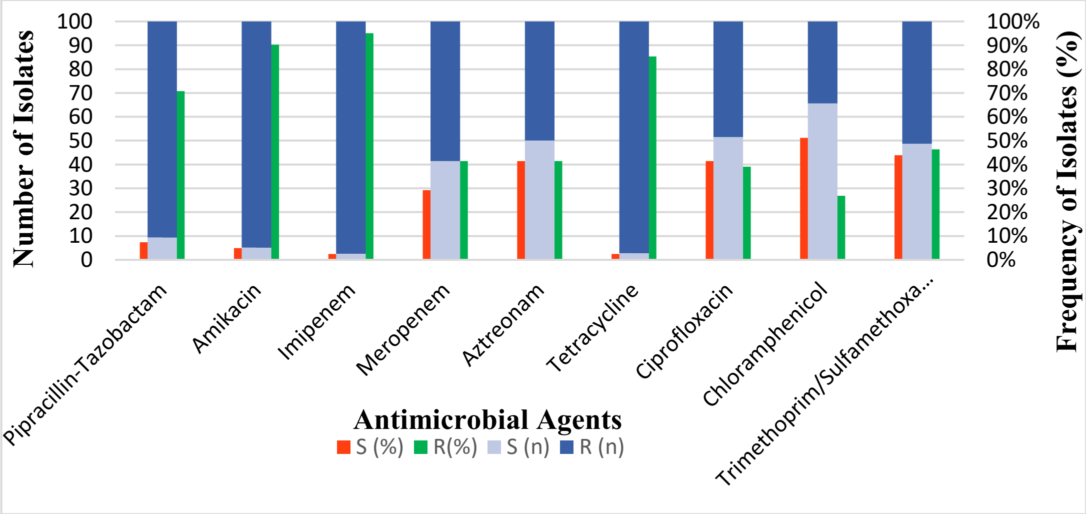

Fig. 4: Comparative Analysis Chart of Antimicrobial Resistance Profiles in *Aeromonas* Isolates

Aeromonas spp. are increasingly known for their resistance to aquatic habitats, where they are able to survive under poor sanitary conditions and may act as reservoirs of virulence and antibiotic resistance (Parker and Shaw, 2011). *Aeromonas* spp. were, significantly, isolated more frequently from environmental than clinical sources, particularly from hospital-associated environments. These observations agree with those of Fernandez-Bravo and Figueras (2020) and Goni-Urriza *et al*. (2000), who documented *Aeromonas* occurrence in hospital water and wastewater installations. Such findings support the suggestion of environmental contamination in dissemination for opportunistic infection.

This study comprehensively investigated the occurrence and phenotypic characteristics of Aeromonas species from clinical and environmental isolates in Duhok City, Kurdistan Region, Iraq.

Among 180 environmental samples tested, 21.6% of isolates were presumptively suspected phenotypically as *Aeromonas*, and 97.4% of them were confirmed by PCR amplification of the GCAT gene, resulting in an overall confirmed environmental prevalence of 21.1%. In contrast, among 207 clinical samples, only 4.8% had presumptive *Aeromonas* phenotypes based on colony morphology and biochemical pattern, and only 1.45% were confirmed by PCR. This wide disparity in prevalence buttresses the environmental predominance of *Aeromonas* species, since in previous studies by Igbinosa *et al*. (2012) ; Janda and Abbott (2010), they reported a high level of *Aeromonas* in untreated and hospital-associated water systems.

The highest contamination environmental sites were the ones collected from sinkholes, especially those associated with healthcare institutions such as Azadi Teaching Hospital and Burn and Plastic Surgery Hospital. For example, 92.3% of the isolates from Azadi sinkholes were positively confirmed by PCR. Similarly, sinkholes of residential towers and Burn Hospital each had 100% positive isolates. These findings illustrate the manner in which semi-closed, nutrient-limited aquatic microhabitats serve as amplification niches for *Aeromonas* proliferation. Goni-Urriza *et al*. (2000) also reported similar patterns of *Aeromonas* colonization of sewers and drew attention to the role of hospital wastewaters in the development of bacterial persistence and antimicrobial resistance. These findings were supported by our results, particularly in hospital sinkholes in which patients have access and which may be underestimated reservoirs for opportunistic pathogens. These microenvironments, with organic material and a continual water source, present a significant risk for immunocompromised patients during standard hygienic practices. These areas of exposure highlight previous evidence that supports the need for focused infection control strategies for the control of environmental reservoirs in healthcare settings.

Other contaminated loci were septic drainage systems, lentic water sources, thoracic device effluents, and sewage conveying networks. Recreational water bodies such as lentic water swabs also showed 100% positivity, therefore pointing up possible public health hazards. Similar issues have been brought up by (Khajanchi *et al*., 2010), who reported *Aeromonas* presence in U.S. public recreational waterways, and Janda and Abbott (2010), who noted the bacterium's ability to live in fluctuating nutrient and oxygen conditions. Children, the elderly, the immunocompromised, and those with open wounds who may come across these waters through recreational or occupational contact are among populations at high risk. Moreover, exposure to septic drainage and thoracic device effluents can lead to gastric, dermal, or respiratory infections, and the fact that *Aeromonas* is found in both healthcare-administered and community-related water systems highlights its involvement in immediate infection and environmental spread of virulence and resistance genes. These findings emphasize the need for routine microbial surveillance of clinical and public water networks, upgrading sewer infrastructure, and implementing localized disinfection practices. An integrated strategy that combines environmental management and public health action is key to reducing the burden of this arising opportunistic pathogen.

The current results indicated that there was no statistically significant association between type of environmental source and occurrence of *Aeromonas* spp. This suggested that *Aeromonas* contamination appeared to be fairly uniformly distributed among the environmental sources in the present study without strong preference or association with any of the environmental source types despite apparent numerical differences in detection rates. Conversely, *Aeromonas* was not detected in 47 samples from relatively sterile systems, including bottled water, tap water, oxygen humidifier systems, and dialysis-associated fluids. These findings strongly suggest that strict sterilization, disinfection, and water treatment protocols inhibit *Aeromonas* contamination efficiently. This was as per previous studies by Kivanc and Yilmaz (2011), who confirmed low *Aeromonas* detection in municipal water systems chlorinated when best practices are maintained. The failure to detect *Aeromonas* from drinking and treated water sources in this study contradicts that of (Kivanc and Yilmaz, 2011), who reported 100% detection from the Porsuk River but not from drinking or tap water. This can be attributed to variations in water treatment efficiency, source water quality, and environmental sanitation. Advanced filtration and chlorination treatment operations and the use of screened groundwater sources presumably reduce microbial load. Conversely, surface waters exposed to poor waste management may have more contaminants. Temperature fluctuations in seasons and local regimes of ecology, including nutrient concentrations and microbial competition, impact *Aeromonas* survival and distribution in bodies of water (Didugu *et al*., 2015). This indicate that there exists a need for ongoing monitoring and adaptive management interventions to help control *Aeromonas* contamination in our environment. Moreover, the presence of *Aeromonas* in public facilities, including gyms and schools, is a concern for possible modes of exposure for community-acquired infections.

Of 207 clinical samples cultured, three (1.45%) were PCR-positive for *Aeromonas* spp., two from diarrheal stool samples at Heevi Pediatric Teaching Hospital and one from a mucoid stool sample at the Central Public Health Laboratory. No *Aeromonas* isolates were isolated from blood or urine samples. These results indicate that while this bacterium can be an enteric pathogen, it is not prevalent in clinical stool samples and not substantial in extraintestinal sites, as evidenced by statements by (Chen *et al*., 2015: Vila *et al*., 2003), and (Senderovich *et al*., 2012). The results of this study also concur with Hashim and Nema (2018) in investigating the prevalence of *Aeromonas* spp. from a group of pediatric patients with diarrhea and analyzing 300 stool specimens from children with diarrhea visiting Fatima Al Zahra and Shaheed al-Sadr hospitals, Iraq. Twelve isolates (4%) were positive for *Aeromonas* spp. in their study, thus confirming its importance as a clinically relevant pathogen in gastrointestinal infections.

Bernabè *et al*. (2023) identified a 0.61% prevalence of *Aeromonas* spp. among 6,570 diarrheal stool specimens from Northeast Italy, and they also found that *Aeromonas* caused 24.7% of bacterial positive cases.

The same was reported by (Chen *et al*., 2015), who cultured *Aeromonas* from 2.5% of diarrheal patients' stools and 3.6% of asymptomatic controls in Southern Taiwan, replicating the current study's 3.5% in diarrheal stools. Such findings project *Aeromonas* both as an opportunistic pathogen and a member of intestinal flora, influenced by environmental exposure, sanitation conditions, and host immune variability. Supporting evidence comprises reports from Spain (Vila *et al*., 2003), noting isolation rates near 2%, and from Switzerland, where (Essers *et al*., 2000) discovered a 4.8% prevalence in children's diarrhea instances consistent with the 3.5% rate seen in the pediatric population sampled here. (Yamada *et al*., 1997) also documented elevated prevalence in symptomatic and healthy individuals in Tokyo.

In contrast, low-resource nation studies such as Peru's report substantially higher rates, for instance, 52.4% in diarrheal infants (Pazzaglia *et al*., 1991), highlighting the impact of water quality, exposure risk, and co-infections on *Aeromonas* epidemiology. It is further to be noted that in this study, no *Aeromonas* was isolated from extraintestinal samples, as seen in reports that bacteremia and urinary tract infections are rare and typically associated with immunocompromised hosts or contaminated medical devices (Ghenghesh *et al*., 2008). This lack can be indicative of successful infection prevention and antimicrobial stewardship in the investigated facilities.

The inability to recover isolates from blood and urine supports the findings observed by (Ghenghesh *et al*., 2008: Janda and Abbott, 2010) that systemic *Aeromonas* infections are infrequent and require predisposing immunocompromising situations or opportunistic nosocomial acquisition.

In addition, the 30% rate of confirmation of PCR-suspected phenotypic clinical isolates is a demonstration of the inadequacies of culture-based diagnosis and supports coupling molecular probes into everyday diagnostics. This hybrid diagnostic approach balances cost with accuracy for monitoring.

Aeromonas spp. continue to be unusual but clinically important enteric pathogens in the Duhok region, in line with global epidemiological data, and should be included in the differential diagnosis of diarrheal disease, particularly in children. The results also implicate environmental reservoirs as essential sources of pathogenic strains and justify the necessity for stringent hygiene practices and infection control measures to avert transmission risk. Molecular typing and virulence profiling of clinical and environmental isolates should be the area of interest for future studies to untangle epidemiological relationships and pathogenicity potential of *Aeromonas* in different reservoirs.

Antimicrobial resistance profiles

The results demonstrated that some groups of antibiotics are more effective than others in inhibiting the growth of bacteria. Resistance to imipenem was the highest (95.12%), which is higher than the 76.9% resistance reported by (Puah *et al*., 2022). This whopping difference may be explained by local selection pressure, environmental exposure to unregulated antimicrobials, or species composition. Amikacin resistance (90.24%) contrasted with the 0% resistance rate reported by (Puah *et al*., 2022) the >80% susceptibility rate reported by (Montalvo *et al*., 2024). This disparity is perhaps accounted for by the prevalence of aminoglycoside-modifying enzymes in local environmental strains, which is fueled by agricultural antibiotic use.

High tetracycline resistance (85.37%) closely aligns with (Sagas, 2024), where a high prevalence of tetracycline resistance genes (*tetA* and *tetE*) was reported in *Aeromonas* from aquaculture facilities. Piperacillin-tazobactam resistance (70.73%) contrasts with (Montalvo *et al*., 2024) who reported over 80% susceptibility, possibly due to local emergence of ESBLs or AmpC-type enzymes as a consequence of frequent hospital use.

Meropenem resistance (41.46%) was closely similar to the 41.9% prevalence in Southeast Asia reported by (Puah *et al*.,2022) suggesting global dissemination of carbapenem-resistant strains, likely through cphA metallo-β-lactamase genes. Aztreonam showed strain heterogeneity with similar susceptibility and resistance (41.46%), likely as a result of genomic adaptability and β-lactamase variability, supporting the finding of (Song *et al*., 2023), who noted the differential resistance patterns seen in the *Aeromonas* strains from the clinical source. Ciprofloxacin resistance (39.02%) exceeded the rates reported by (Zhou *et al*., 2019), which may be due to variations in antimicrobial stewardship, self-medication with fluoroquinolones sold over the counter, or local PMQR gene (*qnr*) and QRDR mutations in *gyrA* and *parC*. These mutations have high-level resistance and are normally underdiagnosed in resource-limited settings.

Chloramphenicol was 51.22% resistant and 26.83% susceptible, likely due to reduced usage related to toxicity concerns. This residual resistance might be sustained by conserved *cat* genes, an evolutionary trend in line with the decline of resistance through nonuse in other nations (Assafi, 2023). Resistance for the trimethoprim-sulfamethoxazole was 46.34% with a susceptibility of 43.90%. This is a moderately low resistance compared to the high 94.8% resistance reported by (Zhou *et al*., 2019) and a much higher rate when looking at (Puah *et al*., 2022) with only 11.1% resistance. The differences might be attributed to local selective pressures and the spread of *sul1*/*sul2* and *dfr* genes by mobile integrous, which are usually propagated via water, food, or hospital settings.

To gain better insight into the trend of antimicrobial resistance in *Aeromonas* environmental isolates, a specific statistical analysis was conducted. More specifically, the analysis was addressed to a selective comparison between two antibiotics to which isolates were most resistant (amikacin and imipenem) and two antibiotics to which the isolates were most sensitive (chloramphenicol and trimethoprim-sulfamethoxazole). This focused approach was taken to define the extremes of the resistance-sensitivity spectrum with a view to determining if resistance and sensitivity patterns differed meaningfully across varying environmental sampling sites. The results presented a p-value of over 0.05 ($p = 0.9999$), showing no statistically significant difference in resistance and sensitivity distribution across the varied environmental sources. The consistent antimicrobial trends across different locations most likely stem from widespread contamination with antibiotics and horizontal gene transfer among microbes, triggering uniform resistance and sensitivity. This level of variation in susceptibility may be a reflection of intrinsic resistance factors inherent in *Aeromonas* species and an effect of selective pressures due to exposure to antibiotics in clinical as well as in environmental settings. Some antibiotic classes have higher resistance levels due to misuse or environmental contamination and hence selecting resistant populations (Assafi *et al*., 2020). This striking disparity emphasizes the necessity of targeted antibiotic susceptibility testing to guide successful antimicrobial therapy of *Aeromonas* infections. Empirical treatment without sensitivity testing could undermine effectiveness due to this resistance variability. The findings underscore the necessity for continuous monitoring to inform both clinical treatment and antimicrobial stewardship efforts. The findings reinforce the importance of constant monitoring in both clinical and environmental settings to inform effective antimicrobial stewardship.

CONCLUSION

This study provides strong evidence that *Aeromonas* bacteria are more prevalent in environmental exposures, chiefly healthcare-associated exposures, than in clinical specimens in Duhok City. Their recovery by phenotypic assays and *GCAT*-targeted PCR reflected high reservoir in sinkholes, wastewater, and recreational water. Clinical prevalence, although lower, confirmed the role of *Aeromonas* as an enteric pathogen with a predominance in pediatric stool samples. The levels of multidrug resistance, particularly to carbapenems and aminoglycosides, emphasize the growing threat that these environmental isolates pose to public health. The fact that this pattern of resistance is duplicated across diverse sources suggests widespread exposure to antibiotic residues and possible horizontal gene transfer. *GCAT*-PCR was a useful genus-level molecular technology for rapid detection that could be applied to both environmental and clinical microbiology laboratories. In conclusion, this study requires immediate integrated surveillance schemes, water management facility upgrades, and judicious use of antibiotics in the healthcare and agricultural settings to fight the spread of resistant *Aeromonas* strains.

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التوزيع وتحليل المقاومة للمضادات الحيوية في أنواع البكتيرية *Aeromonas* متعددة المقاومة المعزولة من مصادر سريرية وبيئية في مدينة دهوك، العراق

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الملخص

جنس ال *Aeromonas* يتضمن ممرضات انتهازية تثير قلقًا متزايدًا سريريًا وبيئيًا، خاصة في البيئات المائية والرعاية الصحية. هدفت هذه الدراسة إلى تحديد مدى انتشار ومقاومة المضادات الحيوية لبكتيريا *Aeromonas* المعزولة من عينات بيئية وسريرية مختلفة مأخوذة من مدينة دهوك، إقليم كردستان، العراق. تم تحليل 387 عينة (180 بيئية و207 سريرية) باستخدام طرق ظاهرية، وبيوكيميائية، ونظام VITEK 2 Compact الآلي، وطرق جزيئية. كانت نسبة العزل في العينات البيئية أعلى (180/38، 21.1%) مقارنة بالعينات السريرية (207/3، 1.45%). بشكل عام، من بين 51 عزلة أولية ظاهرية، تم تأكيد 41عزلة (80.39%) بواسطة تفاعل PCR. بلغ معدل المقاومة المتعددة للأدوية (MDR) الاجمالي 97.56%، حيث سجلت العزلات البيئية معدل MDR بنسبة 97.37%، بينما كانت النسبة 100% في العزلات السريرية. أظهرت البكتيريا أعلى مقاومة لمضادات الإيميبينيم (95.12% إجمالي؛ 94.74% بيئي؛ 100% سريري)، أميكاسين (90.24% إجمالي، 92.11% بيئي، 66.67% سريري)، تتراسيكلين (85.37% إجمالي، 86.84% بيئي،66.67% سريري). كما سجلت مقاومة متوسطة لل تريميثوبريم/سلفاميثوكسازول، ميروبينيم، أزتريونام. في المقابل، كانت أقل معدلات المقاومة ضد سيبروفلوكساسين (39.02%، بيئي: 34.21، سريري: 100%)، كلورامفينيكول(26.83%، بيئي: 26.32%، سريري: 33.33%). تكشف هذه الدراسة عن الانتشار البيئي والسريري للمقاومة المتعددة للأدوية في بكتيريا *Aeromonas*، خاصة في الموائل الدقيقة المرتبطة بالمستشفيات، وأهميتها للصحة العامة. تشير النتائج إلى ضرورة المراقبة البيئية الدورية، وتعزيز مكافحة العدوى، وتبني سياسات مضادة لاستخدام المضادات الحيوية بشكل أكثر تقييدًا في المنطقة.

الكلمات الدالة: *Aeromonas*، الخوازن البيئية، المقاومة المتعددة للأدوية، دهوك.