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Isolation and Antibiotic Susceptibility of *Aeromonas* Spp. from Drinking Water in Al-Khums Libya

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عزل بكتريا *Aeromonas* spp من مياه الشرب في مدينة الخمس، ليبيا ودراسة حساسيتها للمضادات الحيوية

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Abstract:

Background: Aeromonads are frequently found in drinking water and various types of foods. A relationship between the prevalence of different human diseases and the presence of antimicrobial-resistant Aeromonas has been reported (1). Aims: This study aimed to determine the prevalence and investigate the antibacterial susceptibility profiles of *Aeromonas* species in wells, reservoirs, and bottled drinking water in Al-Khoums City, Libya. Methods: Samples of drinking water obtained from deferent water sources in AL-khoums were examined for the presence of Aeromonas and its antibacterial susceptibility using standard microbiological procedures and the BD Phoenix Automated Microbiology System (PAMS, MSBD Biosciences, Sparks, MD, USA), according to the manufacturer instructions. Results: Of 147 water samples examined, 9 (6.12%) were positive for *Aeromonas* spp. these species were isolated from 6.38% (6/94) and 6.97% (3/43) of well water and water reservoir samples, respectively. Of the isolated *Aeromonas* spp., 100% were resistant to ampicillin and amoxicillin-clavulanate,77.8% were resistant to Cefoxitin, while all (100%) were susceptible to other antibiotics such as Amikacin, Meropenem. Conclusion: Potentially pathogenic antibiotic-resistant *Aeromonas* spp. are found in some drinking water sources in Al-khums.

Keywords: Will Water, Aeromonas, Antibacterial susceptibility profile, AL-Khums, Libya.

الملخص:

الخلفية: تُوجد بكتيريا إيروموناس بكثرة في مياه الشرب وأنواع مختلفة من الأطعمة. وقد أُشير إلى وجود علاقة بين انتشار أمراض بشرية مختلفة ووجود سلالات إيروموناس المقاومة للمضادات الحيوية (1). الأهداف: هدفت هذه الدراسة إلى تحديد مدى انتشار أنواع بكتيريا إيروموناس ودراسة أنماط حساسيتها للمضادات الحيوية في الآبار والخزانات ومياه الشرب

المعبأة في مدينة الخمس، ليبيا. الطرق: تم فحص عينات من مياه الشرب المأخوذة من مصادر مياه مختلفة في الخمس - ليبيا، للكشف عن وجود بكتيريا إيروموناس وحساسيتها للمضادات الحيوية باستخدام الإجراءات الميكروبيولوجية الصارمة ونظام BD Phoenix الآلي لعلم الأحياء الدقيقةPAMS) ، MSBD Biosciences، سباركس، ماريلاند، الولايات المتحدة الأمريكية)، وفقًا لتعليمات الشركة المصنعة. النتائج: من بين 147 عينة مياه تم فحصها، كانت 9 عينات (6.12%) إيجابية لبكتيريا إيروموناس. وقد عُزلت هذه الأنواع من 6.38% (94/6) و6.97% (43/3) من عينات مياه الآبار وخزانات المياه، على التوالي. من بين سلالات بكتيريا Aeromonas المعزولة، كانت جميعها مقاومة للأمبيسيلين والأموكسيسيلين-كلافولانات، و77.8% مقاومة للسيفوكسيتين، بينما كانت جميعها (100%) حساسة لمضادات حيوية أخرى مثل الأميكاسين والميروبينيم. الخلاصة: تم العثور على سلالات من بكتيريا إيروموناس المقاومة للمضادات الحيوية، والتي يُحتمل أن تكون ممرضة، في بعض مصادر مياه الشرب في الخمس.

الكلمات المفتاحية: مياه الشرب، اروموناس، حساسية المضادات الحيوية، الخمس، ليبيا.

Introduction:

Aeromonads constitute a group of motile, Gram-negative, rod-shaped bacteria that exhibit facultative anaerobic metabolism, enabling proliferation under both oxic and anoxic conditions. These non–spore-forming microorganisms are autochthonous to aquatic ecosystems and are ubiquitously distributed across diverse freshwater and marine environments (Igbinosa *et al*., 2012). Beyond their natural habitats, *Aeromonas* spp. demonstrate notable ecological versatility, facilitating persistence in a wide range of environmental niches and contributing to their epidemiological significance as opportunistic pathogens in both human and animal hosts.

From a public health perspective, *Aeromonas* species are increasingly recognized as important zoonotic agents, capable of transmission to humans through contact with aquatic organisms or the consumption of inadequately cooked seafood (Moradi *et al*., 2025). In recent years, there has been a marked rise in the incidence of Aeromonas-associated infections, particularly among immunocompromised populations, underscoring their clinical relevance. In developing countries, these organisms have been implicated in a spectrum of pathological conditions, including gastroenteritis, wound infections, septicemia, and respiratory tract infections (Batra *et al*., 2016; Sinclair *et al*., 2023).

Taxonomically, the genus Aeromonas comprises a heterogeneous assemblage of species, encompassing more than fourteen recognized genospecies and phenospecies. Among these, *A. hydrophila*, *A. veronii biovar sobria* (commonly referred to as A. sobria), and A. caviae are the predominant species isolated from clinical, food, and water samples in developing regions (Nashnoush *et al*., 2009a; Moradi *et al*., 2025).

Elevated levels of antimicrobial resistance among *Aeromonas* spp. to commonly prescribed antibiotics have been extensively documented across several developing countries (Subashkumar *et al*., 2006; Nashnoush *et al*., 2009b; Vila and Pal, 2010). This trend reflects a broader and increasingly critical global health challenge, wherein antimicrobial resistance (AMR) exhibits a high potential for rapid dissemination. Resistance emerging in localized settings can readily propagate across national boundaries, driven by factors intrinsic to contemporary globalization, including intensified international trade, population mobility, and migration.

Addressing this escalating threat necessitates the implementation of robust, standardized, and large-scale international surveillance systems to monitor resistance patterns and inform evidence-based interventions. Despite the recognized public health importance of *Aeromonas* spp., epidemiological data concerning their prevalence in Libyan water sources remain notably limited, with existing studies confined to specific regions and lacking comprehensive national coverage (Nashnoush *et al*., 2009b).

Accordingly, the present study was designed to assess the prevalence of *Aeromonas* species and to characterize their antimicrobial susceptibility profiles in diverse potable water sources, including wells, reservoirs, and bottled drinking water, within a semi-urban locality in Libya. This investigation aims to contribute to the existing knowledge base and support the development of targeted public health strategies.

Materials and Methods:

The present investigation was conducted in the Department of Microbiology, Faculty of Pharmacy, Elmergib University, located in Al Khums, over the period from September 2021 to August 2022. A total of 147 drinking water samples were systematically collected from multiple sources, including 94 wells, 43 reservoir supplies, and 10 bottled water samples within and surrounding the Al Khums region. All samples were subjected to microbiological analysis to determine the presence of *Aeromonas* spp. as well as indicator organisms, namely coliform bacteria and Escherichia coli.

For the detection and enumeration of coliforms and *E. coli*, commercially prepared chromogenic media, Compact Dry EC and Compact Dry TC, were employed. Briefly, 1 mL aliquots of each water sample were aseptically inoculated onto the center of the dehydrated culture plates and incubated at 35 °C for 24 hours. Colony differentiation was achieved based on chromogenic reactions, whereby *E. coli* produced blue to blue-purple colonies, while other coliforms exhibited reddish, red-violet, or pink pigmentation (Tosi Robinson *et al*., 2018).

Isolation of *Aeromonas* spp. was performed using an enrichment-based protocol. Specifically, 2.5 mL of each water sample was inoculated into 25 mL of alkaline peptone water (APW; pH 8.6) and incubated overnight at 37 °C. Following enrichment, a loopful of the culture was streaked onto ampicillin blood agar (ABA) plates supplemented with 10 mg/L ampicillin and incubated at 37 °C for 24 hours. Presumptive Aeromonas colonies were identified based on morphological characteristics, including convex colonies measuring approximately 2–3 mm in diameter, with variable hemolytic activity.

Isolates exhibiting Gram-negative staining and positive oxidase reactions were preserved in tryptone soya broth containing glycerol and stored at subzero temperatures for subsequent biochemical characterization (Razzolini *et al*., 2010; Ghenghesh *et al*., 2001). Further identification and antimicrobial susceptibility testing of suspected Aeromonas isolates were conducted using standard microbiological methodologies in conjunction with the BD Phoenix Automated Microbiology System (BD Biosciences, Sparks, MD, USA), in accordance with the manufacturer's protocols.

Results:

Coliform bacteria were detected in 44 (46.8%) and 19 (44.2%) of the total water samples collected from well water and reservoir sources, respectively. Statistical analysis demonstrated that the occurrence of coliforms was significantly higher in well and reservoir water compared to bottled water ($p < 0.05$). In contrast, the highest isolation frequency of *Escherichia coli* was observed in reservoir water samples, accounting for 8 cases (18.6%). Regarding *Aeromonas* spp., no statistically significant variation in isolation rates was observed across the different water sources. The overall prevalence of *Aeromonas* spp. was 6.12% (9/147). Notably, *Aeromonas* spp. were identified in 41 samples (73.2%), whereas *E. coli* was detected in 15 samples (26.8%) of the examined subset. Species-level identification of the Aeromonas isolates revealed that 4 isolates (44.4%) were *Aeromonas veronii biovar veronii*, 3 isolates (33.3%) were *Aeromonas caviae*, and 2 isolates (22.2%) were *Aeromonas veronii biovar sobria*. The distribution and prevalence of *Aeromonas* spp., coliforms, and *E. coli* across well, reservoir, and bottled water samples are summarized in Table 1.

Table (1): prevalence of aeromonads, Coliform and *E. coli* in samples collected from well water, reservoir water, and bottled water.

Microorganisms	Number and % of positive samples			
	Well water (n=94)	Water reservoir (n=43)	Bottled water (n=10)	Total samples (n= 147)
Coliforms	44 (46.8)	19 (44.2)	0 (0.0%)	63(42.86%)
E. coli	9 (9.6)	8 (18.6)	0 (0.0%)	17(11.67%)
Aeromonas spp	6(6.4%)	3(6.9%)	0 (0.0%)	9(6.12%)

A clear seasonal variation was observed in the detection rates of *Aeromonas* spp. with it being detected more frequently in autumn 9/40 (22.5%) than in other seasons ($p < 0.03$). No significant differences were found in the isolation rates of *Aeromonas* spp and coliforms in relation to some factors (Home water connection, Opened or closed tank, and Animal enclosure) ($p > 0.05$). On the other hand, most of *Aeromonas* spp 16.17% (6/36) and coliforms 66.7% (24/36) found to be significantly more isolated from water sources who near sewage water compared with other water samples; *Aeromonas* spp in 2.9% (3/101) and coliforms in 36.6% (36/101) (P< 0.05,). The total bacteria count of the positive cultures in the 147 water samples examined ranged from 1 to 31 cfu/ml. Table 2 shows the typing of gram-negative bacteria isolated from well water and reservoir water samples. The most common gram-negative bacteria identified from water samples was *pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Enterobacter cloacae*.

Among the nine Aeromonas isolates analyzed, a high frequency of antimicrobial resistance was observed. Specifically, 7 isolates (77.8%) exhibited resistance to cefoxitin, while 2 isolates (22.2%) were resistant to gentamicin. Notably, all isolates (100%) demonstrated resistance to both ampicillin and amoxicillin–clavulanate, indicating a consistent resistance profile against β-lactam agents commonly used in empirical therapy. In contrast, complete susceptibility (100%) was recorded for several antimicrobial classes, including amikacin, meropenem, ceftazidime, ceftriaxone, cefepime, aztreonam, piperacillin–tazobactam, trimethoprim–sulfamethoxazole, and ciprofloxacin. These findings highlight a preserved efficacy of these agents against the tested Aeromonas strains. The detailed antimicrobial susceptibility patterns of the isolates are presented in Table 3.

Table (2): Gram-negativebacilli isolated from 137 water samples in El-khoums city

Organism	No (%) positive
Pseudomonas aeruginosa	34 (24.8%)
Klebsiella pneumonia	9 (6.6%)
Enterobacter cloacae	7 (5.1%)
Pseudomonas putida	6 (4.4%)
Acinetobacter baumanni	5 (3.7%)
Pseudomonas pseudoalcaligenes	3 (2.2%)
Cintrobacter farmer	2 (1.5%)
Yersinia pseudotuberculosis	1 (0.7%)
Enterobacter aerogenes	1 (0.7%)
Pseudomonas species	1 (0.7%)
Burkholderia cepacia complex	1 (0.7%)
Achromobacter species	1 (0.7%)

Table (3): Antibacterial susceptibility of *Aeromonas* species isolated from water samples.

Antibiotics	%								
	***A.* Caviae(n=3)**			**A. veronii bv. Veronii (n=4)**			**A. veronii bv. Sobria (n=2)**		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amikacin	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Gentamicin	66.6	0.0	33.3	75	0.0	25	50	50	0.0
Ertapenem	100	0.0	0.0	75	0.0	25	100	0.0	0.0
Imipenem	100	0.0	0.0	75	25	0.0	50	50	0.0
Meropenem	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Cefuroxime	100	0.0	0.0	75	0.0	25	50	0.0	50
Cefoxitin	0.0	0.0	100	25	0.0	75	100	0.0	0.0
Ceftazidime	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Ceftriaxone	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Cefepime	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Aztreonam	100	0.0	0.0	100	0.0	0.0	0.0	0.0	100
Ampicillin	0.0	0.0	100	0.0	0.0	100	0.0	0.0	100
Amox-clav	0.0	0.0	0.0	0.0	0.0	100	100	0.0	0.0
Pipe-Taz	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Tri-sulf	100	0.0	0.0	100	0.0	0.0	50	50	0.0
Ciprofloxacin	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Levofloxacin	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0

Amox-clav= Amoxicillin-clavulanate, Pipe-Taz= Piperacillin-Tazobactam, Tri-Sulf= Trimethoprim-Sulfamethoxazole.

Discussion:

Aeromonas spp. are widely distributed in aquatic environments and are responsible for many diseases in animals and humans. The most common route of infection is through ingestion of contaminated food or water (Didugu *et al*., 2015). In the reviewing the literature, different studies have been conducted worldwide regarding the prevalence of these organisms and relevant risk factors. This is the first study of prevalence of the *Aeromonas* spp. in Al-Khums city. This project set out with the aim of isolating *Aeromonas* spp. in wells, water reservoirs, and bottled water in Al-Khums city, to determine antibacterial susceptibility to some antibacterial agents, owing to changing patterns of antimicrobial resistance, knowledge of recent regional patterns is critical to therapeutic decision-making (Ung *et al*., 2019).

Furthermore. Data regarding antibiotic resistance for Aeromonas in Al-khums is absent. The result of this study demonstrated that, the prevalence of *Aeromonas* spp. was detected in 9 (6.12%) of 147 samples examined. Of the 94 samples tested from well water 6 contained *Aeromonas* spp. (6.38%), and from 43 water reservoir 3 contained *Aeromonas* spp. (6.97%). Contrary to the findings of the present study, a previous study from Tripoli examined drinking water samples and reported rates of 41 (73%) for *Aeromonas* spp. (Nashnoush *et al*, 2009a). A previous study carried out by Ghenghesh *et al*. (2001) in Tripoli reported the isolation of *Aeromonas* spp. from 48.7% of 1000 drinking water samples from wells and other miscellaneous sources of untreated water. In 2007, Ghenghesh and his colleagues isolated *Aeromonas* spp. from 9 (18%) of water samples from mosques in Tripoli, another study in Libya detected 9 (18%) (Nashnoush, 2009b).

In São Paulo, Brazil, the isolation of *Aeromonas* spp. was more than our findings, (36.5%) (Di Bari *et al*., 2007). In addition to, a study conducted in a peri-urban area in Brazil was detected 13 (18.8%) Aeromonas were found in 6 (17.2%) collective reservoirs and 7 (21.9%) well, respectively (Razzolini *et al*., 2010), India 24 (48%) (Didugu *et al*., 2015), Nigeria 31 (25%) (Bello *et al*., 2016), Saudi Arabia 12

(16%) (Elbehiry *et al*., 2019). On the other hand, previous study reported similar findings, 6% of drinking water samples from the general supply network in Tripoli (Ben Ramadan, 2006), 12 (6%) in Brazil (Razzolini *et al*., 2008). Variation in the reported prevalence rates of *Aeromonas* spp in water samples from different regions may be due to differences in geography, source of water samples examined (e.g. from wells, bottled water or Faskia), number of water samples examined, and methods used. These findings highlight the pathogenic potential of *Aeromonas* species and pose a public health concern, and have to be considered while determining the drinking water's quality. In this study, *Aeromonas* spp. Was absent in the bottled water samples, which similar to the findings of El-Emam et al. (2003) in Libya and Di Bari *et al*. (2007) in Brazil.

Also, this samples were not contaminated by coliforms and *E. coli* when used compact dry EC test. Despite the small sample size studied, the study findings showed that bottled water is generally of good quality for drinking, which could be attributed to better hygienic practices in the bottled water industry and being properly protected during bottling and transit. 85% of gastrointestinal problems in people are caused by *Aeromonas caviae* and *Aeromonas veronii* bv. *sobria. Aeromonas veronii* bv. *sobria* and *Aeromonas caviae* are the most often identified cases of so-called traveler's diarrhea and cause enteritis with watery diarrhea (Parker and Shaw, 2011). Globally, only three species (i.e., *A. hydrophila*, *A. veronii biovar sobria* and *A. caviae*) are predominantly isolated from clinical, food and water sources (Ghenghesh *et al*., 2008). In the present study, the most frequent species were *A. veronii* bv. *sobria*, *A. caviae* and *A. veronii* bv. *veronii* were detected at approximately 22%, 33%, and 44%, respectively. Less prevalence rates of these species have been reported from another countries Hofer *et al*. (2006) in Brazil, Evangelista-Barreto *et al*. (2010) in Brazil, and Sadique *et al*. (2021) in Bangladesh. Although, similar findings were reported by Sreedharan *et al*. (2012) in Brazil, Nashnoush *et al*. (2009a) in Libya detected *A. caviae* (24%). These findings show the diversity of *Aeromonas* spp. in water sources.

The result of this study showed that a high prevalence of Aeromonas isolates was found in autumn season (22.5%). Previous study reported the occurrence of Aeromonas during spring and autumn (Sadique *et al*., 2021). While, Ghenghesh *et al*., 2001 isolated Aeromonas at their highest in the winter and lowest in the summer. Vilarruel-Lopez *et al*. (2005), in Mexico, found the largest number of Aeromonas in the summer. However, geographical location, climate zones and rainfall between different regions may play a role (Sinclair., *et al* 2023).

This study showed the prevalence of *E. coli* and coliforms in drinking water samples by using compact dry EC. Of the 147 drinking water samples examined, 42.8% and 11.5% contained coliforms and *E. coli*, respectively. In terms of coliforms, our results (42.8%) are higher than those reported in other studies. (Nashnoush *et al*., 2009b and Pant *et al*., 2016). while, the prevalence of *E. coli* from those studies was higher compared to the current study (Nashnoush *et al*., 2009a and Ghenghesh *et al*., 2007).

Pseudomonas spp. are opportunistic pathogens that have been implicated in water- and food-borne diseases (Fusco et al., 2018 and Bloomfield *et al*., 2024). A previous study carried out in Tripoli reported the *P. aeruginosa* in 40% of 50 water samples collected from water vendors (Nashnoush, 2009b). The organism was detected in nearly 30% of the drinking water samples tested in the present work. The detection of *P. aeruginosa* in drinking water samples from wells and reservoir water in Al-Khums may pose a public health problem for immunocompromised individuals.

Aeromonas spp. usually resistant to penicillins (including their combinations with classical β-lactamase inhibitors), cephalosporins, and carbapenems. Among the β-lactam antibiotics, only fourthgeneration cephalosporins remain almost uniformly active, and some studies have found that previous exposure to antibiotics may be a risk factor for Aeromonas infection (Ghenghesh *et al*., 1999; Moyer, 2006 and Sánchez *et al*., 2025). In the study, antibiotic susceptibility testing was performed with commercially available antibiotics using phoenix. The results obtained showed that all *Aeromonas* spp. are 100% sensitive to Amikacin, Meropenem, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Piperacillin-Tazobactam, Trimethoprim-Sulfamethoxazole, Ciprofloxacin, and Levofloxacin. Although there is 100% resistance to Ampicillin and Amoxicillin clavulanate. *A. caviae* is 100% resistant to Cefoxitin. *A. veronii* bv. *veronii* is 75% resistant to Cefoxitin. *A. veronii* bv. *sobria* is 50% resistant to Cefoxitin. These findings are in line with those reported by Aravena-Román et al., 2012, and other studies from Libya (Ghenghesh *et al*., 2001; Gibreel and Ghenghesh, 2005).

Multiple resistances were observed in *A veronii* bv. *veronii*, *A. caviae*, and *A. veronii* bv. *sobria*. The isolation of multiple resistant aquatic *Aeromonas* spp. from drinking water has been reported previously in other parts of the world (Borrego et al., 1991; Ghenghesh *et al*., 2001; Ghenghesh *et al*., 2007 and Nokhwal *et al*., 2025). Generally, the present investigation warrants the need to take proper measures to prevent the introduction of Aeromonas, which are resistant to these drugs, to water sources used by humans.

Conclusion

The findings of the present study demonstrate the occurrence of potentially enteropathogenic and antibiotic-resistant *Aeromonas* spp. in selected drinking water sources in Al Khums. The presence of these organisms in potable water constitutes a significant public health concern, particularly for vulnerable populations, including infants, the elderly, and immunocompromised individuals, who are at increased risk of infection and associated complications. These results underscore the urgent need for strengthened water quality monitoring and the implementation of effective treatment strategies to ensure the microbiological safety of drinking water. Furthermore, public health interventions led by local health and water authorities in Al Khums and across Libya should prioritize community education initiatives aimed at raising awareness of the hazards associated with the consumption of untreated water. Equally important is the promotion of rational antibiotic use to mitigate the escalation and dissemination of antimicrobial resistance within the community.

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