



Antibiotic Resistance of Pathogenic and Bacterial Contaminants Isolated from Fish Pond Wastewater in Bechar Province, Southwestern Algeria

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ABSTRACT

Background: This study investigates bacterial contaminants and pathogenic species isolated from fish pond wastewater (FPWW) in Bechar province, Southwestern Algeria and assesses their antibiotic resistance profile.

Methods: A total of 21 FPWW samples were analyzed using different culture media to isolate bacterial contaminants via the membrane filter technique. Following purification, all bacterial isolates were identified through biochemical tests and subjected to susceptibility testing by the Kirby-Bauer disc diffusion method on Mueller-Hinton (MH) agar and the broth microdilution technique. Antimicrobial residue detection was also carried out using the microbiological tube test method (MTT).

Result: We successfully isolated 68 bacterial isolates, classified into six bacterial groups: non-*Enterobacteriaceae* (41.18%), *Enterobacteriaceae* (38.24%), *Streptococcaceae* (8.82%), *Pseudomonadaceae* (7.35%), *Erwiniaceae* (2.94%) and *Neisseriaceae* (1.47%). According to the Chi-Square Goodness-of-Fit Test, this distribution was highly significant ($p < 0.001$). The isolated species included *Aeromonas hydrophila hydrophila*, *Streptococcus* spp., *Enterobacter* spp., *Serratia* spp., *Escherichia coli*, *Citrobacter* spp., *Klebsiella* spp., *Plesiomonas shigelloides*, *Kluyvera* spp., *Pseudomonas aeruginosa*, *Pantoea* spp., *Chromobacterium violaceum*, *Salmonella choleraesuis* and *Vibrio* spp. Susceptibility testing of the bacterial contaminants revealed variable responses among the strains, with increased resistance to β -lactam antibiotics. Multidrug resistance results showed that 17 strains (25%) exhibited resistance to three to six different antibiotic classes, with Multiple Antibiotic Resistance (MAR) indices ranging from 0.28 to 0.68. The variability in MAR indices among bacterial groups was statistically significant (ANOVA, $p = 0.0498$). Furthermore, no growth-inhibiting substances were detected in the wastewater samples using the MTT method.

Key words: Antibiotic residues, Bacterial contaminants, Fish ponds, Multidrug resistance, Wastewater.

INTRODUCTION

Aquaculture, the practice of farming fish and other aquatic organisms, is a rapidly growing industry that significantly contributes to global food security and is among the fastest-growing food production sectors (FAO, 2021). With the rising global population, increasing income levels and growing urbanization, aquaculture is expected to surpass traditional fishing by 2030. However, its environmental footprint varies depending on the species being farmed, management practices, location and local conditions (Cretu *et al.*, 2016). One of the major environmental issues in aquaculture is the deterioration of water quality. This degradation can be caused by factors like leftover feed, animal waste and the use of chemical treatments (Nabbou and Benyagoub, 2025). These pollutants negatively impact the water environment, influencing various components such as the water's physicochemical characteristics, sediment composition, plankton and bottom-dwelling organisms (Naylor *et al.*, 2000; Wang *et al.*, 2010). Contamination of both freshwater and saline water by pathogenic bacteria presents a threat to aquatic ecosystems and public health through the food chain (Pandey *et al.*, 2014).

The overuse of antimicrobial agents in aquaculture further exacerbates this issue by promoting the emergence of antibiotic-resistant bacteria (Abedin *et al.*, 2020).

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Despite growing concern, data on bacterial contamination and antibiotic resistance in aquaculture wastewater remain limited, especially in arid regions like Southwestern Algeria.

This study addresses this gap by identifying bacterial contaminants and pathogenic species in wastewater from three fish farms in Bechar and evaluating their antibiotic resistance profiles. To our knowledge, this is the first study

in this region to link aquaculture wastewater contaminants with antibiotic resistance patterns, providing insights critical for environmental monitoring and public health management.

MATERIALS AND METHODS

All experiments were conducted at the University of Bechar (Algeria), over a period of eight months, from January to August 2024.

Study area

This study focused on two fish farms located in Boukais and Taghit and Nif Rhaa-Ouakda, which are situated 50km and 90 km and 15 km, respectively, from the center of Bechar (Fig 1).

These farms are dedicated to cultivating tilapia species in concrete and plastic ponds (Fig 2).

Sampling conditions

Fish pond wastewater (FPWW) samples were collected in sterile glass flasks, following ISO 5667-1 (2023). The frequency and dates of wastewater sampling are provided in Table 1.

Bacterial isolation

The bacterial contaminants and pathogenic bacterial species, such as *Enterobacteriaceae*, *Pseudomonadaceae* and *Streptococcaceae*, were isolated using the membrane filter technique on MacConkey agar, Cetrimide agar and *Salmonella-Shigella* agar by filtering 250 mL of fish pond wastewater, as described by Benyagoub *et al.* (2018), Lipps *et al.* (2022) and Benyagoub (2023a, 2023b).

The isolates were identified following the standard microbiological procedures outlined by Tille (2018).

Biofilm formation

In this study, biofilm formation by *Pseudomonas* spp. strains -recognized as opportunistic pathogens-was assessed using the tube method, as described by Christensen *et al.* (1985) and Kirmusaoglu (2019). This qualitative assay detects biofilm-producing microorganisms based on the formation of a visible film lining the inner surface of a polystyrene test tube containing tryptic soy broth (TSB) medium inoculated with the test isolate and incubated under appropriate conditions.

Detection of antimicrobial residues in fish pond wastewater samples

Detection of antimicrobial residues were carried out using the microbiological tube test method (MTT), a qualitative technique, as outlined by Akshaya *et al.* (2023). Nutrient broth containing the pH indicator bromocresol purple was prepared and an equal volume of fish pond wastewater and bacterial suspension (v/v) was added. The reference bacterial strains tested were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 14579. The tubes were then incubated at 37°C for 18-24 hours. A positive reaction was indicated by tubes

that remained purple, as compared to the positive and negative controls.

Antibiotic susceptibility testing (AST)

The antibiotic resistance profile of bacterial contaminants, including members of *Enterobacteriaceae*, *Pseudomonadaceae* and *Streptococcaceae*, were determined using the Kirby-Bauer disc diffusion method, following the protocols outlined by Kirby *et al.* (1956), Bauer *et al.* (1959), Benyagoub *et al.* (2020a, 2020b), Benyagoub (2022) and Benyagoub (2024). For non-*Enterobacteriaceae* strains, resistance profiles were assessed using the broth microdilution technique, with triphenyltetrazolium chloride (TTC) serving as a viability indicator (Lee *et al.*, 2007; Seghir *et al.*, 2023). Inhibition zones (mm) on agar plates and MIC values on microplates were measured and categorized as susceptible (S) or resistant (R), according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. Species-specific breakpoints for *Aeromonas* spp. were applied where available.

All isolated strains were tested for antibiotic susceptibility. In this study, the following antibiotics were tested against the bacterial contaminants: Gentamicin (CN 10 µg), oxacillin (OX 1 µg), ceftiofur (CX 30 µg), chloramphenicol (C 30 µg), sulfamethoxazole-trimethoprim (SXT 25 µg), erythromycin (E 15 µg), ciprofloxacin (CIP 5 µg), ampicillin (AMP 10 µg), ofloxacin (OF 5 µg), amoxicillin-clavulanic acid (AUG 30 µg), amikacin (AK 30 µg), amoxicillin (AML 2 µg), tetracycline (TE 30 µg), trimethoprim (TM 2,5 µg), aztreonam (ATM 30 µg), cefotaxime (CTX 30 µg), ceftazidime (CAZ 10 µg), piperacillin (PRL 100 µg), ticarcillin-clavulanic acid (TTC 85 µg), fosfomycin (FOS 50 µg), nalidixic acid (NA 30 µg), ceftazidime (CZ 30 µg), tobramycin (TOB 10 µg), vancomycin (VA 30 µg), azithromycin (AZM 15 µg) and imipenem (IMI 10 µg).

Multi-drug resistant (MDR) isolates were defined as those exhibiting resistance to more than three antimicrobial classes tested (Benyagoub *et al.*, 2021a).

Calculation of the MAR index

The MAR index was determined using the formula below (Benyagoub *et al.*, 2021a; Benyagoub, 2024):

$$\text{MAR index} = \frac{a}{b}$$

Where,

MAR = Multiple antibiotic resistance.

a = Count of antibiotics to which the bacterium shows resistant.

b = Total number of antibiotics that were tested.

Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics (version 25, IBM Corp., Armonk, NY, USA) to evaluate differences in the distribution and antibiotic resistance profiles of bacterial groups isolated from fish pond wastewater. A Chi-Square Goodness-of-Fit Test was used to assess the distribution of bacterial groups, while a one-way analysis of variance (ANOVA) was performed to compare the Multiple Antibiotic Resistance (MAR) index

among the different groups. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Bacterial isolation

The bacterial species isolated from wastewater of the fish ponds are categorized based on their Gram stain characteristics (Fig 3) and bacterial groups (Fig 4).

The results of the distribution are consistent with previous findings (Sule *et al.*, 2016; Adebami *et al.*, 2020), which also reported a dominance of Gram-negative bacteria in aquaculture wastewater environments.

Table 1: FPWW sampling frequency.

FPWW samples	Dates of collection
WSb1, WSb2, WSb3	January 21, 2024
WSb4, WSn18, WSn19	February 11, 2024
WSt11, WSt12, WSt13	March 17, 2024
WSt14, WSb5, WSb6	April 14, 2024
WSb7, WSb8, WSt15	May 26, 2024
WSt16, WSt17, WSb9	June 16, 2024
WSb10, WSn20, WSn21	July 21, 2024

WSb1 to WSb10: Wastewater samples from Boukais; WSt11 to WSt17: Wastewater samples from Taghit; WSn18 to WSn21: Wastewater samples from Nif Rhaa-Ouakda.

According to the figure above, the results showed that the bacterial isolates from the wastewater of the fish ponds are categorized into six groups: *Enterobacteriaceae*, non-*Enterobacteriaceae*, *Streptococcaceae*, *Pseudomonadaceae*, *Erwiniaceae* and *Neisseriaceae*, with isolation rates of 41.18%, 38.24%; 8.82%, 7.35%, 2.94% and 1.47%, respectively.

Fish and water are the most reliable sampling sites for pathogen detection in aquaculture environments (Dronen *et al.*, 2022). The predominance of *Enterobacteriaceae* indicates fecal contamination, likely originating from runoff, contaminated water used in fish farming, fish feed, livestock manure, fish excreta, or direct anthropogenic inputs. This suggests inadequate biosecurity and poor waste management practices, which are commonly observed in local fish farms (Degefu *et al.*, 2011; Njoku *et al.*, 2015; Sule *et al.*, 2016; Wisnu *et al.*, 2019). Moreover, the failure to implement proper fishpond management practices can pose health risks to fish by facilitating the spread of harmful pathogens, which may subsequently impact human health (Ajayi and Okoh, 2014; Sule *et al.*, 2016; Opiyo *et al.*, 2018).

While similar bacterial groups-belonging to families such as *Staphylococcaceae*, *Streptococcaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, non-*Enterobacteriaceae*, *Morganellaceae*, *Neisseriaceae* and *Bacillaceae*-have been reported in studies conducted in other parts of Africa (e.g., Nigeria and Zambia) by Ntengwe and Edema (2008), Ajayi

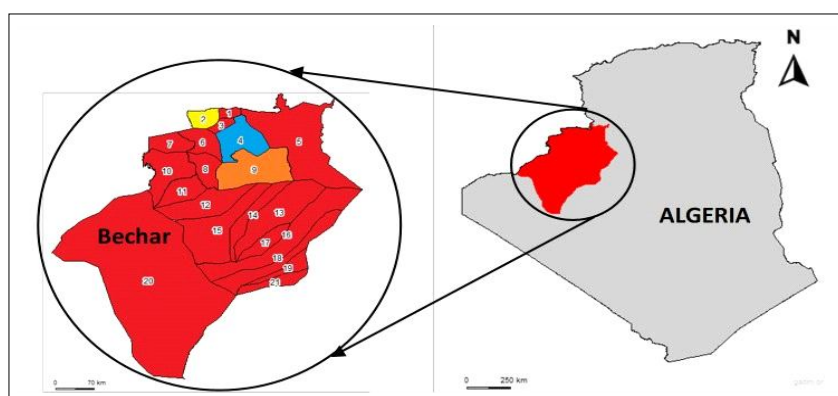


Fig 1: Map showing the geographical locations of Boukais (yellow), Taghit (orange), Bechar city center (blue) and the entire Bechar province (red) in Southwestern Algeria (GADM, 2024).

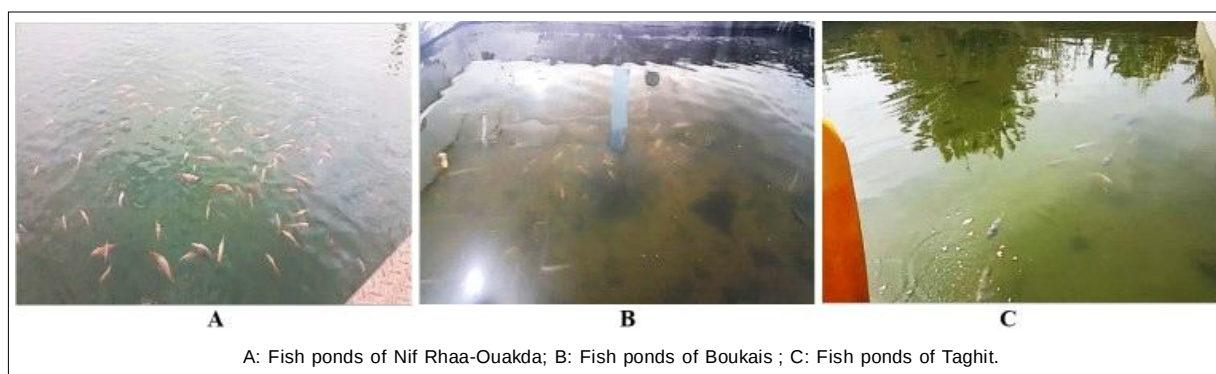


Fig 2: Fish ponds in Bechar province (Southwestern Algeria) (Original, 2024)-Photo by E. Benyagoub.

and Okoh (2014), Njoku *et al.* (2015), Sule *et al.* (2016), Eghomwanre *et al.* (2019) and Adebami *et al.* (2020), our findings provide region-specific microbiological insights from southwestern Algeria, a region where such baseline surveillance remains limited.

Bacterial identification

The results of the identification of the fish pond wastewater isolates are presented in Table 2.

At least 21 unique bacterial species were isolated. These included 14 species from the *Enterobacteriaceae*

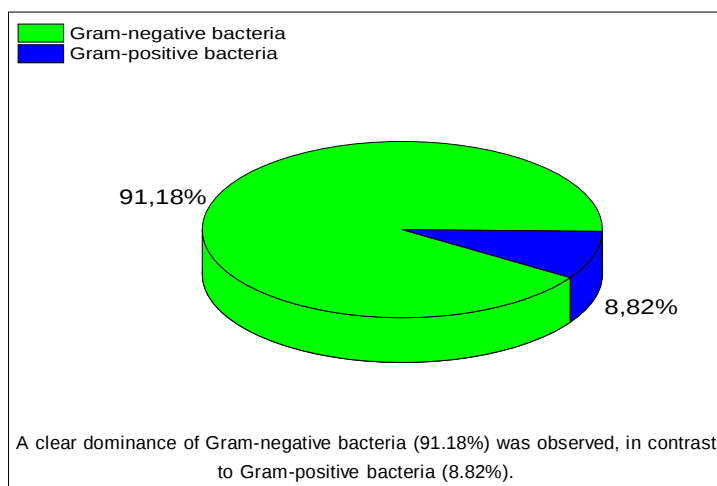


Fig 3: Distribution of isolated bacteria according to Gram type (own elaboration).

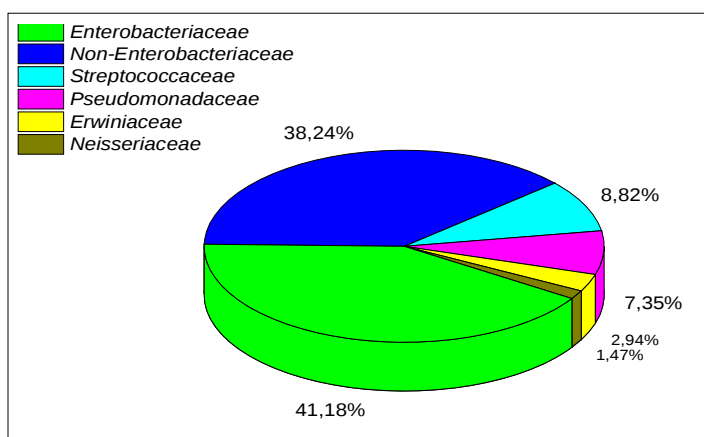


Fig 4: Distribution of bacterial isolates by bacterial group (own elaboration).

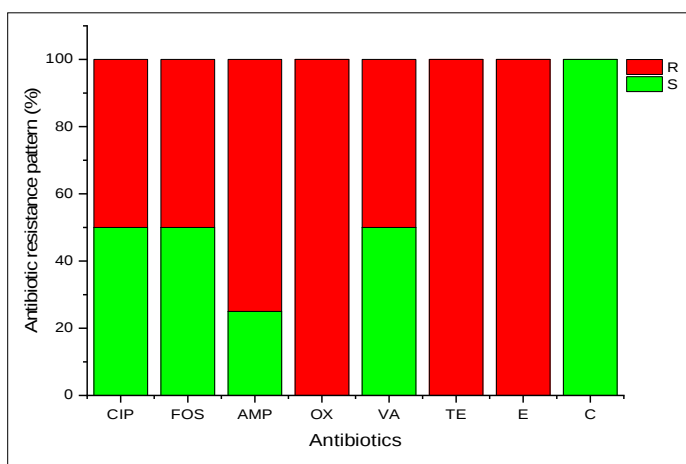


Fig 5: Antibiotic susceptibility test of bacterial isolates belonging to the *Streptococcaceae* family (own elaboration).

family, 3 species from non-*Enterobacteriaceae* families and 1 species each from the *Pseudomonadaceae*, *Erwiniaceae* and *Neisseriaceae* families, as well as unidentified species from the *Streptococcaceae* group.

According to the Chi-Square Goodness-of-Fit Test, the overall distribution of bacterial groups was highly non-uniform (p -value < 0.001), indicating significant variation in prevalence among the groups. This reinforces the dominance of *Enterobacteriaceae* and *Aeromonadaceae* and underlines the selective pressures present in fishpond environments-potentially influenced by temperature, nutrient load and organic matter accumulation.

Antibiotic susceptibility testing (AST)

Streptococcaceae

The results of the antibiotic susceptibility testing for bacterial isolates belonging to the *Streptococcaceae* family are presented in Fig 5.

The antibiotic susceptibility test results of *Streptococcaceae* isolates showed 100% resistance to oxacillin, tetracycline and erythromycin, 75% resistance to ampicillin and 50% resistance to vancomycin. Meanwhile, they were 100% sensitive to chloramphenicol.

Enterobacteriaceae

The results of the antibiotic susceptibility testing for bacterial isolates belonging to the *Enterobacteriaceae* family are presented in Fig 6.

As shown in Fig 6, all isolates exhibited 100% resistance to cefoxitin, cefazolin and ampicillin, with variable resistance to amoxicillin-clavulanic acid. Conversely, all isolates were fully sensitive to ciprofloxacin, ofloxacin, gentamicin, azithromycin, amikacin and tobramycin.

Previous studies, such as those by Sule *et al.*, (2016), Adebami *et al.*, (2020) and Abedin *et al.*, (2020), have similarly reported resistance to amoxicillin-clavulanic acid, amoxicillin and ceftazidime among bacterial isolates from aquaculture wastewater, while sensitivity to ciprofloxacin and ofloxacin was maintained-findings that align with our results. These patterns underscore the role of aquaculture environments as reservoirs of bacteria carrying mobile resistance genes (Del Castillo *et al.*, 2013), which pose a risk of horizontal gene transfer to human pathogens or direct infection (Apenteng *et al.*, 2017). Notably, multidrug resistance-including to critical antibiotics such as cephalosporins and carbapenems-has been detected in *Enterobacteriaceae* from aquaculture systems (Hamza *et al.*, 2020; Custodio *et al.*, 2023), highlighting the potential for interspecies gene transfer within these settings.

Non-enterobacteriaceae (*Aeromonadaceae*)

The results of the antibiotic susceptibility testing for bacterial isolates belonging to the non-*Enterobacteriaceae* family (*Aeromonadaceae*) are presented in Fig 7.

The results obtained showed that *Aeromonadaceae* strains were 100% resistant to ticarcillin-clavulanic acid

Table 2: Identities of bacteria isolated from fish pond wastewater.

Bacterial group	Identity of the isolated strain	Isolation frequency	Occurrence rate (%)
<i>Enterobacteriaceae</i>	<i>Enterobacter aerogenes</i>	1	1.47
	<i>Enterobacter cloacae</i>	2	2.94
	<i>Enterobacter sakazakii</i>	2	2.94
	<i>Enterobacter amnigenus</i>	1	1.47
	<i>Citrobacter freundii</i>	4	5.88
	<i>Escherichia coli</i>	3	4.41
	<i>Klebsiella oxytoca</i>	3	4.41
	<i>Serratia odorifera</i>	3	4.41
	<i>Serratia liquefaciens</i>	2	2.94
	<i>Serratia plymuthica</i>	1	1.47
	<i>Serratia marcescens</i>	3	4.41
	<i>Salmonella choleraesuis</i> ssp <i>arizonae</i>	1	1.47
	<i>Plesiomonas shigelloides</i>	1	1.47
	<i>Kluyvera</i> spp	1	1.47
	<i>Aeromonas hydrophila</i>	21	30.88
N-E <i>Aeromonadaceae</i>	<i>Vibrio fluvialis</i>	4	5.88
	<i>Vibrio cholerae</i>	1	1.47
<i>Pseudomonadaceae</i>	<i>Pseudomonas aeruginosa</i>	5	7.35
<i>Streptococcaceae</i>	<i>Streptococcus</i> spp	6	8.82
<i>Erwiniaceae</i>	<i>Pantoea</i> spp	2	2.94
<i>Neisseriaceae</i>	<i>Chromobacterium violaceum</i>	1	1.47
Total	/	68	100

N-E: Non-enterobacteriaceae.

and, to a lesser extent, to imipenem, ceftazidime and cefotaxime. However, they were 100% sensitive to ciprofloxacin, amikacin, tobramycin, ofloxacin, piperacillin and gentamicin and showed 96% sensitivity to aztreonam and chloramphenicol.

Non-enterobacteriaceae (*Vibrionaceae*)

The results of the antibiotic susceptibility testing for bacterial isolates belonging to the non-*Enterobacteriaceae* family (*Vibrionaceae*) are presented in Fig 8.

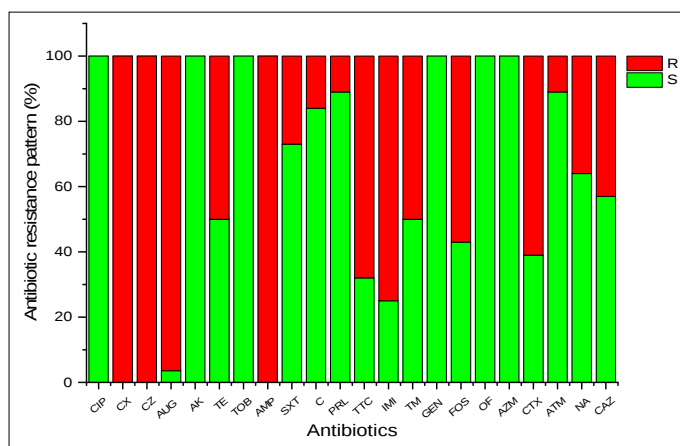


Fig 6: Antibiotic susceptibility test of bacterial isolates belonging to the *Enterobacteriaceae* family (own elaboration).

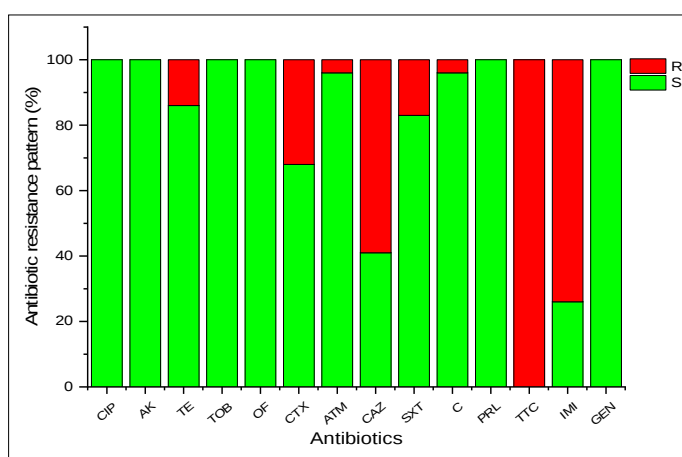


Fig 7: Antibiotic susceptibility test of bacterial isolates belonging to the non-*Enterobacteriaceae* (*Aeromonadaceae*) family (own elaboration).

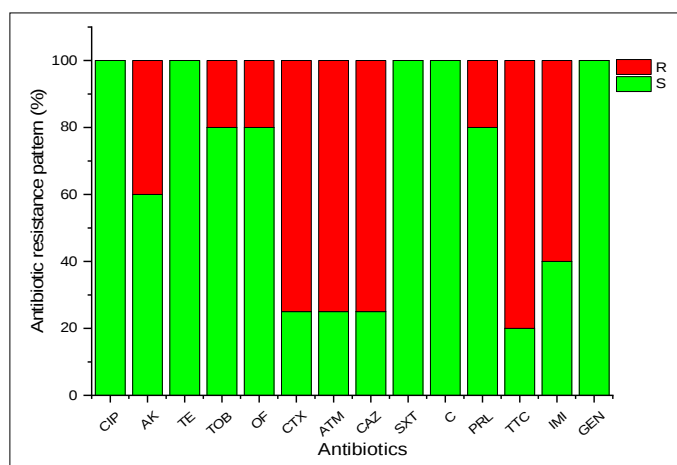


Fig 8: Antibiotic susceptibility test of bacterial isolates belonging to the non-*Enterobacteriaceae* (*Vibrionaceae*) family (own elaboration).

The results obtained showed that the *Vibrionaceae* strains exhibited resistant ranging from 40% to 80% to amikacin, cefotaxime, ceftazidime, aztreonam, imipenem and ticarcillin-clavulanic acid, while the isolates remained 100% sensitive to ciprofloxacin, tetracycline, sulfamethoxazole-trimethoprim, chloramphenicol and gentamicin.

Previous studies have documented varying antibiotic resistance patterns in aquaculture environments. For example, while Manjusha *et al.* (2005) found that *Vibrio* spp. isolated from coastal and tissue samples (shrimp, mussels and cuttlefish) exhibited higher resistance than those from aquaculture farms-likely due to greater antimicrobial use and pollution-our results indicate a similar trend, in that bacterial isolates from inland fish ponds display comparatively lower resistance levels.

In addition, the resistance pattern observed for *Aeromonas* spp. in our study is consistent with earlier reports (Fosse *et al.*, 2003; Henriques *et al.*, 2006; Daood, 2012), which attributed β -lactam resistance to the presence of β -lactamase genes such as *bla*TEM, despite maintained efficacy for antibiotics like ciprofloxacin, gentamicin, amikacin and trimethoprim-sulfamethoxazole. This suggests that reduced antimicrobial application and lower environmental pressures in inland aquaculture may mitigate the

development of resistance. Moreover, the widespread presence of mobile genetic elements (MGEs), reported in previous studies (Lamy, 2012; Del Castillo *et al.*, 2013), supports our hypothesis that horizontal gene transfer is an important driver in the dissemination of resistance determinants in these ecosystems.

Pseudomonadaceae

The results of the antibiotic susceptibility testing for bacterial isolates belonging to the *Pseudomonadaceae* family are presented in Fig 9.

The results obtained showed that the *Pseudomonadaceae* strains were 100% resistant to ticarcillin-clavulanic acid and to a lesser degree, resistant to aztreonam and ceftazidime, with resistance rates of 80% and 40%, respectively. However, they were 100% sensitive to ciprofloxacin, amikacin, tobramycin, ofloxacin, piperacillin and gentamicin.

All *P. aeruginosa* isolates formed biofilms (Fig 10), which enhances resistance and survival in aquatic environments. This biofilm-forming capacity may explain their persistence in low-flow, sediment-rich pond systems.

The *Pseudomonas aeruginosa* strains isolated were 100% sensitive to 6 out of 10 (*i.e.*, 60%) antibiotics tested

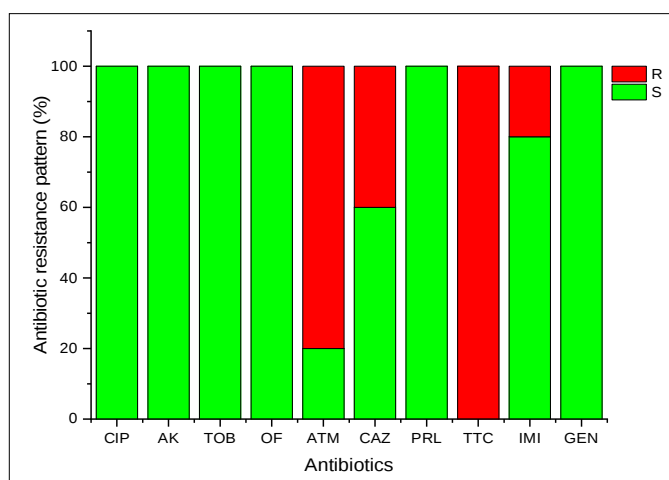


Fig 9: Antibiotic susceptibility test of bacterial isolates belonging to the *Pseudomonadaceae* family (own elaboration).



Fig 10: Detection of biofilm formation using the tube method (Original, 2024)-Photo by E. Benyagoub.

and exhibited lower resistance compared to the *P. aeruginosa* strains isolated in our previous study (Benyagoub, 2023a), which also found that all isolated strains were capable of forming biofilms, a characteristic that enhances the pathogen's adaptability, survival and resistance in various environments (Moradali *et al.*, 2017).

However, due to the absence of standardized guidelines for antibiotic selection and interpretation, we did not include the AST results for *Pantoea* spp. and *Chromobacterium violaceum* strains in this study.

Multidrug-resistant bacterial strains

The strains exhibiting resistance to more than three classes of antibiotics, along with the antibiotic resistance patterns categorized by bacterial group, are presented in Table 3 and Fig 11.

Out of 68 bacterial isolates, 17 (25%) were resistant to more than three classes of antibiotics and exhibited high MAR indices. The variability in MAR indices among bacterial groups was statistically significant (ANOVA, $p = 0.0498$), suggesting that resistance levels differ across bacterial families. The elevated MAR values observed in *Enterobacteriaceae* (above 0.4) suggest sustained antibiotic pressure and fecal contamination, potentially originating from farm runoff, livestock waste, or human sources (Sarkar *et al.*, 2019). Similarly, Rezaul Karim *et al.* (2023) reported non-*Enterobacteriaceae* isolates with MAR indices above 0.2, indicative of antibiotic misuse in both human and veterinary contexts. These findings highlight the urgent need for the implementation of best practice codes among fish farmers and routine monitoring

of fish pond wastewater prior to environmental discharge, in order to prevent the transmission of potential pathogens (Sule *et al.*, 2016).

Comparable results have been reported elsewhere. Snoussi *et al.* (2011) found that *Aeromonas hydrophila* and *Vibrio alginolyticus* strains isolated from marine farm environments were resistant to at least three antimicrobial agents, with MAR indices of 0.71 and 0.68, respectively. Likewise, Abedin *et al.* (2020), identified *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp. as dominant bacteria in various fish species in Bangladesh, reinforcing concerns over antibiotic-resistant pathogens in aquaculture systems.

These results point to a concerning level of resistance to several antibiotic classes, which, according to Adebami *et al.* (2020), could increase the prevalence of infections in fish and potentially trigger disease outbreaks.

Gram-positive bacteria can develop resistance through two main strategies: one involves the enzymatic breakdown of the antibiotic via β -lactamase production, while the other reduces the binding affinity of the antibiotic to its target, the penicillin-binding protein (PBP) (Benyagoub *et al.*, 2020b; Jubeh *et al.* 2020; Benyagoub *et al.*, 2022; Benyagoub, 2024). However, Gram-negative bacteria can resist antibiotics through various mechanisms, such as reducing drug uptake, altering the drug target, inactivating the drug and actively pumping it out. Additionally, these bacteria can acquire resistance factors from other microorganisms (Reygaert, 2018; Benyagoub *et al.*, 2020a; Benyagoub *et al.*, 2021a, 2021b; Gauba and Rahman, 2023).

Table 3: Multidrug-resistant profiles of bacterial strains isolated from fish ponds wastewater.

Bacterial group	Bacterial strains (Code)	Antibiotic resistance profiles	MAR index	ARC (No.)
Enterobacteriaceae	<i>Serratia odorifera</i> (GC L)	CX-AUG-CZ-TE-AMP-SXT-C-TTC-IMI-TM-FOS-CTX-ATM-NA-CAZ	0.68	6
	<i>Citrobacter freundii</i> (SS I noir)	CX-AUG-CZ-TE-AMP-SXT-C-TTC-IMI-TM-FOS-NA-CAZ	0.59	6
	<i>Enterobacter amnigenus</i> (CT j)	CX-AUG-CZ-AMP-SXT-C-TTC-TM-FOS-CTX-NA-CAZ	0.54	5
	<i>Enterobacter cloacae</i> (GC F)	CX-AUG-CZ-TE-AMP-TTC-IMI-TM-FOS-CTX-NA-CAZ	0.54	5
	<i>Citrobacter freundii</i> (SS B c. noir)	CX-AUG-CZ-TE-AMP-SXT-IMI-TM-AZM-CTX-NA	0.5	5
	<i>Klebsiella oxytoca</i> (SS R noir)	CX-AUG-CZ-TE-AMP-SXT-C-TTC-IMI-TM-NA	0.5	5
	<i>Enterobacter aerogenes</i> (CF R)	CX-AUG-CZ-TE-AMP-TOB-CIP-PRL-TTC-IMI-OFX-NA	0.54	4
	<i>Serratia odorifera</i> (SS H noir)	CX-AUG-CZ-TE-AMP-SXT-TTC-IMI-TM-FOS-CAZ	0.5	4
	<i>Escherichia coli</i> (SS F c. noir)	CX-AUG-CZ-AMP-FOS-TM-CTX-ATM-NA-CAZ	0.45	4
	<i>Enterobacter sakazakii</i> (GC O)	CX-AUG-AMP-FOS-TTC-TM-IMI-CAZ-NA	0.4	4
	<i>Enterobacter cloacae</i> (GC P)	CX-AUG-CZ-AMP-SXT-IMI-TM-FOS-CTX-CAZ	0.45	3
	<i>Kluyvera</i> spp (CT L)	CX-AUG-CZ-AMP-TTC-IMI-TM-FOS-CTX-CAZ	0.45	3
	<i>Serratia liquefaciens</i> (CT H)	CX-AUG-CZ-TE-AMP-TTC-IMI-TM-CTX	0.41	3
	<i>Aeromonas hydrophila</i> gr2 (GC A)	TE-SXT-C-TTC-CTX-ATM-CAZ	0.5	4
N-E Aero.	<i>Aeromonas hydrophila</i> gr2 (CT I)	TE-SXT-TTC-CTX-CAZ	0.36	3
	<i>Aeromonas hydrophila</i> gr2 (GC H)	TE-SXT-TTC-IMI	0.28	3
Vib.	<i>Vibrio fluvialis</i> (SS I blanc)	AK-OF-CTX-ATM-CAZ	0.36	3

N-E: Non-enterobacteriaceae, Aero.: *Aeromonaceae*, Vib.: *Vibrionaceae*, MAR: Multiple antibiotic resistance, ARC: Antibiotic resistance classes.

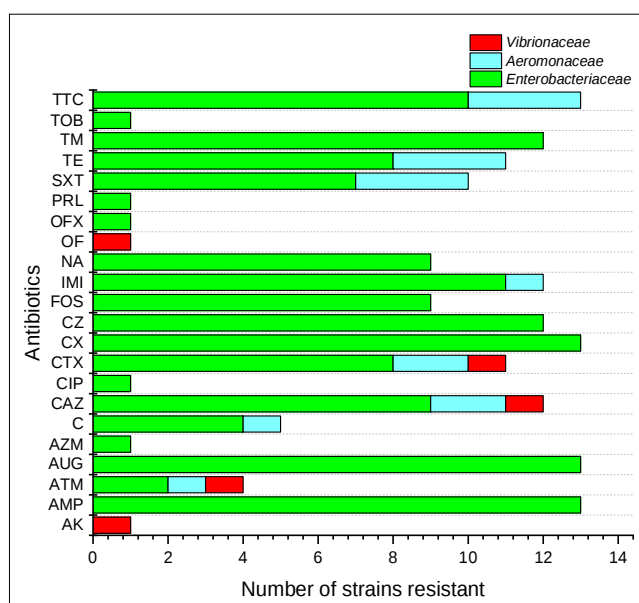


Fig 11: Antibiotic resistance patterns by bacterial group (own elaboration).

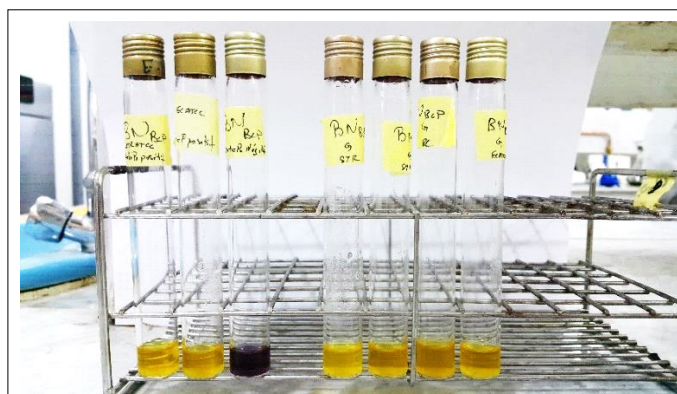


Fig 12: Qualitative detection of antimicrobial residues in fish pond wastewater (Original, 2024)-Photo by E. Benyagoub.

Search for antibiotic residues

The qualitative test (Fig 12) indicated the absence of detectable antibiotic residues in the samples.

This may reflect either the absence of recent antibiotic use or the limited sensitivity of the test method. However, indirect selection pressures-such as low-dose residues from reused water sources or contamination from surrounding agricultural activities-cannot be excluded.

Manjusha *et al.* (2005) and Thiang *et al.* (2021) underscore the threat posed by low-level antibiotic exposure, which can still drive the propagation of resistance genes. The lack of residues should not be interpreted as the absence of selective pressure, especially considering the prevalence of multidrug-resistant bacteria. Effective management of aquaculture wastewater is crucial to reduce antibiotic pollution and prevent the transfer of resistance genes to humans through the food chain. This highlights the need for a deeper understanding of how drug resistance

determinants are spread and transmitted, as well as the importance of enhancing antimicrobial stewardship (Del Castillo *et al.*, 2013, Schar *et al.*, 2020).

CONCLUSION

This study reveals the presence of diverse bacterial communities, including multidrug-resistant and biofilm-forming strains, in fish pond wastewater. The dominance of Gram-negative bacteria and Enterobacteriaceae reflects potential fecal contamination and poor waste management practices common in the study area.

Despite no detectable antibiotic residues, high MAR indices and resistance patterns suggest ongoing selection pressures, likely from environmental or anthropogenic sources. These findings raise concerns about the potential transmission of resistance genes to human pathogens via water and food chains.

To address this risk, we recommend the following practical steps:

- Implement closed-loop or recirculating aquaculture systems (RAS) to reduce water contamination and limit the spread of antibiotic-resistant bacteria.
- Encourage the use of probiotics or approved chemical disinfectants as alternatives to antibiotics for disease prevention and microbial control in aquaculture operations.
- Educate fish farmers on the risks of indiscriminate antibiotic use and encourage adherence to good aquaculture practices (GAQPs).
- Establish local monitoring frameworks for antimicrobial resistance and residue detection.
- Treat aquaculture wastewater before environmental discharge, especially in regions near populated or agricultural zones.

Such measures are essential to enhance antimicrobial stewardship, protect public health and ensure sustainable aquaculture development in Algeria and similar settings globally.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest. No financial support was received for this research.

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