

# Antibiotic Resistance Profiles of Aquatic and Human Pathogens: Implications for Disease Management and Public Health

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DOI: 10.63001/tbs.2025.v20.i03.S.I(3).pp205-208

## KEYWORDS

Aquaculture, Antibiotic resistance, Multidrug-resistant bacteria, Aquatic pathogens, Human pathogens, Disease management, Public health. Received on:

28-05-2025

Accepted on:

29-06-2025

Published on:

03-08-2025

## ABSTRACT

Aquaculture serves as a vital source of global protein but is increasingly threatened by infections caused by both aquatic and human bacterial pathogens present in aquaculture environments. This study evaluates the antibiotic resistance profiles of key aquatic pathogens including *Aeromonas caviae*, *Edwardsiella tarda*, *Vibrio cholerae*, and *Aeromonas hydrophila*, as well as human-associated pathogens such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Antibiotic susceptibility testing revealed widespread multidrug resistance among these bacteria, especially resistance to Methicillin, Oxacillin, Penicillin-G, and Erythromycin, significantly limiting treatment options. Despite this, antibiotics like Chloramphenicol and Ciprofloxacin demonstrated notable effectiveness against certain strains. The presence of multidrug-resistant human pathogens in aquaculture environments underscores the critical public health risks and highlights the potential for the transmission of resistant bacteria through the food chain. These findings emphasize the urgent need for prudent antibiotic stewardship, continuous surveillance, and integrated disease management practices to protect aquaculture productivity, environmental health, and human safety under a One Health framework.

## INTRODUCTION

Aquaculture is one of the rapidly growing industry supplying 15% protein to the global food supply. This sector plays a crucial role in global food security and economic development, particularly in developing countries. However, the presence of aquatic pathogens poses significant challenges to the productivity and health of fish and other aquatic organisms (Mohammed et al., 2025). Antibiotics are widely used in to improve the fish quality and to overcome the disease caused by the pathogens. According to a recent survey, over 60% of the global antibiotic consumption occurs in low- and middle-income countries (Klein et al., 2018). For instance, a 2013 study in China reported the production of 248,000 tons of antibiotics, with 162,000 tons being utilized, 52% of which were used in animals and 48% in humans (Zhang et al., 2015).

However, the over usage of antibiotics resulted in chemical pollution as well as generates selective pressure leading to the emergence of microorganisms that having the ability to resist the antimicrobial drugs (Chinemerem et al., 2022; Ding et al., 2023; Mohammed et al., 2025; Zhao et al., 2019). This selective pressure enables the survival and propagation of strains that can resist commonly used antimicrobial agents, making treatment of infections increasingly ineffective. Since fish, shellfish, and other aquatic foods are a significant source of animal protein in many parts of the world, whether developed (the US, Japan, and the EU) or developing (China, Southeast Asia, India, and Africa), the antibiotic resistance linked to aquaculture is of global importance (Milijasevic et al., 2024). Importantly, resistant bacteria and their

resistance genes can be transferred between aquatic organisms, humans, and terrestrial animals, either through direct contact or via the food chain, water systems, or sediments (Elsabagh et al., 2018; Won et al., 2020).

The over use of antibiotics not only led to antibiotic resistance but also cause various toxic effects like suppression of immune system, decrease in antioxidant enzyme function, cardio vascular malfunction, metabolic abnormalities, developmental defects, decrease in catching, etc., (Limbu et al., 2018; Nguyen et al., 2021; Nunes et al., 2015; Wang et al., 2020; Yang et al., 2020). As the antibiotics are not easily degraded, the bioaccumulate in tissues and transported through food chain to the predators thereby spreading the antibiotic resistance and other side effects (Mohammed et al., 2025). Therefore, it is essential to monitor the patterns of susceptibility of pathogenic bacteria, particularly those that are common in aquatic habitats in order to effectively manage the disease and improve the aquaculture.

Therefore, the current study aims to assess the antibiotic sensitivity patterns of both aquatic and clinical bacterial isolates against widely used antibiotics. This investigation will provide crucial insights into resistance levels and aid in developing evidence-based approaches for the responsible use of antibiotics in aquaculture, contributing to both animal health management and public safety.

### Materials and Methods:

#### Pathogens used:

Eight bacterial pathogens were selected in this study. Four pathogens were aquatic (*Aeromonas hydrophila*, *Edwardsiella*

*tarda*, *Vibrio cholerae*, *Aeromonas caviae*) and another four were human pathogens (*Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). All the bacterial strains were procured from a laboratory. All isolates were sub-cultured on nutrient agar and preserved at 4°C as working stocks.

#### Antibiotic sensitivity test:

The antibiotic sensitivity of the pathogens was tested using standard Kirby-Bauer disc diffusion assay on Mueller-Hinton agar (HiMedia, India). The assay was performed using commercial antibiotics viz., Streptomycin, Ampicillin, Tetracycline, Erythromycin, Penicillin-G, Doxycycline, hydrochloride, Gentamicin, Neomycin, Cloxacillin, Chloramphenicol, Oxacillin, zithromycin, Methicillin, Vancomycin, Amikacin, Polymyxin, Rifampicin, Ciprofloxacin, Kanamycin and Bacitracin. Sterile Mueller-Hinton agar plates were prepared and the bacterial inoculum cultured overnight in nutrient broth was lawned on the agar surface using sterile cotton swab after adjusting the turbidity to 0.5 McFarland standard (Popoola et al., 2024). Sterile antibiotic discs were aseptically placed the surface carefully to avoid overlapping inhibition zones.

#### Inhibition conditions:

The plates inoculated with aquatic pathogens were incubated at 30°C for 24 hours. Plates with human pathogens were incubated at 37°C for 24 hours.

#### Measurement of Inhibition zones:

Following incubation, the plates were observed for the zone of clearance (inhibition). The diameter of the zone of inhibition was measured with a ruler. All the experiments were done in triplicates and measured carefully to ensure the accuracy. The results were interpreted according to the CLSI (Clinical and Laboratory Standards Institute) guidelines and presented as Mean  $\pm$  Standard Deviation.

#### Results:

##### Antibiotic sensitivity of Aquatic Pathogens:

A total of four aquatic bacterial pathogens: *Edwardsiella tarda*, *Aeromonas hydrophila*, *Aeromonas caviae*, and *Vibrio cholerae* were evaluated for their sensitivity antibiotics using the disk diffusion method. The mean inhibition zones (mm  $\pm$  SD) for each pathogen are presented in Table 1.

Antibiotics used	<i>Edwardsiella tarda</i>	<i>Aeromonas hydrophila</i>	<i>Aeromonas caviae</i>	<i>Vibrio cholerae</i>
Streptomycin	13 $\pm$ 0.8	18 $\pm$ 1.2	32 $\pm$ 1.5	20 $\pm$ 0.9
Ampicillin	-	-	32 $\pm$ 1.3	-
Tetracycline	22 $\pm$ 1.1	22 $\pm$ 1.0	21 $\pm$ 0.9	18 $\pm$ 1.2
Erythromycin	-	-	12 $\pm$ 0.5	-
Penicillin-G	-	-	37 $\pm$ 1.4	-
Doxycycline hydrochloride	18 $\pm$ 0.7	20 $\pm$ 1.1	26 $\pm$ 1.3	20 $\pm$ 0.8
Gentamicin	22 $\pm$ 1.0	24 $\pm$ 1.2	26 $\pm$ 0.9	22 $\pm$ 1.1
Neomycin	20 $\pm$ 0.6	20 $\pm$ 0.8	22 $\pm$ 1.0	20 $\pm$ 1.2
Cloxacillin	17 $\pm$ 0.9	16 $\pm$ 0.7	20 $\pm$ 1.4	16 $\pm$ 0.8
Chloramphenicol	28 $\pm$ 1.3	30 $\pm$ 1.1	18 $\pm$ 1.2	30 $\pm$ 0.9
Oxacillin	-	-	24 $\pm$ 0.8	-
Azithromycin	20 $\pm$ 0.7	20 $\pm$ 1.0	16 $\pm$ 0.8	18 $\pm$ 1.1
Methicillin	-	-	-	-
Vancomycin	08 $\pm$ 0.4	10 $\pm$ 0.7	18 $\pm$ 1.1	14 $\pm$ 1.0
Amikacin	18 $\pm$ 1.0	20 $\pm$ 1.3	26 $\pm$ 1.2	20 $\pm$ 0.9
Polymyxin	10 $\pm$ 0.5	12 $\pm$ 0.8	12 $\pm$ 0.6	12 $\pm$ 0.7
Rifampicin	14 $\pm$ 1.1	16 $\pm$ 0.9	18 $\pm$ 1.0	17 $\pm$ 0.8
Ciprofloxacin	26 $\pm$ 1.2	30 $\pm$ 0.9	26 $\pm$ 1.3	30 $\pm$ 1.1
Kanamycin	17 $\pm$ 0.6	18 $\pm$ 1.0	16 $\pm$ 0.7	20 $\pm$ 0.9
Bacitracin	08 $\pm$ 0.3	08 $\pm$ 0.5	08 $\pm$ 0.4	10 $\pm$ 0.6

**Table 1:** Antibiotic sensitivity test against Aquatic pathogens

Among the tested pathogens, *Aeromonas caviae* displayed sensitivity to almost all antibiotics except Methicillin. Other three bacterial strains also showed no sensitivity towards Methicillin. However, they also lack their sensitivity towards other antibiotics viz., Oxacillin, Penicillin-G, Erythromycin and Ampicillin. This shows their Multi-Drug resistance. *Aeromonas caviae* demonstrated notable sensitivity to Penicillin-G (37  $\pm$  1.4 mm), Streptomycin (32  $\pm$  1.5 mm), and Gentamicin (26  $\pm$  0.9 mm). Large zones were observed with Chloramphenicol against *Edwardsiella tarda* (28  $\pm$  1.3 mm) and *Aeromonas hydrophila* (30  $\pm$  1.1 mm), and with Ciprofloxacin against *Aeromonas hydrophila* (30  $\pm$  0.9 mm) and *Vibrio cholerae* (30  $\pm$  1.1 mm).

##### Antibiotic sensitivity of Human pathogens:

The susceptibility of *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* was assessed using the same methodology. The sensitivity pattern observed in this analysis was *Staphylococcus aureus* > *Acinetobacter baumannii* > *Pseudomonas aeruginosa* > *Klebsiella pneumoniae* with *Vibrio cholerae* showing the most sensitivity. Amikacin showed the greatest activity, especially against *Aeromonas caviae* (25  $\pm$  1.2 mm) and *Vibrio cholerae* (25  $\pm$  1.3 mm). Gentamicin and Ciprofloxacin were also highly effective against *Staphylococcus aureus* (24  $\pm$  1.3 mm; 28  $\pm$  1.5 mm, respectively). Neomycin and Tetracycline produced

moderate to strong zones in most isolates. The results of the antibiotic sensitivity assay performed against the Human pathogens were presented in Table 2.

Antibiotics used	<i>Klebsiella pneumoniae</i>	<i>Acinetobacter baumannii</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Streptomycin	12 ± 0.9	16 ± 1.1	16 ± 0.8	22 ± 1.0
Ampicillin	10 ± 0.6	10 ± 0.7	-	-
Tetracycline	13 ± 0.8	18 ± 1.2	16 ± 0.9	19 ± 1.1
Erythromycin	-	22 ± 1.0	10 ± 0.7	14 ± 1.0
Penicillin-G	11 ± 0.7	-	09 ± 0.5	12 ± 0.9
Doxycycline hydrochloride	13 ± 0.9	16 ± 1.0	14 ± 0.8	19 ± 1.1
Gentamicin	19 ± 1.0	22 ± 1.2	18 ± 1.1	24 ± 1.3
Neomycin	14 ± 0.7	18 ± 0.9	18 ± 0.9	22 ± 1.2
Cloxacillin	-	-	-	11 ± 0.6
Chloramphenicol	18 ± 1.0	18 ± 0.9	16 ± 0.8	19 ± 0.9
Oxacillin	-	09 ± 0.6	-	-
Azithromycin	10 ± 0.5	23 ± 1.2	14 ± 0.9	14 ± 1.0
Methicillin	-	-	-	-
Vancomycin	-	15 ± 0.7	14 ± 1.0	18 ± 1.1
Amikacin	18 ± 1.1	21 ± 1.3	25 ± 1.2	25 ± 1.3
Polymyxin	10 ± 0.5	10 ± 0.6	10 ± 0.6	10 ± 0.5
Rifampicin	-	10 ± 0.7	09 ± 0.6	13 ± 0.8
Ciprofloxacin	20 ± 1.0	18 ± 0.9	19 ± 1.0	28 ± 1.5
Kanamycin	19 ± 0.9	17 ± 1.0	14 ± 0.8	18 ± 0.9
Bacitracin	-	-	-	09 ± 0.6

**Table 2:** Antibiotic sensitivity test against human pathogens

## DISCUSSION

The results of the current study clearly show the antibiotic sensitivity of the tested pathogens. It is obvious that the resistance of bacteria to the antibiotics has emerged fast in humans than the aquatic animals. In our study, *Edwardsiella tarda* was found to be resistant against least number of antibiotics. Algamal et al. (2022) observed that *Edwardsiella tarda* showed antimicrobial resistance towards 6 class of antibiotics which includes: ampicillin, streptomycin, tetracycline, ciprofloxacin. Similarly, it was observed in another study that ampicillin, tetracycline and neomycin are resistant towards *E. tarda* (Manzoor et al., 2023). However, in our study, the results are reciprocal all these antibiotics inhibited the bacterial growth. Further, *Vibrio cholerae* is found to resistant to ampicillin which was observed in our study (Traoré et al., 2014). *Staphylococcus aureus* was resistance to three types of antibiotics however all the strains were resistance towards Methicillin. This might be due to the production of PB2a protein secreted by the strains, that blocks the drugs from working properly (Gopikrishnan et al., 2024). In previous studies, *Pseudomonas aeruginosa* was found to resistant towards amikacin, gentamycin, ciprofloxacin, etc., (Avakh et al., 2023; Elfadadny et al., 2024). However, in our study it was found that the strain was sensitive towards these antibiotics. Further research is needed to avoid such ambiguities. The resistance of all pathogens to Methicillin, despite its limited use in aquaculture, could be due to horizontal gene transfer or contamination from anthropogenic sources. On the other hand, the strong

susceptibility of *Edwardsiella tarda* to Gentamicin and Chloramphenicol suggests potential therapeutic utility. These findings highlight the importance of continuous antimicrobial resistance surveillance, stricter regulations on antibiotic use, and the need to explore non-antibiotic alternatives such as vaccines, phage therapy for disease control.

Humans directly intake the antibiotics to combat infections or by consumption of antibiotic contaminated foods and water. Antibiotic resistance will result in increased hospitalization and mortality rates of patients infected with these resistance microorganisms thereby affecting the health and well-being (Berendonk et al., 2015). The antibiotic resistance observed in the aquatic pathogens is likely due to the anthropogenic activities like discharge of antibiotics in considerable amounts through the human waste (Dahunsi et al., 2014). The development and transmission of antibiotic-resistant bacteria (ARB) are significantly influenced by factors associated with the environment. Humans are at risk from water used for drinking, leisure, agriculture, and other household uses. Thus, it is critical to regularly evaluate the patterns of antimicrobial resistance in drinkable water and its sources in order to track its spread and help create preventive measures (Ateba et al., 2020; Popoola et al., 2024).

## CONCLUSION

The study highlights the growing concern of antibiotic resistance among key aquatic pathogens involved in aquaculture. The widespread and often indiscriminate use of antibiotics, especially

in low- and middle-income countries, has contributed significantly to the development of multidrug-resistant bacteria such as *Aeromonas caviae*, *Edwardsiella tarda*, and *Vibrio cholerae*. The results demonstrate that many of these pathogens exhibit resistance to commonly used antibiotics like Methicillin, Oxacillin, Penicillin-G, and Erythromycin, limiting treatment options and posing a serious threat to aquaculture productivity and public health. Notably, some antibiotics including Chloramphenicol and Ciprofloxacin still show effectiveness against certain strains, indicating potential alternatives for treatment. To keep antibiotic resistance from getting worse, it's important to monitor antibiotic use carefully and use these drugs responsibly. In the future, combining different disease management methods and developing new treatments will be key to ensuring aquaculture remains healthy and sustainable for everyone.

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