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Original Article

# Antimicrobial Resistance of Common Bacteria in Water Environments

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***Abstract***

*Antimicrobial resistance (AMR) in water bodies is a rising public health concern, resulting in the increasing detection of resistant bacteria in water bodies. The current study investigates AMR in Salmonella and Vibrio cholerae in different water bodies of Bhiwandi. Resistance patterns of the most commonly prescribed drugs by these microorganisms—including penicillin, rifampicin, chloramphenicol, vancomycin, amikacin, and gentamicin—were considered. The result revealed high resistance, especially in sewage-polluted water, requiring immediate water treatment and regular microbial monitoring. This may also require strict wastewater management and new treatment methods to reduce AMR stressors in water bodies and ensure public health.*

***Keywords:*** *Antimicrobial resistance, water pollution, Salmonella, Vibrio cholerae, public health, environmental microbiology, aquatic ecosystems.*

## Introduction

### Background

The world produces an increasing number of publications which rank and classify AMR as one of the serious threats to human life in the twenty-first century. The overdose and misuse of antibiotics in healthcare and agriculture alike give rise to the emergence of drug-resistant microbes. While AMR is often studied in health care settings, there are opportunities for resistance emergence in water environments. Gradually, our rivers, lakes, oceans, and drinking water are becoming contaminated with antibiotics, animal waste, municipal sewage, and industrial waste, ensuing across them environments for gene exchange among bacteria and spreading resistance to ecosystems and human populations.

Waterborne pathogens, such as *Salmonella* and *Vibrio cholerae*, have shown the ability to develop resistance to antibiotics against the diseases they cause. For example, pathogens of water contamination are particularly troublesome because, should these pathogens develop resistance, treatment may then become ineffective. Marine and freshwater environments can then serve as breeding grounds for resistance in bacteria, with influence exerted by human pollution through numerous ways: for instance, loss of pharmaceuticals into the environment, poor sanitation that allows untreated sewage to be dumped into water bodies, farming runoff, and pollution from urbanization-all these significantly influence the evolution and dissemination of resistance. It is important that, in the general war against AMR, we understand ways by which AMR can be traced and mapped in water environments.

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## Objective

The aim of this study is to analyze the origin and antimicrobial behavior of *Salmonella* and *Vibrio cholerae*, in common water sources. With an emphasis on their response to common antibiotics, prescribed in medical and veterinary contexts. The goals of this study are:-

- To isolate and identify *Salmonella* and *Vibrio cholerae* in a different water sources, such as river, lake, pond, sea
- To analyze the bacteria's resistance to antibiotics, such as penicillin, rifampicin, chloramphenicol, vancomycin, amikacin, and gentamicin; -
- To assess the impact of resistant strains of bacteria on public health and the implications to public supply water.

This study will assist in understanding the antibiotic resistance mediated by the environment and inform public health principles, water regulation, and the management of antibiotics.

## Materials and Methods

### Study Area and Sample Sites

Samples were collected from four types of water environments that were chosen for ease of access, potential for contamination, and relevance to human health:

1. **Juhu Beach, Mumbai (Sea Water):** Since the intertidal zone samples indicate tidal movement influences, coastal pollution, and human activities on water quality
2. **Kamori River, Bhiwandi:** The sampling was done at both rural (less polluted) and urban (closer to industrial discharge) environments.
3. **Pond Water, Bhiwandi:** Sampled from ponds used for household, and potentially influenced by fecal input.
4. **Lake Water, Bhiwandi:** A waterbody that is frequently accessed/used for recreational purposes and for fishing and ganesh vishran festival

### Sample Collection

Sterile 500 mL plastic bottles were employed, and four samples were collected at different times from each site to achieve temporal representation of diurnal variation. Samples were labeled by date, time, and location, stored in ice-filled coolers, and brought directly to the lab for processing.

### Microbial Isolation and Identification

Organisms such as, *Salmonella spp.*, and *Vibrio cholerae* were isolated according to standard microbiological methods.

- **Pre-enrichment**: For *Salmonella* 1mL of water was incubated in 9mL Buffered Peptone Water at 37°C for 24 hours. And for *Vibrio cholerae* 1 mL of water incubated in 9 mL Alkaline Pepton water

- **Selective Enrichment and Plating:**
  - **Salmonella spp.:** Enriched in Selenite Cystine Broth, plated on XLD Agar.
  - **Vibrio Cholera :** Cultured on TCBS agar

### Antimicrobial Susceptibility Testing

Using the Kirby-Bauer disk diffusion method as per CLSI guidelines:

- **Inoculum:** Bacterial cultures were suspended in saline to match 0.5 McFarland standard (~$1 \times 10^8$ CFU/mL).
- **Antibiotic Discs Tested:**
  - Penicillin (10 units)
  - Chloramphenicol (30 µg)
  - Rifampicin (2 µg)
  - Vancomycin (30 µg)
  - Amikacin (10 µg)
  - Gentamicin (50 µg)
- **Procedure:** Mueller-Hinton Agar was streaked with standardized culture. Discs were placed and plates incubated at 37°C for 18–24 hours.
- **Interpretation:** Zones of inhibition were measured and categorized as Sensitive, Intermediate, or Resistant.

## Results

### Bacterial Isolation and Identification

Water samples were collected from various water sources, including river water, lake water, sea water, and pond water reservoirs. The samples were analyzed for the presence of common waterborne pathogens, including *Salmonella spp.* and *Vibrio cholerae*.

### Antimicrobial Susceptibility of Vibrio spp

The antimicrobial resistance profiles for *Vibrio* organisms isolated from water samples were as follows:

- **Rifampicin:** Zone of inhibition = 1 cm (*Resistant*)
- **Chloramphenicol:** Zone of inhibition = 2.5 cm (*Sensitive*)

- **Penicillin:** No inhibition (*Resistant*)
- **Amikacin:** Zone of inhibition = 1 cm (*Resistant*)
- **Vancomycin:** Zone of inhibition = 1 cm (*Resistant*)
- **Gentamicin:** Zone of inhibition = 1.5 cm (*Resistant*)

**Fig 1: Zone of inhibition of *Vibrio cholerae***

For *Vibrio cholerae* isolates from other sampling sites, the resistance patterns were slightly different:

- **Rifampicin:** No inhibition (*Resistant*)
- **Chloramphenicol:** Zone of inhibition = 2.5 cm (*Sensitive*)
- **Penicillin:** No inhibition (*Resistant*)
- **Amikacin:** Zone of inhibition = 1 cm (*Resistant*)
- **Vancomycin:** No inhibition (*Resistant*)
- **Gentamicin:** Zone of inhibition = 2.5 cm (*Sensitive*)

**Fig 2:** Zone of Inhibition of *salmonella*

## Discussion

### Bacterial Prevalence

The identification of Salmonella and Vibrio cholerae in river and sewage water indicates fecal contamination and poor waste treatment. With the potential for high levels of pathogens in untreated environmental oxidative waters, such as sewage, this heightens concern for the use of untreated waters and the overall harmfulness of releasing untreated wastewater.

### Trends in AMR

The alarming resistance to Rifampicin and Penicillin with no noticeable inhibition zones may reveal the potential for transmission of AMR likely through cross-resistance. Low inhibition from Amikacin and Vancomycin only shows partial effectiveness against these bacteria. While the other antimicrobials, including Chloramphenicol and Gentamicin show better inhibition, they do not establish a higher level of functionality; especially given that the resistance to Penicillin points to β-lactamase production. These results drive home the point to pursue non-traditional treatment options and have an ongoing monitoring system for AMR pathogens.

### Public Health Implications

The risks to public health associated with resistant bacterium from contaminated water supplies would be a cause for alarm for vulnerable populations. Contaminated recreational waters used for irrigation or food may result in increased infections in untreated water users, especially for cases where treatment is not feasible and containment has been exceeded.

### Research Suggestions:

- **Genomic Diversity:** The direction of the work is to utilize whole-genome sequencing to better understand the resistance genes

- **Surveillance:** A need for longitudinal work tracking AMR in aquatic environments over time.
- **Treatment Technologies:** There is a need to try new technologies (i.e., biofilters, nanotech) to improve traditional secondary and tertiary waste technologies.
- **Human Health link:** The need for conversations continues in our work that the transfer of AMR associated with consumption of water and environmental exposures on human health needs to be studied.

## Conclusion

This study validates the presence of antimicrobial-resistant Salmonella and Vibrio cholerae in water systems in Bhiwandi, particularly where there are no actions taken for sewage treatment. The bacteria displayed considerable resistance to several antibiotics used in the study, with Chloramphenicol and Gentamicin showing comparatively better results.

The findings highlight the urgent need to regulate wastewater, incorporate advanced treatment processes, and strengthen water quality assessments. The fact that resistant strains were detected in public-use water sources raises important questions about the efficacy of existing water treatment techniques and systems.

Dealing with AMR through advanced water treatments is going to require a multi-faceted, collaborative effort among the intersections of microbiology, public health and environmental governance. Future research should pursue continued investigation into resistance pathways in water environments that inform strategies to protect both human health and ecosystem health.

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## Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

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