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Research Article

# Assessment of Antibiotic Resistance Genes in Drinking Water Bacterial Isolates of Bhopal District

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## ABSTRACT

Antimicrobial resistance (AMR) has developed a serious public health problem, and drinking water is now also seen as a possible source and carrier of antibiotic resistance genes (ARGs). In this study, domestic drinking water from different places in Bhopal district (Madhya Pradesh, India) was checked for tetracycline resistance.Bacteria were first isolated by serial dilution and plating on Nutrient Agar, and then chosen colonies were grown further in Luria broth. DNA from these cultures was extracted using the phenol-chloroform-isoamyl alcohol (PCI) method, and its quality was checked on agarose gel. A PCR test was done with primers specific for the tet(A) gene and the PCR bands were observed on agarose gels. Good quality DNA was obtained from all samples tested. Out of 20 bacterial isolates, one showed a clear positive band for tet(A), which mean tetracycline resistance is present in at least some part of the drinking-water bacteria. These results agree with earlier reports that environmental waters can act as reservoir and route for spreading ARGs, and they point to the need of regular monitoring and better drinking-water treatment to help stop the spread of AMR at household level.

**KEYWORDS:**

antimicrobial resistance (AMR); antibiotic resistance genes (ARGs); tet(A); tetracycline resistance; drinking water; Bhopal District; environmental bacteria; phenol–chloroform DNA extraction

## INTRODUCTION:

Bacterial, viral, fungal, and parasitic diseases persist major global health concern, collectively responsible for millions of deaths each year (Murray et al., 2022). Diarrheal infections, tuberculosis, pneumonia, and septicemia continue to cause significant mortality worldwide (Walker et al., 2013).

Antibacterial agents are small molecule compounds that inhibit or kill bacteria. They can be naturally produced or synthetically developed and serve as defense mechanisms or communication signals among microorganisms and higher organisms (Davies & Davies, 2010). The use antibiotic producing microbes for healing dates back thousands of years, with natural substances applied to wounds long before modern medicine. The discovery of penicillin marked the beginning of the antibiotic era, revolutionizing clinical treatment and saving countless lives (Fleming, 1929). Antimicrobial peptides act by integrating into bacterial membranes

and disrupting their integrity (Zasloff, 2002). However, the widespread and often excessive use of antibiotics has led to the rapid emergence of antimicrobial resistance (AMR). Resistant bacteria can survive exposure to drugs that once inhibited or killed them, making some infections difficult or impossible to treat (WHO, 2014). Resistance arises through genetic changes or the acquisition of resistance genes that encode mechanisms such as enzyme mediated drug inactivation, efflux pumps, or modification of drug targets (Blair et al., 2015). These genes may occur on bacterial chromosomes, plasmids, or bacteriophages. Some species, including Pseudomonas aeruginosa, Escherichia coli, and Enterobacter cloacae, possess naturally low membrane permeability, providing intrinsic resistance to several antibiotics (Poole, 2001).Environmental sources, including raw water, have been identified as reservoirs of antibiotic resistance genes. Studies across multiple regions have detected genes such as sul1 and tetA in untreated water, highlighting the global spread of resistance determinants and the urgent need for monitoring and control (Berendonk et al., 2015).

## METHODOLOGY:

Drinking water samples were collected from domestic points of use in Bhopal district using sterile, autoclaved vials. Microorganisms were screened through serial dilution and plating on Nutrient Agar Medium (NAM). NAM was prepared by dissolving 28 g in 1000 ml distilled water, autoclaved at 121 °C for 15 minutes, and poured into sterile plates. Dilutions from $10^{-1}$ to $10^{-4}$ were prepared, and 100 µl from the final dilution was spread on NAM plates. Plates were incubated at 37 °C for 24 hours, and colony-forming units (CFU) were counted.

Selected colonies were cultured in Luria Broth (LB), prepared by dissolving 20 g in 1000 ml distilled water and autoclaving under similar conditions. Each culture tube contained 25 ml LB broth inoculated with bacterial colonies and incubated at 37 °C for 2–3 days to obtain biomass for DNA extraction.Genomic DNA was extracted using the phenol–chloroform–isoamyl alcohol (PCI) method. Cultures were centrifuged at 5000 rpm for 15 minutes, and the pellet was treated with Solution B and SDS, followed by sodium acetate and PCI. After centrifugation, the aqueous layer was precipitated with chilled isopropanol, washed with 70 % ethanol, dried, and dissolved in MilliQ water. DNA integrity was confirmed by agarose gel electrophoresis Antibiotic resistance was detected by PCR targeting the tetA gene. Each 20 µl reaction contained primers, dNTPs, MgCl, buffer, Taq polymerase, and DNA template. PCR cycling conditions included pre-denaturation at 95 °C for 5 minutes, followed by 36 cycles of denaturation (95 °C, 30 s), annealing (61 °C, 30 s), extension (72 °C, 30 s), and a final extension at 72 °C for 5 minutes. Amplified products were confirmed by agarose gel electrophoresis.

Table 1 : Present table showing Primer details of tet (A) &gyr (A) applied in presents study.

| S. NO. | Resistant against | Mutant gene | Primer sequences | PCR product size | Reference |
|---|---|---|---|---|---|
| 1 | Tetracycline | *tet(A)* | (F) GCGCGATCTGGTTCACTCG | 164 | Aminov et al., 2002 |
| | | | (R) AGTCGACAGYRGCGCCGGC | | |

## RESULTS AND DISCUSSION

Genomic DNA successfully extracted to all bacterial isolates found from drinking water samples from Bhopal District using the phenol-chloroform isoamyl alcohol method, and its integrity was confirmed by agarose gel electrophoresis. PCR amplification revealed the presence of antibiotic resistance genes in several isolates. Specifically, 1 isolates were positive for tet(A), conferring tetracycline resistance among tested 10 isolates. These findings highlight the widespread occurrence of resistance determinants in drinking water bacterial isolates, underscoring the environmental dimension of antimicrobial resistance.

Detection of tet(A) in drinking water isolates is constant to earlier reports that efflux pump-mediated tetracycline resistance is common in environmental bacteria. Thompson et al. (2007) identified novel tetA determinants in Serratiamarcescens from contaminated streams, while Roberts (2012) emphasized that efflux pumps remain the dominant mechanism of tetracycline resistance across Gram negative bacteria. The presence of tet (A) in Bhopal's drinking water isolates therefore reflects the persistence of tetracycline resistance genes in aquatic environments.Mahapatra et al. (2020) reported similar mutations in ciprofloxacin resistant Gram negative isolates in India, while Jose and Joseph (2026) demonstrated gyr A mutations in environmental E. coli from water ecosystems. These findings confirm that fluoroquinolone resistance is not confined to clinical pathogens but is also prevalent in drinking water bacterial isolates, raising concerns about community exposure.Prevalence of resistance genes in Bhopal's drinking water samples underscores the role of water systems as reservoirs of antimicrobial resistance (AMR). Metagenomic studies in India have confirmed that even treated municipal water harbors diverse ARGs, linking environmental microbiomes to clinical resistance pathways (Kumar et al., 2025). The detection of tet(A) in this study adds to the growing evidence that domestic drinking water points of use are critical reservoirs of ARGs, with possible for horizontal gene transfer to pathogenic bacteria.

From a public health perspective, the presence of ARGs in drinking water is alarming. Waterborne bacteria carrying resistance determinants can colonize humans directly or transfer genes to commensal microbiota, thereby increasing the risk of multidrug-resistant infections. This aligns with one health agenda, which emphasizes the interconnectedness of human, animal and environmental

health in tackling AMR (Robinson et al., 2016)

Conclusion : the detection of tet(A) gene in drinking water bacterial isolates from Bhopal District demonstrates the urgent need for continuous surveillance, improved water treatment practices, and integrated AMR management strategies. These findings reinforce the importance of monitoring environmental reservoirs to mitigate the spread of resistance genes into clinical settings.

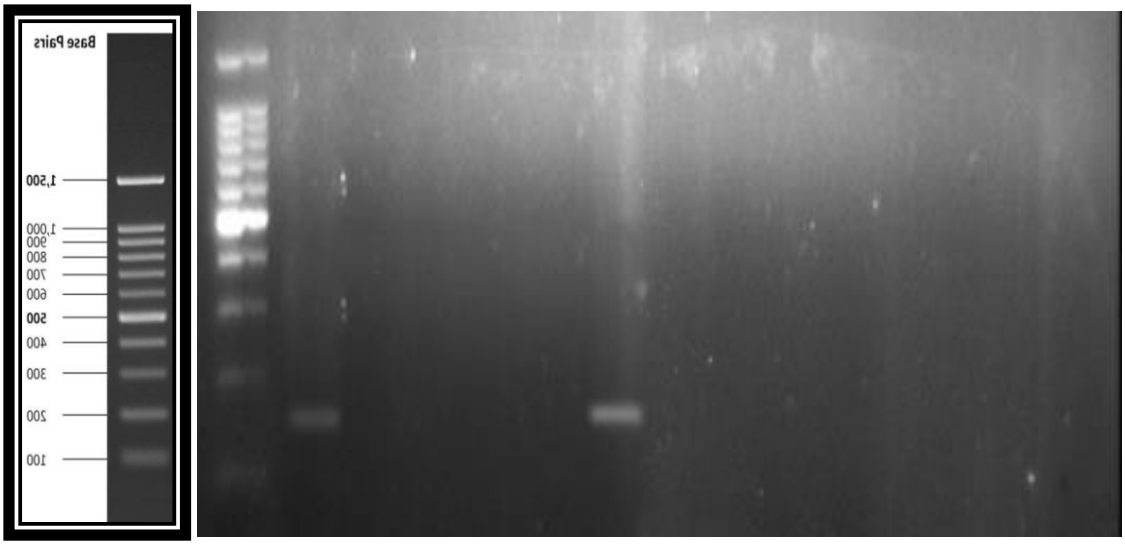


Figure 4.24: PCR detection of the *tet(A)* gene using established primers. Lane 1: DNA ladder; Lane 2: positive control; Lane 5: bacterial isolate showing *tet* resistance gene.

| S. NO. | Resistant against | Mutant gene | Gene reported in 20 colonies of Bactria species |
|---|---|---|---|
| 1 | Tetracycline | *tet(A)* | 1 |

Table 2 Frequency of Antibiotic Resistance Genes Detected in Drinking Water Bacterial Isolates from Bhopal District

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