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Assessment of Antibiotic Resistance in Bacterial Isolates from Drinking Water Sources in Sehore District, Madhya Pradesh

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ABSTRACT: This study investigates the issue of antibiotic-resistant bacteria in drinking water from various sources within the Sehore district of Madhya Pradesh, highlighting a critical public health concern. Access to clean water is a vital necessity for human health, yet ensuring its provision remains a challenge in many developing regions. The research employs rapid detection methods to identify antibiotic-resistant bacterial strains. Samples were gathered from taps, borewells, water tanks, and the Narmada River in different villages and were cultivated on Nutrient Agar at 37°C to encourage bacterial growth.

Three types of bacterial isolates *Pseudomonas* sp., *Escherichia coli,* and *Salmonella* sp. were previously identified and subjected to antibiotic susceptibility testing using the disc diffusion method. The analysis revealed significant antibiotic resistance among the isolates, with *Escherichia coli* showing resistance to ampicillin, norfloxacin, and ciprofloxacin. *Pseudomonas* sp. demonstrated moderate resistance in some instances, while the no *Salmonella* sp. cases identified remained sensitive to the antibiotics tested. The study emphasizes the need for persistent monitoring of drinking water to detect antibiotic-resistant bacteria, thereby safeguarding public health. It calls for the development of efficient strategies to manage and mitigate waterborne bacterial threats. The evidence presented points to the necessity for enhanced water quality management in the Sehore district, addressing both environmental and public health challenges posed by antibiotic resistance.

Keywords: Antibiotic resistance, *Pseudomonas* sp., *Escherichia coli, Salmonella* sp., Madhya Pradesh.

INTRODUCTION

Antibiotic resistance Antibiotic Resistance (ABR) has emerged as a significant global public health challenge, primarily driven by the excessive and often in appropriate use of antibiotics in healthcare and agriculture (Ventola, 2015). The indiscriminate and excessive prescription of antibiotics has accelerated the evolution of antibiotic-resistant bacteria, compromising the efficacy of treatments and threatening to reverse the medical advancements achieved over the past century. This growing crisis particularly endangers vulnerable populations in developing countries, where healthcare systems may lack the necessary resources and infrastructure to effectively manage and treat resistant infections (Levy & Marshall 2004). The World Health Organization (WHO) has highlighted that antibiotic resistance could lead to millions of deaths annually if not addressed (WHO, 2019). In developing regions, the prevalence of resistant bacteria is exacerbated by factors such as limited access to quality healthcare, poor infection control practices, and the unregulated sale of antibiotics (Al-Worafi, 2023).

These issues are further compounded by the high burden of infectious diseases, making the management and treatment of antibiotic-resistant infections particularly challenging (Levy, 2002; Narwade *et al.*, 2023). Access to safe drinking water is essential for human health, yet many regions, especially in low- and middle-income countries, face significant challenges in ensuring water quality (WHO, 2019). Contaminated water sources can act as reservoirs for antibiotic-resistant bacteria, leading to serious health complications in affected communities. The interconnection between water quality and antibiotic resistance is increasingly recognized as a critical public health concern. Poor water sanitation and hygiene practices can facilitate the spread of antibiotic-resistant bacteria, particularly in densely populated urban areas with inadequate sanitation infrastructure (Ashbolt, 2004). Rapid urbanization, combined with insufficient investment in water infrastructure, creates an

environment conducive to the proliferation and transmission of resistant pathogens (Pereira *et al.,* 2009). The Sehore district of Madhya Pradesh presents a critical case for investigation, given its reliance on various water sources, including borewells, taps, and rivers. Pathogenic bacteria such as *Escherichia coli*, *Salmonella* sp., and *Pseudomonas* sp. are frequently found in water supplies, contributing to waterborne diseases and showing increasing resistance to common antibiotics, which complicates treatment strategies (Baquero *et al.,* 2008). The geographic and socio-economic characteristics of the Sehore district make it a representative example of the broader challenges faced by similar regions in managing antibiotic resistance in water sources. Studies have documented the presence of antibiotic-resistant bacteria in various water sources, emphasizing the role of environmental reservoirs in the dissemination of resistance genes (Ashbolt, 2004). The dissemination of these resistant bacteria through drinking water poses a significant threat to public health, as it can lead to outbreaks of water borne diseases that are difficult to treat with available antibiotics. Moreover, the presence of antibiotic residues in water bodies, resulting from human and agricultural waste, can further select for resistant strains, creating a vicious cycle of resistance development (Pereira *et al.,* 2009). This study aims to assess the prevalence of antibiotic-resistant bacteria in drinking water sources in the Sehore district. Water samples will be collected from diverse sources, including household taps, borewells, water tanks, and the Narmada River, and analyzed using standard microbiological techniques (CLSI, 2020). The antibiotic susceptibility of the isolated strains will be tested using the disc diffusion method to elucidate the resistance patterns of the identified bacterial species (Jorgensen & Turnidge 2015). This methodological approach ensures that the findings are robust and can provide a comprehensive understanding of the prevalence and distribution of antibiotic-resistant bacteria in the district. The findings of this research are expected to provide valuable insights into the public health risks associated with antibiotic-resistant bacteria in drinking water and underscore the critical need for improved water quality management strategies. By highlighting the intersection of water safety and antibiotic resistance, this study aims to inform public health policies and contribute to efforts focused on safeguarding community health in the Sehore district. Effective water quality management strategies are essential to mitigate the spread of antibiotic-resistant bacteria and ensure the provision of safe drinking water to all populations (WHO, 2019). Addressing the challenge of antibiotic resistance in water sources requires a multi-faceted approach. Policies need to emphasize the regulation of antibiotic use in both human and veterinary medicine to minimize the selection pressure for resistant strains. Public health initiatives must focus on improving water, sanitation, and hygiene (WASH) practices to reduce the transmission of antibiotic-resistant bacteria. Additionally, there is a need for ongoing surveillance and monitoring of water sources to detect and respond to emerging resistance patterns promptly (Baquero *et al.,* 2008). Educational campaigns targeting healthcare providers, farmers, and the general public are crucial to raise awareness about the prudent use of antibiotics and the importance of maintaining water quality. Collaborative efforts between governmental agencies, non-governmental organizations, and the private sector can enhance the implementation of effective water quality management practices and antibiotic stewardship programs (Ventola, 2015). The interplay between antibiotic resistance and water quality is a critical public health issue that requires urgent attention (Serwecińska, 2020). The Sehore district of Madhya Pradesh provides a pertinent context to investigate the prevalence of antibiotic-resistant bacteria in drinking water sources and their implications for community health. The results of this study will contribute to the understanding of the environmental dimensions of antibiotic resistance and inform strategies to mitigate its impact on public health. By addressing the root causes and implementing comprehensive management strategies, it is possible to curb the spread of antibiotic resistance and safeguard the health of communities worldwide.

MATERIALS AND METHODS

Sample Collection A total of 80 drinking water samples were collected from various sources in the Sehore district of Madhya Pradesh, India. The sources included household taps, borewells, water tanks, and the Narmada River. Samples were gathered from different villages within the district to ensure a comprehensive assessment. Each sample was collected in gamma-sterilized bottles to prevent contamination and maintain sterility. Water Sample Inoculation and Bacterial Isolation To isolate bacteria from the water samples, the serial dilution method was employed. Each water sample was serially diluted and inoculated onto different selective media, including MacConkey agar, Eosin-Methylene Blue (EMB) agar, cetrimide agar, and Xylose Lysine Deoxycholate (XLD) agar. The inoculated plates were incubated at 37°C for 24 to 48 hours to allow for bacterial growth. Colony Selection and PCR Identification Randomly selected bacterial colonies from each plate were transferred to individual autoclaved PCR tubes. The bacterial samples were then incubated at 96°C for 7 minutes to lyse the cells and release the DNA. The resulting lysates were used as templates for colony PCR. Gene-specific primers were utilized to detect the presence of *Escherichia coli, Pseudomonas* sp., and *Salmonella* sp. This PCR process allowed for the accurate identification of the bacterial isolates.

Antibiotic susceptibility testing three types of bacterial isolates (*Pseudomonas* sp., *Escherichia coli*, and *Salmonella* sp.) identified from the water samples were subjected to antibiotic susceptibility testing using the

disc diffusion method. A panel of antibiotics, including ampicillin, ciprofloxacin, and tetracycline, was used to determine the resistance profiles of the isolates. Preparation of Bacterial Suspensions: Each bacterial isolate was cultured overnight in nutrient broth at 37°C. The bacterial cultures were adjusted to match the turbidity of a 0.5 McFarland standard.

Inoculation of Agar Plates: Mueller-Hinton agar plates were inoculated with the bacterial suspensions using a sterile cotton swab, ensuring even distribution across the entire surface of the agar. Placement of Antibiotic Discs: Antibiotic discs containing ampicillin, ciprofloxacin, and tetracycline were placed on the inoculated agar plates using a disc dispenser. The plates were then incubated at 37°C for 18 to 24 hours.

Measurement of Zones of Inhibition: After incubation, the zones of inhibition around each antibiotic disc were measured in millimeters. The results were interpreted based on standardized criteria provided by the Clinical and Laboratory Standards Institute (CLSI, 2020). Reproducibility of Results to ensure the reliability of the findings, all experiments were conducted in duplicate. This approach helped to confirm the reproducibility and accuracy of the results.

RESULTS AND DISCUSSION

The antibiotic susceptibility results of bacterial isolates from drinking water sources in Sehore district, Madhya Pradesh, are summarized in Table 1. The table provides a clear indication of the resistance profiles observed among the isolates.

Table 1: Antibiotic Resistance Profiles of Bacterial Isolates from Drinking Water Sources.

Sr. No.	Bacterial Species	Tetracycline	Ofloxacin	Ciprofloxacin	Ampicillin
1.	*Escherichia coli* sp. (n=40 isolates / 80 samples)	2	1	1	6
2.	*Pseudomonas* sp. (n=20 isolates / 80 samples)	-	1	-	1
3.	*Salmonella* sp. (n=4 isolates / 80 samples)	-	-	-	-

The analysis revealed significant antibiotic resistance among the bacterial isolates, with *Escherichia coli* showing notable resistance to multiple antibiotics. Specifically, out of 40 isolates of *E. coli*, 2 displayed resistance to tetracycline, 1 to ofloxacin, 1 to ciprofloxacin, and 6 to ampicillin. This high resistance rate to ampicillin is particularly concerning, as it highlights the growing ineffectiveness of this commonly used antibiotic against *E. coli* in water sources (Ventola, 2015).

Pseudomonas sp. isolates exhibited moderate resistance, with 1 out of 20 isolates showing resistance to both ofloxacin and ampicillin. Although the resistance observed in *Pseudomonas* sp. was less extensive compared to *E. coli*, it still poses a significant public health risk, given the pathogenic potential of *Pseudomonas* species and their known ability to acquire and disseminate resistance genes (Levy & Marshall 2004). Interestingly, the *Salmonella* sp. isolates, though fewer in number (n=4), hence no demonstrated resistance against any antibiotics. This suggests that, while *Salmonella* sp. in this region may not be as resistant as *E. coli*, there is still a presence of antibiotic-resistant strains that could complicate treatment options for waterborne infections (Baquero *et al.*, 2008).

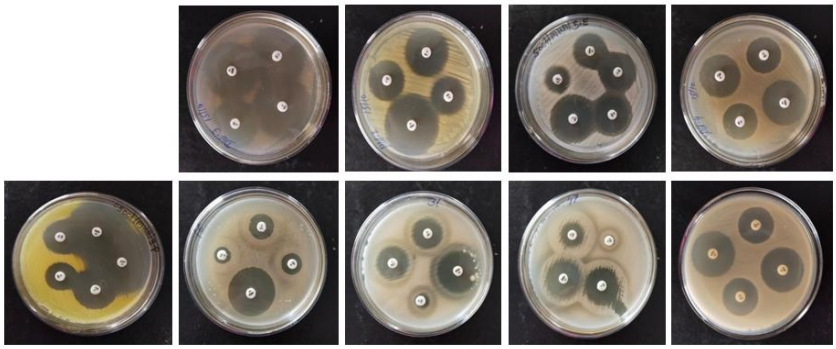

Fig. 1. Antibiotic susceptibility disc diffusion Test was performed in present study.

The findings of this study underscore the critical need for continuous monitoring of drinking water sources to detect antibiotic-resistant bacteria, thereby enhancing public health surveillance and intervention strategies. The significant resistance observed in *E. coli* isolates suggests that these bacteria are exposed to high selective pressures, likely due to the misuse and overuse of antibiotics in both human medicine and agriculture. This resistance can be propagated through water systems, leading to widespread public health challenges (WHO, 2019). The presence of antibiotic-resistant *Pseudomonas* sp. and *Salmonella* sp. further highlights the complexity of managing waterborne bacterial infections. *Pseudomonas* sp., known for its intrinsic resistance mechanisms and ability to survive in various environments, poses a continuous threat. The resistance

not observed in *Salmonella* sp., cannot be overlooked as it underscores the potential for these pathogens to acquire further resistance through horizontal gene transfer (Ashbolt, 2004). The study's findings suggest a dire need for improved water quality management in the Sehore district to address the environmental and public health issues caused by antibiotic resistance. Ensuring safe drinking water involves not only treating and monitoring water sources but also implementing stricter regulations on antibiotic use. Public health policies must prioritize the reduction of antibiotic contamination in the environment, which can be achieved through proper waste management and the regulation of agricultural practices (Pereira *et al.*, 2009; Marita *et al.*, 2024). Educational initiatives targeting healthcare providers and the general public are crucial to promote the prudent use of antibiotics. These efforts should be complemented by robust surveillance systems that can detect and respond to emerging resistance patterns promptly (Levy, 2002). Future research should focus on exploring the molecular mechanisms underlying the observed antibiotic resistance to develop targeted interventions. Investigating the genetic basis of resistance and the role of mobile genetic elements in the dissemination of resistance genes will provide deeper insights into combating antibiotic resistance in environmental settings (Jorgensen & Turnidge 2015). Additionally, expanding the scope of the study to include a larger number of samples and a wider range of antibiotics could offer a more comprehensive understanding of the resistance landscape. Collaboration with local authorities and international organizations can enhance the effectiveness of interventions aimed at mitigating the spread of antibiotic-resistant bacteria in drinking water sources (CLSI, 2020).

CONCLUSIONS

The study highlights the alarming prevalence of antibiotic-resistant bacteria in drinking water sources in the Sehore district. The significant resistance observed among *E. coli* isolates, along with the presence of resistant *Pseudomonas* sp., underscores the urgent need for continuous monitoring and effective water quality management strategies. By addressing these challenges, it is possible to safeguard public health and mitigate the impact of antibiotic resistance on community health in Sehore and similar regions.

FUTURE SCOPE

Future research should expand surveillance for antibiotic-resistant bacteria in diverse water sources to enable early detection. Widening the geographical scope of sampling will enhance understanding of resistance patterns. Molecular studies to uncover genetic mechanisms can lead to targeted interventions. Additionally, exploring innovative water treatments, understanding agricultural impacts, and educational initiatives on prudent antibiotic use are crucial. Collaboration among governments, academics, and the private sector is vital for developing strategies to manage water quality and antibiotic resistance, protecting public health and antibiotic efficacy.

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