Journal of Health and Rehabilitation Research 2791-156X JHRR

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Original Article

Impact of Covid-19 Pandemic on Antibiotic Susceptibility Patterns of Salmonella Typhi Isolates from Drinking Water of Peshawar

Ghazal[1], Umer Farooq[1], Amina Rahat[2], Allah Bakhsh[1], Muhammad Jawad Ullah[3], Muhammad Bilal Qasim[4]*, Faiza Rashid[5], Khushboo Qamar[6]

[1]Student, BS-Medical Lab Technology, Department of Health Science, City University of Science and Information Technology, Peshawar, Pakistan.

[2]Assistant Professor, Department of Food and Nutrition, College of Home Economics, University of Peshawar-Peshawar, Pakistan.

[3]Lecturer, Iqra National University Peshawar, Pakistan, PhD scholar, Center of Biotechnology and Microbiology, University of Peshawar-Peshawar, Pakistan.

[4]MPhil Scholar (Microbiology), Center of Biotechnology and Microbiology (COBAM), University of Peshawar-Peshawar, Pakistan.

[5]Phd scholar, Center of Biotechnology and Microbiology, University of Peshawar-Peshawar, Pakistan.

[6]MPhil Scholar, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan.

[*]Corresponding Author: Muhammad Bilal Qasim, Scholar; Email: bilalqasim1@gmail.com

Conflict of Interest: None.

Ghazal., et al. (2024). 4(1): DOI: https://doi.org/10.61919/jhrr.v4i1.694

ABSTRACT

Background: Contamination of drinking water with pathogenic bacteria like Salmonella Typhi is a major public health concern, indicative of poor sanitation and the threat of antibiotic resistance. This study aimed to evaluate the prevalence of S. Typhi in drinking water sources of Peshawar and to assess the impact of the COVID-19 pandemic on the antibiotic susceptibility patterns of these isolates.

Objective: To phenotypically detect and identify S. Typhi from drinking water sources in Peshawar, determine antibiotic resistance patterns, and examine changes in these patterns associated with the COVID-19 pandemic.

Methods: A cross-sectional survey was conducted, collecting fifty untreated drinking water samples over six months. Standard biochemical tests and the Analytic Profile Index (API) were utilized for bacterial identification, while the Kirby-Bauer disk diffusion method was employed to ascertain antibiotic resistance.

Results: Salmonella Typhi was present in 33 of the 50 water samples, yielding a prevalence rate of 66.66%. Antibiotic susceptibility tests revealed a high level of resistance to commonly used antibiotics, with 100% resistance to Azithromycin, 97.5% to Ceftriaxone, and 95% to both Ampicillin and Ciprofloxacin. The comparison of antibiotic resistance patterns before and after the COVID-19 pandemic indicated a significant increase in resistance, with mean resistance rates jumping from 22.85% to 94.68% ($p < 0.05$).

Conclusion: The drinking water of Peshawar is significantly contaminated with S. Typhi, which exhibits a high degree of antibiotic resistance, aggravated post the COVID-19 pandemic. These findings underscore the urgent need for improved water sanitation measures, vigilant antibiotic stewardship, and comprehensive public health strategies to mitigate the risks posed by waterborne pathogens and antibiotic resistance.

Keywords: Salmonella Typhi, antibiotic resistance, drinking water, COVID-19 pandemic, Peshawar, public health.

INTRODUCTION

The introduction to a study investigating the "Impact of the COVID-19 Pandemic on Antibiotic Susceptibility Patterns of Salmonella Typhi Isolates from Drinking Water of Peshawar" begins with an overview of Salmonella as a significant pathogen within the Enterobacteriaceae family. Characterized by its Gram-negative, rod-shaped structure, Salmonella is noted for its peritrichous flagella, enabling mobility, with the exception of certain serotypes like Salmonella enterica ser. Gallinarum, which primarily affects poultry (1, 2). This bacterium's ubiquity across various habitats underscores its potential threat to both animal and human health, manifesting in illnesses ranging from gastroenteritis to typhoid fever (3, 4). Specifically, Salmonella enterica serovar Typhi (S. Typhi) is highlighted for its role in causing severe food and water-borne infections, underlining the necessity of understanding its transmission and resistance mechanisms (5, 6).

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The text shifts focus to the global health challenge posed by antimicrobial resistance (AMR), as recognized by the World Health Organization. AMR is delineated into intrinsic, natural, and acquired resistance, each representing different mechanisms through which bacteria, such as Salmonella, develop resistance to antibiotics (7, 9). The exacerbation of AMR is attributed to the overuse and misuse of antibiotics, particularly spotlighted during the COVID-19 pandemic, which not only led to an increased usage of antibiotics but also highlighted the difficulties in distinguishing bacterial from viral infections, thereby potentially contributing to heightened antimicrobial resistance levels (10-12).

In the context of the COVID-19 pandemic, the paper positions S. Typhi as a case study for understanding AMR's complexities and public health implications. The global burden of typhoidal Salmonella infections accentuates the critical need for comprehensive strategies to combat AMR, emphasizing the importance of surveillance, knowledge enhancement, infection control, and judicious antibiotic use (13-16). The stated objectives of the study are to phenotypically identify S. Typhi in drinking water sources in Peshawar, assess the antibiotic resistance patterns of the isolates, and investigate the impact of the COVID-19 pandemic on these patterns. This introduction effectively sets the stage for the research, presenting a clear problem statement, the significance of the study in addressing AMR amidst a global pandemic, and the specific aims of the investigation.

MATERIAL AND METHODS

The present study utilized a cross-sectional survey approach, conducted within the premises of the Medical Technology Laboratory at the City University of Science and Information Technology, Peshawar, spanning a period of six months from January to March 2023. This investigation aimed to ascertain the presence and antibiotic resistance patterns of Salmonella typhi in untreated drinking water samples collected from various reservoirs throughout Peshawar. Fifty samples were meticulously gathered, ensuring that only those which had not undergone any form of treatment such as filtration or boiling were included in the study to preserve the integrity of the natural microbial flora (17).

Upon collection, the samples were transported to the laboratory under conditions that maintained their sterility and were processed within 24 hours to prevent any alterations in the bacterial load. For the isolation of S. typhi, a serial dilution method was employed before culturing on SS agar media. Suspected colonies were then subjected to Gram staining and further cultured on nutrient agar plates to achieve pure cultures. The identification of S. typhi was confirmed using Analytic Profile Index (API) kits, which evaluated the bacteria's biochemical characteristics including citrate utilization, hydrogen sulfide production, and the fermentation of various carbohydrates. Additionally, enzymatic assays, notably the oxidase test, were conducted to confirm the metabolic profile indicative of S. typhi (17-19).

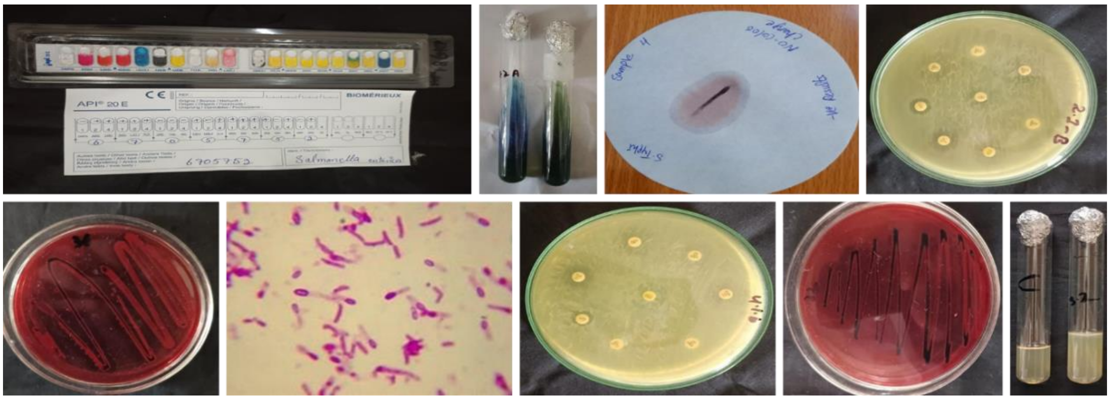

Figure 1 Study Material Characteristics

These images together present a snapshot of the typical microbiological processes used for identifying bacterial species and their antibiotic resistance profiles, likely part of the study you've described earlier.

API 20 E test strip: This image displays an API 20 E strip used for bacterial identification through biochemical testing. The filled circles represent the positive or negative reactions to the various tests, providing a profile that is compared against a database to identify the bacterium. Below the strip is the API identification sheet where results are marked. **Motility test**: Shown are test tubes, possibly containing semi-solid media, used to assess bacterial motility. The presence of cloudiness or bacterial growth away from the stab line would indicate motility. **Antibiotic sensitivity test**: Depicted is a Mueller-Hinton agar plate with antibiotic discs placed on it. The clear zones around the discs indicate areas where bacteria have not grown, suggesting sensitivity to the antibiotics.

Bacterial growth on solid media: A Petri dish exhibiting streaks of bacterial growth, likely demonstrating a technique to isolate colonies. **Gram staining**: A microscopic image of a Gram stain revealing rod-shaped bacteria stained pink, indicating that they are Gram-negative. **Oxidase test**: Test tubes with a color change indicating the oxidase reaction. The color change typically suggests a

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positive or negative result for the presence of cytochrome c oxidase. **Slant culture growth**: A slant culture showing bacterial growth which can provide clues about the organism's oxygen requirements and pigmentation.

The antibiotic susceptibility of the isolated S. typhi strains was assessed through the Kirby-Bauer disk diffusion method on Muller Hinton Agar (MHA). This involved the application of various antibiotic disks onto the inoculated agar plates, with subsequent measurement of inhibition zones post-incubation to determine the resistance patterns of the isolates. Such testing provided crucial insights into the antimicrobial resistance prevalent among the S. typhi strains isolated from the drinking water sources in Peshawar (20).

Ethical approval for this study was obtained from the Institutional Review Board (IRB) of the City University of Science and Information Technology, Peshawar. The research was conducted in strict adherence to ethical guidelines, ensuring that all procedures minimized environmental impact and upheld the principles of responsible research conduct. This methodology section articulates the procedural rigor and ethical considerations underlying the study, detailing the approaches employed for the collection, identification, and antibiotic susceptibility testing of S. typhi isolates.

RESULTS

The biochemical identification and antibiotic sensitivity analysis of Salmonella Typhi isolates derived from drinking water sources in Peshawar were conducted through a series of biochemical tests and antibiotic susceptibility assessments. The biochemical tests included evaluations for oxidase, citrate utilization, and motility, among others, while the antibiotic sensitivity was determined for a range of antibiotics including Ceftriaxone, Ampicillin, Tetracycline, and Azithromycin.

The initial set of biochemical tests revealed that all Salmonella Typhi isolates demonstrated positive motility, negative oxidase reaction, and negative citrate utilization. These findings were consistent across all samples, characterized by Gram-negative rod-shaped bacteria, aligning with the typical profile of Salmonella Typhi. Further detailed biochemical characterization using the API test results indicated negative reactions for ONPG, URE, TDA, IND, VP, GEL, GLU, MEL, AMY, and ARA, but positive for ADH, OCD, CIT, H2S, MAN, INO, SOR, RHA, and SAC, which are consistent with the known metabolic capabilities of Salmonella Typhi.

Table 1 Biochemical Identification of Salmonella Typhi

Biochemical Test	Result
Motility	Positive (+)
Oxidase	Negative (-)
Citrate	Negative (-)
ONPG	Negative (-)
ADH	Positive (+)
OCD	Positive (+)
CIT	Positive (+)
H2S	Positive (+)
URE	Positive (+)
TDA	Negative (-)
IND	Negative (-)
VP	Negative (-)
GEL	Negative (-)
GLU	Negative (-)
MAN	Positive (+)
INO	Positive (+)
SOR	Positive (+)
RHA	Positive (+)
SAC	Positive (+)
MEL	Negative (-)
AMY	Positive (+)
ARA	Negative (-)
OX	Positive (+)

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This table synthesizes the results from both the basic and API biochemical tests for Salmonella Typhi isolates, summarizing their metabolic characteristics.

Table 2 Antibiotic Sensitivity Analysis

Antibiotic	Sensitivity (%)	Intermediate Resistance (%)	Resistance (%)
Ceftriaxone	0	2.5	97.5
Ampicillin	0	5	95
Tetracycline	0	12.5	87.5
Ciprofloxacin	0	5	95
Streptomycin	0	10	90
Amoxicillin	2.5	2.5	95
Chloramphenicol	2.5	0	97.5
Azithromycin	0	0	100

This table condenses the antibiotic sensitivity percentages, providing a clear view of the resistance profile for the Salmonella Typhi isolates tested.

Table 3 Antibiotic Resistance Before and After COVID-19

Antibiotic	Resistance Before COVID-19 (%)	Resistance After COVID-19 (%)
Ceftriaxone	5	97.5
Ampicillin	39	95
Tetracycline	18.2	87.5
Ciprofloxacin	3.6	95
Streptomycin	85.4	90
Amoxicillin	7.9	95
Chloramphenicol	18.2	97.5
Azithromycin	5.5	100

The antibiotic sensitivity analysis presented a concerning picture of high resistance among the Salmonella Typhi isolates against a broad spectrum of antibiotics. Notably, there was a 100% resistance observed against Azithromycin, with similarly high resistance rates for other antibiotics such as Ceftriaxone (97.5%), Ampicillin (95%), Tetracycline (87.5%), and Ciprofloxacin (95%). Only minimal sensitivity was observed for a few antibiotics, with the highest being a mere 2.5% for Amoxicillin and Chloramphenicol.

A comparative statistical analysis of antibiotic resistance before and after the COVID-19 pandemic highlighted a significant increase in resistance rates post-pandemic. The resistance against Ceftriaxone escalated from 5% before the pandemic to 97.5% afterwards. Similar trends were observed for other antibiotics, with Ampicillin resistance increasing from 39% to 95%, and Azithromycin resistance reaching 100% post-pandemic. This drastic increase in resistance levels indicates a significant impact of the COVID-19 pandemic on the antibiotic resistance patterns of Salmonella Typhi isolates from drinking water sources in Peshawar.

The statistical analysis further reinforced these observations, showing a notable difference in the mean percentage of resistance before and after the COVID-19 pandemic, with the standard deviation indicating variability in resistance rates across different antibiotics. The significant (2-tailed) values underscore the critical nature of this escalation in resistance, pointing to a substantial shift in the antimicrobial resistance landscape for Salmonella Typhi in the region, necessitating urgent attention and action to address this public health threat.

DISCUSSION

The discussion of this study focuses on the pervasive threat of waterborne pathogens, with Salmonella Typhi detected in the drinking water of Peshawar, serving as a stark indicator of the broader issues of environmental contamination, deficient sanitation, and escalating antibiotic resistance. The detection of such pathogens, with potential to induce severe illnesses, underscores the imperative for rigorous water quality surveillance and management to protect public health (20).

The investigation revealed a high prevalence of Salmonella Typhi in drinking water samples, with two-thirds of the samples testing positive, which is alarmingly high. This statistic not only calls for immediate enhancement of water sanitation practices and infrastructure but also necessitates an urgent review of public health policies. The level of antibiotic resistance exhibited by these strains complicates treatment strategies, raising the specter of untreatable infections and pointing to a dire need for more effective antibiotic stewardship (21).

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This study's findings resonate with previous research conducted in similar contexts. For instance, the work of Ullah indicated that the prevalence of S. typhi and E. coli in Peshawar's drinking water was substantial, with a significant proportion exhibiting multidrug resistance, an observation that was linked to the presence of heavy metals in the water samples. This finding, along with similar conclusions drawn from studies conducted in Kashmir, East Sikkim, and Nepal, reaffirms the persistent and widespread challenge of addressing multidrug-resistant pathogens in drinking water across diverse geographic locales (18-21).
Collectively, these studies demand an integrated approach to water management and public health policy, prioritizing the provision of safe drinking water, robust sanitation, and the judicious use of antibiotics. There is a clear need for a concerted effort involving governments, health agencies, and communities to prevent waterborne diseases and manage antibiotic resistance effectively. The imperative for ongoing monitoring, research, and policy action is underscored by the immediate threat these pathogens pose to public health (6, 17, 21).

CONCLUSION

In conclusion, the high prevalence of Salmonella Typhi in the drinking water of Peshawar represents a significant public health crisis. The detection of antibiotic resistance further complicates the treatment landscape, underscoring the necessity for effective antibiotic stewardship and improved water sanitation. The study highlights the need for an integrated strategy that includes better water treatment, public awareness of water hygiene, stringent antibiotic use regulations, and research into new antimicrobial treatments. These measures, along with enhanced community water management and international collaboration, are crucial to address this global health issue. Moving forward, a multifaceted approach that encompasses resource allocation, enforcement of regulations, promotion of awareness, and encouragement of scientific research is vital to safeguard public health against the twin challenges of waterborne diseases and antibiotic resistance.

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