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UNSAFE SIPS: PREVALENCE OF PATHOGENIC BACTERIAL DIVERSITY IN DRINKING WATER OF KHAIRPUR CITY PAKISTAN

Original Research

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ABSTRACT

Background: Access to clean drinking water is vital for public health, yet contaminated water remains a leading cause of morbidity and mortality worldwide. In Pakistan, approximately 30% of diseases and 40% of deaths are waterborne, disproportionately affecting children in vulnerable environments such as schools. Poor water quality poses a significant risk to children's health, necessitating studies to assess microbial contamination and antimicrobial resistance in drinking water.

Objective: To evaluate the microbial quality of drinking water in government primary schools in Khairpur, Pakistan, by identifying bacterial diversity, prevalence of pathogenic bacteria, and antimicrobial resistance patterns.

Methods: A total of 42 water samples were collected from primary schools across six union councils in Khairpur. Total viable counts (TVC) were determined using serial dilution methods, and coliforms were detected using the membrane filtration method in accordance with WHO protocols. Bacterial isolates were identified and subjected to antimicrobial susceptibility testing using the MicroScan WalkAway 96 plus system. Sensitivity profiling was performed to determine resistance patterns, including multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL) production.

Results: Microbial contamination exceeded acceptable limits in all samples, with TVC ranging from 9,900 to 23,300 CFU/mL. Coliforms were detected in 57% of samples, and *Escherichia coli* was the most prevalent pathogen, constituting 47% of identified isolates. Among the 28 coliform isolates, 21.4% were identified as MDR, and 10% were ESBL producers. Antibiotics such as tigecycline, meropenem, and imipenem demonstrated the highest efficacy, whereas ampicillin and amoxicillin were the least effective.

Conclusion: This study highlights the poor microbial quality of drinking water in Khairpur's primary schools, posing significant health risks to children. Immediate measures, including improved water treatment and regular monitoring, are crucial to mitigating the burden of waterborne diseases.

Keywords: Antimicrobial Resistance, Bacterial Contamination, Drinking Water, Enterobacteriaceae, Microbial Diversity, Primary Schools, Waterborne Diseases.

INTRODUCTION

Access to clean and safe water is a fundamental requirement for a healthy life, as water constitutes approximately 60 percent of the human body. Despite its critical importance, many individuals, particularly in developing countries, lack access to microbiologically safe drinking water. Contaminated water, often clear and free of detectable odor or taste, poses significant health risks due to the presence of harmful pathogens (1). Safe drinking water must be free from microbial contamination, odor, and turbidity to be considered fit for consumption (2). The issue of groundwater contamination has become increasingly severe in some regions of Pakistan, including Khairpur, where immediate interventions are essential to prevent further degradation of the nation's water resources (3). Bacterial contamination of drinking water systems can arise from various sources, including human and animal waste, agricultural runoff, septic systems, and inadequately maintained residential lagoons. These sources introduce pathogenic bacteria that are not visible to the naked eye and can only be detected through laboratory analysis. The presence of coliform bacteria, a key indicator of water quality, signifies inadequate water treatment and potential health risks. Coliforms are Gram-negative, rod-shaped bacteria that thrive in aerobic or facultatively anaerobic environments, producing gas by fermenting lactose at specific temperatures (9). They are commonly associated with faecal contamination and are linked to a range of waterborne infections, which remain a leading cause of morbidity and mortality worldwide (5). Among these, diarrhoeal diseases claim approximately 1.8 million lives annually, as reported by the World Health Organization (WHO) in 2005, with children being particularly vulnerable due to their underdeveloped immune systems (4).

Waterborne diseases are caused by a variety of enteric pathogens, including bacteria, viruses, and parasites, which are typically transmitted through ingestion of contaminated water (6). The infectious dose varies among pathogens, with some requiring as few as 100 cells to cause disease (7). Comprehensive identification of each waterborne pathogen is challenging, costly, and impractical for routine water quality analysis (8). However, the presence of faecal coliforms provides a reliable indicator of contamination. Previous studies conducted in Khairpur, Sindh, have reported coliform contamination in drinking water, underscoring the urgent need to investigate water quality, particularly in environments frequented by children, such as schools (11). Globally, the burden of waterborne diseases is often underestimated due to underreporting and the limitations of health surveillance systems, particularly in low- and middle-income countries (12). In contrast, high-income nations, such as the United States, have established robust surveillance systems like the Waterborne Disease and Outbreak Surveillance System. This has facilitated extensive research on outbreaks linked to drinking water contamination, identifying pathogens such as *Legionella*, *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., *Cryptosporidium*, *Giardia intestinalis*, and norovirus as major threats (13). Such initiatives emphasize the importance of rigorous monitoring and reporting mechanisms to address waterborne diseases effectively.

This study aims to assess the prevalence and diversity of pathogenic bacteria in drinking water in Khairpur City, Pakistan, providing critical insights into the public health risks associated with microbial contamination. By identifying key sources and patterns of bacterial pollution, the findings will contribute to evidence-based strategies for ensuring safer water quality and protecting community health.

METHODS

The study was conducted in Khairpur Mirs' City, where water samples were collected from various primary government schools in each union council. Water samples were obtained using standardized protocols to ensure accuracy and reliability (14). For bacteriological analysis, samples were collected in sterile 100 mL containers, while separate containers were used for physiochemical analysis. Physiochemical parameters were analyzed onsite using portable instruments and meters to maintain the integrity of the data. To detect total and fecal coliforms, the Most Probable Number (MPN) method was utilized. This involved inoculating a series of fermentation tubes with five tubes for each dilution, varying the sample-to-medium ratio by inoculating 1 mL of the water sample into 10 mL of sterilized medium. Lauryl tryptose broth and EC broth were employed as the recommended media for quantifying total and fecal coliforms in the presumptive test. Fermentation tubes containing 10 mL of sterilized media were inoculated with water samples and incubated at 37°C for 48 hours. Gas production in Durham's tubes was recorded as a positive presumptive test, indicating the presence of coliforms.

Bacterial isolates were tentatively identified based on colonial morphology and Gram staining. Further identification and antimicrobial sensitivity testing were conducted using the MicroScan Walk Away 96 plus System. The initial oxidase test was performed manually, followed by automated identification and sensitivity testing. Pure cultures were used to prepare inoculum, which was then transferred to trays and subsequently loaded into panels with the help of Ronix. These panels were placed into the MicroScan analyzer, and results were recorded after 16 to 18 hours of incubation. The MicroScan Walk Away 96 plus is an advanced automated analyzer designed for bacterial identification and antimicrobial sensitivity testing. It employs techniques such as colorimetric, photometric, and fluorometric analyses, allowing for the simultaneous processing of standard, rapid, and specialized panels on a single automated platform. This system is particularly effective in detecting emerging antimicrobial resistance, including pathogens such as Vancomycin-intermediate *Staphylococcus aureus* (VISA), Vancomycin-resistant *S. aureus* (VRSA), Methicillin-resistant *S. aureus* (MRSA), and Extended Spectrum β-lactamase (ESBL)-producing bacteria. Additionally, the MicroScan Walk Away 96 plus system has proven to be a valuable diagnostic tool for identifying carbapenemase-producing *Enterobacteriaceae*, which are often associated with multidrug resistance (16). In the United States, systems such as Vitek, MicroScan, and BD Phoenix are widely used for bacterial identification and antibiotic profiling, highlighting their clinical significance (17).

RESULTS

A total of 42 water samples were analyzed for coliform contamination, of which 24 (57%) were found positive for coliforms, while the remaining 18 (43%) tested negative. Samples were collected from various government primary schools across union councils in Khairpur City. The frequency of coliform-positive samples varied among the union councils, reflecting substantial microbial contamination in several areas. Advanced microbial identification using the MicroScan Walk Away 96 plus system revealed the presence of 28 coliform isolates. Among these, *Escherichia coli* was the most prevalent species, accounting for 47% (n=13) of the isolates, followed by *Klebsiella pneumoniae* (18%, n=5), *Citrobacter freundii* (14%, n=4), *Klebsiella aerogenes* (7%, n=2), *Enterobacter intermedius* (7%, n=2), and *Enterobacter cloacae* (7%, n=2). The findings highlight *E. coli* as a dominant contaminant in the analyzed water samples.

Table 1. Coliform screening of drinking water from study sites.

S.NO	NAME OF UC	SCHOOLS	Sample ID	COLIFORM TEST
		GBPS - POLICE LINE	1	Positive
		GBPS - SAIDA GOTH	2	Positive
		GBPS - CENTRAL JAIL	3	Negative
1	JILLANI	GGPS - JILANI MOHALA	4	Positive
		GBPS - MADARS FAZUL QURAN	5	Negative
		GBPS - KHOKHAR MOHALLA	6	Positive
		GBPS - SUMING(GARI-PULL)	7	Positive
		GBPS - KASHMIR	8	Negative
		GBPS - OSTA GAHI	9	Positive
		GBPS - SHAMAN ALI SHAIKH	10	Positive
2	BUKHARI	GGPS - SANWALO JAMALI	11	Positive
		GBPS - HUSSAIN BUX SHAIKH	12	Negative
		GBPS - SANWILO JAMALI	13	Positive
		GBPS - SALEEM ABAD	14	Negative
		GBPS - MUMTAZ COLONY	15	Positive
		GBPS - ANWAR UL MUJTABA	16	Negative
		GBPS - PACCA SHADE CITY KHAIR PUR	17	Positive
3	BHURGRI	GBPS - NOOR MUSTAFA	18	Positive
		GBPS - PERAL MOREJO(BRANCH)	19	Positive
		GBPS - STAFF QUARTER	20	Negative
		GBPS – PHAT	21	Negative

S.NO	NAME OF UC	SCHOOLS	Sample ID	COLIFORM TEST
		GBPS – HAMADIA	22	Positive
		GBPS - MADERSA HUSSAINI	23	Negative
		GGPS - SADORO JANWARI	24	Positive
4	SHAH LATIF	GBPS - UNAR COLONY	25	Negative
		GBPS - NAZAR ALI JAMALI	26	Negative
		GBPS - MITHO MARI	27	Positive
		GBPS - MAHER TARAYOON	28	Negative
		GBPS - MUHAMMAD USMAN BHAYO	29	Positive
		GBPS - MITHO MARI	30	Negative
		GBPS - GUJO PHUL POTO	31	Positive
5	NIZAMANI	GBPS - ALI MUHAMMAD ABRO	32	Negative
		GBPS – SIMING	33	Positive
		GBPS - IMTIAZ HUSSAIN PHULPOTO	34	Positive
		GBPS - MISRI KATOHAR	35	Positive
		GBPS - ANWAR UL HAIDERIA	36	Negative
		GBPS - MADARSA AL-QADIRIA LUQMAN	37	Positive
		GBPS - MUHAMMAD JANWARY	38	Positive
6	LUQMAN	GBPS- SINDHI LUQMAN	39	Negative
		GBPS - MUHAMMAD QASIM PULPOT0	40	Positive
		GBPS - BAHER NOONARI	41	Negative
		GBPS - MANGAN HAR	42	Negative

Antimicrobial sensitivity profiling of the isolates showed diverse resistance patterns across different bacterial species. Resistance to amoxicillin-clavulanic acid was observed in 61% of *E. coli*, 60% of *K. pneumoniae*, and 100% of *K. aerogenes*. Alarmingly, 100% of *E. coli*, *K. pneumoniae*, *K. aerogenes*, *E. intermedius*, and *E. cloacae* demonstrated resistance to ampicillin, emphasizing the prevalence of antimicrobial resistance in these coliforms. Additionally, 84% of *E. coli* and 100% of *C. freundii* exhibited resistance to aztreonam. Carbapenem resistance, indicated by resistance to imipenem and meropenem, was relatively low, ranging between 0% and 40%, depending on the isolate. Resistance to tigecycline was found in 20% of *K. pneumoniae* and 100% of *K. aerogenes*, underscoring the emerging challenges of multidrug resistance (MDR).

Table 2. Frequency of coliform screened through MicroScan machine

S. No.	Isolate according to MicroScan Database	Frequency (%)
1	Escherichia coli	13 (47%)
2	Klebsiella pneumoniae	05(18%)
3	Citrobacter freundii	04 (14%)
4	Klebsiella aerogene	02(7%)
5	Enterobacter intermedius	02(7%)
6	Enterobacter cloacae	02(7%)
Total		28

Among the 28 coliform isolates, six (21.4%) were identified as multidrug-resistant (MDR) bacteria, while three (10.7%) were extended-spectrum β-lactamase (ESBL) producers. Notably, *E. coli* constituted the majority of MDR and ESBL-producing isolates, with specific strains, such as MS-O14 and MS-O23, showing resistance to multiple antibiotic classes. Similarly, MDR and ESBL-producing *K.*

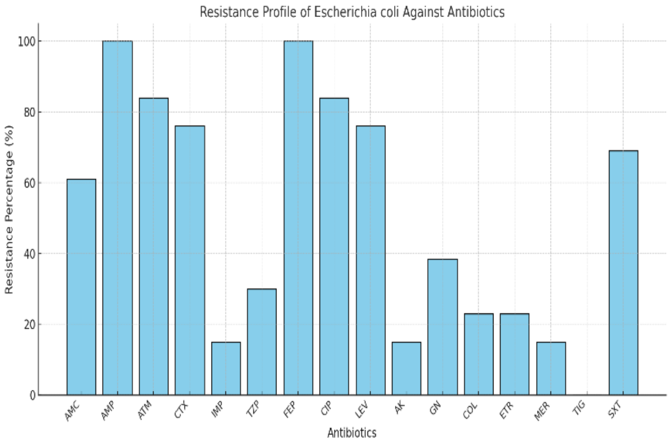
pneumoniae isolates were also detected, highlighting their clinical significance in public health. These results indicate that a significant proportion of the drinking water samples in the study area harbored pathogenic bacteria with notable resistance to antibiotics. The high prevalence of *E. coli* and the emergence of MDR and ESBL-producing isolates underscore the critical need for improved water treatment and monitoring strategies to mitigate public health risks associated with microbial contamination.

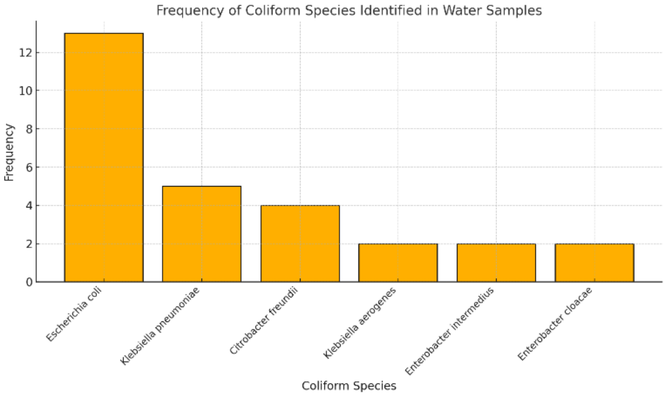
Table 3. Summary of overall resistance profile of organisms towards antibiotics

S/No	Antibiotics	E. coli N= 13	K. pneumoniae N= 05	C. freundii N=0 4	K. aerogenes N= 02	E. intermedius N= 02	E. cloacae N= 02
1	AMC	61.00%	60%	25.00%	100%	50.00%	100.00%
2	AMP	100%	100.00%	50.00%	100%	100.00%	100.00%
3	ATM	84%	80%	100.00%	50.00%	50.00%	100.00%
4	CTX	76%	80%	50.00%	100%	50.00%	0.00%
5	IMP	15%	40%	0.00%	0.00%	0.00%	0.00%
6	TZP	30%	40%	0.00%	50%	0.00%	0.00%
7	FEP	100.00%	80.00%	75.00%	100%	0.00%	50.00%
8	CIP	84%	100.00%	0.00%	100.00%	0.00%	50.00%
9	LEV	76.00%	80.00%	0.00%	50%	0.00%	50.00%
10	AK	15%	80%	50%	50.00%	0.00%	0.00%
11	GN	38.40%	60%	25%	0.00%	0.00%	50.00%
12	COL	23.00%	0.00%	25.00%	100%	0.00%	50.00%
13	ETR	23.00%	40.00%	25.00%	0.00%	0.00%	50.00%
14	MER	15.00%	40.00%	0.00%	0.00%	0.00%	0.00%
15	TIG	0.00%	20.00%	0.00%	100%	0.00%	0.00%
16	SXT	69%	100.00%	100.00%	0.00%	100.00%	0.00%

Table 4. MDR and ESBL producer isolated from drinking water

S.No	Isolate Code	Isolate	Drug Resistance
1	MS-O4	E. coli	ESBL
2	MS-O14	E. coli	MDR
3	MS-O15	E. coli	ESBL
4	MS-O18	E. coli	MDR
5	MS-O19	E. coli	MDR
6	MS-O23	E. coli	MDR
7	MS-O24	E. coli	MDR
8	MS-O6	K. pneumoniae	ESBL
9	MS-O11	K. pneumoniae	MDR





DISCUSSION

The primary objective of this study was to evaluate pathogenic bacterial contamination in drinking water samples collected from schools in Khairpur, focusing on coliform bacteria as indicator organisms. Water samples were processed following standard protocols, with serial dilution and subsequent inoculation onto nutrient agar for total viable counts (TVC) and into lactose broth for coliform detection. When coliforms were not detected in 1 mL samples, the membrane filtration method was employed as recommended by WHO, processing 100 mL water samples with a 0.45 µm filter. Among the 42 water samples analyzed, 24 (57%) were positive for coliforms. Bacterial isolates were identified and subjected to antimicrobial profiling using the MicroScan Walk Away 96 plus system, a robust and efficient tool that provides bacterial identification and antimicrobial sensitivity results within 24 hours (18). The bacterial counts in all samples exceeded the acceptable threshold of <500 cfu/mL set by WHO and EPA. The minimum bacterial load recorded was 900 cfu/mL, while the maximum was 23,000 cfu/mL. These findings align with earlier studies conducted in different regions, which also reported high microbial contamination in water supplies, particularly in developing areas. Coliform bacteria, commonly used as markers for water contamination, were isolated in significant quantities, indicating environmental exposure and inadequate water treatment. This highlights a persistent public health challenge, especially in areas where water treatment systems are insufficient or absent (19).

The study identified six coliform species, with *Escherichia coli* being the most prevalent (46%). This aligns with previous research indicating the widespread occurrence of *E. coli* in contaminated water supplies. Other species identified included *Klebsiella pneumoniae*, *Citrobacter freundii*, *Klebsiella aerogenes*, *Enterobacter intermedius*, and *Enterobacter cloacae*. The predominance of *E. coli* reflects its ecological prevalence and association with fecal contamination. These findings corroborate earlier studies conducted globally, which also reported high rates of coliform contamination in water samples (19, 20). Antimicrobial profiling revealed alarming resistance patterns among the isolates. Multidrug resistance (MDR) was identified in 6 (21.4%) isolates, while 3 (10.7%) were classified as extended-spectrum β-lactamase (ESBL) producers. The MDR and ESBL-producing isolates included *E. coli* and *K. pneumoniae*, consistent with findings from studies conducted in other countries where these pathogens have been identified as major contributors to waterborne diseases. Resistance to commonly used antibiotics, such as ampicillin and amoxicillin, was widespread, while drugs such as tigecycline, imipenem, and meropenem were found to be the most effective. These results highlight the growing challenge of antimicrobial resistance in environmental isolates, which has significant implications for public health and treatment strategies (16, 18).

The results of this study demonstrate substantial microbial contamination in drinking water from schools, posing serious health risks to children. The presence of coliform bacteria and high bacterial loads in the majority of samples underscores the need for improved water quality management and stricter enforcement of safety standards. Children, due to their underdeveloped immune systems, are particularly vulnerable to waterborne diseases, including diarrhea, which remains a leading cause of morbidity in this age group. The findings emphasize the critical need for regular monitoring, effective water treatment, and targeted interventions to protect vulnerable populations (18, 20). This study provides valuable insights into the microbial quality of drinking water in Khairpur schools. However, certain limitations must be acknowledged. The study focused solely on bacterial contamination and did not assess other potential contaminants, such as heavy metals or viruses, which may also impact water quality and public health. Additionally, the sample size, though adequate for preliminary conclusions, could be expanded in future studies to enhance the generalizability of the findings. Despite these limitations, the study serves as a baseline for further research and offers actionable data to health authorities and policymakers.

The strength of this study lies in its comprehensive approach, employing advanced microbial identification tools and adherence to standardized protocols. The use of the MicroScan Walk Away 96 plus system allowed for precise identification of pathogens and detailed antimicrobial resistance profiling, which is critical for understanding the scope of the problem. By highlighting the prevalence of MDR and ESBL-producing pathogens in school water supplies, this study underscores the urgent need for immediate corrective measures to ensure the safety of drinking water and protect the health of schoolchildren (15). The findings provide a strong basis for health management authorities to prioritize water safety interventions. Safe drinking water is fundamental to public health, and addressing contamination in schools is particularly vital to safeguard the well-being of children, who represent the foundation of future societal strength. The study calls for coordinated efforts to enhance water treatment facilities, implement rigorous monitoring protocols, and raise awareness about waterborne diseases to mitigate the risks associated with microbial contamination.

CONCLUSION

The study evaluated the bacterial quality of drinking water in government primary schools in Khairpur, Pakistan, focusing on the prevalence of coliform contamination and antimicrobial resistance. The findings revealed significant microbial contamination in the majority of water samples, with *Escherichia coli* identified as the most prevalent pathogen. The study also highlighted the presence of multidrug-resistant and extended-spectrum β-lactamase-producing bacteria, emphasizing the critical health risks posed by contaminated water. While certain antibiotics, such as meropenem and tigecycline, were found effective, widespread resistance to commonly used antibiotics underscores the growing challenge of antimicrobial resistance. These findings emphasize the urgent need for improved water treatment and regular monitoring to ensure safe drinking water in schools, ultimately safeguarding the health of children and fostering a healthier future generation.

Author Contribution

Author	Contribution
Mohsin Raza Gambhir	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sham Lal	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Sadam Hussain Shaikh	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Syed Ahmed Saeed Zaidi	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Farhaz Ahmed Khuhro	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Asif Raza Kanhar	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Tabish Akbar Phul	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Rooma Khuhro	Writing - Review & Editing, Assistance with Data Curation
Rasheeda Rustam Mailto	Writing - Review & Editing, Assistance with Data Curation
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