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ANTIMICROBIAL RESISTANCE PROFILE OF ENTEROBACTERIACEAE ISOLATED FROM DRINKING WATER

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Abstract

Water contamination with pathogenic microorganisms is one of the serious threats to human health, particularly in developing countries. In Pakistan, due to a lack of awareness about water-borne diseases and poor infrastructure water-borne diseases are common. Additionally, contamination of surface freshwater sources increases the frequency of waterborne disease outbreaks such as diarrhea, cholera, typhoid fever, intestinal infections, and jaundice. Therefore, the purpose of this present work was to study the quality of water in District Barkhan, Quetta Pakistan, which included identification and bacteriological characterization of antibiotic-resistant bacteria found in drinking water. A total of 500 drinking water samples were randomly collected from district Barkhan for analysis of pathogenic bacteria. Out of 500 drinking water samples, 334 (66.8%) were found contaminated with pathogenic bacteria where the prevalence of Salmonella typhi was 28%, followed by E. coli (22.4%) and P. aeruginosa (16.4%) respectively. According to our result, all S.typhi isolates were highly resistant to Ampicillin (95%), Chloramphenicol (90%), Ceftriaxone (85%), Gentamicin (85%), Sulfamethoxazole/ Trimethoprim (80%), E. coli isolates were resistant against Ampicillin (90%) followed by Ceftriaxone (85%), Chloramphenicol (80%), Azithromycin (75%) and Sulfamethoxazole/ Trimethoprim (70%), while P.aeruginosa isolates were found resistance against Ampicillin (100%), Ceftriaxone (95%), Chloramphenicol (90%), Azithromycin (75%) and Sulfamethoxazole/ Trimethoprim (70%), and while all pathogenic isolates were highly sensitive to Imipenem. The study concluded that continuous screening and treatment of water sources is essential for managing and preventing infectious diseases caused by pathogenic microorganisms. Therefore, prompt and consistent bacteriology research is needed to address the crisis caused by poor water quality.

Keywords: *Growth, Levels, Phosphorus, Wheat, Yield*

INTRODUCTION

Water is one of the most essential elements for survival. According to the World Health Organization (WHO), having access to water is a "prime tool for improving public health because it can greatly improve hygiene and avert many diseases. According to the International Monetary Fund (IMF), Pakistan ranks third among countries with poor sanitation and inadequate water supplies, with 2.1 million without access to clean water (1). In 2017, about 2.5 million deaths were recorded in Pakistan where literature reported that consumption of contaminated water is the main cause of 50% of illnesses and 40% of deaths in the country (2). Numerous contaminants comprise different types of microbes, feces, metallic toxins, household as well as factorial waste, antibiotics, and various hazardous drugs that make water contaminated. Innumerable types of harmful bacteria with enteric origins have been identified in water sources representing a broad spectrum of diversity. They comprise *Shigella spp, Campylobacter jejuni, Salmonella typhi, enterotoxigenic. Escherichia coli, enterotoxigenic Bacteroides fragilis Vibrio cholerae O1, Aeromonas* *spp, Pseudomonas aeruginosa, enteropathogenic E. coli,* and *V. cholerae O139* (3). Contamination of surface freshwater sources increases the frequency of waterborne disease outbreaks such as diarrhea, cholera, typhoid fever, intestinal infections, and jaundice. As a result, in developing countries, this is an ongoing issue for the environment as well as for public health (4-5).

In the lower gastrointestinal tracts of warm-blooded animals as well as humans, *Escherichia coli* bacteria are more frequently found (6). Detection of *E. coli* in potable water is a typical microbiological indicator of water quality and fecal contamination (7). The most common pathogenic bacteria, Enteritis *Salmonella typhi*, primarily affects people living in low- and middle-income nations with inadequate sanitary facilities for disposing of waste and clean, drinkable water (8). Globally, each year there are about 21 million cases of enteric fever and 200,000 deaths are reported by typhoid fever (9). Pakistan has an annual incidence of 493.5 cases per 100,000 people, making it one of the highest-burden countries. *Pseudomonas aeruginosa* is naturally found in water and it is also one of the most common causes of HAIs (such as skin and soft tissue infections, ear and eye infections, respiratory and urinary tract infections, and bacteremia), which primarily affect patients with compromised immune system (10-11).

Since their discovery in the early 20th century, antibiotics have been used extensively to treat a broad spectrum of illnesses and infections caused by pathogenic bacteria. Furthermore, antibiotic resistance developed as a result of misappropriation and overuse. Consequently, over time bacteria acquired resistance genes through either horizontal gene transfer or mutation (12). It is estimated that due to antimicrobial resistance (AMR) infections, approximately 10 million people will die each year (13-14). Additionally, new infections that are challenging to treat with the previous medication of choice for the same infection have emerged as a result of this antibiotic resistance. Concerns regarding future resistance to currently available antibiotics have been raised by antibiotic resistance, particularly if treatment is not appropriately addressed (15). The biggest hazard to public health is the transmission of resistance genes from environmental bacteria to pathogenic and clinically relevant microorganisms. The main cause for concern is the contamination of drinking water and the resulting infections, which can eventually cause diseases and even death (16-17). The purpose of the current study was to determine the prevalence of pathogenic bacteria in drinking water as well as the pattern of antibiotic resistance against these pathogens.

MATERIALS AND METHODS

STUDY AREA DESCRIPTION

Barkhan is the eighth-smallest district of Balochistan and has an area of 3410 square kilometers with 171025 populations. Barkhan District lies between 69°3'-70° 4' East longitude and 29°37'-30°21' North latitude. Barkhan's hot, semi-arid climate results in sweltering summers and moderate winters.

COLLECTION OF WATER SAMPLE AND PRESERVATION

A total of 500 samples of drinking water were randomly collected from district Barkhan (Rarkan, Rakni, Barkhan city, Nahar kot, and Baghao) in 200 ml sterile collection bottles and the caps were replaced immediately to prevent air bubbles. After that, the water samples were transported in ice boxes to the CASVAB laboratory where they were kept cold at 4 °C until examination (18).

TIME PERIOD

The present study was conducted in Barkhaan district of Balochistan from April 2022 to September 2022.

IDENTIFICATION OF BACTERIA

Water samples were processed according to the International Organization for Standardization (ISO) 5667-5:2006 with some modifications. All the water samples were cleared out with the aid of a membrane filtration approach. In this method, the water sample could be passed through the cellulose nitrate sterile membrane. After the analysis of microbial growth, the filter paper will be placed on differential media, followed by selective media for suspected bacteria including salmonella-shigella (SS) agar for *salmonella typhi,* eosin methylene blue (EBM) agar for *E. coli,* and cetrimide agar for *Pseudomonas aeruginosa* and was incubated at 37°C for 24hrs (19-20).

CONFIRMATION OF ISOLATES

From selective media typical colonies were chosen and then subjected to gram staining, microscopic examination, biochemical testing, and then finally PCR.

MOLECULAR IDENTIFICATION

A DNA purification kit (Thermos Scientific) was used to extract DNA from the culture. DNA templates were kept at -20 °C for future use. The specific primers were used for each three pathogenic bacteria to amplify their respective. PCR fragments as shown in Table I. The PCR reaction mixture for the three isolates was subjected to thermal cycles including initial denaturation at 94°C for 5 minutes, final denaturation at 94°C for 1 minute, annealing has a specific temperature and time based on their primers, initial extension for 1 min at 72°C, and final extension for 10 min at 72°C followed by 30 cycles for three isolates. The final product was observed using gel electrophoresis on a 1.5% agarose gel under UV light.

Table I. Specific primers used for the confirmation of pathogenic microbes

P. aeruginosa	Forward Reverse	5`-ATG GAA ATG CTG AAA TTC GGC-3` 5`-CTT CTT CAG CTC GAC GCG ACG-3`	oprLgene	21 (Khattb *et al.*, 2015)
S. typhi	Forward Reverse	5`-TATGCCGCTACATATGATGAG-3` 5`-TTAACGCAGTAAAGAGAG-3`	fliC gene	22 (Song *et al.*,1993)
E. coli	Forward Reverse	5`-ACA CTG GAT GAT CTC AGT GG-3` 5`-CTG AAT CCC CCT CCA TTA TG-3`	SLTI	23 (Gannon *et al.*, 1992)

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The Analytical Profile Indexing (API) was used to identify the bacteria. Antimicrobial susceptibility was tested against eight different antimicrobials using the Kirby Bauer disk diffusion method. The antibacterial drugs were chosen because due to their regular use against these pathogens. They included Gentamicin (30ug), Ampicillin (10ug), Ciprofloxacin (5ug), Ceftriaxone (30ug), Chloramphenicol (30ug), Azithromycin (15ug), Sulfamethoxazole/trimethoprim (25ug) and Imipenem (10ug). The agar plates were supplemented with commercially made antimicrobial discs, and the clear zones of bacterial growth inhibition were measured after incubation (24-19).

RESULTS

The current study evaluated the microbiological quality of 500 drinking water samples that were randomly picked from different locations within the Barkhan district. The frequency of bacterial contamination in drinking water samples is presented in Table II. Out of 500 drinking water samples, 334 (66.8%) were found contaminated with pathogenic bacteria where the prevalence of *Salmonella typhi* was 28%, followed by *E. coli* (22.4%) and *P. aeruginosa* (16.4%) respectively. The suspected positive samples were confirmed by colony morphology, microscopy, or biochemical tests like, catalase, oxidase, citrate, methyl red, voges- proskauer, indole, and sugar fermentation tests, the results are shown in Table III, and molecular identification was done by PCR.

By using the Kirby-Bauer method, antibiotic sensitivity was performed against *S. typhi, E. coli* and *P. aeruginosa* as shown in Table IV**.** In our findings all *S.typhi* isolates were highly resistant to Ampicillin (95%), Chloramphenicol (90%), Ceftriaxone (85%), Gentamicin (85%), Sulfamethoxazole/ Trimethoprim (80%) and sensitive to imipenem (90%), azithromycin (85%) and Ciprofloxacin (70%). *E. coli* isolates were resistant against Ampicillin (90%) followed by Ceftriaxone (85%), Chloramphenicol (80%), Azithromycin (75%), Sulfamethoxazole/ Trimethoprim (70%), and sensitive to Imipenem (95%), Gentamicin (90%), Ciprofloxacin (85%) to while *P.aeruginosa* isolates were found resistance against Ampicillin (100%), Ceftriaxone (95%), Chloramphenicol (90%), Azithromycin (75%), Sulfamethoxazole/ Trimethoprim (70%), and sensitive to Imipenem (90%), Gentamicin (75%), Ciprofloxacin (70%).

Table II. The prevalence of identified pathogenic bacteria in drinking water

Sample	Sample size	Number of samples positive	Samples of positive percentage prevalence
Drinking water	500	334	66.8%
S. typhi	334	140	28%
E. coli		112	22.4%
P. aeruginosa		82	16.4%

Table III. Biochemical characterization of pathogenic bacteria

Selective media used for isolated Bacteria			
Growth of selective media	***E. coli***	***S. typhi***	***P. aeruginosa***
	On EMB agar *E. coli* are circular shaped, and are 1-3mm in size. The green metallic sheen indicates *E. coli* can ferment lactose to produce strong acid end-products.	On SS agar, colonies of *Salmonella typhi* are smooth and opaque or colorless and are 2-3mm in size	*P. aeruginosa* colonies on Cetrimide agar, medium range in size from 1 to 3 mm and can appear blue, blue-green, or unpigmented
Gram staining results of isolated bacteria			
Gram staining	*E. coli* is a Gram-negative, rod-shaped, about 1.5 µm long and 0.5 µm wide	*Salmonella typhi* is rod-shaped about 1–3 µm × 0.5–0.6 µm and *Salmonella typhi* is arranged singly or in pairs	*P. aeruginosa* is a gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium. The size of a cell is about 1-5 µm long and 0.5-1.0 µm wide

Biochemical tests result of isolated bacteria from drinking water						
Organism	**Catalase**	**Oxidase**	**Citrate**	**Indole**	**MR**	**Vp**
S. typhi	+	_	_	_	+	_
E. coli	+	_	_	+	+	_
P. aeruginosa	+	+	+	_	_	_
Sugar fermentation tests						
Organism	**Glucose**	**Lactose**	**Sorbitol**	**Mannitol**	**Sucrose**	**Xylose**
S. typhi	+	-	+	+	_	+
E. coli	+	+	+	+	+	+
P. aeruginosa	_	_	_	+	_	_

Table IV. Antibiogram of isolated pathogenic bacteria

Antibiotic	**Conc.**	***S. typhi*** **(20) isolates**		***E. coli*** **(20) isolates**		***P. aeruginosa*** **(20) isolates**	
		R	**S**	**R**	**S**	**R**	**S**
Ampicillin	10ug	19/20 (95%)	1/20 (5%)	18/ 20 (90%)	2/20 (10%)	20/20 (100%)	0/20 (0%)
Ceftriaxone	30ug	17/20 (85%)	3/20 (15%)	17/20 (85%)	3/20 (15%)	19/20 (95%)	1/20 (5%)
Chloramphenicol	30ug	18/20 (90%)	2/20 (10%)	16/20 (80%)	4/20 (20%)	18/20 (90%)	2/20 (10%)
Gentamicin	30ug	17/20 (85%)	3/20 (15%)	3/20 (15%)	17/20 (85%)	5/20 (25%)	15/ 20 (75%)

Ciprofloxacin	5ug	6/20 (30%)	14/20 (70%)	4/20 (20%)	16/20 (80%)	6/20 (30%)	14/20 (70%)
Azithromycin	15ug	3/20 (15%)	17/20 (85%)	15/20 (75%)	5/20 (25%)	15/20 (75%)	5/20 (25%)
Sulfamethoxazole/ trimethoprim	25ug	16/20 (80%)	4/20 (20%)	14/20 (70%)	6/20 (30%)	14/20 (70%)	6/20 (30%)
Imipenem	10 ug	2/20 (10%)	18/20 (90%)	1/20 (5%)	19/20 (95%)	2/20 (10%)	18/20 (90%)

DISCUSSION

The occurrence of antibiotic-resistant bacteria and contaminated drinking water sources raises the risk to public health. It is very important to have comprehensive information about such issues. According to published research, inadequate water sanitation and hygiene account for 13.6% of all deaths in Pakistan. In certain remote areas where individuals usually depend on surface reservoirs without any quality assessment, the magnitude of the disease is higher and incalculable (25). Hence, this present study aimed to examine the pathogenic bacteria in the drinking water of Barkhan city. According to our findings out of 500 drinking water samples 334 (66.8%) were found contaminated with pathogenic bacteria where the prevalence of *Salmonella typhi* was 28%, followed by *E. coli* (22.4%) and *P. aeruginosa* (16.4%) respectively. In agreement with the study by Ni *et al*., several biochemical tests were conducted to determine the conformation of pathogenic bacteria (26). This suggests that the drinking water in Barkhan city was highly contaminated which raises the risk of illnesses caused by these bacteria. In contrast to our findings a research study conducted in Lahore, the outcomes of this research work exhibited that 76% of water samples were contaminated with pathogenic bacteria, where the occurrence of *E. coli* was 22%, *Pseudomonas aeruginosa* 12% which shows a higher prevalence and *S. typhi* was not detected from any water sample (20).

In addition, antibiotics remained the first drug of choice for treating microbial diseases. Likewise, *E. coli* is one of the most important bio-indicator models for surveillance studies on antibiotic resistance because of its propensity to develop resistance. Using the disc diffusion method, antibacterial resistance testing was carried out against *S. typhi, E. coli,* and *P. aeruginosa* using eight different antibiotics. According to our result, all *S.typhi* isolates were highly resistant to Ampicillin (95%), Ceftriaxone (85%), Chloramphenicol (90%), Gentamicin (85%), Sulfamethoxazole/ Trimethoprim (80%), *E. coli* isolates were resistant against Ampicillin (90%) followed by Ceftriaxone (85%), Chloramphenicol (80%), Azithromycin (75%) and Sulfamethoxazole/ Trimethoprim (70%) while *P.aeruginosa* isolates were found resistance against Ampicillin (100%), Ceftriaxone (95%), Chloramphenicol (90%), Azithromycin (75%) and Sulfamethoxazole/ Trimethoprim (70%) while all pathogenic isolates were highly sensitive to imipenem.

Compared with the current study Anjum *et al.,* obtained data about the antimicrobial resistance profile of *S. typhi,* which exhibited high resistance rates of 98.7% against Ampicillin, 96% against Trimethoprim-sulfamethoxazole and Chloramphenicol, 94.7% to Ceftriaxone and 82.2% to Ciprofloxacin, 2.63% to Azithromycin (27). Similarly, the previous study published by Khan *et al*., documented that *S. typhi is* resistant against Trimethoprim-sulfamethoxazole and Ampicillin with a ratio of 89%, and 82% respectively (28). However, all the isolates of *S. typhi* were sensitive against Imipenem. In late 2000, since the 1st line antibiotics for *S.typhi* showed resistance, Fluoroquinolones, and 3rd-generation Cephalosporin were used in many endemic places and countries including Pakistan (29).

Furthermore, in agreement with our findings, the result of previous research work revealed that *E. coli* isolates were resistant to Chloramphenicol (94.29%), Ampicillin (82.86%), and Trimethoprim-Sulfamethoxazole (65.71%) (30). Another study also shows that *E. coli* isolates were resistant to (82.6%) against Ampicillin followed by Chloramphenicol, Trimethoprim-Sulfamethoxazole (32.7%), Ceftriaxone (30.7%), and Ciprofloxacin (21.1%) respectively (31).

Currently, high resistance rates of *Pseudomonas aeruginosa* with Ampicillin, Chloramphenicol, Cotrimoxazole, and Tetracycline are frequently observed (32). Another study of *P. aeruginosa* isolated from river water showed higher sensitivity to Ciprofloxacin and Imipenem and showed resistance to

Erythromycin and Trimethoprim/sulfamethoxazole (33). Likewise, Okafor *et al*., also reported that isolates of *P. aeruginosa* showed complete resistance to Amoxicillin (100%) while sensitive to Ciprofloxacin (69.89%) and Gentamicin (91.97%) was also observed (33).

The differences in results of all studies discussed above may be due to several reasons including geographical differences, types of sample collection and isolation methods, socioeconomic conditions, number of microorganisms that were isolated, and type of water samples. Additionally, a broken water supply system may cause drinking water contamination. Likewise, overflow from the sewer collection system is another source of contamination of drinking water. Worldwide reports of antimicrobial susceptibility are wide-ranging due to the impact of geographical location and variations in antibiotic use.

CONCLUSION

The findings of the current study revealed that the water of Barkhan City is tainted with numerous pathogenic bacteria and unfit for human consumption. Unscrupulous dumping of domestic wastes, poor drainage facilities, Open defecation, and a water-logging environment are the main causes of deterioration of water quality in this city. The study also displayed that widespread water-borne illnesses are common in this region. Thus, this study concluded that globally accepted guidelines for treatment and prevention policies should be implemented through national authorities and at the individual level. Awareness of the use of clean water must be emphasized. In addition, filtration and boiling of tap and boring water reduces the number of pathogens in it, making it safer and better for human consumption.

Conflict of Interest:

The authors have no conflict of interest.

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