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Isolation and Identification of *Escherichia coli* and *Klebsiella* species using Chromogenic Agar from Drinking Water of District Thar, Sindh, Pakistan

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ABSTRACT

Most of the studies do not conduct bacteriological analysis of the Thar region drinking water, except for transportation and travel problems. **Objectives:** To identify the bacteriological profile of water at various regions of the Thar Desert, Sindh, Pakistan, concerning the presence of the indicator Coliform, i.e. *E coli* and *Klebsiella* spp., on Rapid Media Chromogenic agar. **Methods:** A total of 70 drinking water samples were collected in 5 talukas of District Tharparkar, namely, Islamkot, Nangrpar, Kalohi, Dahri, and Diplo between December 2021 and May 2021. Water samples were tested right after the collection, with the help of the membrane filtration method. MacConkey agar and Chromogenic agar and plates were inoculated with filters and incubated aerobically in 35-37°C, respectively, and kept for 24 hours. Bacterial identification tests were performed on the colonies on MacConkey agar plates and *E. coli* and *Klebsiella* spp. produced turquoise-blue colony and Mergenda color colony on chromogenic agar, respectively. **Results:** The findings of the bacteriological test revealed that the water samples are not potable, and 47 (67%) samples contained bacteria. There were 28 (59%) coliform and 19 (41%) non-coliforms. *Escherichia coli* (18; 40%) was most common, followed by *Klebsiella* spp. (12; 27%), *Pseudomonas* spp. (9; 20%), *Salmonella* spp. and *Proteus* spp. were the least common (3; 6%) each. **Conclusions:** Chromogenic agar is a fast and convenient way of presumptive identification of bacterial contaminants in drinking water. *E. coli* and *Klebsiella* spp. were the largest bacterial communities in drinking water specimens of the Thar region.

INTRODUCTION

Tharparkar is the largest region of the desert region of Sindh, located in Pakistan. Tharparkar is found in 69°3'35 E and 71° 7 47 E longitudes and 24° 9 35 N and 25° 43 6 N latitudes [1]. The only significant drinking water source is groundwater, which is a significant source of domestic, livestock, and agricultural usage. These groundwater sources in Southern Sindh, such as Tharparkar, contain a significant number of coliform bacteria, which implies that the groundwater poses a health hazard to the locals [2].

The polluted drinking water is the cause of numerous water-borne illnesses, most commonly dysentery, vomiting, and gastroenteritis [3]. Polluted water has also been reported in various parts of Pakistan that harbor coliform bacteria and other pathogenic microorganisms such as *Escherichia coli*, Vibrio cholera, *Salmonella* spp., and *Pseudomonas* spp; that cause gastroenteritis, cholera, typhoid fever, diarrhea, and other enteric diseases in children and adults who consume it [4-6]. The

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contamination bacteria (Total coliforms and *Escherichia coli*) of various cities of Sindh, such as Nagarparkar, have been reported, signifying the intrusion of sewage water into the drinking water system [7-9]. Therefore, various procedures involving agar and liquid media employing various techniques such as the membrane-filtration method, multiple tube or most probable number (MPN) methods are well elaborated to identify the water contamination to isolate indicator microorganism in water [10, 11]. The traditional procedures are tedious and require more time to detect bacterial contamination [12, 13]. Nevertheless, the use of agar with the aid of chromogenic agar makes it quicker, and even the process of identifying indicator microorganisms in drinking water is easier. Safe drinking water is important to the health of the people, particularly in arid areas such as Tharparkar, where the primary source of water is through groundwater [14]. The scarcity of literature in the field of bacteriological quality in this area demonstrates that the new surveillance with the help of fast techniques is necessary. This paper sought to determine the quality of drinking water in the District of Tharparkar by identifying bacterial contamination and coliform bacteria, which are indicators of the presence of pathogens, through rapid chromogenic agar media.

In the Tharparkar region of Sindh, Pakistan, safe drinking water is scarce, with groundwater being the primary source for domestic use, yet it is frequently contaminated with coliform and pathogenic bacteria such as *Escherichia coli* and *Klebsiella* species, posing significant public health risks. Despite the known prevalence of waterborne diseases in the area, there is limited bacteriological surveillance, particularly using rapid detection methods, and the antibiotic resistance profiles of these contaminants remain largely unexplored. This study aimed to address these gaps by isolating and identifying coliform and non-coliform bacteria in drinking water using chromogenic agar, assessing their distribution, and evaluating antibiotic susceptibility patterns to inform effective water safety interventions and public health strategies.

METHODS

A cross-sectional descriptive study of coliform contamination and antibiotic resistance in drinking water from five talukas of District Tharparkar. The samples included a total of five talukas of District Tharparkar, with each taluka comprising 70 (n=70) water samples (250 ml each) collected randomly in December 2021 and May 2021. The samples were aseptically packed into the sterile bottles and transported to the laboratory in ice boxes and then immediately processed or stored at 4°C 24-48hours in the event of delayed analysis. To isolate Gram-negative bacilli, such as the coliforms, in the sample water collected, the Filtration Assembly Method (FAM) was carried out on each sample [11]. The filtration process was done through the membrane filtration assembly (Millipore, USA) unit of a funnel, vacuum pump, filter flask, and filter membrane (Merck, USA) with pore sizes of 0.45 micrometer (µm). An autoclave was used to sterilize the membrane filtration assembly at 121°C for up to 15-20 minutes. Assembled aseptically Sterile membrane filter unit was assembled. With Filtration Assembly, the water samples (100 ml) were filtered with the assistance of sterile forceps, removing the membrane filter and placing it on Chromatic™ Coli Coliform (Liofilchem, Italy) agar and on MacConkey (Oxoid, UK) agar plates separately. The Plates were incubated aerobically at 37°C between 24-48 hours. This was done to all the water samples. The plates were then incubated, and the appearance of colored colonies was noted, which validated the findings of the Chromogenic agar of the specific bacteria that were recommended. Moreover, the antimicrobial susceptibility profile was performed based on the principles of Clinical and Laboratory Standards Institute (CLSI 2020) [15] using Kirby-Bauer disc diffusion on Muller-Hinton agar. The antibiotic discs used were Oxoid (UK) which included Amoxicillin plus clavulanic acid (AMC,30+ 10 µg) or augmentin (AMC) and Ceftazidime (CAZ, 30 µg) and Cefotaxime (CTX, 30 µg) were both 3rd generation cephalosporin, Aztreonam (ATM, 30 µg) and Ciprofloxacin (CIP, 5 µg). Selection of bacterial colonies was done and bacterial lawn was prepared on Muller Hinton agar plates. After the antibiotic discs had been placed, the plate was incubated at 37°C for 24 hours. The outcome of the antibiotic susceptibility test, as given by the CLSI guidelines, is expressed as sensitive (S) or resistant (R).

RESULTS

Pure bacterial cultures were first isolated from drinking water using chromogenic agar. The filter paper placed onto the chromogenic agar plate surface indicates the growth of specific bacterial colonies. Indications of the appearance of turquoise-blue colonies of *E. coli* and for *Klebsiella* mauve colonies were seen on chromogenic agar (Chromatic™ Coli Coliform) as mentioned by the manufacturer. While some other colorless colonies (Creamy/off-white color) of other bacterial species belong to the family Enterobacteriaceae (Figure 1).

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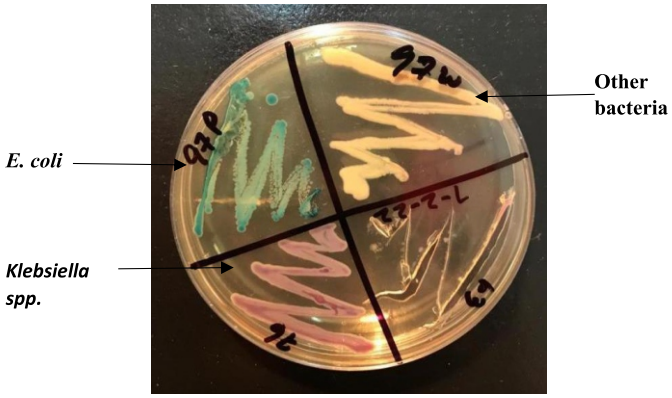

Figure 1: Isolation of Pure Bacterial Culture on Chromogenic Agar

As a result of overnight incubation after FAM, growth was observed. The colonies of lactose-fermenting Coliform group of bacteria appeared as medium to large pink colonies. However, non-lactose fermenting non-Coliform bacteria appeared as colorless colonies that need further identification based on biochemical characterization. From these colonies, pure cultures were obtained by the streak plate method (Figure 2).

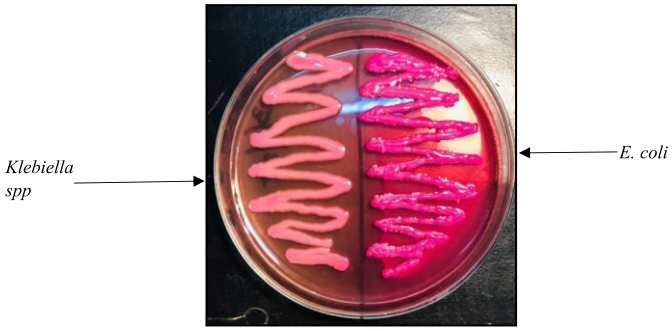

Figure 2: Growth on MacConkey Agar and Subculture of Pure Isolates on MacConkey

Out of 70 samples collected from 5 different talukas of the district Thararkar, 47 (70 %) samples were positive for bacterial growth, whereas 23 samples (30%) were found negative. Among 47 positive samples, 28 samples (60 %) were positive for the presence of Coliform, and 19 (40%) samples showed the presence of non-coliform bacteria (Figure 3).

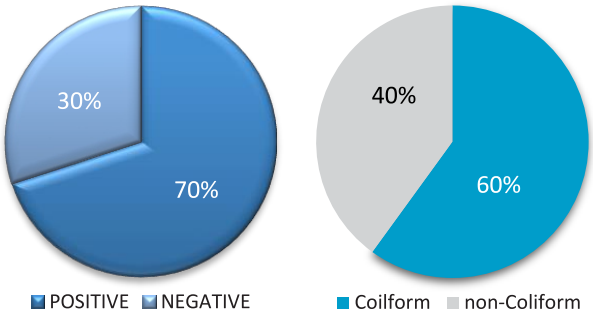

Figure 3: Distribution of Water Samples for Bacterial Contamination (A) Bacterial Distribution of Water Samples (B) Coliform and Non-Coliform Distribution

Other than Coliform bacteria, non-lactose-fermenting, Gram-negative bacteria were also isolated, including *Salmonella* species. Further investigation, biochemical analysis of isolated pure culture results showed that five types of Gram-negative bacilli were isolated, including *E. coli* 18 (40%), *Klebsiella* species 12 (27%), members of coliform and *Pseudomonas* spp. 9 (20%), *Salmonella* species, 3 (6%), and *Proteus* spp. 3 (6%) members of the non-coliform group of bacteria (Table 1).

Table 1: Distribution of Gram-negative Bacilli Isolated

Coliform (n=30)		Non-Coliform (n=15)		
E. coli	*Klebsiella spp*	*Salmonella spp*	*Pseudomons spp*	*Proteus spp*
18 (40%)	12 (27%)	3 (6%)	9 (20%)	3 (6%)

For antibiotics sensitivity pattern, following 24-h incubation, 29 (93%) isolates were resistant to Amoxicillin, 21 (68%) were resistant to Cefotaxime and 19 (60%) were resistant to Ceftazidime with 2 (7%), 10 (32%) and 11 (40%) being sensitive, respectively. However, Imipenem, Ciprofloxacin, and Fosfomycin showed less or no resistance with 93% sensitivity and 7% resistance (Table 2).

Table 2: Sensitivity Pattern of Bacterial Isolates

Sr. No.	Antibiotics	Resistant (R)	Percentage (%)	Sensitive (S)	Percentage (%)
1	AMC	29	93	2	7
2	CAZ	19	60	11	40
3	CTX	21	68	10	32
4	ATM	19	60	11	40
5	CIP	2	7	29	93
6	IPM	2	7	29	93
7	FOS	2	7	29	93

DISCUSSION

Chromatic™ Coli Coliform has chromogenic that is used by the bacteria to produce colored colonies, in which substrate is a chromogen, X-glucuronidase, and through which we can be able to observe different bacterial species. The colonies of *E. coli* were observed as blue in color since the X-glucuronidase cleaved the enzyme chromogen, X-glucuronidase. This medium is specifically applicable in the identification of coliforms and the identification of *E. coli* and *Klebsiella* spp. In our study, 47 samples were positive with bacterial growth of the total 70 samples of water found in the Thar area, and 28 of the 47 positive samples were coliforms. A similar trend was observed in other research, wherein 71 percent of *E. coli* isolated in drinking water out of 155 samples [15]. Sood, N conducted a study on the Prevalence of pathogenic bacteria on fecal coliforms. 50 water samples were sampled; *E. coli* was isolated from these samples 12 [16]. A similar study was conducted locally in Aga Khan university Karachi, Pakistan, and 42 water samples were sampled, in

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which 62.96% of the samples were found to be *E. coli* [17]. Antibiotic, levofloxacin, has been reported to be most sensitive to the *E. coli* and *Salmonella*, and the most resistant was reported to be against antibiotics clindamycin and amoxicillin [18, 19]. Similar findings have been brought to light in this study, with maximum resistance (93%) against the more popularized antibiotic Augmentin/Amoxicillin, and moreover against third-generation cephalosporin members like cefotaxime and ceftazidime, 68% and 60% respectively. This is a worrying scenario to Thar people who have fewer hospitals and a deficiency of medicine. Our water isolates have a high prevalence of resistance to frontline antibiotics such as amoxicillin, which is also similar to the critical resistance patterns maintained in clinical pathogens. An investigation into Uganda surgical site infections indicated that 90.9 percent of Enterobacteriaceae isolates were multidrug-resistant, and ampicillin resistance was up to 95.1 percent [20]. This analogy of a compromised ecology of Thar and a compromised health of Uganda reveals a universal dilemma of resistance caused by the improper use of ordinary antibiotics in healthcare and the household sphere. The principal risk to human health is from drinking contaminated water since it is the most crucial parameter to consider. Out of 70 samples, which were tested in 5 different regions of the district Tharparkar, 47 samples (70 percent) tested positive for coliform/bacterial contamination. It has been noted that the total viable count test conducted on microbial analysis and the sample got excessively contaminated with microbial growth of fecal coliforms and *Escherichia coli* [3]. Again, 18 samples in this research demonstrated the existence of *E. coli*, which is the hallmark of the existence of fecal coliform. Further, the health of the water in the area is not good because it has the Coliform group of bacteria, which is threatening, in case of positive results with coliform presence. The infections may be caused by contaminated water and may be fatal in poor areas as a result of poor medical facilities. In this study, three types of bacteria, which are *E. coli* 18 (40%), *Klebsiella* spp. 12 (27%) and *Salmonella* spp. 3 (6%). The bacteria are causative bacteria of water-borne illness, such as Diarrhea, Dysentery, and Typhoid fever, and can lead to urinary tract infections (UTI). In Pakistan, major illnesses because of contaminated drinking water encompass diarrhoea, gastroenteritis because of Coliform contamination, and typhoid because of *Salmonella typhi*, giardiasis because of intestinal worms *Giardia* spp., and viral hepatitis, like hepatitis A caused by the hepatitis A virus. [5, 10, 21]. It has been observed and hypothesized in recent research that the presence of *E. coli* in water is not necessarily due to feces contamination but may survive and propagate in tropical water. Moreover, the Coliform test remains a useful test of microbiological analysis of drinking water. Species-level identification of other non-coliform bacterial species to be used as part of the current study is a useful tool in the identification of the nature of contamination of water. *Salmonella* spp. was also identified in current research, that are harmful enteric pathogen and not part of the coliform group of bacteria.

This study is limited by its cross-sectional design and focus on only five talukas within Tharparkar, which may not fully represent the district's diverse water sources. The reliance on chromogenic agar, while rapid, may not detect all bacterial species, and the absence of molecular confirmation for isolates limits strain-level characterization. Seasonal variation in water quality was also not assessed, as samples were collected only between December and May. Future studies should incorporate longitudinal sampling across different seasons and expand to more talukas to better understand temporal and spatial contamination patterns. Molecular methods such as PCR or sequencing could be used for precise identification and antibiotic resistance gene profiling. Additionally, public health interventions—including community education on water safety and pilot water treatment projects—should be implemented in high-risk areas identified in this study.

CONCLUSIONS

This study shows the presence of coliform bacteria in drinking water of Thar district samples and proves that water samples were not safe for drinking, bathing, or washing purposes, as *E. coli* is a major causative agent for Urinary tract infections. *Escherichia coli* determination and its application in the identification of the major animal source of fecal contamination have been used as an indicator of water fecal contamination, such as *Salmonella* and Giardia. Use of Chromogenic agar proves to help in fast recovery and identification of *E. coli.*

Authors' Contribution

Conceptualization: SJ
Methodology: SJ, BA
Formal analysis: JML, NQ, HN, SN
Writing and Drafting: JML
Review and Editing: BK, JML, SJ, NQ, HN, BA, SN

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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