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Isolation and Characterization of Bacterial Contamination from Water Sample of Kharar, Punjab, India

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ABSTRACT

Background: Water is one of the most important natural resources for industry, agriculture, and human nourishment. This research focuses on the quality of water, its effects on public health, and techniques for identifying potential contamination.
Objective: The objective of this study was to identify any pathogenic bacteria in water samples that were taken from several sites from in and around Kharar, Punjab, India.
Methods: A total of six bacterial species, such as E. coli, Klebsiella, Proteus, Salmonella sp. Pseudomonas sp., and Staphylococcus aureus, were identified from 6 different drinking water samples. Isolates were cultured on nutrient agar medium, and characterization of the isolates was done based on their morphological characteristics, biochemical properties, and antibiotic susceptibility testing.
Results: The colonies were seen to be tiny, translucent, mucoid, smooth, round, and disc-like. The isolated water samples, designated W1 and W2, underwent additional testing to determine their susceptibility to different antibiotics. While W1 showed no resistance to any antibiotic, isolate W2 shown resistance to both ceftriaxone and ciprofloxacin. In W1, it was discovered that Ampicillin and Vancomycin had intermediate susceptibility, whereas in W2, Amoxyclav, Vancomycin, Ampicillin, and Levofloxacin also displayed intermediate susceptibility.
Conclusion: This research found antibiotic-resistant strains of E. coli and other hazardous microorganisms in water samples from the Kharar area. These results emphasize how important it is to monitor water quality more closely in order to protect public health.

Keywords: Drinking Water, Bacterial Contamination, Phenotypic Characterization, Antibiotic Susceptibility.

INTRODUCTION

Accessibility to secure drinking water is essential for human survival; however, a substantial section of the global populace faces water scarcity and pollution. Approximately 4 billion people experience acute water scarcity, and 771 million people globally lack access to potable drinking water. [1] Chronic public health issues become worse from pathogenic contamination (bacteria, viruses) of water sources. The number of kids who pass away

from waterborne illnesses rises every year. [2] Water contamination occurs by pathogenic as well as non-pathogenic organisms, for example, *Escherichia coli, Campylobacter sp. Salmonella, Staphylococcus aureus, Clostridium botulinum*, *Pseudomonas aeruginosa, and Vibrio cholera.* [3] Fecal water contamination is indicated by *Clostridium perfringens, fecal streptococci, and Klebsiella.* [4] It can spread *Entamoeba histolytica, Cryptosporidium spp., Dientamoeba fragilis, Giardia duodenalis, and Blastocystis hominis* in both human and animal excrement/ [5] However, in sewage samples, *Salmonella enteritidis, Salmonella typhi, Shigella spp., Proteus spp., and Salmonella typhimurium* are the most prevalent. [6] Enteric pathogens that can cause cholera, typhoid, dysentery, and hepatitis, like *Escherichia coli*, *Salmonella typhimurium, and Vibrio cholera,* also transmit through water. [7] The following enteric viruses are associated with diarrhea: enterovirus, rotavirus, astrovirus, adenovirus, Norwalk virus, and picobirnavirus. [8] The discovery of *Cholera vibrio and Salmonella enterica serovar Typhi* in the 19th century led to a better understanding of waterborne diseases. This knowledge has helped to reduce the spread of these and save lives. [9] A waterborne illness outbreak in the U.S.A was primarily caused by Shigella bacteria, in which *Campylobacter* and *Pseudomonas* were also involved. [10] Waterborne diseases can cause various symptoms, including skin problems, respiratory disorders, bladder disease, severe diarrhea, dizziness, vomiting, and hepatitis. It's crucial to remember that everyone is not equally susceptible; young people with weaker immune systems and elderly people are often more vulnerable. [11] *Coliform bacteria* include not just Escherichia coli but also all *lactose-fermenting bacteria* belonging to the genera *Klebsiella, Citrobacter, Enterobacter, Hafnia, and Serratia.* The most often used measure for evaluating the microbiological purity of water free from feces includes assessing the coliform or thermotolerant E. coli count, enteric viruses, and protozoa, which are more resistant to disinfection. The absence of other pathogenic organisms cannot be guaranteed by a negative E. coli test; it would be advantageous to additionally test for more resistant microbes such as coliphages, Clostridium perfringens spores, or intestinal enterococci (*E. hirae, Enterococcus faecalis, E. faecium, and E. durans*). [12] According to International Organization for Standardization (ISO) guidelines, the enrichment approach recommended for detecting fecal streptococci and clostridia is membrane filtration for the detection of fecal indicator bacteria in water. [13] To screen drinking water for opportunistic pathogenic bacteria, such as *Aeromonas, Flavobacterium, Acinetobacter, Klebsiella, Moraxella, Pseudomonas, and Xanthomona*s, heterotrophic plate counts are advised. [14] The objective of the study was to identify any pathogenic bacteria in water samples that were taken from several sites from in and around Kharar, Punjab.

MATERIALS AND METHODS

Study Area:

Kharar has an essentially flat topography, with its immediate surroundings characterized by minimal elevation changes within a few miles, and it sits within the broader alluvial plains of the Indo-Gangetic basin. The land is predominantly covered by cropland, with local variations in soil types from loam to sandy loam. Key Topographical Features are: Flat Terrain - Within a few miles of Kharar, the topography is described as essentially flat, with very little variation in elevation, Alluvial Plains - The area is part of the Indo-Gangetic Quaternary basin, which is characterized by alluvial plains formed by deposits of sand, silt, and clay. The population of Kharar in 2024 is estimated to be around 103,000, according to projections from Census 2011. [15]

Fig.1. Municipal Council – Kharar [15]

Sample Collection:
Water samples were gathered in an aseptic manner from the different locations near Kharar by using sterile containers to prevent contamination. The samples were refrigerated after being delivered to the lab at the appropriate temperature.

Isolation of bacteria:
We isolated the microorganisms from the different water samples on nutrient agar plates. The samples were cultured by the streak plate method. These petri dishes were allowed to incubate at 37^0C for the whole day. The colonies appeared on the nutrient agar plates after 24 hours. Different colonies grew differently on agar plates. They were further analyzed morphologically and microscopically and maintained at -4^0C for further examinations. [16]

Phenotypic Characterization:
Phenotypic characterization of all isolates was performed based on their morphological and biochemical characterization.

Gram Stain:
Once the bacteria were isolated on nutrient agar plates, they were further characterized using gram staining. Initially, we placed a tiny layer of bacterial culture onto the slide, and then we carefully heated the slide over the flame to fix the bacterial culture. Next, we applied crystal violet (primary stain) for 60 seconds and an iodine solution that acts as a mordant for 60 seconds. decolorization with 95% ethanol for 20 seconds and safranin as a counterstain for 40 seconds. After finally being cleaned with distilled water, it was examined under a microscope. [17]

Biochemical Examination:
Numerous biochemical analyses, including methyl red (MR), oxidase, catalase, indole, and Voges-Proskauer (VP), were performed to determine the types of bacteria found in the collected water samples.

Catalase Test:
A test for catalase was conducted on every isolate. A small portion of the isolated colony was spread onto a spotless slide using an inoculating loop. A drop of hydrogen peroxide was applied to assess the activity of the enzymes. Bubble production indicates the breakdown of hydrogen peroxide by bacteria. Bubbles that emerge as a consequence of the production of oxygen gas hence signify catalase-positive bacteria. On the other hand, the lack of bubbles suggests catalase-negative bacteria. [18]

Indole Test:
After 24–48 hours of growth, 4-5 Kovac's reagent droplets were added. After gentle shaking, the results were examined. A positive result indicates a red-colored ring in tryptone broth containing bacterial isolates, and a negative result shows either medium

color or no color (the original color of Ehrlich and Kovac's reagent). [19]

Methyl Red:
The MRVP broth containing the test organism is left to incubate for 24 hours at 37°C. Following that, it was put into two sterile test tubes. Subsequently, 5 ml of broth was mixed with 6-7 drops of methyl red reagent and shaken well. When a color changes to red, it signifies a positive response, and yellow is a negative one.

Voges Proskauer:
After incubating the test organism in MRVP broth for a full day, it was transferred into two sterile test tubes. Barrett's reagents A and B were added to the two test tubes. A positive test result is indicated by the formation of a reddish-pink tint on the medium's surface.

Oxidase Test:
A well-isolated colony of bacteria was picked up from prepared culture and applied upon the oxidase disk and then observed for color change. [20]

Antibiotic Susceptibility:
Every isolate underwent a disc-diffusion antibiotic sensitivity test. They were cultured on nutrient agar for the entire night and then suspended in physiological saline solution (0.9% w/v NaCl) using a sterile wire loop until reaching 0.5 McFarland turbidity standards. Equal amounts of sterile and non-toxic cotton-tipped applicators, soaked in a uniform bacterial suspension, were applied to MHA (Mueller-Hinton agar) plates. [21] After that, the resulting isolates were examined using the following 18 antibiotics: amikacin, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, amoxyclav, gentamicin, cefoperazone, levofloxacin, meropenem, cefixime, piperacillin/ tazobactam, ampicillin, tetracycline, netilmicin, trimethoprim-sulfamethoxazole, nitrofurantoin, and vancomycin. Antibiotic disks were then aseptically placed using sanitized forceps, and after a 24-hour incubation period at 37°C, the inhibition zone for each antibiotic was noted on each plate.

RESULTS AND DISCUSSION

Phenotypic characterization of the isolates was done by using 100 x magnifications. The colonies were observed as small, smooth, circular, mucoid and translucent discs like colonies.

Fig 2. Colonies grown on nutrient agar media

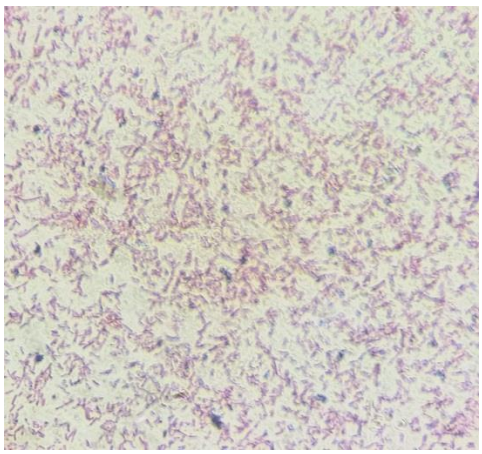

Fig 3. Gram staining of isolates

Biochemical Characterization of Obtained Isolates:
Table 1 lists the various biochemical assays performed on the isolates, including methyl red, VP, oxidase, catalase activity, and indole synthesis.

Table 1- The biochemical properties of isolated bacteria

Samples	Bacterial isolates	Gram stain	Catalase examination	Test for indole	Methyl red (MR) test	Voges Proskauer (VP)	Oxidase assessment
W1	Escherichia coli	-	-	+	+	-	-
W2	Klebsiella	-	+	-	-	+	-
W3	Proteus	-	-	+	+	-	-
W4	Salmonella sp.	-	+	+	-	+	-
W5	Pseudomonas sp.	-	+	-	-	-	+
W6	Staphylococcus aureus	+	+	+	+	-	-

Antibiotic Sensitivity Results:

The isolated water samples, named W1 and W2, were further tested for antibiotic susceptibility against various drugs, as shown in Table 2. The isolate W2 exhibited resistance to Ceftriaxone and Ciprofloxacin, whereas W1 showed no resistance against any antibiotic. Ampicillin and Vancomycin were found to be intermediate in susceptibility in W1, while Amoxyclav, Vancomycin, Ampicillin and Levofloxacin showed intermediate susceptibility in W2.

Table 2-Antibiotic sensitivity result of W1 isolate

	Antibiogram (W1)	
Antibiotics	**Zone size (mm)**	**Interpretation**
Amikacin	21	Sensitive
Cefepime	22	Sensitive
Ceftazidime	17	Sensitive
Ceftriaxone	18	Sensitive
Ciprofloxacin	28	Sensitive
Amoxyclav	25	Sensitive
Gentamicin	20	Sensitive
Cefoperazone	20	Sensitive
Levofloxacin	25	Sensitive
Meropenem	19	Sensitive
Cefixime	18	Sensitive
Piperacillin/Tazobactum	25	Sensitive
Ampicillin	14	Intermediate
Tetracycline	22	Sensitive
Netilmicin	24	Sensitive
Trimethoprim-sulfamethoxazole	21	Sensitive
Nitrofurantoin	15	Sensitive
Vancomycin	13	Intermediate

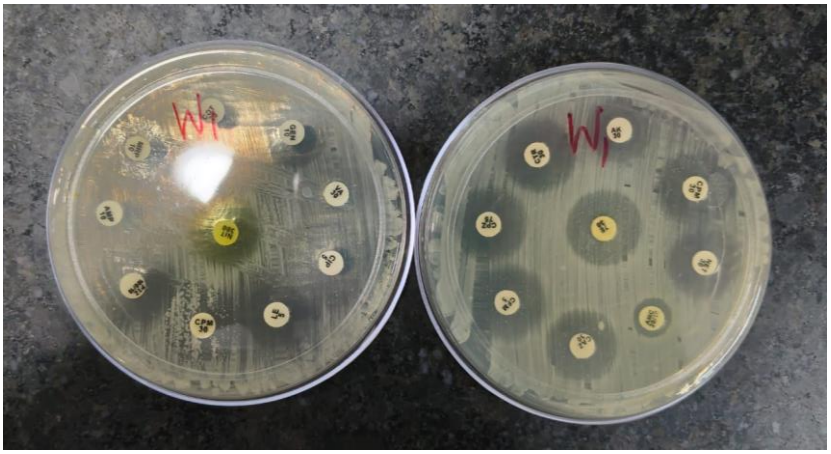

Fig 4. Antibiotic sensitivity on isolate W1

Table 3-Antibiotic sensitivity result of W2 isolate

	Antibiogram (W2)	
Antibiotics	**Zone size (mm)**	**Interpretation**
Amikacin	24	Sensitive
Amoxiclav	12	Intermediate
Ampicillin	12	Intermediate
Cefepime	32	Sensitive
Cefixime	20	Sensitive
Cefoperazone	25	Sensitive
Ceftazidime	22	Sensitive
Ceftriaxone	02	Resistance
Ciprofloxacin	02	Resistance
Gentamicin	15	Sensitive
Levofloxacin	14	Intermediate
Meropenem	23	Sensitive
Nitrofurantoin	26	Sensitive
Netilmicin	25	Sensitive
Nitrofurantoin	26	Sensitive
Netilmicin	25	Sensitive
Trimethoprim-sulfamethoxazole	22	Sensitive
Vancomycin	10	Intermediate

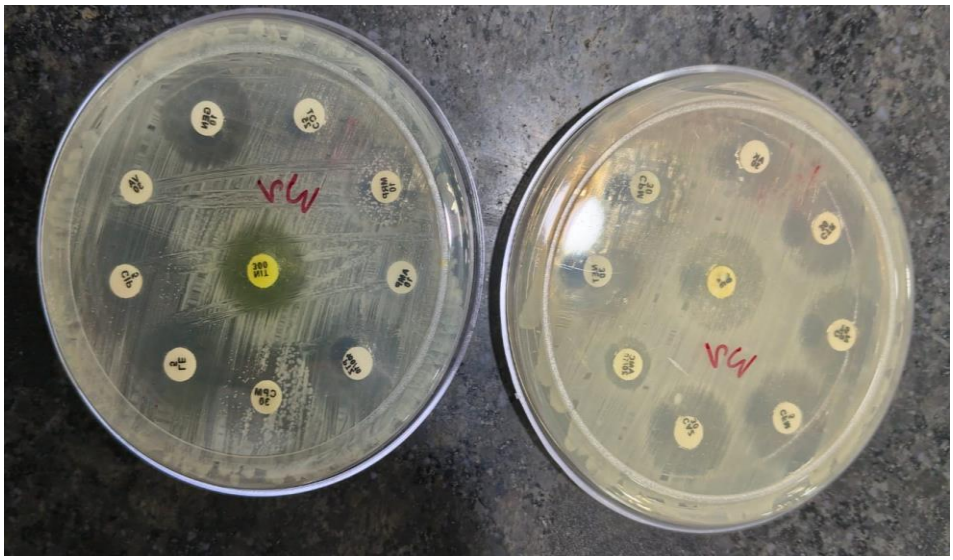

Fig 5. Antibiotic sensitivity on isolate W2

CONCLUSION

In this study, a total of six bacterial species, such as *E. coli, Klebsiella, Proteus, Salmonella sp., Pseudomonas sp., and Staphylococcus aureus,* were identified from drinking water samples that were collected from different locations near Kharar. This study highlights the critical significance of bacterial contamination isolation and characterization for environmental preservation, public health, and scientific advancement in water samples. Moreover, the study revealed the critical need for proactive methods to protect public health, inform water quality monitoring, address environmental concerns, and improve epidemic identification and treatment strategies. The main objective of this study is to raise public awareness of the issue and provide suggestions for countermeasures against bacterial contamination of water sources, ensuring that everyone gets access to clean, and safe drinking water.

Declaration by Authors:

Ethical Approval: Approved.

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Conflict of Interest: The authors declared no conflict of interest.

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