Original Research Article

IDENTIFICATION AND CHARACTERIZATION OF WATER BORNE PATHOGEN

ABSTRACT

Aims: To carry out isolation and characterization of bacteria from water samples.
Study design: Collection of water samples from different water sources, isolation of bacteria and establishment of pure culture, identification of bacteria
Place and Duration of Study: Banasthali Vidyapith, Rajasthan.
Methodology: For initial screening of bacterial population, standard methods namely serial dilution and streaking methods were used, for identification, colony morphology and molecular characterization (16s rRNA gene) was done.
Results: The bacteriological analysis revealed that *Enterobacter cloacae* were present in contaminated.
Conclusion: Presence of *E.cloacae*in tap water necessitates recommendation of frequent sanitation, treatment, management of water sources, and routine regular bacteriological assessment of all water sources for consumption as a drinking water.

Keywords: Enterobacter cloacae, *16s rRNA gene, potable water, water borne pathogen, water borne diseases*

1. INTRODUCTION

Waterborne diseases have emerged out as the most prevalent threat in developing and developed nations. According to the WHO, 1.1 billion people do not have access to safe drinking water, and 3.4 million people die each day as a result of water-borne diseases caused by contaminated water [1]. According to a recent United Nations report, more than 3 million people, including 1.2 million children, die each year as a result of drinking contaminated water[2].

Due to poor drinking water quality, water-borne illnesses such acute, cholera, diarrhea, gastroenteritis, and UTI etc. primarily break out every year during the summer and rainy seasons especially in developing countries [3].

Since the last few decades, a variety of antibiotics have been widely employed to treat both acute and long-term bacterial infections. However, it has been well documented that these agent causes serious adverse effects and thus further, worsen the situation for recipients. In addition, the development of resistance in microbial species has now become one of the major threats to physicians and researchers and has been considered as most important factor in an exponential rise in the burden of microbial diseases and associated mortality. The continuous progression of bacterial resistance to presently accessible antibiotics has emerged as a necessity to investigate some novel and effective therapeutic agents for the management of bacterial infections.The present study was carried out for isolation and characterization of bacteria from water samples for identification of potent disease causing organisms.

2. MATERIAL AND METHODS

2.1 Collection and preservation of water sample

On the basis of previous study conducted at our laboratory[4], the Hingotiya and Palai village of Tonk district, Rajasthan were reported to have high chances of water contamination especially by enteropathogenic bacteria. As such, drinking water samples were collected from the hand pump located in both sites. The collected sample was stored in sterilized screw-cap glass bottles in duplicates at 4ºC.

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2.2 Isolation of bacterial species from water samples

For initial screening of bacterial population, standard methods namely serial dilution and streaking methods were used.

2.2.1.Serial dilution method

A known quantity (1mL) of the collected freshwater sample was serially diluted in distilled sterile water (9mL) from 10-1 to 10-6 dilution. The diluted water samples were cultured on nutrient agar medium plates and incubated at 37°C [5].

2.2.2. Streak for isolation of bacteria

A sterile inoculating loop was used to select representative sample colonies of each visually distinguishable bacterium. Each colony was streaked onto nutrient agar medium plates using an aseptic technique and an inoculation loop. The streaked plates were kept at 37°C for 24h [6]. At this point, each bacteria isolate's initial observations, such as visual properties, were recorded. As a pure culture, isolated single colonies were selected and streaked onto selective nutrient agar medium plates, and they were characterized using standard morphological and biochemical tests.

2.3Biochemical charecterisation of isolated bacterial strain

The following biochemical tests were carried out for the pre-confirmation of isolated bacterial species: Methyl Red Test [7],Voges Proskauer test [7], Indole test[7], Citrate test [8],

Catalase test [9], Ammonia production [8], HCN production [10], Urease test [11], Nitrate reduction and Nitrate test [12], Motility test [13], Triple sugar iron test [14].

2.4 Molecular characterizationof isolated bacterial strain

GenElute Bacterial Genomic DNA Kit (Sigma, Catalogue number NA2110-1KT) was used to isolate the bacterial DNA. PCR amplification was done with the help of universal 16S rRNA gene primers fd1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rd1 (5'-AAGGAGGTGATCCAGCC-3'). To design the conserved regions of the 16S rRNA genes are used and are capable of amplifying nearly full-length 16S rDNA.

2.5 Antibiotic assay of the isolated bacterial strain

Antibiotic sensitivity profiles of isolated bacteria were investigated using the disc diffusion method against various antibiotics according to[15] and National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 2005). Isolated bacteria were grown in nutrient media for 24 h at 37°C. Ampicillin (25µg), amikacin (30µg), cephaloridine (10µg), erythromycin (30µg), streptomycin (µg), and tetracycline (30µg) antibiotic discs were placed on the agar medium with sterile forceps and incubated at 37^{o}C for 18-24 h. The zones of inhibition (ZOI) formed around the antibiotic disc are indicative of the bacterial isolate's sensitivity or resistance to the respective antibiotic. The inhibition zone's diameter was measured. The results in terms of sensitivity or resistance were interpreted in accordance with the manufacturer's guidelines.

2.6 Statistical analysis

All experiments were repeated at least thrice. The data represented are Mean ± standard deviations (SD) of all the three replicates. Difference between values significantly ($p<0.05$) are compared using LSD following ANOVA.

3. RESULTS AND DISCUSSION

3.1 Isolation and morphology characterization of bacteria from water samples

Bacterial isolation was done within 24h after water sample collection by conducting serial dilution (10-1 to 10-6). 100µl of water samples were spread over solidified nutrient agar plates and incubated at 37^{o}C for 24h. The different bacterial colonies were observed. Colony morphology observed and colonies with whitish, circular with slime/mucoid nature similar to Enterobacteriaceae was selected for pure culture as mentioned in Bergey's Manual of Systemic Bacterialogy, 2005. Gram staining was conducted and observed that isolate out of the several isolates, Enterobacteriaceae is Gram negative and rod-shaped bacteria.

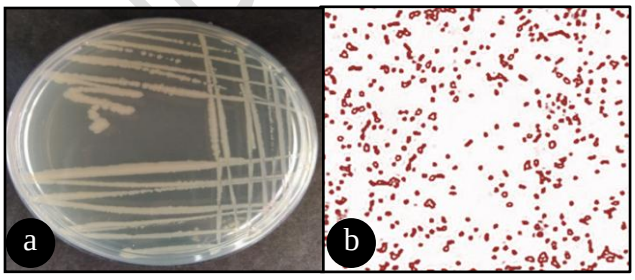

Figure1. Growth and Gram staining of bacterial isolate (a) Growth on nutrient agar (b) Gram staining.

3.2 Biochemical characterization of the selected bacterial isolate

The inoculatedbacteria changed the red colourof methyl red dye which indicates a positive result for the methyl red test.Theinoculatedbacteria changed the red colourof the dye after a

few min of incubation indicates a positive result for the Voges-Proskauer test. In the Indole test, the inoculated bacteria did not change the colorwhich indicates negative result. In the citrate test, the inoculated bacteria changed the colour from greenish to blue, indicating a positive result for the citrate utilisation test. In Catalase test, 3% hydrogen peroxide was used and when it came in contact with isolated culture, bubble formation occurred. This results show that the cultureis aerobic in nature. In the test for Ammonia production, the inoculated bacteria changed the colour from brown to yellow which indicates a positive result for ammonia production. In the test for HCN production, the inoculated bacteria changed the colour from pale yellow to brown which indicate positive results for HCN production. In the Urease test, the inoculated bacteria changed the colour from red or dark pink which indicates the presence of urease activity. In the Nitrate reduction and Nitrate test, the inoculated bacteria changed the colour from distinct red or pink colour indicates a positive result for the nitrate reduced into nitrite.

A positive motility is indicated by a red turbid area that extends away from the line of inoculation. The triplesuger test indicated that the isolated bacteria can use sucrose and lactose, acid production will continue and the colour of the media will remain yellow.If it cannot use sucrose or lactose, the bacteria begin to use amino acids, which cause the medium to become alkaline which will change the colour of the medium into red due to phenol red. If the bacteria are strict aerobes, the colour of the butt will not change, and the reaction will take place only in the slant. The reaction will occur in both if the bacteria are a facultative anaerobe. Rising or breaks of agar medium indicate the production of hydrogen peroxide gas by many species of bacteria via thiosulfate reduction.

3.3 Molecular characterization

The molecular characterization of isolated bacterial strain was performed by 16S rRNA sequencing. Genomic DNA of isolated bacterial strain was isolated) and PCR amplification was done using fd1 rd1 universal primers After sequencing of 16S rRNA gene, the partial sequence was obtained and analysed using NCBI BLAST tool for identification of isolated bacterial strain and submitted as *Enterobacter cloacae* BVDWH 38 and submitted to NCBI (Accession no. OQ772170).

3.4 Growth curve of isolated bacteria

The growth curve of *E. cloacae* BVDWH 38 sis represented in Figure2.

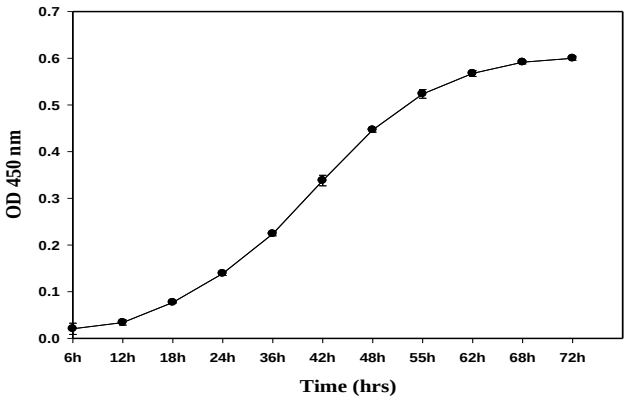

Figure 2Growth curve of isolated bacteria *E. cloacae* BVDWH 38. Error bar indicates standard error of mean

3.5 Antibiotic susceptibility analysis

The antibiotic sensitivity assay, bacterial resistance and sensitivity were evaluated by disc diffusion method. *E.cloacae* BVDWH38 was resistant to Cephaloridine, Ampicillin, and

Erythromycin while sensitive to Amikacin, Streptomycin, and Tetracycline (Table.1, Fig.3). The whole results show inhibition of *E. cloacae* occurs in dose-dependent manner with susceptible antimicrobial agents [16].

Table1. Antibiotic resistance/sensitive study of selected *E. cloacae* BVDWH 38

Antibiotic disc	Resistance/Sensitive	ZOI (cm)
Amikacin	Sensitive	4.5±0.35
Streptomycin	Sensitive	4.7±0.21
Tetracycline	Sensitive	4.1±0.07
Cephaloridine	Resistance	-
Ampicillin	Resistance	-
Erythromycin	Resistance	-

4. CONCLUSION

In developing countries, the most common cause of UTI, diarrhea and gastroenteritis, which affects humanity, is due to lack of safe and clean drinking water supply. In the present study, the pathogenic bacteria were isolated from nearby villages. The bacteriological analysis revealed that *E. cloacae* were present in water. Therefore, this type of research can be useful in bringing awareness both to the public regarding the safety precautions and measures to be taken to prevent contamination of water resources.

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