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ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM HUSSAIN SAGAR LAKE, HYDERABAD AND THEIR ANTIBIOTIC SENSITIVITY

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ABSTRACT

In the present study water samples were collected from two different sites of Hussain Sagar lake (Hyderabad), and analyzed microbiologically using standard methods. The pathogenic bacteria isolated were identified to be *Escherichia coli*, *Staphylococcus aureus*, *Bacillus species*, *Proteus species*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and actinomycetes member. The presence of pathogenic bacteria can be attributed to discharge of untreated effluents and sewage into the lake. Antibiotic sensitivity test result for four antibiotics (ampicillin (A) −10 µg, Streptomycin (S) 10 µg, Pencillin (P) 10 µg, and Gentamycin (G) 10 µg) were variable, most species were resistant to Pencillin, Ampicillin and Streptomycin but susceptible to Gentamycin. A better understanding of pathogens that cause infections is important to select appropriate antimicrobials.

Key words: Pathogenic bacteria, Water sample, Isolation, Antibiotics, Resistance

INTRODUCTION

Water is an important nutrient and plays a vital role in life processes since it is essential to all physiological processes of animals.[1,2] Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases.[3] In developing countries, pollution of surface and underground water by liquid and solid wastes is widespread, thereby rendering them unsuitable for human use.[4,5] Contaminated water with pathogenic bacteria severely affects the performance of humans. Human pathogens such as *Salmonella*, *Camphylobacter sp*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium botulinum*, *Vibrio cholerae* and *Escherichia coli* are responsible for water contamination.[6] Microbial contamination of water should be prevented to protect human health. Bacterial infections are one of the major public health problems and a leading cause of morbidity and mortality in many developing countries.

Uncontrolled and excessive use of antibiotics by human and animals results an increase in antibiotic resistance [7] and cause the spread of resistance genes in environmental samples. Therefore, antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats. Higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats, indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment. A better understanding of pathogens that cause infections is important to select appropriate antimicrobials. Due to the inappropriate use of antibiotics there is a spread of bacterial resistance. The present study is designed to isolate pathogenic bacteria from polluted lake water of Hussain Sagar, Hyderabad and identify the pathogenic bacteria and their sensitivity to some commonly prescribed antibiotics. The determination of bacterial contamination of water carried out in this study was necessary in safeguarding public health. This is because water which is contaminated with faecal material before or during harvest may cause outbreak of intestinal infectious diseases.

The Study Area

It is an artificial lake that holds water perennially fed by canals from Musi river. Hussain Sagar was the main source of water supply to Hyderabad before Himayat Sagar and Osman Sagar were built on river Musi. Now it has become a reservoir for Ganesh idol immersion.

MATERIALS AND METHODS

Isolation of bacteria from water:

Water samples were taken in clean sterile glass bottles, and transported from the Hussain Sagar Lake to the laboratory within 30 min in ice bags during the months of june and july, 2015. All collected samples were transported and prepared for immediate bacteriological test. The physicochemical parameters: electrical conductivity, turbidity, salinity, total organic carbon, phosphate, nitrate, total nitrogen and sulphate content of the samples were analysed as described by APHA. [8] (1998-data not shown).

A 100 mL of serial ten-fold dilution sample was filtered through a 0.5 µm Millipore membrane, which was then placed on plate count agar and incubated aerobically at 37°C for 24–48 hours. After incubation, based on colony morphology representative colonies were picked and sub-cultured on different selective and differential media such as blood agar, EMB agar, MacConkey agar, and pseudomonas agar. After obtaining pure colonies and recording important features such as Starch hydrolysis, lactose fermentation, haemolysis on blood agar isolated organisms were identified biochemically in a systematic way following standard methods.

Sterilization of materials

All the glass ware used was washed, dried and sterilized in hot air oven at a temperature of 160°C for 1 h according to the standard methods. Culture media used were sterilized in an autoclave at a temperature of 121°C for 15 min. The wire loop was also sterilized using spirit lamp.

Identification of Bacteria

Preliminary identification of bacterial strains obtained in pure culture was based on gram staining, respiration, fermentation tests and biochemical tests (IMViC test).

Test for antibiotic resistance

Muller Hinton agar medium was used for drug sensitivity test. Now-a-days it is more commonly used in conjunction with high potency antibiotic discs for determination of antibiotic sensitivity patterns of bacteria. All the bacterial isolates were tested for their sensitivity to antibiotics by means of disc diffusion method test as per the Method.[9] The following antibiotic discs (Hi media) with the final concentrations that are indicated were used: Ampicillin (A)-10 µg, Streptomycin (S)-10 µg, Pencillin (P)-10 µg, and Gentamycin (G)-10 µg. These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

RESULTS AND DISCUSSION

The water samples collected from Hussain sagar lake, Hyderabad were processed to identify the microorganisms present. A total of 8 strains were isolated from the samples collected from the two sites of lake waters (Table 1). The results for microscopic examination such as Gram staining reactions, cell shape, motility and colony morphology were checked after obtaining pure cultures. Table - 2 gives a summary of the physiological and biochemical properties differentiating the isolates.

Table 1. Morphological and cultural characteristics of the organisms isolated from lake water samples.

S. NO.	Sample	Gram Staining	Motility	Nutrient Agar
1.	Site I	G +ve rods G +ve cocci G -ve rods G -ve rods	Motile / Non motile	White rhizoidal Golden yellow , Large transparent, greyish white, entire round or undulate
2.	Site II	G –ve rods G + ve rods	Motile/ Non motile	Irregular white powdery, Golden yellow, Yellow, Mucoid

Table 2. Biochemical characteristics of the isolated organisms from lake water samples

S. No	Indole	Methyl Red	Voges Proskauer	Citrate utilization	Coagulase	Catalase	Oxidase	Identified organisms
1	+	+	-	-	-	+	-	*E. coli*
2	+	+	-	-	-	+	-	*Staphylococcus aureus*
3	+	+	-/+	-	-	-	+	*Staphylococcus sp.*
4	+	+	-	+	-	-	-	*Proteus sp*
5	-	-	-	-	-	-	-	*Bacillus megatherium*
6	+	+	-	-	-	+	-	*Actinomycetes sp*
7	-	-	+	+	-	+	-	*Klebsiella pneumoniae*
8	-	-	-	+	-	+	+	*Pseudomonas aeruginosa*

All the isolates were found to be sensitive against Ampicillin, Streptomycin and Gentamycin (10µg), out of the 4 antibiotics studied maximum resistance was shown for pencillin and ampicillin and minimum for streptomycin. The resistance and susceptibility of the isolated strains against the above antibiotics are tabulated as follows (Table 3).

Table 3. Antibiotic sensitivity levels of the isolated organisms

S. No	Isolated organism	A	S	G	P
1	*E. coli*	s	s	s	r
2	*Bacillus megatherium*	r	s	s	r
3	*Staphylococcus aureus*	r	r	s	r
4	*Staphylococcus species*	r	s	s	r
5	*Proteus species*	r	r	s	r
6	*Actinomycete*	s	s	s	r
7	*Klebsiella pneumonia*	r	s	s	r
8	*Pseudomonas aeruginosa*	r	r	s	r

(A) Ampicillin -10 µg, (S) Streptomycin -10 µg, (P) Pencillin -10 µg, and (G) Gentamycin -10 µg.

s= susceptible r= resistant

In the present study an evaluation of the bacteriological quality of water confirmed the presence of various bacterial species including *Escherichia coli, Staphylococcus aureus, Staphylococcus sp., Proteus sp, Bacillus megatherium, Actinomycetes sp, Klebsiella Pneumoniae* and *Pseudomonas aeruginosa.* The determination of bacterial contamination of lake water carried out in this study was necessary in safeguarding public health. It has clearly indicated that the lake area is highly contaminated by bacteria. These organisms were resistant to several classes of antibiotics. Undesirable properties of water quality caused by the presence of drug-resistant bacteria can pose a negative impact on human health. To protect the consumer's health it is of crucial importance to establish water quality standards and criteria that are chemically balanced and medically safe.

Bacteriological contamination of surface water has remained as one of the major problems in the country in rural as well as urban areas. Many researchers have reported the occurrence of pathogenic bacteria in drinking water, municipal water and lake waters. [10, 11, 12, 13] The emergence of antibiotic resistance in bacteria has been a problem throughout the world. [14] Several studies have reported previously the resistance of bacteria to antibiotics of clinical interest. [15, 16, 17] This study is therefore intended to provide basic information about the lake water, which is contaminated with microorganisms, likely to play a role in the transmission of potential harmful organisms that cause dreadful water borne diseases.

CONCLUSION

The presence of pathogenic bacteria can be attributed to discharge of untreated effluents and sewage into the lake. The lake act as a reservoir of human pathogens and the presence of these highly pathogenic agents such as *Escherichia coli, Staphylococcus aureus, Bacillus species, Proteus species, Klebsiella pneumoniae, Pseudomonas aeruginosa* and actinomycetes species is a potential health risk to human beings and may cause diseases to susceptible individuals.

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