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Original Article

Screening, biochemical characterization and antibiotics resistance/susceptibility of bacteria isolated from native soil and water samples

Triagem, caracterização bioquímica e resistência/suscetibilidade a antibióticos de bactérias isoladas de amostras nativas de solo e água

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Abstract

The present study was conducted to isolate and characterize bacteria from water and soil sample taken from the Lahore Canal at different sites *i.e.* Mall Road, Mohlanwal and Khera site. Isolated bacterial strains were identified on the basis of morphological and biochemical tests. Identification was confirmed by culturing bacteria on selective media. Antibiotic resistance test was also performed to observe the resistance of bacteria against different antibiotics. Blood agar test was performed for identification of different pathogenic bacteria. The result revealed that water and soil samples of Lahore Canal Lahore from different sites were contaminated with *Escherichia coli*, *Salmonella* sp., *Vibrio* sp., *Bacillus* spp., *Enterococcus* sp. and *Staphylococcus* spp. Due to presence of these pathogens, this water is not suitable for any domestic and irrigation use. Study also revealed that water of the Lahore Canal is harmful for human health as it is contaminated with bacteria that can cause severe disease e.g., *Escherichia coli* can cause gastroenteritis, *Bacillus* spp. can cause nausea and vomiting, *Enterococcus* may infect urinary tract, *Salmonella* sp. is responsible for Bacteremia, *Staphylococcus* spp. can cause mild fever and *Vibrio* sp. can be the reason of cholera. Thus it is rendered unfit for any kind of human use even other than drinking like swimming, bathing, washing etc., until and unless some remedial measures are employed to eradicate pathogenic microorganisms by WASA and LWMS according to standards of WHO. Similarly, it is quite harmful, when and where ever it is used for irrigation without proper treatment.

Keywords: bacterial pathogens, antibiotics, antibacterial agents, characterization, water borne bacteria.

Resumo

O presente estudo foi realizado para isolar e caracterizar bactérias de amostras de água e solo retiradas do Canal Lahore, em Lahore, em diferentes locais, ou seja, Mall Road, Mohlanwal e Khera. As cepas bacterianas isoladas foram identificadas com base em testes morfológicos e bioquímicos. A identificação foi confirmada por cultura de bactérias em testes de meios seletivos. O teste de resistência aos antibióticos também foi realizado para observar a resistência das bactérias a diferentes antibióticos. Foi realizado o teste de ágar sangue para identificar diferentes bactérias patogênicas. O resultado revelou que amostras de água e solo do Canal Lahore, Lahore, de diferentes localidades estavam contaminadas com *Escherichia coli*, *Salmonella* sp., *Vibrio* sp., *Bacillus* spp., *Enterococcus* sp. e *Staphylococcus* spp. Por causa da presença desses patógenos, essa água não é adequada para qualquer uso doméstico e de irrigação. O estudo revelou que a água do Canal Lahore é prejudicial à saúde humana, pois está contaminada com bactérias que podem causar doenças graves, por exemplo: *Escherichia coli* pode ocasionar gastroenterite; *Bacillus* spp. pode causar náuseas e vômitos; *Enterococcus* sp. pode infectar o trato urinário; *Salmonella* sp. é responsável pela bacteremia; *Staphylococcus* spp. pode causar febre leve; e *Vibrio* sp. pode ser a razão da cólera. Assim, torna-se imprópria para uso humano, como natação, banho, lavagem etc., até que algumas medidas corretivas sejam empregadas para erradicar microrganismos patogênicos por WASA e LWMS de acordo com os padrões da OMS. Da mesma forma, é bastante prejudicial, quando usada para irrigação sem tratamento adequado.

Palavras-chave: patógenos bacterianos, antibióticos, agentes antibacterianos, caracterização, bactérias transmitidas pela água.

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1. Introduction

Microorganisms are ubiquitous and form communities in different natural habitats. Most hostile environments like deep sea, rocks, geysers, deserts and even the poles are the habitats of microorganisms. The activity of these microbial communities has very important role in ecological systems as well on human health (Szewzyk et al., 1994).

Generally, these ubiquitous microorganisms do not prove to be a threat to human health rather most of them are actually quite beneficial. Particularly, presence of certain bacteria in and on our bodies actually proved to be initial defense like against invading pathogens. After prolonged treatment of antibiotics, normal flora is disturbed offering opportunistic microorganisms a chance to establish and risks of developing diseases are increased. Many soil inhabiting organisms play important roles in geochemistry and provide vital elements like nitrogen and phosphorus, thus ensure availability for other living organisms. However, certain other microorganisms in our surroundings prove to be harmful and cause many diseases in plants and animals including humans (Bryson and Szybalski, 1952).

World Health Organization estimates that 88% of diseases are caused by contaminated water. Infections of microorganisms usually result due to washing, drinking, bathing etc. Some diseases are caused by bacteria present in fresh water, e.g., botulism is caused by "*Clostridium botulinum*", cholera is caused by "*Vibrio cholera*" and dysentery is spread by the number of species belonging to genera "*Shigella*" and "*Salmonella*". Many viruses and protozoa in fresh water also cause different disease.

The Lahore Canal runs through the main city Lahore, the provincial capital of Punjab province, Pakistan. It previously provided a means of aesthetic pleasure for citizens of Lahore, has now become a dumping site. It flows between the BRB Canal start point near Jallo and flows through the city ending at Bhaipheru. Its length is 82 kilometers and accommodates about 350 cusec water. Purpose of the canal, initially, was to facilitate irrigation of agricultural land in district Lahore and Kasur. Pollution level around the canal is increasing tremendously due to improper drainage system of housing societies and industrial that are established along the canal bank.

Lahore BRB (Bombanwala Ravi Bedian) canal is an important source of water for irrigation and is an important watershed as well. However, its water is not regularly monitored for its quality. The water bodies like oceans, rivers, canals and streams are usually self-sufficient in their ability to keep themselves clean systematically, but continuously added waste-water of our urban areas reducing their ability to purify themselves rendering unfit for other inhabitants belonging to them. Animals and plants dwelling these water bodies get poisoned due to harmful metals and other chemicals. Use of waste water for agricultural irrigation is a very common in arid regions but poses many public health issues as well as environmental side effects. The effluent are very often contaminated with elevated levels of various salts, toxic metals, detergents and pathogens (Faryal et al., 2007). Irrigation of vegetables and other crops with such contaminated waters thus proves to become detrimental for livestock as well as humans (Shainberg and Oster, 1985).

Level of pollution is continuously growing in the canal water and poses a serious threat to health of human beings. It also leaves the water not suitable for irrigation purposes. Many researchers have worked to analyze its water quality. In an earlier study conducted on the BRBD (Bhambanwala Ravi Badian Depalpur) link canal, it was concluded that industrial effluents and sewage and industrial effluents that continuously flow into the canal water, deteriorate the water quality in canal (Ahmad, 1978). The pollution level increases in the canal water due to uncontrolled growth of population which in turn causes increased leaching effect on the ground water (Saleemi, 1993)

The Lahore canal faces many waste-water drains and overflowing gutters thus receives industrial as well as domestic wastewaters, respectively. Some reports when compared to data obtained from PCRWR, WAPDA, FAO, and NEQS conclude that the level of pollution in canal water is not much higher except having higher level of cadmium as well as turbidity. The turbid water is not liked of any recreational use or any other esthetic enjoyment. Also, if such water is used for swimming, it can adversely affect the skin of sensitive people (Aftab et al., 2011). Canal water becomes unfit for use in human recreational activities due to high microbial load in its water. Various pathogens have been reported in vegetables that were irrigated with such water (Baghel et al., 2005). High level of total coliform was also observed in the mud at the bottom of Lahore canal (Amin et al., 2005). The samples of the canal water are observed to have excessive limits of sulfide, biochemical oxygen demand, chemical oxygen demand (COD), total dissolved solids, total suspended solids, chlorine and sulphate; a disturbed pH balance and several other imbalances. All of the pollutants exceed beyond the limits set by national environment quality standards. Regrettably, many citizens swim in that water and some even use it for other house hold activities, oblivious to the fact that this polluted water can cause diseases like hepatitis and various skin diseases. Hence, present study was planned and executed to investigate the incidence of microorganism in BRB canal water of Lahore, Pakistan.

2. Materials and Methods

2.1. Sampling

Samples of water and soil were collected from the canal water and canal bank site (Mall Road, Mohlanwal and Khera) Lahore, Pakistan during summer season. Grab sampling technique was used for collection of water sample. The temperature was measured on the site of sampling by dipping a standard thermometer inside water for few minutes. Soil and water samples were collected aseptically in 500 ml sterile autoclavable plastic (polypropylene) bottles from three different points (100 meters apart) and transferred in an icebox to the laboratory. These were immediately stored at 4°C. Microbiological analyses were carried out within 24 h of sampling.

2.2. Isolation of bacteria from soil and water samples

For isolation of bacteria, 1 g of soil was mixed in 50 ml of autoclaved distilled water. The mixture was filtered using filter paper (Whatmann paper, 20µm). Autoclaved Nutrient agar medium (Merck) was poured in sterilized petri plates and allowed to stand for 5-10 min to solidify. Soil filtrate was added with micropipette in different quantities (20µl, 50µl, and 100µl) and spread evenly. Plates were then incubated at 37°C, for 24 h. Isolation of bacteria was performed on nutrient broth (Merck) medium by using spread plate technique. For isolation of bacteria from water samples, the Nutrient agar plates were prepared. 100 µl of the water sample was spread on already prepared nutrient agar plates and incubated at 37°C, for 24 h. In a given plate, all the isolates with differential colony morphology were selected (Bopp et al., 1999).

2.3. Morphological and biochemical characterization

After isolation, the isolates were purified and characterized by Gram stain examination. A number of biochemical tests were performed for the identification of bacterial isolates with the help of Bergey's Manual and also using ABIS 7 (Bacterial identification software) online software. The chemicals and media used for bacterial identification were company based (Merck). The principal tests used for this purpose are lactose fermentation test (LAC), methyl red test (MR), Voges-Proskauer Test (VP), citrate utilization test (CIT), urease test (URE), catalase test (CAT), and hydrogen sulphide (H_2S) production test. For LAC test, lactose broth was inoculated and incubated at 37°C for 24 h. After incubation, a positive result was noted as change of color to yellow while no color change was observed in negative results. MR test was performed by inoculation of the glucose phosphate peptone water broth in a screw capped tube, incubation for 24-48 h and then addition of 5 drops of methyl red where the change in color of the medium to cherry red was considered as positive. VP test was performed by inoculating glucose phosphate peptone water broth with the microbial isolates in a screw capped tube, incubating for 24-48 h, then adding of 0.6 ml of alpha-naphthol solution and 0.2 ml of potassium hydroxide solution. The tubes were then allowed for 5-10 min after shaking well. The red color formation was taken as the positive result. For the CIT test, the Simmons citrate agar slants were inoculated and incubated at 37°C for 24-48 h. The positive slants were noted to change color from green to blue. For URE test, urea broth was inoculated and incubated at 37°C for 24 to 48 h. The change of color of the broth from yellow-orange to bright pink was considered as positive. CAT test was performed by adding a small amount of bacterial isolate into freshly prepared 1% hydrogen peroxide, and the bubbles of oxygen if appeared the isolate was considered as positive for CAT test. H_2S test was used to differentiate species of the family Enterobacteriaceae. This test was used to determine the ability of an organism to reduce sulfur into H_2S. Sufur, idole, motility (SIM) media was used for the H_2S production test. SIM media contains the sulfur containing amino acid, sodium thiosulfate, cysteine, and ferrous sulfate. The SIM media was inoculated with bacterial cultures by stabbing SIM media with inoculating needle. The tubes were then incubated at 35°C for 24 h. After incubation, a positive result was indicated by a black precipitate formed because of the reaction of H_2S with the iron or ferrous sulfate; while the negative result was indicated by no black precipitate (Bergey and Holt, 2000).

2.4. Susceptibility/ resistance of isolates against antibiotics

Antibiotic resistance test were also done. To check antibiotics resistance/susceptibility, antibiotics disc method was used. Nutrient agar plates were prepared and bacteria were spread on agar plates. The antibiotic discs of known concentration were put on these nutrient agar plates. These plates were then incubated at 37°C and results were observed after 24h. Clear zones showed the sensitivity to antibiotics and no clear zone indicated no inhibition of bacteria.

2.5. Use of selective media for bacterial identification

Different types of selective media were used to confirm the identification of isolated bacteria from water and soil samples. For this purpose five types of Selective media were usedL: Mac-Conkey Agar, EMB Agar, *Pseudomonas* Agar, *Staphylococcus* selective medium.

3. Results and Discussion

In the present study, different types of bacteria were isolated from water and soil samples and cultured in laboratory. Results are presented in Table 1a, 1b and Table 2a, 2b. These bacterial strains were identified by different morphological and biochemical tests as *Pseudomonas* sp., *Staphylococcus* spp., *Micrococcus* sp., *Klebsiella* sp., *Vibrio* sp., *Enterococcus* spp., *Salmonella* spp., *Escherichia coli, Bacillus* sp. and *Proteus* sp. *Staphylococcus* spp. were most abundantly present both in water and soil samples of canal. These are Gram-positive cocci. These are common pathogenic bacteria and are normally present on the skin and mucous membranes of humans and other organisms. Madigan and Martinko (2005) also identified that it caused a wide variety of infections in humans and other animals. *Staphylococcus* spp. also produced toxins in food and caused food poisoning (Cenci-Goga et al., 2003). On blood agar test it showed gamma as well as beta hemolytic activity indicated harmful effect on humans. Similar response was reported by Skalka (1988) who studied the hemolytic activity of different species of *Staphylococcus* and observed both beta as well as gamma hemolysis. These bacteria might have been introduced to the canal water through domestic waste and sewage water that enter at so many places without proper treatment, at the site under study and areas near it. In our study, *Staphylococcus* showed maximum resistance against Penicillin while showed maximum sensitivity against Teicoplainin. Carter et al. (2000) also studied the effects of different antibiotics and concluded that many antibiotics are resistance to *Staphylococcus* sp. showed resistance against Penicillin.

Table 1a. Physical and Biochemical characteristics of bacterial strains isolated from water sample of Lahore Canal, Lahore.

Bacterial Strains	Shape/ type	Gram staining	Endospore staining	Urease test	Lactose	Glucose	Sucrose	H_2S production test	Citrate test	Species identified
WSBS1Z	cocci	+ve	-ve	-ve	+ve (A)	-ve	+ve (A)	-ve	-ve	*Enterococcus sp.*
WSBS2Z	Bacilli	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	*Vibro* sp.
WSBS3Z	Cocci	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	*Staphylococcus* sp.
WSBS4Z	Rod	-ve	-ve	-ve	-ve	+ ve (A)	+ve (A)	-ve	-ve	*Aeromonas* sp
WSBS5Z	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve (A)	-ve	-ve	Staphylococcus sp.
WSBS6Z	Rod	-ve	-ve	+ve	+ve (A)	-ve	+ve (A)	-ve	-ve	*Klebsiella* sp.
WSBS7Z	rod	-ve	-ve	-ve	+ve (A)	-ve	-ve	-ve	-ve	*Aeromonas* sp.
WSBS1A	Bacilli	-	-ve	-ve	-ve	+ve (A)	-ve	-ve	-ve	*Escherichia coli*
WSBS2A	Cocci	+	+ve	-	-	+ (A)	-	-	-ve	*Staphylococcus* sp.
WSBS3A	Bacilli	+	-ve	+ve	+ve (A,G)	+ve (A)	+ve(A,G)	-	-ve	Bacillus sp.
WSBS4A	Bacilli	-	-ve	-ve	-ve	+ve (A)	-ve (G)	+ve	+ve	*Salmonella* sp.
WSBS5A	Bacilli	+	-ve	-ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Bacillus* sp.
WSBS6A	Cocci	+	-ve	-ve	+ve (A)	+ve (A)	-ve	-	-ve	*Staphylococcus* sp.
WSBS7A	Vibrio	-	-ve	-ve	+ve (A,G)	+ve (A)	+ve (A)	-	-ve	*Vibrio* sp.
WSBS8A	Cocci	+	-ve	+ve	-ve	+ve (A)	-ve	-	-ve	*Staphylococcus* sp.
WSBS1M	Cocci	+ve	-ve	+ve	-ve	+ve (A)	+ve	+ve	-ve	*Staphylococcus* sp.
WSBS 2M	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve	-ve	-ve	*Staphylococcus* sp.
WSBS 3M	Cocci	+ve	-ve	+ve	+ve	+ve (A)	-ve	-ve	-ve	*Staphylococcus* sp.
WSBS 4M	Rod	-ve	-ve	-ve	-ve	+ve (A)	+ve	-ve	-ve	*Aeromonas* sp.

Table 1a. Continued...

Bacterial Strains	Shape/ type	Gram staining	Endospore staining	Urease test	Lactose	Glucose	Sucrose	H_2S production test	Citrate test	Species identified
WSBS 5M	Rod	-ve	-ve	-ve	-ve	+ve (A)	+ve	-ve	-ve	*Vibrio* sp.
WSBS 6M	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve	-ve	-ve	*Staphylococcus* sp.
WSBS 7M	Rod	-ve	-ve	-ve	+ve	+ve (A)	-ve	-ve	-ve	*Escherichia coli*
WSBS 8M	Cocci	+ve	-ve	-ve	+ve	+ve (A)	+ve	-ve	-ve	*Staphylococcus* sp.
WSBS 9M	Cocci	+ve	-ve	+ve	-ve	+ve (A)	+ve	+ve	-ve	*Staphylococcus* sp.

Table 1b. Physical and Biochemical characteristics of bacterial strains isolated from water sample of Lahore Canal, Lahore.

Bacterial Strains	Catalase test	MR-VP test		Litmus milk test	Triple sugar iron test	Mac-conkey agar	Staphylococcus agar	Pseudomonas agar	Blood agar test	EMB agar test
		MR	VP							
WSBS1Z	+ve	+ve	-ve	Curd formation + clear zone (Stormy fermentation)	+ve	-ve	-ve	-ve	γ	-ve
WSBS2Z	+ve	+ve	-ve	C, + clear zone (Stormy fermentation)	-ve	-ve	-ve	-ve	γ	+ve
WSBS3Z	+ve	+ve	-ve	C, clear zone (Stormy fermentation)	+ve	-ve	+ve	-ve	γ	-ve
WSBS4Z	+ve	+ve	-ve	C, clear zone (Stormy fermentation	+ve	-ve	-ve	-ve	α	+ve
WSBS5Z	+ve	+ve	-ve	C, clear zone (Stormy fermentation)	+ve	-ve	+ve	-ve	β	-ve

Table 1b. Continued...

Bacterial Strains	Catalase test	MR-VP test		Litmus milk test	Triple sugar iron test	Mac-conkey agar	Staphylococcus agar	Pseudomonas agar	Blood agar test	EMB agar test
		MR	VP							
WSBS6Z	+ve	+ve	-ve	A, no curd formation	+ve	-ve	-ve	-ve	γ	+ve
WSBS7Z	+ve	+ve	-ve	C,A	-ve	-ve	-ve	-ve	γ	+ve
WSBS1A	+ve	+ve	-ve	C, A	+ve	+ve	-ve	-ve	α	Shiny green
WSBS2A	+ve	+	-	C, K	+ve	-ve	+	_	γ	-ve
WSBS3A	+ve	+ve	-ve	C, A	+ve	+ve	-ve	-ve	α	-ve
WSBS4A	+ve	+ve	-	C, A	-ve	-ve	-ve	-ve	γ	+ve
WSBS5A	+ve	+ve	-	C. A	-ve	-ve	-ve	-ve	α	-ve
WSBS6A	+ve	+ve	-	C, A	-ve	-ve	+ve	-ve	γ	-ve
WSBS7A	+ve	+ve	-	C, A	-ve	+ ve	-ve	-ve	β	+ve
WSBS8A	+ve	+ve	-	C, A	-ve	-ve	+ve	-ve	γ	-ve
WSBS1M	+ve	+ve	-ve	C	+ve	-ve	+ve	-ve	B	-ve
WSBS 2M	+ve	+ve	-ve	C,A	+ve	-ve	+ve	-ve	γ	-ve
WSBS 3M	+ve	+ve	+ve	C,K	-ve	-ve	+ve	-ve	B	-ve
WSBS 4M	+ve	+ve	-ve	C	+ve	+ve	-ve	-ve	γ	+ve
WSBS 5M	+ve	+ve	-ve	C	-ve	+ve	-ve	-ve	γ	-ve
WSBS 6M	+ve	+ve	-ve	C,K	-ve	-ve	+ve	-ve	γ	-ve
WSBS 7M	+ve	+ve	-ve	C.A	+ve	+ve	-ve	-ve	α	+ve
WSBS 8M	+ve	+ve	-ve	C,K	-ve	-ve	+ve	-ve	γ	-ve
WSBS 9M	+ve	+ve	-ve	C,A	+ve	-ve	+ve	-ve	γ	-ve

Table 2a. Physical and Biochemical characteristics of bacterial strains isolated from water sample of Lahore Canal, Lahore.

Bacterial Strains	Shape/ type	Gram staining	Endospore staining	Urease test	Lactose	Glucose	Sucrose	H_2S production test	Citrate test	Species identified
SSBS1Z	Cocci	+ve	-ve	+ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBSZ2	rod	-ve	-ve	-ve	-ve	+ve (A)	-ve	-ve	-ve	*Pseudomonas* sp.
SSBS3Z	Cocci	+ve	-ve	+ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS4Z	Cocci	+ve	-ve	+ve	+ve (A)	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS5Z	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Enterococcus* sp.
SSBS6Z	Cocci	+ve	-ve	-ve	+ve (A)	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS7Z	Cocci	+ve	-ve	+ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Micrococcus* sp.
SSBS1A	Cocci	+ve	-ve	-ve	+ve (A)	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS2A	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS3A	Cocci	+ve	-ve	+ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS4A	Cocci	+ve	-ve	+ve	+ve (A)	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS5A	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS6A	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Enterococcus* sp.
SSBS7A	Cocci	+ve	-ve	-ve	-ve	+ve (A)	+ve (A)	-ve	-ve	*Staphylococcus* sp.
SSBS 1M	Cocci	+ve	-ve	-ve	-ve	+ (A)	+ (A)	-ve	-ve	*Staphylococcus* sp.

Table 2a. Continued...

Bacterial Strains	Shape/ type	Gram staining	Endospore staining	Urease test	Lactose	Glucose	Sucrose	H_2S production test	Citrate test	Species identified
SSBS 2M	Cocci	+ve	-ve	+ve	-ve	+ (A)	+ (A)	-ve	-ve	*Staphylococcus* sp.
SSBS 3M	Rod	+ve	-ve	+ve	-ve	+ (A)	+ (A)	-ve	-ve	*Bacillus* sp.
SSBS 4M	Cocci	+ve	-ve	+ve	-ve	+ (A)	+ (A)	-ve	-ve	*Staphylococcus* sp.
SSBS 5M	Rod	-ve	-ve	+ve	-ve	+ (A)	+ (A)	-ve	-ve	*Proteus* sp.
SSBS 6M	Cocci	+ve	-ve	+ve	-ve	+ (A)	-ve	-ve	-ve	*Staphylococcus* sp.
SSBS 7M	Rod	-ve	-ve	-ve	+ (A)	+ (A)	-ve	-ve	-ve	*Escherichia coli*
SSBS 8M	Cocci	-ve	-ve	+ve	-ve	+ (A)	+ (A)	-ve	-ve	*Klebsiella* sp.

Table 2b. Physical and Biochemical characteristics of bacterial strains isolated from soil sample of Lahore Canal, Lahore.

Bacterial Strains	Catalase test	MR-VP test		Litmus milk test	Triple sugar iron test	Mac-conkey agar	Styphylococus agar	Pseudomonas agar	Blood agar test	EMB agar test
		MR	VP							
SSBS1Z	+ve	+ve	-ve	A,no Curd formation	-ve	-ve	+ve	-ve	β	-ve
SSBSZ2	+ve	+ve	-ve	C,A	-ve	-ve	-ve	+ve	γ	-ve
SSBS3Z	+ve	+ve	-ve	C,A	-ve	-ve	+ve	-ve	α	-ve
SSBS4Z	+ve	+ve/ -ve	-ve	C,A	+ve	-ve	+ve	-ve	B	-ve
SSBS5Z	+ve	+ve	+ve	C, clear zone	-ve	+ve	-ve	-ve	β	-ve
SSBS6Z	+ve	+ve	-ve	A, no curd formation	+ve	-ve	+ve	-ve	γ	-ve
SSBS7Z	+ve	+ve	-ve	C,A	-ve	+ve	-ve	-ve	B	-ve
SSBS1A	-ve	+ve	-ve	C, K	-ve	-ve	+ve	-ve	β	-ve
SSBS2A	-ve	+ve	-ve	C, K	-ve	-ve	+ve	-ve	γ	-ve

Table 2b. Continued...

Bacterial Strains	Catalase test	MR-VP test		Litmus milk test	Triple sugar iron test	Mac-conkey agar	Styphylococus agar	Pseudomonas agar	Blood agar test	EMB agar test
		MR	VP							
SSBS3A	-ve	+ve	-ve	C, K	+ve	-ve	+ve	-ve	β	-ve
SSBS4A	+ve	+ve	-ve	C, A	-ve	-ve	+ve	-ve	β	-ve
SSBS5A	+ve	+ve	-ve	C, K	+ve	-ve	+ve	-ve	γ	-ve
SSBS6A	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	γ	-ve
SSBS7A	+ve	+ve	-ve	C, K	-ve	-ve	+ve	-ve	γ	-ve
SSBS1M	-ve	+	-	C,A	-ve	-ve	+ve	-ve	γ	-ve
SSBS2M	-ve	+	-	C,A	-ve	-ve	+ve	-ve	β	-ve
SSBS3M	-ve	+	-	C,K	-ve	-ve	-ve	-ve	α	-ve
SSBS4M	-ve	+	-	C,A	-ve	-ve	+ve	-ve	γ	-ve
SSBS5M	+ve	+	-	C,K	-ve	+ve	-ve	-ve	β	-ve
SSBS6M	-ve	+	-	C,A	+ve	-ve	+ve	+ve	γ	-ve
SSBS7M	+ve	+	-	C,A	+ve	+ve	-ve	-ve	α	-ve
SSBS8M	+ve	-	+	C,A	+ve	+ve	-ve	-ve	α	+ve

Pseudomonas sp. are Gram-negative, rod shaped, non-endosporic bacteria, commonly found in soil, water, faeces and sewage (Jay, 2000). These are common pathogenic bacteria causing infectious diseases in humans like on burn wounds, urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bone and joint infections, gastrointestinal infections and septicaemia *etc*. Immune suppressed people may have severe infection in their eyes due to *Pseudomonas* sp. High counts of this organism in canals and swimming pool water can caused rashes and superficial infections of the outer ear canal (Calderon and Mood, 1982). It also causes a lot of disease in plants, such as bacterial blotch and drippy gill in cultivated mushrooms (Young, 1970). The basic source of these bacteria in canal water is domestic waste and sewage water that may enter at so many places without proper treatment at and near the site under study.

Aeromonas sp. is Gram-negative, rod-shaped and non-endosporic bacteria. It is isolated from a variety of aquatic environments, including freshwater, estuarine, brackish, and salt waters and foods, including red meats, poultry, produce, fish, and shellfish (Janda and Duffey, 1988).

It showed alpha hemolytic activity on blood agar. On antibiotic resistance test it was resistive to Ampiciline, Cloxacilline and Penicillin and only sensitive against Teicoplanin so, it can easily transmittable from one person to another. Similar response was studied by Awan et al. (2009) on *Aeromonas* species isolated from food.

Klebsiella sp. is Gram-negative and rod-shaped bacteria. *Klebsiella* species are ubiquitous in nature. These bacteria have been recovered from aquatic environments receiving industrial wastewaters (Caplenas et al., 1981), plant products, fresh vegetables, food with a high content of sugars and acids, frozen orange juice concentrate, sugar cane wastes, living trees, plants and plant by-products. It can cause many diseases like pneumonia, urinary tract infections, septicemia, and soft tissue infections.

In present study, it showed gamma hemolytic activity on human blood. However, Albesa et al. (1985) studied the hemolysis by *Klebsiella* on rabbit blood agar. It showed maximum resistance against Penicillin and maximum sensitivity against Teicoplanin.

Vibrio sp. is Gram-negative, rod shape bacteria. It is commonly present in water and considered to be pathogenic and causes many harmful diseases in humans. *It* can cause cholera, which is an acute, diarrheal illness that can result in severe dehydration and even death within a matter of hours. These bacteria might have been introduced to the canal water through domestic waste and sewage water.

Enterococci sp. is Gram-positive cocci and non-endospore forming bacteria. It was isolated from soil as well water. *Enterococcus* bacteria are considered as one of the preferred indicator of fecal contamination form animals. It is pathogenic in nature and causes many infections included urinary tract infections, bacteremia etc. (Fisher and Phillips, 2009). On blood agar test, it showed gamma and beta hemolytic activity. It showed maximum resistance against Penicillin, Ampiciline and Cloxacillin. Detection of *Entrococcus* sp. in the canal water shows that water is unhygienic and can lead to many harmful diseases if it is used untreated.

Micrococcus sp. is Gram-positive and endosporic bacteria. Smith et al. (1999) reported that the death of immune compromised patients has occurred from pulmonary infections caused by *Micrococcus.* It may be involved in other infections, including recurrent septic shock, septic arthritis, endocarditic, meningitis, and pneumonia etc. On blood agar test it showed beta hemolytic activity. It showed resistance against Penicillin, Cloxacillin and Ampicilline and showed sensitivity against Teicoplanin. From results it is concluded that this bacteria is resistant to above mentioned antibiotics so can easily transmittable and can cause severe diseases.

Bacillus sp. is Gram-positive, rod shaped bacteria. The primary source of *Bacillus* species and other Gram-positive bacilli are soil, water, dust, air, feces, vegetation, wounds and abscesses. *Bacillus cereus* can cause two distinct types of illnesses; a diarrheal illness and an emetic (vomiting) illness. Symptoms due to *Bacillus* include abdominal cramps, Watery diarrhea, Nausea and Vomiting. Incidence of *Bacillus* sp. in Canal water is due to fecal contamination and domestic waste. If the water is used without proper treatment, it can cause life threatening diseases.

Escherichia coli are Gram-negative, rod shaped, non-spore forming bacteria. They are very common bacteria present in fresh water. *E. coli* is major cause of enteric illness including enteropathogenic, enteroinvasive, enterotoxigenic and enterohaemorrhagic strains (Bopp et al., 1999). *E. coli* are also responsible for Gastroenteritis and produce large quantities of toxins that can cause mild diarrhea to hemorrhagic colitis. They are most specific indicator for faecal contamination; occur only in the feces of warm-blooded mammals. So the major source of entrance of *E. coli* in Canal water is cattle feces, birds, and pets especially dogs from the nearby areas.

Salmonella spp. are Gram-negative, rod shaped, non-spore forming bacteria. They are widely distributed in environment and enter in water bodies through faecal contamination, drainage waters and incompletely treated waste discharges. *Salmonella* sp. causes serious health problems in developing countries through a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia. The majority of human salmonellosis is caused by the consumption of contaminated eggs, poultry, beef and milk products (Zaki et al., 2009). The site under study, receives domestic waste along with traffic pollution. Fecal contamination might have introduced these *Salmonella sp.* in canal water and may cause life threatening diseases if used without proper disinfectant applications. Occurrence of *Salmonella* in freshwater due to fecal contamination was also reported by Floyd and Jones (1954) and these bacteria were reported to cause infections in the animals found associated with these water bodies. Thus canal water is quite unhygienic and even diving and bathing is quite hazardous.

Klebsiella sp. is inherently environmental organisms that survive and sometimes multiply in suitable waters. They are associated with roots of plants and can grow to high levels on the leaves of vegetables. They are frequently present in raw waters, and can increase to high levels in waters containing pulp mill wastes. *Klebsiella* sp. have been

identified as important common pathogens for nosocomial pneumonia, septicaemia, urinary tract infections, wound infections, intensive care unit (ICU) infections and neonatal septicaemias (Janda and Abbott, 2006). Presence of *Klebsiella* sp. in the canal water and soil samples indicates that domestic waste is its source and it may become a risk factor if this water is used for irrigation purpose as the bacteria will contaminate the crops especially vegetables and will cause diseases in consumers. Diving and bathing in this water is also a health hazard.

Proteus sp. belonging to the family Enterobacteriaceae (Ronald, 2003) generally range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length (Abbott, 2007). They are widespread in the environment, including animals, soil and polluted water. They are part of normal flora of human gastrointestinal tract. They are the second most common cause of urinary tract infections, and a major cause of nosocomial infections. In the present study they showed resistance against Ampicillin (AMP) and penicillin (P). Presence of *Proteus* sp. in canal water and soils shows many harmful effects on the human health if it is used without disinfectant application.

4. Conclusion

At the end, it is concluded that the water and soil of the Lahore Canal is highly contaminated with *Pseudomonas* sp.*, Staphylococcus* spp., *Micrococcus* sp.*, Klebsiella* sp., *Vibrio* sp. *and Enterococcus* spp. which indicates that it is highly polluted and unhygienic and may cause spread of various diseases like Pneumonia, Typhoid fever, Cholera, food poisoning, Wound infections, Gastroenteritis, Bacillary dysentery, Infectious hepatitis, Urinary tract infections, Dermatitis, Soft tissue infections, bone and Joint infections, Gastrointestinal infections and Septicaemia *etc*. Thus it is rendered unfit for humans use like swimming, bathing, washing and even other recreational purposes, until and unless some remedial measures are employed to stamp out pathogenic microorganisms by authorities like WASA and LWMS according to standards of WHO. Similarly, it is quite harmful, when and where ever it is used for irrigation without proper treatment.

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