RESEARCH ARTICLE

Bacteriological and Physiochemical Analysis of Drinking Water from Different Areas of District Gujrat, Pakistan

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Abstract

The contribution of water in causing water borne bacterial infections is continuously neglected and much focus is made on treating infections rather than preventing infections. The human body is easily exposed to rich bacterial diversity of water and it necessitates its bacteriological and physiological analysis to made awareness about its careful use. The quality of water is deteriorating very rapidly due to number of factors. Drinking water samples from different areas of district Gujrat, Pakistan were subjected to bacteriological and physiochemical analysis. In addition to physical parameters like pH, presence of foul odor and suspended particles, temperature of samples, the potential of water to cause infection was also analyzed by studying microbial count. Morphological attributes such as color, shape, surface, elevation and opacity of bacterial colonies were observed. Gram staining and biochemical tests (oxidase test and catalase test) on pure bacterial cultures isolated from drinking water samples, exposed the presence of Staphylococcus, Bacillus, Streptococcus, Streptomyces, Escherichia, Enterococcus and Citrobacter. Furthermore, the biofilm forming capacity of the isolated species was also estimated to establish its contribution in inducing infections in the local community. Growths of these strains on Congo red supplemented nutrient agar have shown their biofilm forming ability and represented different morphotypes including rough dry and red (rdar) and smooth and white (saw) due to binding expression of Congo red dye with extracellular components of bacterial strains. Citrobacter and Escherichia were present in 37.5% samples and 62.5% water samples contain Staphylococcus strains. Biofilm forming characteristic is shown by all isolated bacterial strains.

Keywords: Bacteriological, Physiochemical, Drinking Water, Morphotypes, Staphylococcus,

Introduction

Water is crucial for all forms of life and is required in almost all human activities. It is now universal human right to gain access to clean and safe freshwater (United Nations Committee on Economic Social and Cultural Rights, 2003). About 70% of people in Pakistan use ground water for domestic usage (Malik et al, 2010). High population growth rate, expansion in industries and factories, discharge of poorly treated wastes and chemical effluents into canals and other water sources are major causes of water pollution. According to recent studies, the quantity of available water in developing regions of South Asia, Middle East and Africa is decreasing sharply while quality of water is deteriorating rapidly due to fast urbanization, deforestation, and land degradation (Annachhatre, 2006).

The increase in organic pollutants promotes the growth of microorganisms. Microorganisms are widely distributed in aquatic bodies where they become cause of many water borne diseases (Hans et al, 2003). Unfortunately, water borne diseases are most common in developing countries like Pakistan due to discharge of hazardous industrial

wastes including toxic synthetic organic chemicals, heavy metals, pesticide products, municipal wastes and untreated sewage water in natural water bodies. 20 to 30% of all hospital cases and 60% infant deaths are due to water borne diseases (Government of Pakistan, 1999).

Pathogens present in drinking water include many viral, bacterial and protozoan agents that cause 2.5 million deaths from endemic diarrheal disease each year. The prevalence of diarrhea, typhoid fever, bacillary dysentery, infectious hepatitis, vomiting, nausea, trachoma and other enteric infections are common and are transmitted through contaminated water (Kahlown et al, 2006).

The tests for water safety in practice today are directed at identifying particular indicator organisms or a bio indicator, living within natural communities to monitor the impact of disturbance communities. Bio indicators are used to observe and measure the effect of pollutants on specific environment (Fatma et al, 2009). Bio indicators may include organisms like lichens, insects, birds and bacteria (Akanksha et al, 2010).

Indicator bacteria are those bacteria that are used to determine and measure the level of fecal contamination of water. For water quality analyses, it is not necessary to analyze all the pathogens. *Escherichia coli*, total coliforms and fecal coliforms are commonly used as indicator bacteria. They are not harmful to human health, but their presence indicates health risk for humans (Frost et al, 1996). Simple and rapid methods are used to detect fecal indicator bacteria. Hence, Escherichia, Klebsiella, Enterobacter and Citrobacter genera are most commonly targeted to indicate water pollution. Similar to the work on coliforms, a group of Gram-positive coccoid bacteria known as fecal Streptococci (FS) were being investigated as important pollution indicator bacteria. Fecal Streptococc*i* are relatively high numbers in the excreta of humans and other warm blooded animals. They are abundantly present in waste-waters and polluted waters and are persistence in environment without multiplication, which makes them better bio indicators (Rhodes and Kator, 1999).

When biofilm forming strains of *S. typhimurium*, *E.coli* and some other members of Enterobacteriaceae are grown on diazo dye Congo Red (CR) supplemented in agar plates, dark, purple and wrinkled colony morphology results called red dry and rough phenotype (rdar) due to the expression of cellulose and curli (Romling U, 2005). This phenotype is the consequence of Congo Red binding with the extracellular matrix components that alters the spectral properties of CR. Cellulose producing strains appear pink dry and rough (pdar) on CR plates and production of curli fimbrae results in brown, dry and rough (bdar) colony morphology. A smooth, glossy and white phenotype results if none of the matrix component expressed and named as smooth and white (saw). The current studies focus on the biofilm capabilities of the bacterial population that would be helpful in future to fight antibiotic resistance and establish a strong relation biofilm based infections in the local community of district Gujrat, Pakistan. Communities or groups of microorganisms that attach to the surfaces of living or nonliving objects are known as biofilms. Biofilm communities are found almost in every environment. Microbes become less susceptible to antibiotics when they are in form of communities (Jeff, 2009). Biofilms indicate about formation and living mechanisms of bacteria in multicellular communities. On the other side, biofilm formation causes considerable problems in medical and industrialized settings, as bacteria in biofilms can resist antibiotic treatment, host immune responses, and biocide treatments (Harmsen et al, 2010). Biofilm formation signifies a secure mode of growth that allows cells to survive in hostile environments and also to disperse and colonize new niches (Launne et al, 2004).

Pakistan is ranked in the list of water stress countries, with the availability of about 1200 m^3 per capita, which is swiftly declining with time (Malik et al, 2010). Being a developing country, Pakistan needs to deal with an immense group of problems regarding water availability, quality, usage and deaths caused by water-borne diseases. According to an estimate, 40% of all deaths are caused by poor quality of water bring used (Global Water Partnership, 2000).

The present study was conducted from district Gujrat, which is included in one of the populous and industrial cities of Punjab, Pakistan. Drinking water sources were preferred for sampling purpose, to analyze the quality of water. According to the information in district Gujrat 96,160 patients were registered in Government hospitals as victims of water borne and hygiene related diseases from Jan-Jun 2010 (Directorate General Health Services Punjab, 2010).

Material and Methods

Water samples were collected from different areas of district Gujrat, Pakistan. Water sources included dug wells, tube wells, bore wells, hand pumps and surface water, filter plants. Drinking water from 16 sources was collected aseptically to minimize contamination and kept in vials of 15ml. Temperature and pH readings were noted at the sample site.

Sterilized Eppendorf tubes autoclaved at 121°C for 20 minutes were used for sample storage and transportation. Samples were temporarily stored in refrigerator at 4°C, whenever required.

Nutrient agar medium was prepared, autoclaved and poured in 60mm petri plates. Plates were inoculated with their respective label samples using following spread plate procedure (Jett et al, 1997). 10µl of each water sample was poured on nutrient medium and spread all over the plate, priory heating on flame to avoid contamination. Plates were taped and incubated at 37° C for 72 hours. Growth of microbes was observed every 24 hours.

Different colonies with distinct morphologies were observed and pure cultures were prepared on morphological basis. Sample colony was introduced on medium using streaking technique. Three phase streaking technique, known as T-streaking (Finegold and Sweeney, 1960) was used for this purpose. Following tests were formed on the prepared pure cultures.

Congo Red Assay

For this analysis, Congo Red supplemented nutrient media was prepared in flask. 6ml Congo red solution was prepared by dissolving 12mg Congo red in 6ml distilled water in measuring cylinder. Syringe sterilized Congo Red solution was dissolved in autoclaved nutrient agar media and mixed by shaking. When agar media containing Congo red solution was cooled down at room temperature, it was then poured in petri plates and allowed to solidify. Each pure culture of bacterial strain was dissolved in 1ml autoclaved distilled water in eppendorf tube to make a turbid solution. Each pure culture was then inoculated on Congo Red supplements agar media by dropping and subsequent spreading 4µl inoculum. Plates were incubated at 37°C for 48 hours and growth was observed at an interval of 24 hours.

Staining Reaction

The Gram staining procedure was performed for each pure culture (Gephart et al, 1981).

Biochemical Tests

Catalase Test

Catalase test is mainly performed for differentiation between bacterial genera. A drop of 3% hydrogen peroxide is taken on microscopic slide. A small amount of freshly prepared 18-24 hours pure colony is taken with help of sterile inoculating loop and placed on microscopic slide, observed the slide for bubble formations (MacFaddin, 2000). Bubble formation indicates positive results and confirms the presence of catalase enzyme in bacteria. This test also differentiates between gram similar strains, which are in other way dissimilar in catalase test results (Mahon, 2011).

Oxidase Test

This test helps to indicate the presence of cytochrome oxidase enzyme in bacteria (MacFaddin, 1972). For this purpose, filter paper test method was used (Kovács, 1956), in which a piece of filter paper was soaked in 1% Kovács oxidase reagent and let it dry. A freshly prepared 18 to 24 hours pure culture was placed on filter paper by using sterile inoculation loop and observed for color changes. Organisms are oxidase positive, when the colorless oxidase reagent changes to dark purple within 5 to 90 seconds. If the color not changes, then the organism lacks oxidase enzyme (Lui and Jurtshuk, 1986).

Results and Discussion

Physical Analysis of Water Samples

The physical analysis done on each sample site is reported in Table 1.

Water sample	Temperature	pH	Solid suspended particles	Foul Odor
1	29°C	6.89	Present, black in color	Present
2	29°C	7.39	Absent	Absent
3	30°C	7.38	Absent	Absent
4	32°C	7.64	Absent	Absent
5	29°C	8.04	Absent	Absent
6	31°C	7.71	Absent	Absent
7	32°C	7.56	Absent	Absent
8	29°C	8.4	Absent	Absent
9	30°C	8.4	Absent	Absent
10	30°C	8.11	Present, black in color	Absent
11	28°C	7.82	Absent	Absent
12	29°C	7.74	Absent	Absent
13	31°C	8.37	Absent	Absent
14	31°C	8.23	Absent	Absent
15	29°C	6.24	Present, brown in color	Present
16	28°C	6.82	Present, green in color	Present

Table 1: *Physical analysis of water samples*

Growth of Microbes on Nutrient Media

Observing morphologies, 07 distinct colonies were targeted following growth on nutrient agar medium out of 16 samples. The colonies were named for convenience as shown in Table 2.

Morphologically, 71% of colonies were of white color, pale yellow comprise 14% and remainning 15% colonies were of yellow color. 86% of the colonies had smooth surface and 14% had rough surface. The morphological attributes are tabulated in table 3.

Colony	Name	Source Sample
1	ABM-1	3, 4, 6, 11, 14
2	ABM-2	1, 2, 5, 8, 12, 15
3	ABM-3	5, 8, 9, 10, 13, 15
4	ABM-4	2, 3, 5, 7, 12, 16
5	ABM-5	2, 3, 4, 6, 7, 11, 12
6	ABM-6	5, 8, 9, 10, 13, 14
7	ABM-7	1-16

Table 02: *Isolated colonies with their sample source*

Name	Color	Shape	Surface	Elevation	Opacity
ABM-1	White	Circular	Smooth	Convex	Yes
ABM-2	White	Filamentous	Smooth	Flat	Yes
ABM-3	Pale yellow	Circular	Smooth	Flat	Yes
ABM-4	White	Circular	Smooth	Convex	No
ABM-5	White	Irregular	Smooth	Elevated	No
ABM-6	Yellow	Circular	Smooth	Convex	Yes
ABM-7	Yellow	Circular	Smooth	Raised	Yes
ABM-8	Green	Irregular	Smooth	Raised	No

Table 3: *Morphologies of isolated colonies*

Congo Red Supplemented Growth

Bacterial isolated showed different morphotypes, due to the Congo red binding activity with the extracellular matrix components of bacterial strains, when grown on Congo red supplemented nutrient agar plates.

Rough, Dry and Red (Rdar) and Smooth and White (Saw) Morphotype

Rough, dry and red phenotype appeared due to the resulting expression of cellulose and curli, by binding of Congo red dye to the extracellular matrix components of bacterial strains. Smooth and white (saw) morphotype appeared as none of the matrix components expressed on binding with the Congo red dye. Different strains have shown different rdar morphotypes at 24, 48 and 72 hour intervals (AMB-1-AMB-7).

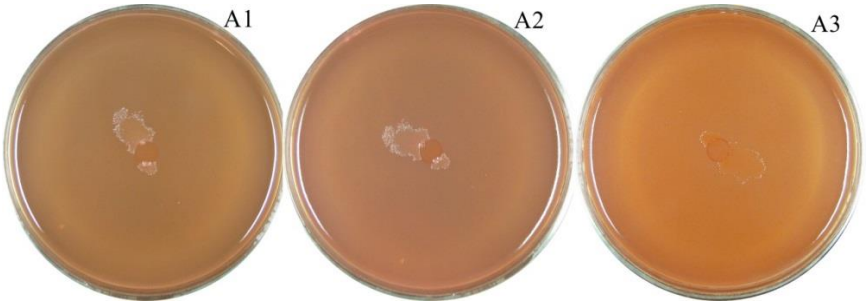

Figure 1: *ABM-1 rdar morphotype at 24 (A1), 48 (A2) and 72 (A3) hour intervals*

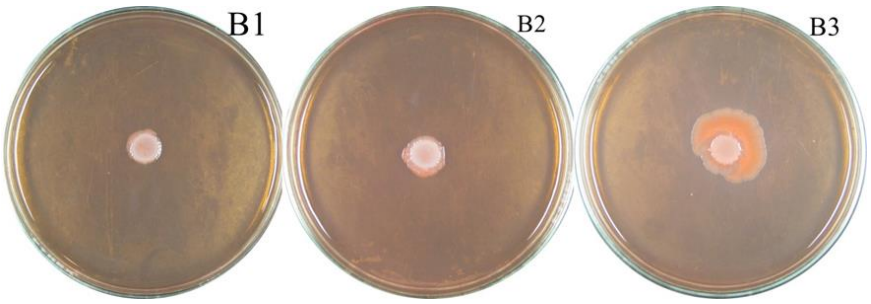

Figure 2: *ABM-2 saw morphotype at 24 hours (B1), and 48 hours (B2) and rdar morphotype at 72 hours (B3)*

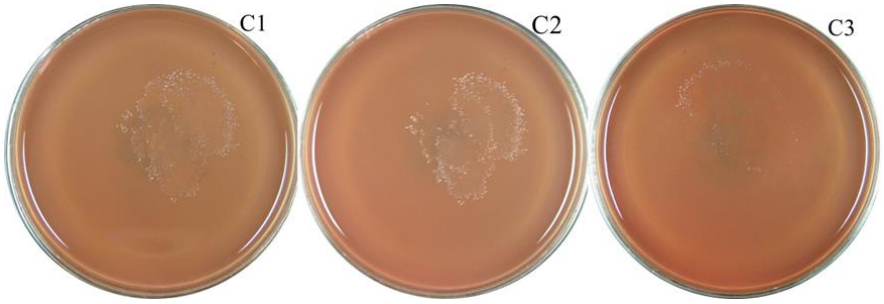

Figure 3: *ABM-3 saw morphotype at 24 (C1), 48 (C2) and 72 (C3) hour intervals*

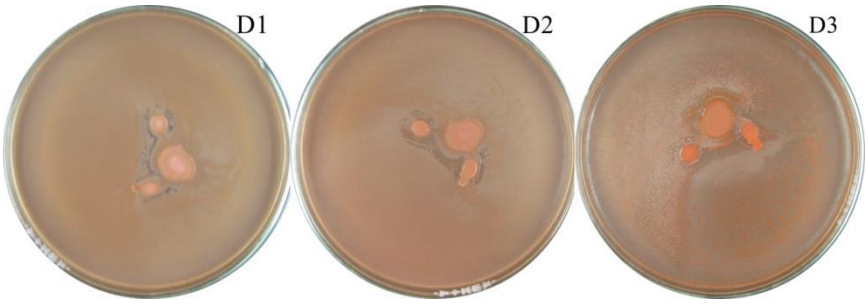

Figure 4: *ABM-4 rdar morphotype at 24 (D1), 48 (D2) and 72 (D3) hour intervals*

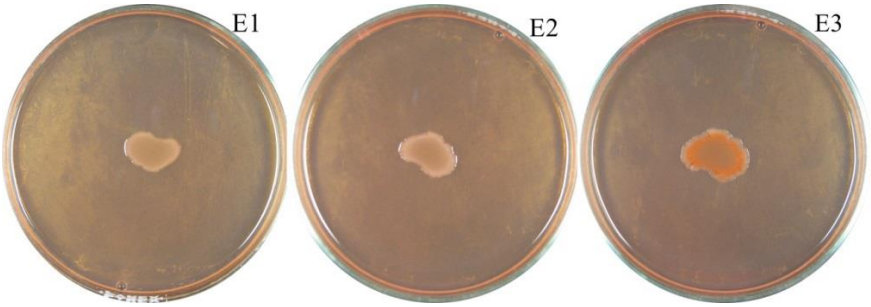

Figure 5*: ABM-5 saw morphotype at 24 hours (E1), and 48 hours (E2) and rdar morphotype at 72 (E3)* hours

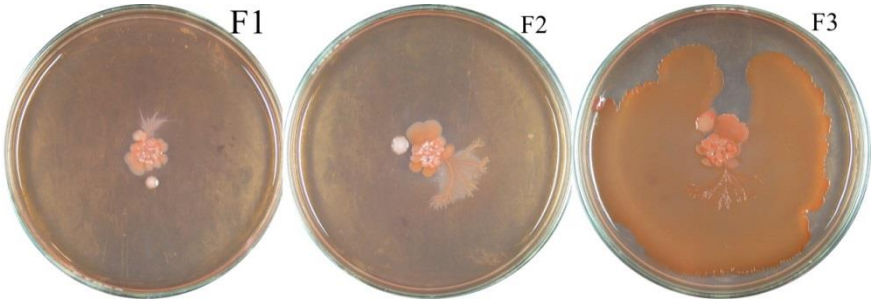

Figure 6: *ABM-6 rdar morphotype at 24 (F1), 48 (F2) and 72 (F3) hour intervals*

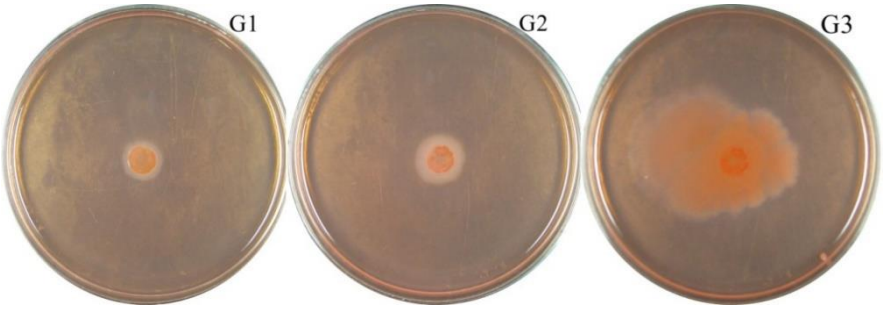

Figure 7: *ABM-7 rdar morphotype at 24 (G1), 48 (G2) and 72 (G3) hour intervals*

Gram Staining and Biochemical Tests

Gram positive bacterial genera have shown purple color and negative genera gave pink color on staining. Those strains which were oxidase positive, gave blue color on filter paper with oxidase reagent (Figure 8), other remained colorless. As a result of catalase test, rapid bubble formation (Figure 9) due to gas (O_2) production, confirms the strain for presence of catalase enzyme. Catalase negative strains gave few scattered bubbles (Figure 10). Bacterial strains have shown positive and negative results towards oxidase and catalase tests (Ruangpan and Tendencia, 2004), which are tabulated below:

Strain	Gram stain	Oxidase test	Catalase test	Genera	References
ABM-1	Gram positive	Positive	Negative	Enterococcus	Albert and Anicet, 1999
ABM-2	Gram positive	Negative	Positive	Streptomyces	Houssam, 2011
ABM-3	Gram positive	Negative	Positive	Staphylococcus	Doaa and Salwa, 2012
ABM-4	Gram positive	Negative	Positive	Streptococcus	Patricia And Laura, 2013
ABM-5	Gram negative	Negative	Positive	Citrobacter	Michael et al, 1994
ABM-6	Gram negative	Negative	Positive	Escherichia	Doaa and Salwa, 2012
ABM-7	Gram positive	Positive	Positive	Bacillus	May, 2011

Table 4: *Staining and biochemical test results of bacterial colonies*

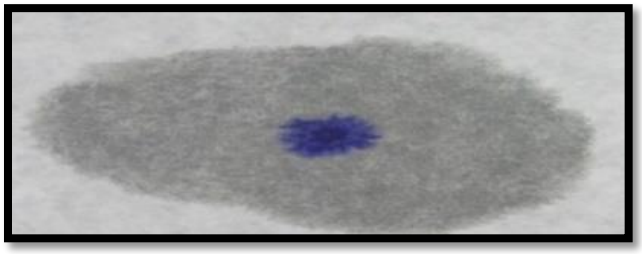

Figure 8: *Oxidase positive Bacillus strain showing color change*

Figure 9: *Catalase positive Streptococcus strain showing bubble formation*

Figure 10: *Catalase negative Enterococcus strain showing few scattered bubble*

Majority of the water samples comprise of Enterococcus, Streptococcus and Staphylococcus strains. Significant clinical infections caused by Enterococcus include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis (Fisher and Philips, 2009). Sensitive strains of these bacteria can be treated with ampicillin, penicillin and vancomycin (Pelletier, 1996), but this genus is known for high level of intrinsic antibiotic resistance.

Streptococcus present in water is mainly associated with the pneumonia, which is inflamematory condition of lungs (McLuckie, 2009).

Staphylococcus is responsible for Staphylococcus enteritis. It is the inflammation and swelling of the small intestine, usually caused by the bacterium toxin that settles in the small intes-tine. This infection in turn can cause abdominal pain, cramping, fever, diarrhea, and dehydration (Vorvick et al, 2010).

Conclusion

Present study conducted on water samples of different areas from Gujrat demonstrated that pH of water samples having foul odor was less as compared to other water samples. This study also revealed the presence of pathogenic bacterial strains including Staphylococcus, Streptomyces, Bacillus, Escherichia, Citrobacter, Enterococcus and Streptococcus drinking water samples. Among these strains, Enterococcus and Streptococcus were present in all water samples. 6.25% and 43.75% of samples contain Streptomyces and Bacillus respect-tively. Citrobacter and Escherichia were present in 37.5% samples and 62.5% water samples contain Staphylococcus strains. Biofilm forming characteristic is shown by all isolated bacterial strains. Citrobacter have shown smooth and white morphotype, and Streptococcus, Bacillus, Enterococcus and Escherichia appeared in red, dry and rough morphotype, when grown on Congo red supplemented nutrient agar plates. All isolated bacterial strains were Gram positive, which appeared in purple color except Escherichia and Citrobacter, which gave pink color on staining. Enterococcus and Bacillus strains were oxidase positive, which contains cytochrome oxidase while remaining strains have shown negativity towards oxidase test. All strains were positive for the presence of catalase enzyme in their cellular structure except Enterococcus.

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