QUALITATIVE AND QUANTITATIVE ANALYSIS OF MICROBIOLOGY AND ANTI-MICROBIAL RESISTANCE OF GHARO WATER SAMPLES (KARACHI I– PAKISTAN); A CASE STUDY.

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ABSTRACT: *Gharo is a city in Thatta District, Sindh. The people of the district are deprived of basic civic facilities like drainage features, portable water, and sanitation facilities that can be a major cause of contamination in the nearby rivers, lakes and ponds that are the only source of water available domestic and industrial use in the area. 60 samples of drinking water (1 liter) were collected from various sourced in the city and were then transferred to the Biological Lab of Department of Biosciences, SZABIST (Karachi). Each sample was analyzed by Membrane Filtration Techniques on a variety of differential and microbiological media. For the next phase, all the potential pathogens were identified using conventional and rapid (QTS 10) methods and then were run through the Kirby Bauer method for antibiotic susceptibility. Further, Minimum Inhibitory Concentration was found using the microdilution method. Almost each of the tested samples was found to be positive for the presence of potential gram-positive and negative microbes with Escherichia coli being the most abundant found in more than half of the samples. In the second phase, potential microbes were found to be resistant to a number of antibiotics with Doxycycline being the least effective and also showing a result of 99% resistance. The analysis clearly shows that a variety of potential microbes are present in the water that can be a major cause of GI diseases and the presence of E. coli indicated the contamination of water through faecal matter. Plus, a high level of antibiotic resistance among potential pathogens is a matter of great concern for public health.*

Keywords: Contamination, Doxycycline, Escherichia coli, Membrane filtration.

1. INTRODUCTION

Water is one of the most essential requirements for sustenance of life. Since all living organisms need water for survival, it should not be contaminated with different inorganic or organic chemicals as well as microorganisms. The presence of some of the microbes may pose a great threat to other living organisms that consume this water, resulting in many harmful enteric water-borne diseases. Water can be contaminated through many different sources, like disposing harmful chemicals from the factories or disposing of human and cattle excretory materials which could alter the natural microbiota of the water present in the lake, rivers, and wells, etc. Such factors tend to increase the ratio of pathogenic microorganisms exponentially [1].

Although Pakistan is a country that has been blessed with a large number of surface and groundwater sources but due to some reasons like increasing population, the rapidly multiplying industries and negligence of proper sanitary and drainage system have shown adverse and overwhelmed effects on the available sources of clean, consumable drinking water. According to the water status quality of Pakistan 2008, the freshwater availability has fallen from 5600 m^3 to 1000 m^3 making it a great concern for the countries welfare. The major effects of contaminated water could be seen in the small cities around large industrial setups that release their waste in the nearby water bodies [2,3].

Gharo is a city in the Thatta district, Sindh, approximately 70 Km East from Karachi the industrial capital city of Pakistan. It has a population of approximately 20,000 people. The inhabitants of this region are deprived of the availability of the basic civic facilities which like pure and clean drinking water and proper sanitation facilities, which becomes the major cause of the waterborne disease outbreak. The inhabitants of the city mainly depend upon the limited and scarce supply of water from the nearby lakes and rivers that too is harbouring predominant microbes like *Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella typhi, and Aeromonas hydrophila* and gram- positive include *Staphylococcus aureus and Staphylococcus epidermidis* that can easily cause waterborne and gastrointestinal diseases like cholera, diarrhoea and typhoid [4].

Another worst part is that they have become resistant to a number of antimicrobial compounds. Hence decreasing the option to cure those who have already have been infected. These diseases not only make people more susceptible to malnutrition but also adds up in the total number of deaths per annum [1,2,5].

2. METHODOLOGY

Sample Collection

A SCRC team was sent to the main Gharo city where they collected 60 samples of 1000 mL which people were consuming on a daily basis. All the samples were collected in 1000 mL polyethylene sterile sampling bottles and were then carefully transported to the Biological Lab of Department of Biosciences, SZABIST(Karachi). For more precaution, each of the 1000 mL was kept in dark insulated sterilized box that had pre-checked for any microbial growth [1].

Membrane Filtration Technique

100 mL of each of the 60 samples was separated in sterile falcon tubes for analyzing the presence of microbes. The samples were filtered using a 47 mm diameter and 0.45 µm pore size cellulose acetate MFT grid. The rundown samples were then poured in MacConkey agar and Muller Hinton agar and were left to incubate at 37 ºC for 24 hrs. The next day, each of the colonies was isolated and identified using the rapid and convention QTS method [1].

Antibiotic Susceptibility Testing (Kirby Bauer Method)

Once the microbes were identified in all the 60 samples a panel of antibiotics was determined that could be used in order to treat the inhabitants that are being exposed to a variety of microbes for a long period of time. In our analysis, we chose Amoxicillin, Ampicillin, Cephalexin, Ceftrizoxime, Chloramphenicol, Ciprofloxacin, Doxycycline, Erythromycin, Gentamicin, Novobiocin, Ofloxacin, and Tetracycline. All the isolated microbes were inoculated in Muller Hinton broth and then were again poured on the MacConkey agar plate. Discs of each antibiotic were placed on the agar plate. The agar plate was left to incubate 18 hrs. Once incubated the diameter of the clear rings around the discs was measured [1].

Minimum Inhibitory Concentration

The minimum inhibitory concentration of the antibiotics was found by the micro-dilution method in which a wide range of concentrations of the antibiotics was tested. The antibiotics were diluted by the ratio of two-fold and then the inoculum maintained to 0.5 McFarland Turbidity was added in the tubes containing the antibiotics. The tubes were incubated for 20 hours and then the results were analyzed [6,7].

3. RESULTS

Membrane Filtration Technique

All samples showed positive results for Gram-Negative organisms mostly *Escherichia coli* and *Enterobacter aerogenes* and Gram-Positive bacteria like *Staphylococcus Aureus* and *Staphylococcus Epidermidis.* Figure 1 and Figure 2 show the presence of all the major gram-negative and positive bacteria found in the 60 samples respectively

Antibiotic Susceptibility Testing

After conducting the Kirby Bauer method, the microbes which were identified through the QTS method were found to be highly resistant against the 12 selected antibiotics. The greatest level of resistance was seen against Doxycyclin. Table 1 shows the results obtained from the Antibiotic Susceptibility Testing and figure 4 gives the graphical representation of the obtained data.

Minimum Inhibitory Concentration

Table 2 is depicting the minimum concentration of antibiotics required to inhibit the pathogenicity of microorganisms. It was observed that the antibiotics used are acting at two or more types of bacteria at the same time.

4. DISCUSSION

Gharo drinking water is not suitable for consumption by humans and animals as it has been inhabited by numerous antimicrobial organisms. In order to find more about this severe problem, we conducted a quantitative and qualitative analysis of water by taking 60 samples from the different places in Gharo and then applying different methods to study the alteration that has occurred in the properties of drinking water in the wells and lakes of Gharo. The water analysis showed the extent to which the water was contaminated by bacteria; this analysis showed the presence of *E. coli*, *E. aerogenes* and *P. aeruginosa* in great quantities which were alarming results depicting that it is highly unsafe for consumption. The antimicrobial susceptibility test concluded that microbial organisms developed a high resistance against some of the antibiotics like Doxycyclin, Novobiocin, Cephalexin, and Cephtrizoxime. While showing a comparatively lower resistance to the antibiotics like Ampicillin, Amoxicillin, Chloramphenicol, Gentamicin, Ofloxacin, Ciprofloxacin, Erythromycin, and Tetracycline. Resistance among bacterial isolates to certain antimicrobial agents is increasing in a number of incidences worldwide. Conducting all these tests depicts that there is a great necessity for water treatment in Gharo. Some treatment methods may include chlorination, heat treatment and other methods that would purify and eradicate all the harmful pathogens. Educating the public regarding the proper disposal of waste and excretory materials will decrease the contamination of water not only in Gharo but also in other places where the water is unfit for usage.

5. CONCLUSION

By analyzing different water samples from Gharo. It was concluded that a high amount of *E. coli*, as well as other pathogens, were found in the samples derived from the different regions of Gharo. By conducting antibiotic susceptibility tests, results showed that a high level of resistance of bacteria against most of the antibiotics used. This is a very critical situation as most of the common antibiotics prove to be futile against these pathogens. Hence, considered a matter of great concern for the Public Health Sector.

Antibiotics	No. of isolates resistant of drinking water	Percentage
Ampicillin	66	9%
Amoxicillin	41	6.1%
Cephalexin	80	12%
Chloramphenicol	40	6%
Ceftrizoxime	95	14.2%
Ciprofloxacin	20	3%
Doxycyclin	99	15%
Gentamicin	30	4.4%
Erythromycin	48	7.1%
Tetracycline	48	7.1%
Ofloxacin	30	4.4%
Novobiocin	70	10.4%

Table 1: Resistance pattern of bacteria against 12 antibiotics in percentages

	Enterobacteriaceae	Pseudomonas spp.	Satphylococci
Amoxicillin	0.25-128 mg/L	No effect	0.03-128 mg/L
Ampicillin	0.25-128 mg/L	No effect	0.03-128 mg/L
Ceftrizoxime	0.004-128 mg/L	No effect	1-128 mg/L
Cephalexin	0.25-128 mg/L	No effect	0.5-128 mg/L
Chloramphenicol	0.25-128 mg/L	No effect	2-16 mg/L
Ciprofloxacin	0.004-128 mg/L	0.015-128	0.06-128 mg/L
Doxycyclin	No effect	No effect	0.06-128 mg/L
Erythromycin	No effect	No effect	0.06-128 mg/L
Gentamicin	0.03-128 mg/L	0.06-128 mg/L	0.008-128 mg/L
Ofloxacin	0.06-128 mg/L	0.25-8 mg/L	0.12-128 mg/L

Table 2: MIC values of some commonly used antibiotics against the three main groups of bacteria.

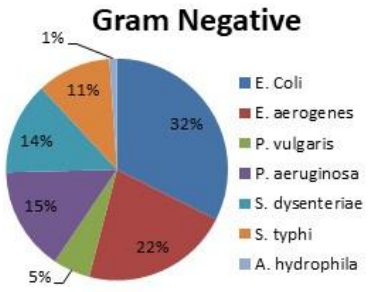

Figure 1: Gram Negative Bacteria by percentage

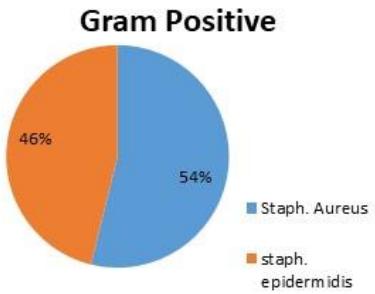

Figure 2: Gram Positive Bacteria by Percentage

Figure 3: Kirby Bauer Testing

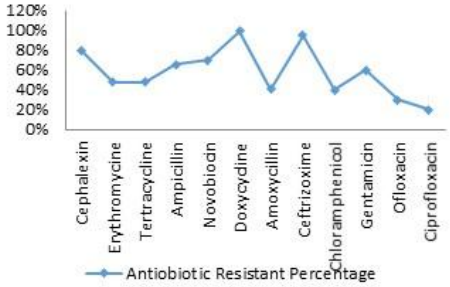

Figure 4: Graphical Representation of Kirby Bauer Results

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