Bacterial Analysis and Antimicrobial Susceptibility of Bacteria Found in Different Water Sources in Karachi

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ABSTRACT

Background: In Pakistan, the bacteriological quality of drinking water is not closely monitored and prevalence of waterborne diseases due to the contamination of drinking water is among the most common problems faced in urban and rural areas of Pakistan. The bacteria inhabiting these water sources carry genes which render them resistant to many antimicrobials. These genes can be transmitted to other non-resistant bacteria as well making the diseases caused by them hard to treat.

Objective: To test the isolated bacteria from collected drinking water samples for their antibiotic susceptibility profile for 14 commonly used antimicrobials.

Methods: The study involves collection of 100 samples of drinking water from four water sources, namely boring water, tap water, filtered water and boiled water, randomly collected from different parts of Karachi using a sterile method. They were tested to determine the bacterial isolates present in them using the analytical profile indexing (API). The antimicrobial susceptibility profile of these bacteria was done using the Kirby Bauer disk diffusion method.

Results: It was estimated that *Klebsiella* is the most commonly found organism in the drinking water samples, followed by *Pseudomonas* and *E.Coli, Staphylococcus Aureus* being the least common. The isolates were found to be most resistant to Ampicillin (99%) and least resistant to Amikacin (1%) while they were found to be most sensitive to Amikacin (96%) and Imipenem (96%).

Conclusion: Better quality and standards of drinking water are necessary for healthy human life. Filtration and boiling of tap and boring water decreases the number of pathogens present in it making it better and healthier for human consumption.

KEY WORDS: *Frequency, Bacteriological Analysis, Antimicrobial Susceptibility, Drinking Water.*

Cite as: Amin S, Abdulla FE, Usman G. Bacterial analysis and antimicrobial susceptibility of bacteria found in different water sources in Karachi. Pak J Med Dent 2014; 3(3):62-67.

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INTRODUCTION

Water of good drinking quality is the most important and basic requirement of human life. Better quality water is necessary for human development, better health and wellbeing.[1] There are certain physical, chemical and microbiological standards which govern the safety and suitability of water for human consumption.[2] It should be free of any color, odor, unpleasant taste or any microbiological organism.

The developing countries face the problem of safe water provision to its population.[3] Pakistan, being a developing country, faces this problem as well and clean water is available only to 40-60% of its entire population.[4] In Pakistan much work has not been done on water microbiology and it continues to be a much neglected topic. A few studies which have been conducted in some urban and rural regions indicate that the drinking water contamination ratio is really high.[5] According to one such study most of the drinking water supplies in Pakistan are fecal contaminated.[6] It is a common knowledge that many infectious diseases (ID) are transmitted to humans via fecal contaminated water.[5] According to World Health Organization (WHO) half of the population of developing countries is sufferer of waterborne IDs round the year and 3.4 million people die annually due to consumption of focally contaminated water, among these children and infants are a majority.[7]

The developed countries have policies of monitoring the safety level of drinking water due to its importance but several outbursts of waterborne diseases still occur throughout the year in developing countries.[5] Several diseases like cholera and typhoid, are linked to unhygienic drinking water consumption.[8] In Pakistan approximately 30% of all the patients coming to hospitals and 40% of all the deaths occurring have contaminated drinking water as their cause.[6] Also, repeated bouts of diarrhea occurring due to fecal contamination of drinking water leads to malnutrition which renders a person more susceptible to disease causing pathogens.[9] These findings have made it clear that drinking water from any original source contains many pathogens which if consumed can lead to devastating effects on human life hence it is unfit to consume such untreated water.[2] It is a common knowledge that in-house treatment of drinking water via filtration, boiling or chlorination improves the quality of water and makes it safer for consumption.[10]

Therefore, 100 drinking water samples from four different water sources namely, boring water, tap water, filtered water and boiling water, from different areas of Karachi were collected in order to determine the prevalence of microorganisms in these water sources.

Today, almost all bacteria exhibit some resistance to almost all the known antimicrobials. The bacteria which show resistance against greater than two antimicrobials are termed as multiple antibiotic resistant (MAR) bacteria. This multiple drug resistance is a result of multiple mutations occurring in the bacterial genome. These mutations are attributed mainly to excessive use, improper use and noncompliance of the patients to the drugs[11], hence it is seen more commonly in regions where excessive use of antibiotics is common. But new studies reveal that these antibiotic resistant bacteria are also inhabitants of surface and ground water alike.[12] The genes which govern this resistance can be transferred to other non-resistant microorganisms through R-factor plasmid vectors in lateral transmission or to the next generation of the resistant organisms via vertical transmission.[13,14,15]

Transmission of these R-factor plasmids from the resistant organisms to the non-resistant normal flora of the body can lead to devastating effects if ever these R-factor positive flora cause disease in an immune-compromised person. Therefore, the isolated bacteria from these drinking water samples were tested for their antibiotic susceptibility profile for 14 commonly used antimicrobials.

METHODOLOGY

Hundred water samples were collected from different areas of Karachi using sterile cap glass bottles (200 ml), sterile pipettes (1, 10, 100 ml), gram stain, sodium thiosulphate and durham tubes. Samples were collected from four sources which were boring water, tap water, boiled water and filtered water. The water samples were collected according to the standard procedure.The media used included

chocolate agar, CLED, EMB, SS, SIMS, TSI and McConkey broth (Oxford, UK). The samples were collected from 1st May, 2012 to 31st October, 2012.

Samples were collected in 200 ml sterile collection bottles having the caps replaced promptly. The color, odor and transparency were of the samples was noted. We added 0.1 ml of fresh 1.8% (w/v) aqueous solution of sodium thiosulphate to each bottle of 100 ml to neutralize the bactericidal effect of any chlorine or chloramines in the water. Calibrated lapful's of water were streaked separately on half plates of CA, CLED, EMB and SS and incubated at 37 C for up-to 48 hours.

The bacteria were identified using the API. API is a bacterial classification which is based on experiments. The clinically significant bacteria can be identified rapidly and easily using API.

The antimicrobial susceptibility profile for the isolates against fifteen antimicrobials was determined using the standard Kirby Bauer disk diffusion method (Bauer *et al*., 1966). The drugs with antibacterial effects were chosen due to their frequent use against these microorganisms in our setting. They included Amikacin, Gentamicin, Ampicillin, Augmentin, Ciprofloxacin, Cephradine, Cefuroxime, Cefixime, Ceftriaxone, Imipenem, Meropenem, Doxycycline, Chloramphenicol, Cotrimoxazole and Erythromycin. Commercially prepared antimicrobial discs were added to the agar plates and after incubation the clear zones of bacterial growth inhibition were measured.

RESULTS

In this study the bacteriological quality of 100 drinking water samples, randomly obtained from four different sources from Karachi, was determined. Out of these, 60 were obtained from boring water, 23 from tap water, 15 from filtered water and 2 from boiled water. The samples were found to be positive for *Klebsiella, Pseudomonas aerugenosa, Escherechia coli and Staphycococcus aureus*. Table # 1 shows the number of water samples positive for either of the four bacterial isolates.

Figure: 1, summarizes the percentages of bacterial isolates in the (A) boring water samples, (B) tap water samples, (C) filtered water samples and (D) boiled water samples. Boiled water was found to be least contaminated by bacteria hence it is most suitable for drinking which is in opposition to the boring water which harbors the heaviest load of bacteria and hence is most unfit for drinking.

When tested for resistance the bacterial isolates were found to be most resistant to ampicillin (99%) while the resistance to Imipenem was found to be none. The antimicrobial susceptibility profile of the bacterial isolates for all the tested antimicrobials is summarized in Table 2.

Table1: Number of water samples inhabiting bacteria

Isolates Samples	Boring Water	Tap Water	Filtered Water	Boiled Water	Total
Klebsiella	29	18	7	2	56
P. Aerugenosa	28	3	7	0	38
S. Aureus	2	0	0	0	2
E. Coli	1	2	1	0	4

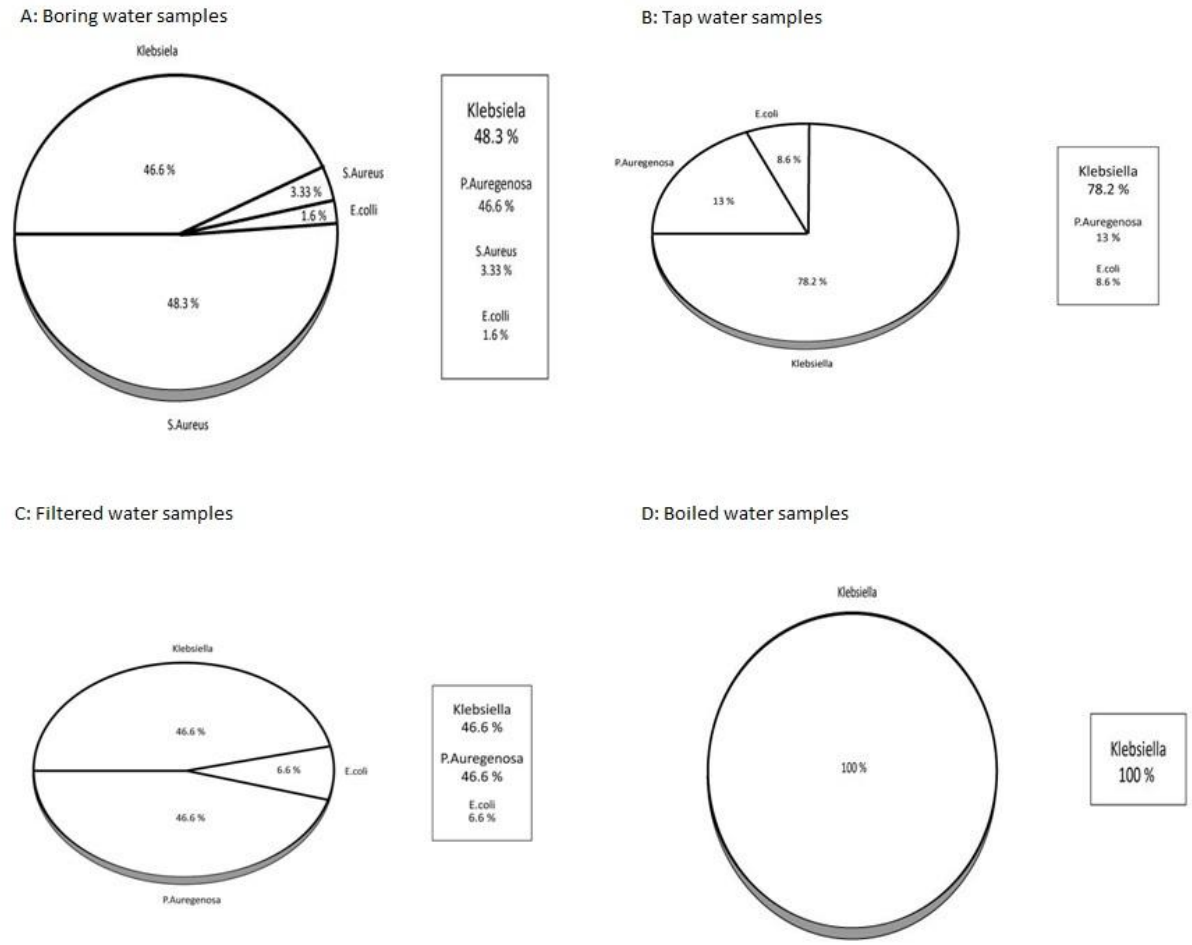
Table 2: Susceptibility of organisms to antibiotics

Drugs	Total Percentage		Percentage of Klebsiella		Percentage of Pseudomonas		Percentage of E. Coli		Percentage of Staph. Aureus	
	R	S	R	S	R	S	R	S	R	S
Amikacin	5	95	3	97	0	100	0	100	4	96
Gentamicin	20	80	21	79	0	100	50	50	20	80
Ampicillin	98	2	100	0	100	0	100	0	99	1
Augmentin	57	43	100	0	75	25	50	50	74	26
Ciprofloxacin	4	96	16	84	0	100	50	50	9	91
Cephradine	57	43	95	5	50	50	50	50	71	29

Cefuroxime	75	25	100	0	50	50	100	0	90	10
Cefixime	55	45	100	0	75	25	50	50	73	27
Imipenem	2	98	5	95	0	100	50	50	4	96
Doxycyclin	25	75	97	3	50	50	0	100	53	47
Chlorampehnicol	39	61	100	0	50	50	50	50	63	37
Cotrimoxazole	32	68	97	3	0	100	100	0	57	43
Erythromycin	57	43	100	0	100	0	100	0	75	25

R= Resistance, S= Sensitivity

Figure 1: Summarized the percentages of bacterial isolates in the drinking water



DISCUSSION

Having the access to safe and healthy drinking water is among the basic needs of a society.[3] Unhygienic drinking water is the foremost cause of waterborne diseases which are especially prevalent among the two extremes of age, the immune-compromised and the low socio economic people.[10,16] The pollution and contamination of water is a major concern worldwide since it is due to drinking water contamination that two children lose their lives every minute across the globe.[17] and approximately five billion children annually, making it an alarming problem especially for the developing countries.[18]

Pakistan has water supplies enough only to fulfill drinking needs of 79% of its total population and a major part of that water supply is from ground (boring) water which they consume for drinking.[6] Of that 79%, 59% of water is unfit for human consumption which is an alarming ratio.[4] According to the government of Pakistan estimated ratio of contamination of drinking water by bacteria is one of the most grievous problems the country is facing. It is also estimated that 14% of diarrheal diseases among the children of 5 years or under of age and 40% of all the diseases occurring in the country are due to drinking water contamination.[19]

It is of much importance that the safety and bacterial profile of drinking water should be monitored from time to time. Fecal contamination of drinking water can be very detritus for human health and the isolation coliforms is taken as an indicator of fecal contamination of water.[20]

According to a study, 82% of all the drinking water samples were positive for *E.Coli* which is a marker of fecal contamination and were classified as being unfit for human consumption[11]. *E.Coli* is taken as the indicator of fecal contamination because it is found in the feces of human and almost all other mammals.[21] *Pseudomonas* can multiply in a wide variety of aquatic habitats. It is not always found in feces hence is not a good indicator of fecal pollution. According to a study in Punjab, among the 15 most common ID's diarrhea is marked as the second highest in its prevalence among children under 5 years of age. Clearly indicating water contamination in that region of Pakistan as well.[6]

Boiling or filtration of tap and boring water before drinking decreases the number of pathogens in them. The filtration chamber of a filter traps the bacteria within itself while boiling kills the pathogens in water. *E.Coli* is most stable to heat and is not easily killed at lesser temperatures than 65^0C. *Pseudomonas* is lesser stable to heat and is killed easily while *Staphylococcus Aureus* and *Klebsiella* are extremely heat labile. The optimum temperature for killing all the microbes in water is 55 to 65^0C and makes the water healthier for human consumption.[22] Therefore, consumption of boiled water for drinking leads to lesser incidences of water borne diseases as compared to the consumption of either boring water, tap water or filtered water.

Pseudomonas is unique in that it has natural resistance to most antibiotics available even though it carries lesser R-plasmids than *Klebsiella*. Even the drug susceptible strains of *Pseudomonas* have certain defenses in them rendering them resistant.[23] Antibiotic resistance spreading among the microorganism is a huge problem worldwide. The R-plasmids, responsible for this resistance, are believed to be originated in some pathogens and passed on to other pathogens, including the normal flora of body, via either vertical or horizontal gene transmissions. This prolongs the survival of pathogens in body, increasing the duration of disease as well making these diseases refractory to regular antimicrobials.[24]

CONCLUSION

It is a fundamental requirement of humans to have access to safe and healthy drinking water. Consumption of contaminated water has many adverse consequences on human health. These include life threatening diarrhea especially among the children. This risk can be decreased by efficient boiling of tap and boring water before consumption. Boiling of drinking water decreases the amount of bacteria present in it and makes the water healthier for human consumption.

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