

## Prevalence of Bacteria in Drinking Water in Karachi and their Antimicrobial Susceptibility

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### ABSTRACT

**Introduction:** 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.

**Methodology and Results:** We collected 100 drinking water collected from four water sources, namely boring water, tap water, filtered water and boiled water, randomly collected from different parts of Karachi by 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. 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:** Bacteria, drinking water, antibiotic resistance, antibiotic susceptibility.

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### INTRODUCTION

Water of good drinking quality is the most important and basic requirement of human life. Its better quality is necessary for human development, good health and well being.<sup>1</sup> There are certain physical, chemical and microbiological standards which govern the safety and suitability of water for human consumption.<sup>2</sup> 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.<sup>3</sup> Pakistan, being a developing country, faces this problem as well and clean water is available only to 40-60% of its entire population.<sup>4</sup> 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.<sup>5</sup> According to one such study most of the drinking water supplies in Pakistan are fecally contaminated.<sup>6</sup> It is common knowledge that many infectious diseases (ID) are transmitted to humans via fecally contaminated water.<sup>5</sup> 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 fecally contaminated water, among these children and infants are a majority.<sup>7</sup> 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.<sup>5</sup> Several diseases, e.g cholera and typhoid, are linked to unhygienic drinking water consumption.<sup>8</sup> 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.<sup>6</sup> Also,

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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.<sup>9</sup> 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.<sup>2</sup> It is a common knowledge fact that in-house treatment of drinking water via filtration, boiling or chlorination improves the quality of water and makes it safer for consumption.<sup>10</sup>

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. 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 these drugs.<sup>11</sup> 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.<sup>12</sup> 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.<sup>13-15</sup>

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.

## MATERIALS & METHODS

### Study sites:

Hundred water samples were collected from different areas of Karachi.

### Sample collection:

#### Material used:

Sterile cap glass bottles (200 ml) , Sterile pipettes (1, 10, 100 ml) , Gram stain, Sodium thiosulphate and Durham tubes.

### Source of water:

Samples were collected from four sources which were boring water, tap water, boiled water and filtered water.

### Media used:

Chocolate agar, Cysteine Lactose Electrolyte Deficient agar (CLED), Eosin-methylene Blue (EMB), Salmonella Shigella agar (SS), Sulfide Indole Motility (SIM), Triple Sugar Iron agar (TSI) and McConkey broth (Oxoid, UK).

### Time Period:

The samples were collected from 1<sup>st</sup> May, 2012 to 31<sup>st</sup> October, 2012.

### Collection and culture procedure:

Samples were collected in 200 ml sterile collection bottles having the caps replaced promptly. The color, odor and transparency of the samples were 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. One loop of water was streaked separately on half plates of CA, CLED, EMB and SS and incubated at 37 C for upto 48 hours.

### Identification of bacteria and Antimicrobial susceptibility testing:

The bacteria were identified using the Analytical Profile Indexing (API).

The antimicrobial susceptibility profile for the isolates against fifteen antimicrobials was determined using the standard Kirby Bauer disk diffusion method. The drugs whose antibacterial effects were chosen due to their frequent use against these microorganisms in our setting. They included, coamoxiclav 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.

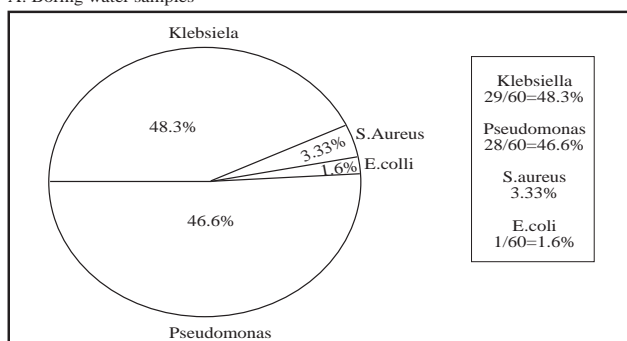
## RESULTS

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 aeruginosa, Escherichia coli and Staphylococcus aureus. Table 1 shows the number of water samples positive for either of the four bacterial isolates.

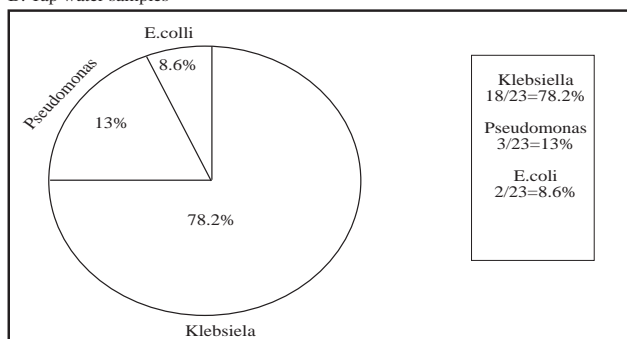
Table1: Number of water samples inhabiting bacteria

Isolates	Boring Water	Tap Water	Filtered Water	Boiled Water	Total Samples
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

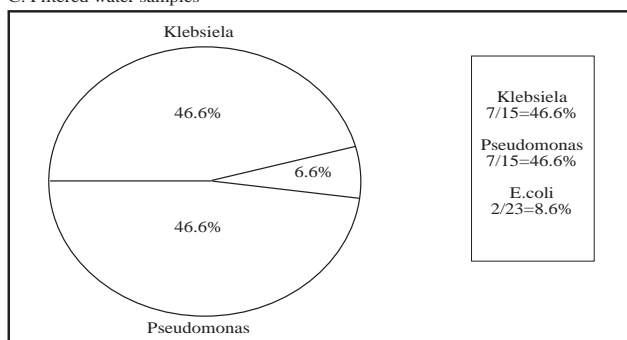
A: Boring water samples



B: Tap water samples



C: Filtered water samples



D: Bioled water samples

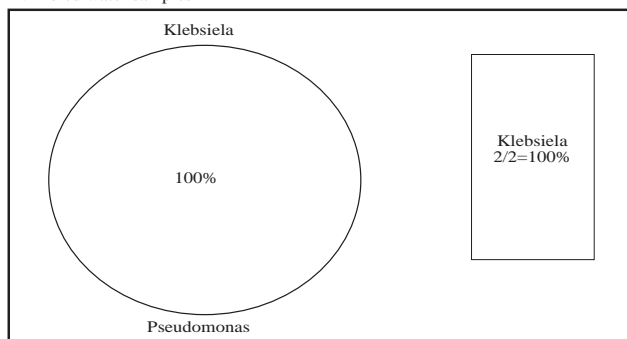


Figure 1 summarizes the percentages of bacterial isolates in the ( A ) boring water samples, ( B ) tap water samples, ( C ) filtered water samples and ( D ) boiled water samples. Boiled water was found to be least contaminated by bacteria hence it is most suitable for drinking which is in opposition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

Table 2: Susceptibility of organisms to antibiotics

Drugs	Percentage of Klebsiella		Percentage of Pseudomonas		Percentage of E. Coli		Percentage of Staph Aureus		Total Percentage	
	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
Cephadrine	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
Chloramphenicol	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

## DISCUSSION

Having the access to safe and healthy drinking water is among the basic needs of a society.<sup>3</sup> Unhygienic drinking water is the foremost cause of waterborne diseases which are specially prevalent among the two extremes of age, the immune-compromised and the low socio-economic people.<sup>10,16</sup> 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<sup>17</sup> and approximately five billion children annually, making it an alarming problem especially for the developing countries.<sup>18</sup>

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.<sup>6</sup> Of that 79%, 59% of water is unfit for human consumption which

is an alarming ratio.<sup>4</sup> 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 of age or under and 40% of all the diseases occurring in the country are due to drinking water contamination.<sup>19</sup> 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 detrimental for human health and the isolation coliforms is taken as an indicator of fecal contamination of water.<sup>20</sup>

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.<sup>11</sup> E.Coli is taken as the indicator of fecal contamination because it is found in the feces of human and almost all other mammals.<sup>21</sup> 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.<sup>6</sup> Boiling or filtration of tap and boring water before drinking decreases the number of pathogens in them. The filtration chamber of a filter traps 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<sup>0</sup>C. 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<sup>0</sup>C and makes the water healthier for human consumption.<sup>22</sup> 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 defences in them rendering them resistant.<sup>23</sup> 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 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.<sup>24</sup>

## 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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