



## **Survey of Gram Negative and Gram Positive Bacteria in Drinking Water Supplies in Karachi, Pakistan**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author NAK conceived the study. Authors FAY and RS designed and conducted all experiments under the supervision of author NAK. Authors FAY, RS, and NAK contributed to the writing of the manuscript. All authors read and approved the final manuscript.*

**Short Communication**

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### **ABSTRACT**

More than 20,000 children die annually in the city of Karachi alone, majority of whose death are thought to be associated with waterborne pathogens. Drinking water and recreational exposure to polluted water pose a significant public health threat including gastroenteritis, paralysis, meningitis, hepatitis, respiratory illness and diarrhoea. The aim of this study was to determine the presence of bacterial contamination in drinking water supplies in Karachi, Pakistan. A total of fifty two domestic tap water samples were collected from different areas of Karachi, between May to June 2011 and analyzed for bacterial presence based on biochemical testing. The results revealed a high prevalence of *Bacillus* spp. (86.84%), followed by *Pseudomonas* spp. (57.14%), *Citrobacter* spp. (14.28%) *Serratia* spp., *Enterobacteriaceae* species (14.28%), *Cornebacterium* (10.52%), and *Acinetobacter* spp. (2.63%). These findings disclose bacterial contamination in drinking water supplies, many of which are pathogenic and can produce serious as well as life-threatening infections. Future studies will determine whether bacterial contamination of drinking water occurred post-source contamination. It is recommended that household water treatment interventions should be introduced to improve water quality.

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## 1. INTRODUCTION

The drinking water contaminated with pathogenic microbes is associated with adverse health consequences, especially in urban communities [1,2,3]. Infectious diseases account for 68% of global child mortality, with vast majority occurring in the developing countries. Several lines of evidence suggest diarrhoea as the leading cause of morbidity and mortality in children in Africa and South Asia [4,5,6,7,8,9,10,11,12].

Like many other developing countries, Pakistan faces serious water scarcity and contamination problems [13]. Given that water and sewage pipelines are situated next to each other, sewage waste and toxic chemicals may enter the water distribution systems through leakage pipes, valves, joints and contamination of tap by the end users [14,15,16,17]. The drinking water supplies to major cities like Karachi, Lahore, Rawalpindi, Peshawar, Faisalabad, Qasur, Sialkot and Gujrat is not fit for human consumption as it is contaminated with infectious agents and/or toxic chemicals [18,19,20,21]. Ninety-four percent of untreated waste water in Karachi is discharged into Liyari and Malir rivers destroying the aquatic life. According to a survey conducted by Pakistan Council of Research in Water Resources (PCRWR) around 84 – 89% of water supplies from around the country have water quality below the recommended standards for human consumption [22]. In this study, the occurrence of bacteria in drinking tap water supplies in different localities of Karachi, Pakistan was investigated.

## 2. METHODS

One litre water samples were collected from domestic tap water supplies in sterile bottles. A total of 52 samples were collected from different areas of Karachi from May to June 2011. Among 52 water samples tested, 43 were sourced from households where water is normally stored in large tanks, 6 were sourced from households where water is normally stored in wells, and one household were receiving mixed water, i.e., taken from well and then stored in water tank. Each sample was collected into a polypropylene bottle, and stored at 4°C until subsequent analysis within one week.

One liter of each water sample was filtered through a sterilized 0.45µm pore size cellulose filter under vacuum. The filters were inverted on 1.5% nutrient agar plates. The plates were incubated at 37°C for up to 72 h. Plates were examined every 24 h, visually, for the presence of bacterial colonies. The genetic level of the bacterial strains was identified following Bergey's manual of determinative bacteriology [23].

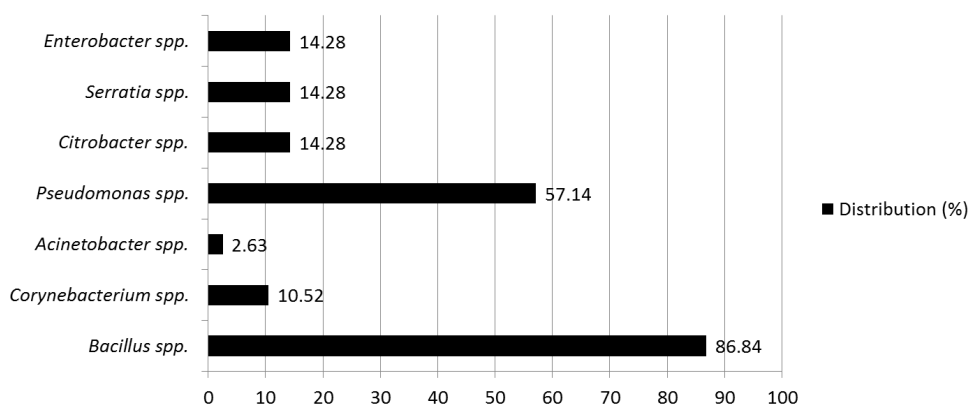
## 3. RESULTS AND DISCUSSION

Among 52 water samples tested, 50 samples (96.15%) showed the presence of bacteria. Gram staining revealed the presence of both Gram positive and Gram negative bacteria. Thirty eight samples (76%) contained only Gram positive bacteria, 7 samples (14%) contained only Gram negative bacteria, while 5 samples (10%) contained mixed populations of both Gram positive and Gram negative bacteria (Table 1). Based on Bergey's manual of determinative bacteriology, 50 bacterial isolates could be identified as seven bacterial species. These included Gram positive *Bacillus* spp. (86.84%), *Cornebacterium* spp. (10.52%), and *Acinetobacter* spp. (2.63%), Gram negative *Pseudomonas* spp. (57.14%),

*Enterobacter* spp. (14.28%) *Serratia* spp. (14.28%) and *Citrobacter* spp. (14.28%) (Fig. 1), which are potentially pathogenic and can cause serious as well as life-threatening infections in humans and animals. Notably, species of the genera, *Pseudomonas* and *Acinetobacter* have been heralded as the 'super-bugs' of the future demonstrating considerable intrinsic resistance to many antibiotics.

**Table 1. Distribution of bacteria species in domestic tap water supplies in Karachi, Pakistan**

Bacteria	Distribution (%)
Gram negative	76
Gram positive	14
Mixed	10



**Fig. 1. The distribution of bacterial species (Gram positive and Gram negative) in drinking tap water supplies in the city of Karachi, Pakistan**

Pakistan has a population of 180.8 million in 2009 and is projected to reach 208 million by 2020. Karachi has a metro area population of 18 million people and it is estimated that more than 30,000 people, 20,000 of who are children die every year because of unsafe water. The sewage management in Karachi is in frantic state. The British Empire built the sewage system around 100 years ago, during the colonial days. This system has not been restored by any government of Pakistan and is in a pitiable condition. Scores of hospitalizations are accredited to breakage of sewage line and seepage of sewage into drinking water lines [24,25].

In the present study, we report the occurrence of potentially pathogenic bacterial species in drinking tap water supplies in Karachi. More than 96% of water samples tested were contaminated with bacterial species including *Bacillus* spp., *Corneybacterium* spp., *Acinetobacter* spp., *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., and *Citrobacter* spp.

These findings are consistent with other studies from developing countries. For example, [26] identified 10 bacterial species: *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella* spp., *Proteus vulgaris*, *Alcaligenes faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus lactis* and *Micrococcus luteum* in water samples in

Rajasthan, India. Several species of *Pseudomonas* have been considered as opportunistic pathogens that can colonize animal and human tissues resulting in serious infections [27,28]. The results of our study show 57.14% distribution of *Pseudomonas* spp., in drinking water supplies. Similar studies in Bangladesh revealed up to 91% distribution of *Pseudomonas* spp., from household-based rainwater harvesting systems in Bangladesh [29,30], while water supplies in Canada have also shown the presence of *Pseudomonas* spp., and *Enterobacter* spp [31]. Water treatment plant in Zurich revealed distribution of *Pseudomonas aeruginosa* in 30% of samples tested [32], and in Macedonia, non-coli forming bacteria belonging to the genus *Bacillus* were found [33,34]. Potable water samples from various rural and urban sources located in the Lublin region of Eastern Poland were shown to possess 10 bacterial species of Gram negative bacteria including *Legionella* spp., *Pseudomonas* spp., *Aeromonas* spp., *Acinetobacter* spp., *Chryseomonas* spp., *Flavimonas* spp., *Flavobacterium breve*, *Pseudomonas aeruginosa*, *Stenotrophomonas* spp., *Vibrio* spp., and *Xanthomonas* spp. [33,34]. Accordingly, the quality of drinking water in Karachi is poor and indicates a potential source of human exposure to pathogenic bacterial species that can lead to the development of severe diseases caused by these organisms. In addition to the contamination by broken sewage pipelines that affects water quality, the environmental, climatic and locality factors play a major role in the rate of bacterial contamination. Additional problems arise as the majority of the residents in Karachi have water storage tanks, as the water supplies are intermittent. The water storage tanks are not properly maintained, i.e., not cleaned properly, not tightly covered, and could easily be exposed to environmental pathogens. Such water storage tanks provide additional sanctuaries to the microbial pathogens. The general public should be made aware of the health risks that are associated with the use of storage tanks. The storage tanks should be well maintained and cleaned properly by routine scrubbing of the internal surfaces of the tank, disinfection by chlorination (using high-strength calcium hypochlorite [HSCl]), disinfection of the hoses and pump, disposing of the disinfecting water as it will contain a high concentration of chlorine and fill the tank with drinking water.

#### 4. CONCLUSIONS

The occurrence of bacterial pathogens should be considered a potential health risk associated with human activities in domestic tap water supplies in Karachi, Pakistan.

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#### CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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