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Prevalence of Pathogenic Microorganisms in Drinking Water of Rawalpindi and Islamabad

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Abstract: Most common bacterial contaminations associated with drinking water are fecal *Coliforms.* These bacteria were classically used as indicators of fecal contamination of waters. The aim of this study was to find out Associations of total coliforms with the waterborne diseases. Study design for this study was cross sectional and research study was included nine different areas of Rawalpindi and Islamabad. Respondent water samples were analyzed using most probable numbers (MPN/100ml) technique. Sample size was 130 for this study (N=130). Out of 130 water samples 92 samples were taken from different areas of Rawalpindi and other 38 samples were taken from areas of Islamabad. Out of 130 samples 56.1% (n= 74) water samples were unsatisfactory and the other 43.9% (n=56) samples were satisfactory. Out of unsatisfactory (n=74) samples 58.7% (n=54), 52.6% (n=20) were from Rawalpindi and Islamabad respectively. Prevalence of coliforms organisms in unsatisfactory samples (n=74) for total coliforms, fecal coliforms and *E. coli* was 12.3%, 23.8%, 20% respectively. The WASA supply (n=38) was found to be the most contaminated for followed by CDA (n=21), BORING water (n=33) and least contamination was Tanker water (n=38), Ranges from 2.2-240+/100ml of confirmed fecal coliforms count/100ml (MPN/100ml) in Rawalpindi and Islamabad were 48.8% (n=43), 39.5% (n=14) respectively, ranges of from 2.2-240+/100ml confirmed *E. coli* count/100ml (MPN/100ml) in Rawalpindi and Islamabad were 16.3%(n=16), 26.3% (n=10) respectively and Prevalence of waterborne diseases, The conclusion from this data indicate that waterborne diseases are more prevalent and are strongly associated with prevalence of fecal coliforms (P=.001).

Key words: MPN · Coliforms · Waterborne Diseases · WASA · CDA

INTRODUCTION

The most common and widespread risk associated with drinking water is Bacterial contamination that mostly are *Coliform, fecal coliform*, *E. coli.* Fecal Coliform are subset of total coliform, these are again not a single bacteria but a group of specie. These are just like total coliform can ferment lactose with gas production but in 24 hours at 44.5 °C [1]. These bacteria were classically used as indicators of fecal contamination of waters. *E. coli* is a member of fecal coliform group, being more specific indicator for the presence of fecal contamination [2].

Drinking water is a major source of microbial pathogens in developing regions, although poor sanitation and food sources are integral to enteric pathogen exposure. Gastrointestinal disease outcomes are also more severe, due to under-nutrition and lack of intervention strategies in these regions [3].

Illnesses caused by major waterborne pathogens are of greater concern Gastroenteritis is a common disease caused by *Salmonella* spp., *Shigella* spp. and *Campylobacter jejuni. E. coli is also a cause of* Gastroenteritis but also cause of hemolytic uremic syndrome. *Vibrio cholera* is a major cause of Cholera (Dehydration and kidney failure) [4].

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In 2001, infectious diseases accounted for an estimated 26% of deaths world-wide [5]. Fourteen to thirty thousand people, mostly young children and the elderly, die every day from water related diseases globally. Estimated of water-related mortalities were reported per year as more than 5 million people die of waterborne diseases [6].

In Pakistan, there are several potential sources to contaminate drinking water. Bacteriological contamination of drinking water has been reported to be one of the most serious problems throughout the country in rural as well as urban areas such contamination is attributed to leakage of pipes, pollution from sewerage pipes due to problem within the distribution system, intermittent water supply and shallow water due to human activities [7-10].

There is a lack of drinking-water quality monitoring and surveillance programmes in the country [11]. The principal source of drinking water for the majority in Pakistan is groundwater. Domestic waste containing household effluent and human waste is discharged directly to a sewer system is a serious problem and associated with a host of illnesses such as diarrhea, typhoid, intestinal worms and hepatitis Poor water and sanitation is a major public health concern [12].

Outbreaks of emerging disease from contaminated drinking water have become a worldwide threat. There is an average of 10 to 15 outbreaks in the United States (U.S) per year associated with drinking water [13]. The total number of drinking water-related illness in the USA has been estimated at 19 million/year [14]. Various estimates in North America suggest up to 15–30% of gastrointestinal disease may come via water [15].

Approximately 3.1% of annual deaths (1.7 million) and 3.7% of the annual health burden (Disability adjusted life years [DALYs]) world-wide (54.2 million) are attributable to unsafe water, sanitation and hygiene. In developing regions, where there is a higher rate of endemic (background) gastrointestinal disease and pathogen concentrations in wastewater [16, 17].

The overall aim of this study was, to determine the prevalence of pathogenic microorganisms in drinking water in Rawalpindi and Islamabad and to find out Associations prevalence of Microorganisms (Total coliforms, fecal coliforms and *E. coli*) with the waterborne diseases. Examination of water for the control and assessment of pollution is the main objective of our study. In this project basic and important bacteriological water quality parameters were reviewed and determined in different selected areas of Rawalpindi and Islamabad. The prime objective of demographic survey was to evaluate the attitude and practices of respondents of study areas. And secondly prevalence of these fecal coli form in drinking water helps us in developing of disinfection techniques to reduce the coli form level in drinking water.

MATERIALS AND METHODS

Study Design and Duration: The study design employed in this study was cross sectional in nature. The time duration for this study was six months.

Settings: The Laboratory work for this study was done at the Microbiology Section Public Health laboratory Division (PHLD); National Institute of Health (NIH) Islamabad. This laboratory of Microbiology Section receives Water samples for testing total coliform, fecal coliforms and *E. coli.*

Sample Size: The estimated sample size that is 130 (N=130) calculated based on estimated numbers of samples that was analyzed considering the estimated proportion of waterborne disease, precision value 0.05 and confidence interval of 95%.

Sampling Technique: Random sampling technique was employed for this study. Nine areas of Rawalpindi and Islamabad were randomly selected for this research study. Collection of tap water was made from the respondent's home in both, Rawalpindi and Islamabad areas, the level of Total Coliform count, fecal Coliform count and confirmed *E. coli* count in the domestic water supplies, therefore it was important to obtain samples from eachparticipating household at the time of the study. The representative sample of drinking water was collected and analyzed according to WHO guidelines and the Standards laid down by American Public Health Water Works Association (APHA) and Pakistan Standard Quality Control Authority (PSQCA).

Sampling Bottles: Sterile Glass Bottles used for sample collection.

Sample Preservation and Storage: Examination of bacteriological water samples should be performed immediately after collection. So samples was transported to laboratory within 2-3 hours maintaining the temperature at 4°C or less. As the maximum recommended holding time for fecal coliform samples from

wastewater is 6 hours. (WHO Guidelines for drinking water quality.

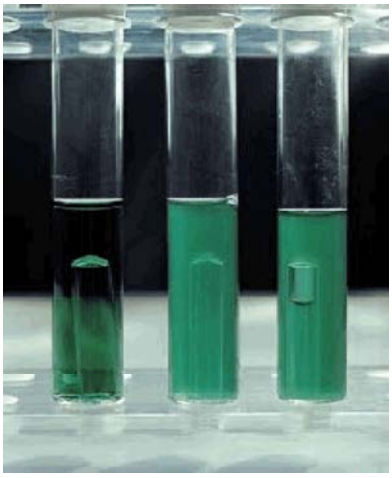
Inclusion Criteria: All the portable and drinking water sample received from selected areas of Rawalpindi and Islamabad were included for the study purpose and then examined and analyzed accordingly.

Exclusion Criteria: Samples of drinking water received other than the study area and the sample received from study area but not used as drinking water like recreational water, lake water and river water were not included for the research study purpose.

Data Collection Methods: The 18th Edition of "Standard Methods for the Examination of Water and Wastewater approved method multiple tube fermentation (MPN) procedure was employed for the confirmed count of coliforms. MacConkey broth was used in both Single strength and double strength for the isolation of coliforms). Brilliant green lactose bile (BGLB) broth was used as confirmatory media for Fecal Coliforms and Tryptone water medium was used for *E. coli* count.

MPN Presumptive test MacConkey Broth: Tube 1: Positive for fecal coliforms. Note (1) the gas present in the Durham tube and (2) the color change from red to yellow as acid end-products react with the pH indicator. Tube 2: Negative for fecal coliforms. Note the absence of gas in the Durham tube. Even though the pH indicator has changed from red to yellow, gas must be produced for a positive result. Tube 3: Negative for fecal coliforms.

Confirmatory Test for Fecal Coliform in Brilliant Green Broth: Tube 1: Negative for fecal coliform as only color change due from green to colorless but no gas production, Tube 2: Negative for fecal coliform as no effect on Broth media, Tube 3: Positive for fecal coliform no color change but gas produced



Date Analysis: Results were analyzed by using MS Excel 2007 and a statistical tool SPSS 18 was also employed for the data analysis. Pie diagram and Bar graphs are generated on the MS Excel 2007 and the cross tabulation and Chi-square test was done to find out associations on SPSS18.

RESULTS

This test clearly indicated that there was strong association between both variables. Having significant association.

DISCUSSION

Water pollution is affecting the lives of many people throughout the world as organic and inorganic pollution as well as fecal matters load in natural water is being increased [18]. In Pakistan, the bacteriological contamination of drinking water has been reported to be one of the most serious problems. It can lead to water borne diseases [19]. In Punjab 90% of the people suffer from water borne diseases e.g. dysentery, typhoid, cholera and diarrhea [20]. In our study we found associations of hygienic status and waterborne diseases with the prevalence of microorganisms (Coliforms).

We also found that the population of Rawalpindi and Islamabad with poor hygienic status was more prone to waterborne diseases as here was a strong association between hygienic status and waterborne diseases (P=0.02) and this work is in lined by Bangladesh Rehabilitation Assistance Corporation [13].

This study also indicated that there was a strong association between prevalence of waterborne diseases and prevalence of microorganisms (Coliforms) (P=0.01) diarrhea was observed to be the major waterborne disease

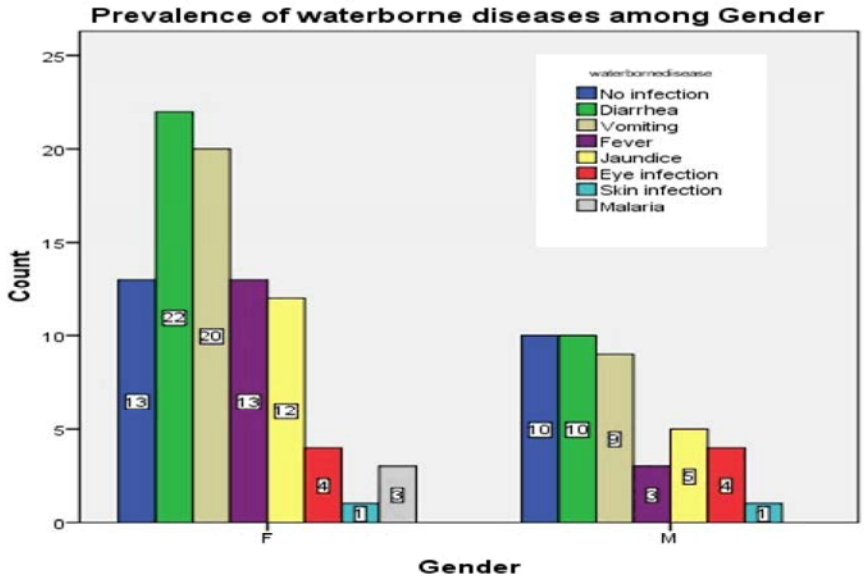


Fig. 1: Showed the Gender frequency distribution of the cases with water-borne diseases. The table indicates that out of total percentage 85.23% (n=75) the water-borne diseases victims were females and else 14.77% (n=13) had no infection, whereas 76.20% (n=32) were males with waterborne diseases and else 23.80% (n=10) had no infection. The reason for this distribution was hypothesized to be due to the fact that women's activities involve water usage in many ways, e.g. cooking washing and bathing and mostly stay inside their homes so they are not immune to any poor hygienic condition so whenever contamination comes they ill while Males mostly eats outside so they are immune to such poor hygienic condition. As a result women found to be prone to these diseases.

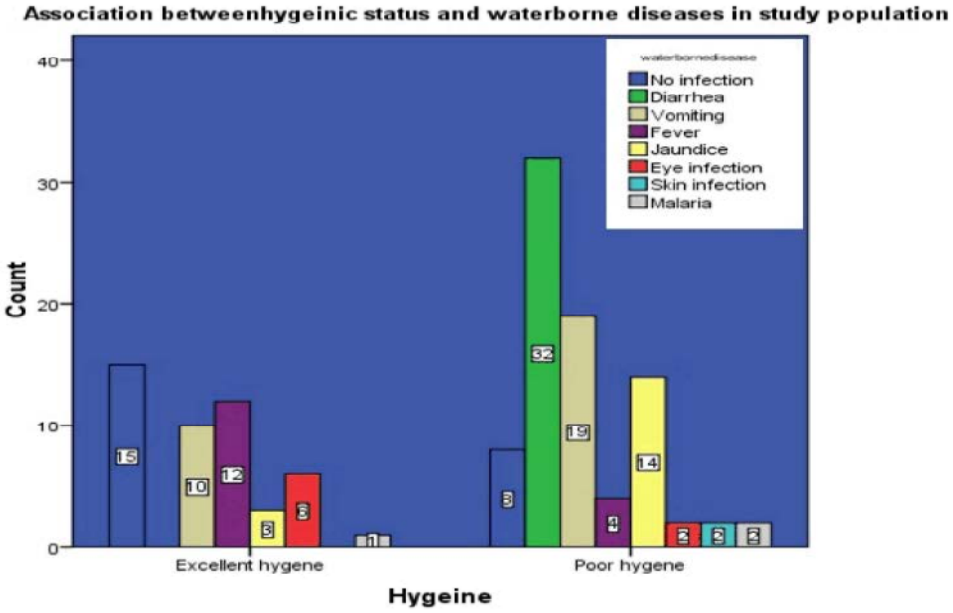


Fig. 2: Clearly indicated that study populations with poor hygienic (treatment with drinking water and types of water container using) condition were more prone to the waterborne diseases as compared to the population having excellent hygienic condition. Diarrhea (n=32) was observed to be the major waterborne disease contributed 100% (n=32) in population with Poor hygiene (n=83), whereas no Diarrhea was observed in population with excellent hygiene (n=47).

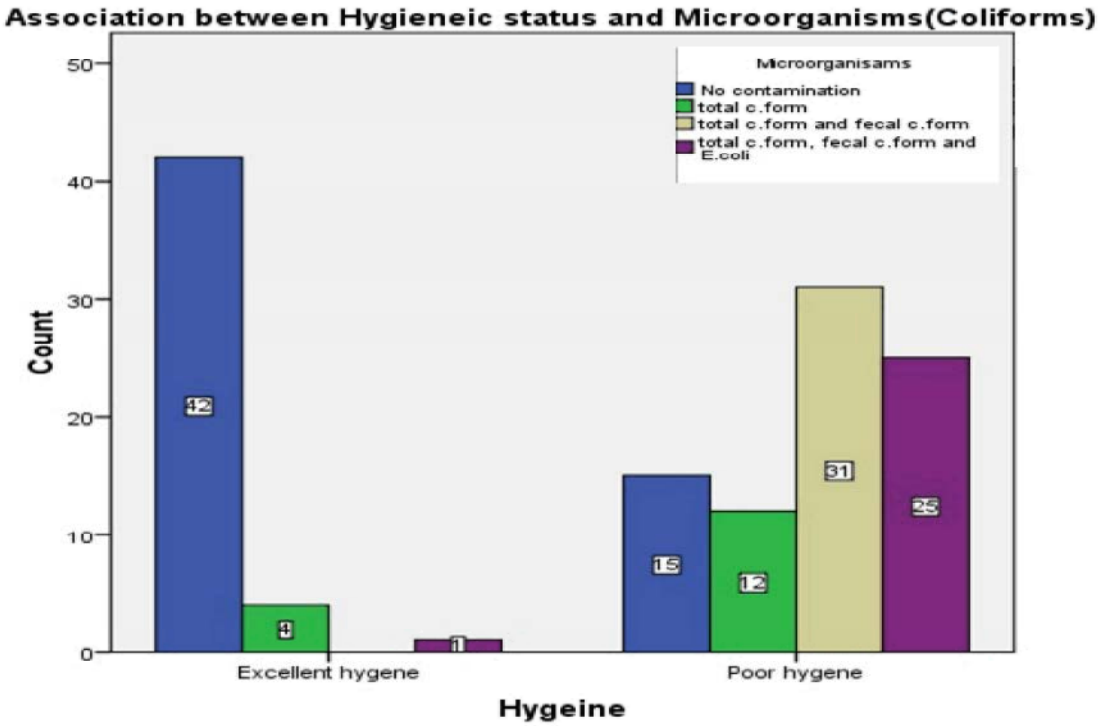


Table 1: Association between hygienic status and Microorganisms (Coliforms)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	64.955[a]	3	.000
Likelihood Ratio	77.943	3	.000
N of Valid Cases	130		

Bacteriological examination of Drinking water (N=130)

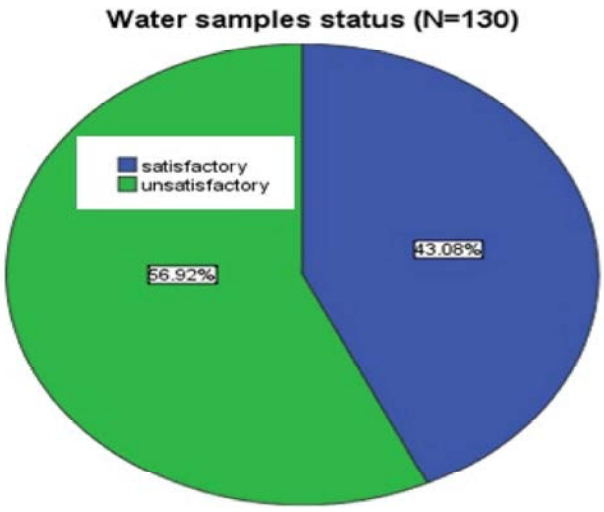


Fig. 3: Illustrates that 56.92% (n=74) of the drinking water samples were contaminated with coliforms and remarked as unsatisfactory for drinking purpose, while rest of the 43.08% (n=56) water samples were not contaminated with fecal matters or any other contaminantsAnd were remarked as satisfactory for drinking purpose.

Bacteriological examination of Drinking water (N=130)

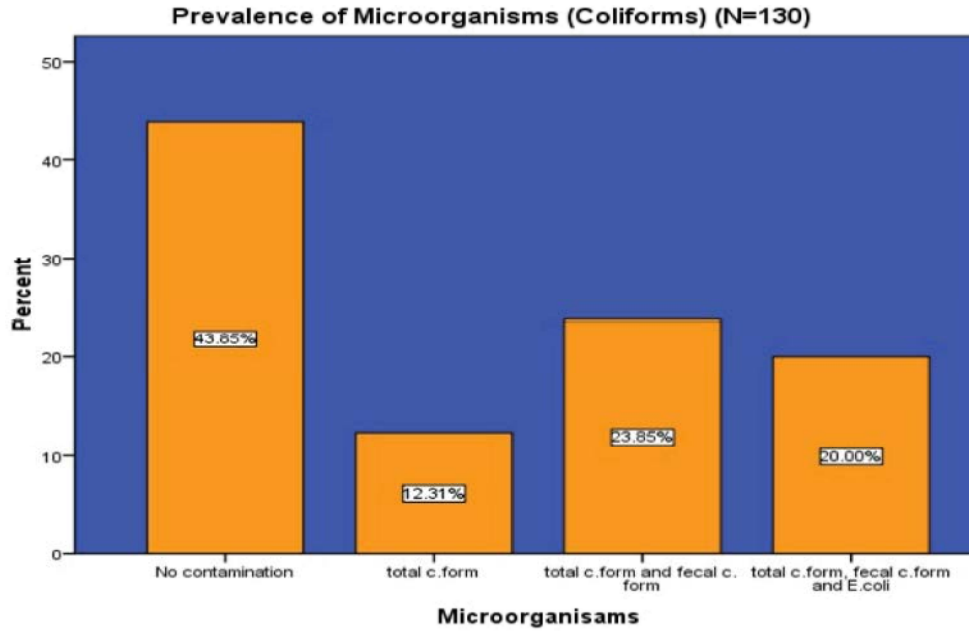


Fig. 4: Illustrates that out of sample size (N=130) 43.9% (n=56) samples were not contaminated else 56.1% (n=74) were contaminated with fecal matters in which different proportions of total coliforms, fecal coliforms and *E. coli* were present, in which contributions of only total coliforms 12.31% (n=16), total coliforms and fecal coliforms 23.8% (n=31), "total coliforms, fecal coliforms and *E. coli* 20% (n=26). Prevalence of overall total coliform 56.92% (n=74), total coliforms and fecal coliforms 43.84% (n=57), total coliforms, fecal coliforms and *E. coli* 23.84% (n=57), Presence of *E. coli* in water was significant as the presence of *E. coli* confers 100% fecal contaminations

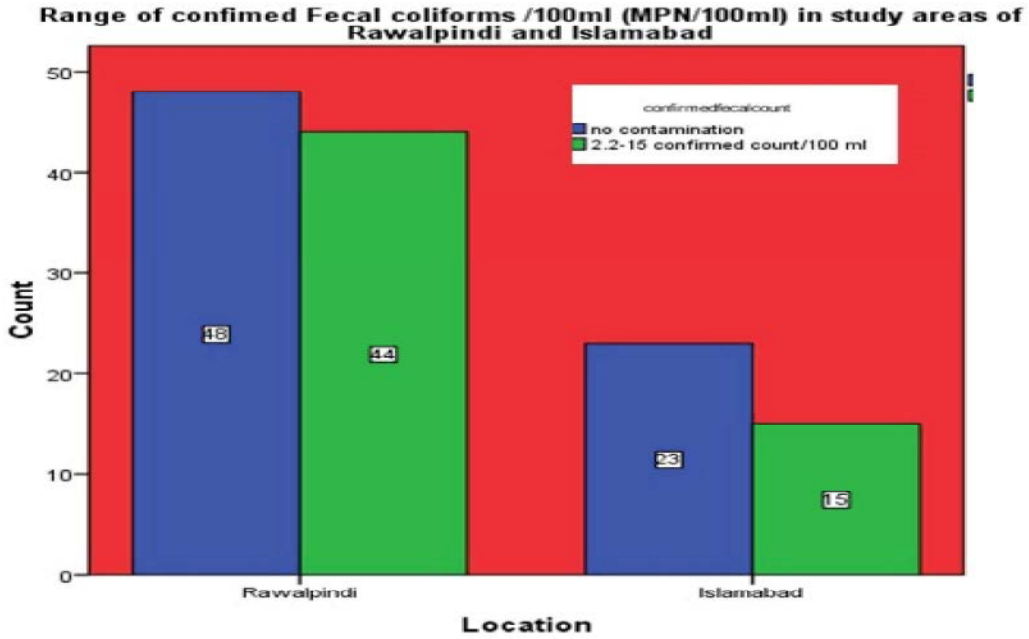


Fig. 5: Clearly indicated that in areas of Rawalpindi maximum ranges of confirmed fecal coliforms count were calculated. Confirmed fecal coliforms count ranges from 2.2-240+/100ml (MPN/100) in areas of Rawalpindi and Islamabad were calculated 48.8% (n=44), 39.5% (n=15) respectively.

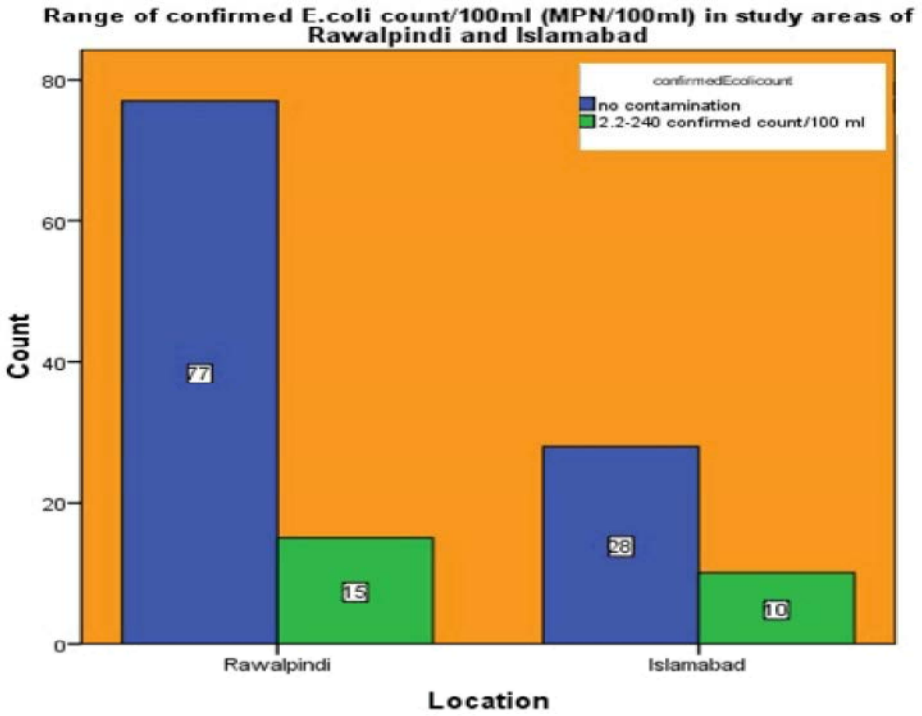


Fig. 6: Clearly indicated that in areas of Rawalpindi maximum ranges of confirmed *E. coli* count were calculated. Confirmed *E. coli* count ranges from 2.2-240+/100ml (MPN/100) in areas of Rawalpindi and Islamabad were calculated 16.3% (n=15), 26.3% (n=10) respectively.

as High prevalence of Diarrhea in the study area may indicate the prevalence of its infective agent (*E. coli*) more than the infective agents of other water-borne diseases. This work showed accordance with the work done by Figueras and Borego [21]. As *E. coli* is and indicator organism of fecal contamination there might be a chance of cross contamination of water supply pipes with the seepage system.

In our study we found that the only 43.9% water samples were found to be satisfactory for drinking purposes whereas 56.1% water samples were unsatisfactory for drinking purpose. These results indicated that only more 40% population having excess to the safe drinking water as WASA and CDA supply is the major water supply sources this study is in line by the work done in Lahore city from the total drinking water samples (n = 100), 30% (n = 30) samples were found to be excellent, 7% (n = 7) were satisfactory, 4% (n = 4) were suspicious and 59% (n = 59) were unsatisfactory [22] this shows thatwater quality status became more worse in recently 2 years no care given on such serious issue.

In our study we found that in study areas of Rawalpindi and Islamabad prevalence of fecal coliform and *E. coli* was 28.7% and 20% respectively as according to WHO the coliform count should be below 2.2/100ml of water a detectable limit whereas our this study is in accordance with another study in done in Lahore in a total of 100 samples, 42% (n = 42) revealed growth of *E. coli*.

CONCLUSION

The main conclusions from these data indicate that three major microorganisms are responsible for water-borne disease in areas of Rawalpindi and Islamabad and these are *fecal coliforms* and more importantly *E. coli* because it is termed as fecal contamination indicator was identified as the major cause of water-borne diseases Diarrhea within the study area.

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