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PATHOGENS FROM DRINKING WATER;

Isolation and antibiogram of pathogenic organisms from drinking water in Quetta city

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ABSTRACT… Objectives: Main objective was to check drinking water for pathogenic bacterial load, their resistance to antimicrobials and to create awareness among the people of Quetta city about safe drinking water. **Place and Duration of Study:** The study was conducted in Department of Microbiology Bolan Medical Complex Hospital Quetta during the hot season in Quetta City (June- September 2013). **Methodology:** One hundred and twenty five (125) tape water samples were collected aseptically in 200 ml sterile caped glass bottles from different localities of Quetta city. Samples were passed through mille pore assembly containing 0.45 µm-pore-size cellulose nitrate sterile membrane filter (MF).Viable count technique was used for enumeration of water samples having high bacterial burden. Serological tests and analytical profile index API-20E (Biomerieux France) were used to identify pathogens according to the manufacturer's directions. Standardized antibiotic sensitivity test was performed on Mueller Hinton agar using disc diffusion Kirby Bauer technique and McFarland Turbidity Standard method 0.5 following CLSI protocols. **Results:** Out of hundred and twenty five (125) tape water samples 110 (88 %) showed highly pathogenic bacterial load, in which the most prominent organism was E.coli 36 (28.8 %), followed by Enterobacter 35 (28 %), Klebsiella 24 (19.2 %), Pseudomonas, 10 (08 %), and Salmonella 05 (04 %). All pathogens in this study expressed a high level of resistance to antimicrobials that are commonly used in clinical medicine i.e. Tetracycline, Gentamycin, Sulphamethaxazole, Piperacillin, Ampicillin, Augmentin and Imipenam etc. Only 15 (12 %) samples were pathogens free. **Conclusion:** Among drinking water samples the presence of pathogenic bacteria (88%) is alarming for public health authorities. The emergence of resistance and decreasing level of susceptibility of pathogens to a wide spectrum of antimicrobials is a matter of great concern, because it may limit the availability of antimicrobials for clinical management of water born outbreaks in future.

Keywords: Drinking Water, Pathogens, Quetta.NDM.

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INTRODUCTION

Fresh water is a source of transmission of pathogens like viruses, bacteria and protozoa. The assessment of drinking water for fecal contaminants is important information for health authorities. The degree of fecal contamination in rivers, lakes, reservoirs and groundwater aquifers is variable[1,2] . Instead of global efforts and latest techniques water born diseases are still a challenge[3]. Many studies have been conducted for the assessment of contamination of drinking water and prevention of the water borne diseases world wide[4,5].

In Pakistan, common people use subjective quality criteria like brackish, foul smelling, bad tasting, and turbid or colored water to determine that it is not suitable for drinking. The agencies responsible for monitoring of water quality perform periodic checks of the basic water parameters against certain recommended standards. In order to ensure that consumers throughout the country are receiving quality water, research-based standards and guidelines for quality drinking water must be available to monitoring agencies[6]. 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[7,8,9]. China has improved the rate of use of safe drinking water upto 89% as it was 67% in 1990[10]. According to the WHO calculated data approximately 3.2% deaths worldwide are because of unsafe water due to poor sanitation and hygiene, that is a critical issue especially in rural areas of developing countries[11]. Total coliforms and E. coli have the capability to live longer in biofilms of water treatment industry. False sensing of these organisms and their release in water from biofilms mask the recent fecal contamination in water treatment industry. Long-term survival of total coliforms and E.coli in distribution system biofilms is proved from several documentations[12,13]. Antimicrobials have significantly changed the treatment of infectious disease and enhanced agricultural output throughout the world. Random use of antibiotics result in emergence of resistant mutants in bacterial population, due to which multi drug resistant pathogens are found in the hospital and community[14,15].

This study was designed to check the drinking water for contamination with pathogenic bacteria and their susceptibility to commonly used antibiotics in clinical medicine.

MATERIALS AND METHODS

Collection of water samples

One hundred and twenty five (125) tap water samples in sterile 200 ml caped glass bottles from different localities of Quetta city were collected aseptically. The localities were divided into five zones; East, west, North, South and Center of the city.

Processing of samples

All the samples were transported to the laboratory in cold chain and were processed within 6 hours of collection. Approximately 100 ml of water sample was passed aseptically in BSL-II cabinet through mille pore assembly containing 0.45 µm-pore-size cellulose nitrate sterile membrane filter (MF). The membrane filter, funnel and flask were autoclaved before each experiment. Viable count technique was used for enumeration of water having heavy bacterial burden[16]. All counts were expressed as total number of bacterial colonies per 100 mL of water. All water samples having one or more pathogenic bacteria per 100 ml were judged to be of poor quality, based on the zero tolerance level for coliform in water that the EPA advocates[17].

Isolation and Identification

Cellulose nitrate membrane filter containing the microorganisms was aseptically transferred to the MacConkey agar plate and was incubated at 37^0 C for 24 hours to obtain bacterial colonies. Colonies of all the samples were confirmed by Gram staining[18]. Triple cloned single colonies of suspected bacterial pathogens were further confirmed by serological tests and analytical profile index API-20E (Biomerieux France), according to the manufacturer's directions.

Antibiogram of isolates

Standardized antibiotic sensitivity test was performed on Mueller Hinton agar using disc diffusion Kirby Bauer technique and McFarland Turbidity Standard method 0.5 following CLSI protocols[19]. Isolates were considered as sensitive, intermediate and resistant to a particular antimicrobial agent on the basis of inhibitory zone that match the criteria of the manufacturer's interpretive table which follow the recommendations of National Committee for Clinical Laboratory Standards[20].

RESULTS

One hundred and twenty five (125) drinking water samples were collected from different zones of Quetta city as, East 18 (14.4 %), west 15 (12 %), North 24 (19.2 %), South 29 (23.2 %) and Center 39 (31.2 %) as shown in figure 1.

The results of drinking water samples processed through Membrane Filtration (MF)[21] and Viable count for those showing too numerous to count (TNTC) containing high bacterial load are shown in table-I.

No and % age of isolates.

Five (05) different types of pathogenic bacteria

were isolated and identified through manipulation of different media/methods, biochemical & serological tests. Figure 2

Antibiogram

Antibacterial susceptibility testing of all the isolates was done using commercially available standard antibiotic discs (Oxide UK) by Kirby-Bauer disk diffusion method. The zones of inhibition were recorded following CLSI guidelines. Table-II

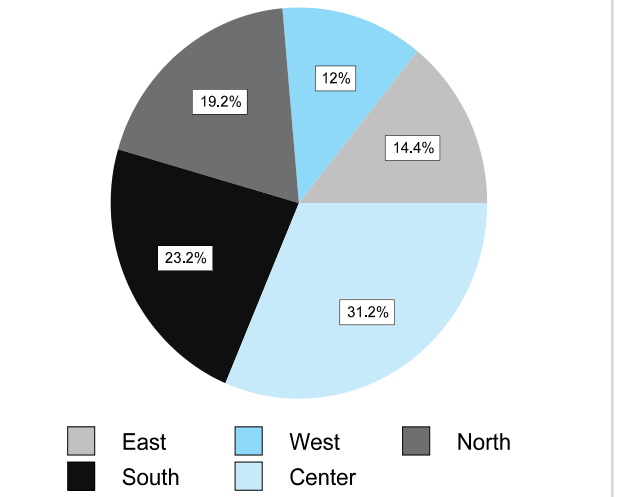

Figure-1. Number & %age of samples collected from different areas of Quetta city (n=125)

No of Samples	Method of Processing	Dilution Factor	Quantity of Water Processed	No of bacterial Colonies Obtained	Result (colonies/100ml)
46	Membrane Filtration	Nil	100 ml	0-10	0-10
31	Viable Count	1:1	50 ml	60-70	120-140
17	Viable Count	1:5	20 ml	30-40	180-240
11	Viable Count	1:10	10 ml	20-25	220-275
08	Viable Count	1:20	05 ml	10-20	210-420
07	Viable Count	1:50	02 ml	5-10	255-510
05	Viable Count	1:101	01 ml	1-8	101-808

Table-I. Samples Containing High Load of Pathogens.

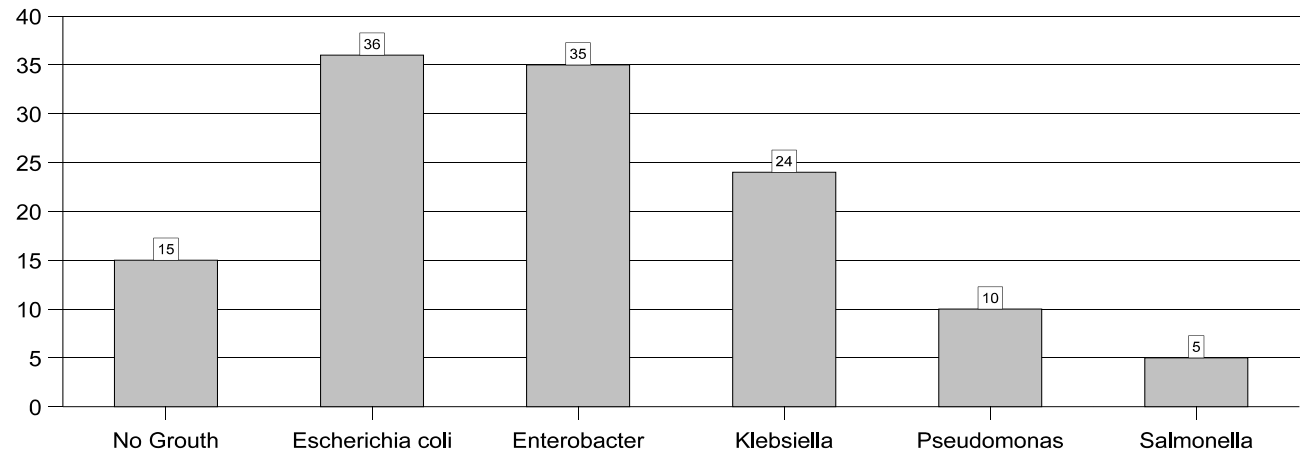

Figure-2. Number & % age of isolated pathogens in drinking water samples. (n = 125)

Antibiotics	E. coli	Citrobacter	Klebsiella	Pseudomonas	Salmonella
AMC (10 ug)	65.38 %	77.3 %	96.4 %	77.9 %	76.8 %
SXT (25 ug)	53.84 %	58.5 %	88.9 %	81.9 %	79.6%
CIP (05 ug)	11.53%	42.8 %	38.8 %	16.8 %	27.3 %
CN (10 ug)	57.69%	52.7 %	62.9 %	37.8 %	NT
C (30 ug)	NT	NT	NT	NT	12.5 %
CRO (30 ug)	21.46 %	NT	24.6 %	NT	13.8 %
CFM (05 ug)	NT	NT	NT	NT	24.3 %
CAZ (30 ug)	NT	NT	NT	18.3 %	NT
NA (30 ug)	NT	NT	NT	NT	32.6 %
TZP (100/10ug)	46.14 %	29.4 %	12.6 %	07.5 %	NT
IMP (10 ug)	00 %	00 %	20.0 %	00 %	NT
AK (10 ug)	27.9 %	NT	15.6 %	16.9 %	NT
AMP (10 ug)	NT	74.6 %	NT	NT	87.1 %
T (25 ug)	NT	23.4 %	NT	NT	NT

Table-II. Antibiotic resistance% age in isolated pathogens.

Abbreviations: *NT= Not Tested, AMC=Augmentin, SXT=Cotriamoxazole, Cip= Ciprofloxacin, CN=Gentamycin, C=Chloramphenicol, CRO= Ceftriaxone Sod. CFM= Cefixime, CAZ= Ceftazidime, NA= Nalidixic acid, TZP= Piperacillin/Tazobactam, IPM= Imipenam, AK= Amikacin, AMP= Ampicillin, T=Tetracycline.*

DISCUSSION

A vast majority of diarrheal diseases are due to unsafe drinking water, improper sanitation and poor hygiene. Analysis for fecal-indicator bacteria provides a sensitive, although not the most rapid, indication of pollution in drinking water supplies. The level of coliform/Pathogenic bacteria should be zero in a 100 ml sample of water directly intended for drinking or in treated water entering a distribution system. However, certain drinking water standards allow the presence of 10-100 fecal coliform/100 ml in drinking water[22,23].

Our findings indicated that water distribution system may be polluted by fecal contaminants, leaking sewage lines, human and animal excreta flowing into open drain system may be the common potential source of contamination in defective drinking water distribution system .Out of 125 drinking water samples taken from Houses, Hotels, Clinics, Hospitals etc., only 15 (12%) samples showed no bacterial growth and were fulfilling the WHO standards of potable drinking water. These samples were taken from houses and the residents of these houses were using different methods for the treatment of drinking water i.e. some of them were using boiled drinking water, while others were using WHO recommended water purification tablets (B.N.522381), and few of them had maintained proper hygienic conditions of their drinking water stores. 110 (88 %) samples contained high load of pathogenic bacteria, which exceeded the standard permissible limits recommended by various regulatory bodies for drinking water[24].

Our findings also indicated that pathogens we recovered in this study expressed a high level of resistance to antimicrobials that are commonly used in clinical medicine like Cephradine, Tetracycline, Gentamycin, Sulphamethaxazole, Piperacillin, Augmentin and Imipenam. The

emerging resistance of water born pathogens to a wide range of antibiotics is a matter of concern and needs an immediate attention from the concerned authorities, because it may lead to the decreased availability of antibiotics which are in clinical practice against water born diseases in near future.

Moreover a Klebsiella pneumoniae showed sensitivity zone 17mm to imipenam/meropenem. The standard sensitivity zone of Imipenam/ meropenem is ≥ 23mm. This is an indication of NDM (New Delhi Metallo ß lactamase) producing organism in drinking water, which is an emerging superbug and a serious threat to the public health worldwide.

CONCLUSION

Among drinking water samples the presence of pathogenic bacteria (88%) is alarming for public health authorities. The emergence of resistance and decreasing level of susceptibility of pathogens to a wide spectrum of antimicrobials is a matter of great concern, because it may limit the availability of antimicrobials for clinical management of water born outbreaks in future. In spite of small sample size, the results of the present study emphasize the human health risk associated with exposure to the contaminated drinking water due to the presence of multi antimicrobial resistant organisms.

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