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Original Research

A Study on the Microbiological and Chemical Profile of Water Sources in a Teritiary Care Hospital, Hyderabad

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ABSTRACT

Introduction: Hospital water and water delivering systems may serve as a reservoir of water borne pathogens. Water in hospital can get contaminated with microorganisms, which may be due to favourable conditions supporting bacterial growth and complexity of water system and machinery used in hospital facilities.

Aim: This study is intended to identify bacteriological and chemical profile in different water samples from different sources in hospital and to describe their relation with patient morbidity.

Materials and methods: This is a Prospective study conducted from July 2023 to October 2023 in Department of Microbiology,Osmania General Hospital, Hyderabad. A total of 18 samples collected from various water sources which were used for patient care as part of diagnosis and treatment werecollected and subjected to both chemical and bacteriological analysis. The samples are inoculated in the Peptone water,MacConkey broth, Eosin - Methylene blue agar, Brilliant green bile lactose broth and their growth was analysed after 24 hrs of incubation.

Results: Out of 18 samples, 7 samples are Unsatisfactory (39%) showing growth of Escheria coli and 11 samples are culture Satisfactory (61%) with no growth. Chemical analysis of all the samples are in accordance to National standards.

Conclusion: This study shows various water sources which were used for patient care could be the reason for long hospital stay of the patients leading to increase in morbidity. A proper surveillance, identification and Disinfection measures could help in prevention of infections in the hospital.

Keywords : Water analysis of hospital environment, Microbiology analysis of water, Hospital acquired infection

INTRODUCTION

Emerging water-borne pathogens pose a significant health risk in both developed and developing countries because they can spread quickly and affect large populations. Patients with immunocompromised states e.g., organ transplantation, dialysis, malignancies are particularly vulnerable to severe nosocomial infections caused by waterborne pathogens mostly bacteria which can result in significant morbidity.[1,2]Waterborne infections can occur from proximal (central pipes) or distal (points of use) contamination of the hospital water supply(5).Waterborne pathogens can infect patients through a variety of routes, including direct aerosol transmission from water droplets to patients, indirect transmission from fomites , improper use of non-sterile water for oral or tracheostomy care of ventilated patients, rinsing respiratory and endoscopic equipment in tap water, hand washing with contaminated water, exposure of implanted devices to contaminated water (e.g., bathing with an improperly covered central venous catheter), use of contaminated water for hemodialysis.[1,3-7]

The microbial contamination of water in health care settings are mainly due to enteric pathogens and others such as Legionellae, Multi drug resistant (MDR) Gram-negative bacteria, Non-tuberculous mycobacteria, etc. The common indicator of water contamination are intestinal commensal bacteria which are excreated in large number by man and animals. The presence of this indicating organism in water supply suggestive of fecal contamination indicate failure of disinfection measures.

The hospital environment may allow contamination by waterborne pathogens, in part because water temperatures are suitable for bacterial growth, and the

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complex structure of hospital water systems often leads to stagnation, corrosion, and biofilm formation.[8,9]Biofilms occur in the pipes and the points of exit of the water distribution system. Stagnation promotes the ideal conditions for biofilm formation and give protection against disinfection and transfer the antimicrobial resistance genetic elements.[6,10] There are limited resources to identify hospital acquired infection by water contamination.

MATERIALS AND METHODS:

This is a Prospective study done for a duration of 4 months from July 2023 to October 2023. A total of 18 water samples were collected from RICU, ICCU, NSICU, Distilled water from CSSD, EOT, MOT, CTOT, NSOT, Endoscope rinse water, Burns ward, Metro water main sump, one of Overhead tanks, Microbiology lab, Reverse osmosis plant, Dialysis water, common drinking water, Diet room drinking water, Direct borewell sump.

Sample Collection

For Bacteriological analysis 100ml of water samples collected in a wide mouthed screw capped sterile container and for Chemical analysis 2 liters of water collected in a container.

Source of water	Location of sample collection
Distilled water	RICU, ICCU, NSICU, Distilled water of CSSD
Metro water	EOT, MOT, CTOT, NSOT, Endoscope rinse water, Burns ward, Metro water main sump, one of Overhead tank, Microbiology laboratory
Reverse Osmosis water	Reverse osmosis plant, Dialysis water, common drinking water plant, Diet room drinking water
Borewell water	Direct borewell sump

Sample Processing

The water sample for bacteriological analysis was collected post-flush aseptically in sterile containers and immediately transferred for processing.

All samples subjected to the Total coliform count by multiple probable index (MPN) per 100ml by inoculating ontoMacConkey broth and Nutrient agar (Total Plate count) for 48 hours at 37 degree celsius. After 48hours, positive samples from Mackonkey broth are inoculated into Eosin-Methylene blue agar, Brilliant Green Bile broth and Peptone water. Inoculated Eosin-Methylene blue agar incubated at 37 degree celsius for 24 hours. Inoculated Brilliant Green Bile broth and Peptone water incubated at 40 degree celsius at water bath for 24 hours.

Persumptive Test

MPN index is a Persumptive test to detect Coliform bacteria. The series of tubes consist of three groups of three tubes in each. A series of lactose broth tubes are inoculated with measured amounts of the water sample to be tested. Water sample to be tested is taken with tubes of lactose broth with 10 ml, three tubes with 1.0 ml and three tubes with 0.1 ml with inverted Durham test tube. Incubate all tubes at 37 degree celsius for 48 hours. Acid and Gas production in any one of the tubes is presumptive evidence of the presence of Coliforms. The most probable numberof Coliforms in 100 ml of the water sample can be estimated by the number of positive tubes based on theMcCardy's table (given below).

Confirmed Test

In order to confirm the presence of Coliforms, inoculated in EMB (Eosin Methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram-positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. *E. coli* colonies are small and have a green metallic sheen. *E. coli* is an indicator of fecal contamination, since it is not normally found in water or soil.

Completed Test

The test is completed by inoculating colony from EMB agar to Brilliant Green Bile broth (BGBL) and Peptone water, then placed in water bath at 40 degree celsius for 24 hours. Brilliant green and dehydrated bile present in the BGBL medium inhibit gram-positive bacteria including lactose fermenting Clostridium. Production of brilliant green acid and gas by lactose fermentation is detected by incorporating inverted Durham's tube indicates a positive evidence of faecal Coliforms since nonfaecal coliforms growing in this medium do not produce gas. When Kovac's reagent added to inoculated Peptone water form indole ring due to hydrolyze of tryptophan, which confirm presence of Escherichia coli in sample. Chemical parameters such as Colour (Hazen units), Turbidity (NTUS), Odour, PH at degree celsius, Conductivity, Alkalinity, Total hardness, Calcium Hardness were measured according to the National Standards for drinking water.

RESULTS

Out of 18 samples, 7 samples are unsatisfactory (39%) and 11 samples are satisfactory (61%). Total coliform count shows >1609 MPN index per 100ml in all Unstatisfactory samples &Nil in Satisfactory samples. Unsatisfactory samples (39%) in MPN index suggestive of presence of Coliform bacteria in the water samples from EOT, MOT, Burns ward, Microbiology laboratory, Metro water main sump, direct borewell water and overhead tank. Most of the water contamination in our study is seen with Metro water source which indicate fecal contamination with

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it. The presence of Escherichia coli seen with water sample from CTOT, NSOT and Microbiology laboratory. Total Plate count method shows numerous bacterial counts in unsatisfactory samples.

Chemical parameters of sample collected from all the water source are in limits according to the National Standards of drinking method.

MPN and Confidence Limits for Various Combination of Positive Results When Three 10 ml Portions, Three 1.0 ml Portions and Three 0.1 ml Portion are used.

OF Tubes giving positive reaction out of					
3 of 10 ml	3 of 1.0 ml each	3 of 0.1 ml each	MPN of coliform Bacteria	95 % Confidence Limit.	
				Lower Limit	Upper Limit
0	0	1	3	0.5	9
0	1	0	3	0.5	13
1	0	0	4	0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	149
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	379
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	38	130
3	3	1	460	71	240
3	3	2	1100	150	4800
3	3	3	≥ 1609	---	---

Sl. No	Source of Water	Collection site	Sample Collected	Result
1.	Distilled water	RICU	1	Satisfactory
		ICCU	1	Satisfactory
		NSICU	1	Satisfactory
		Distilled water of CSSD	1	Satisfactory
2.	Metro water	EOT	1	Unsatisfactory
		MOT	1	Unsatisfactory
		CTOT	1	Satisfactory
		NSOT	1	Satisfactory
		Endoscope rinse water	1	Satisfactory
		Burns ward	1	Unsatisfactory
		Metro water main sump	1	Unsatisfactory

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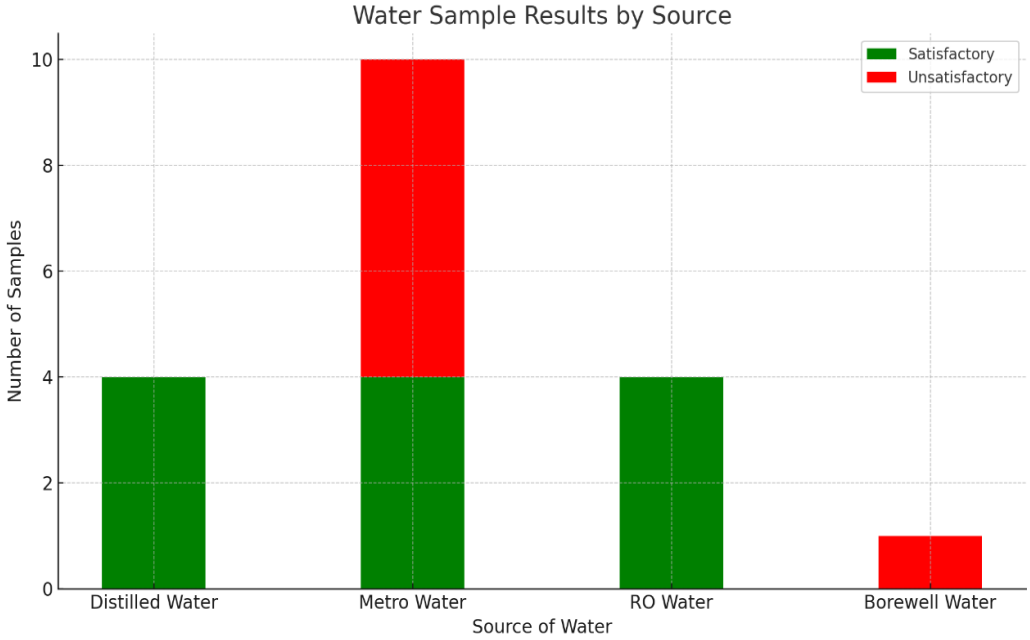
		One of Overhead tank	1	Unsatisfactory
		Microbiology laboratory	1	Unsatisfactory
3.	Reverse Osmosis water	Reverse osmosis plant	1	Satisfactory
		Dialysis water	1	Satisfactory
		Common drinking water plant	1	Satisfactory
		Diet room drinking water	1	Satisfactory
4.	Borewell water	Direct borewell sump	1	Unsatisfactory



MacConkey broth - Multiple probable index

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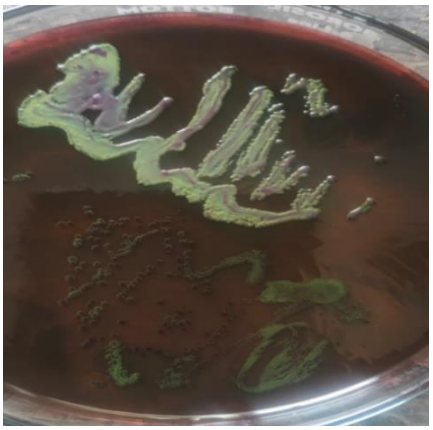
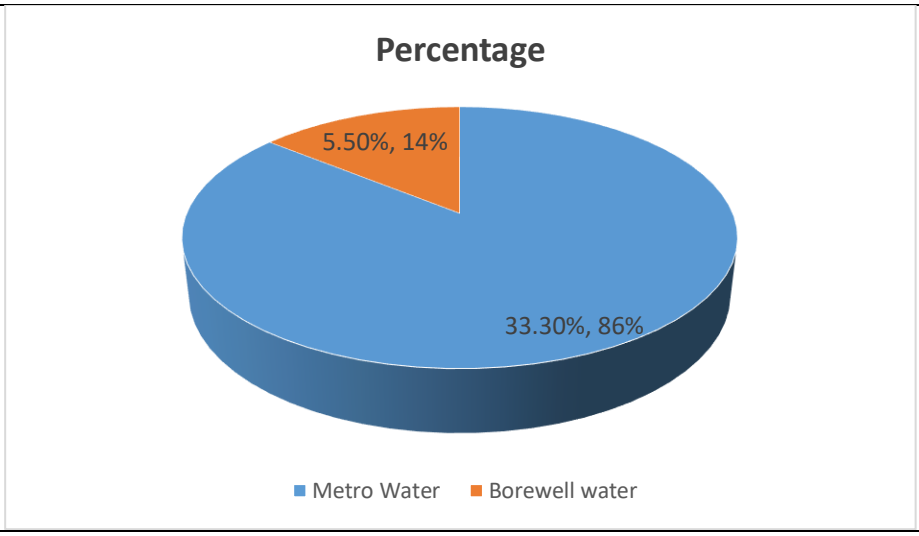
Total plate count method - showing numerous growth



Eosin-methylene blue(EMB) agar - showing Esherichia coli growth

A) Peptone water with indole formation confirm E.coli
B) BGBL medium

Source of Sample	Sample Positive (n = 39%)
Metro water	6 (33.3%)
Borewell water	1 (5.5%)



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DISCUSSION

The purpose of the microbiological examination of hospital water supplies is to protect the patients as well as the health care workers from nosocomial illnesses and outbreaks brought on by contact with or consumption of water that may be contaminated with pathogens. This study was conducted with the objective of identifying the different sources of water which are contaminated with pathogens.

In the course of our study we discovered about 11(61%) samples from different locations were contaminated .Metro water and Bore well water collected in our studies shows more contamination of up to 33.3% & 5.5% respectively when compared to other water supplies. This is comparable to the finding of Lipika Gaur et al in 2023 in India[1] and MairaAleem et al in 2021 in Pakistan[3] who reported contamination at hospital water.

Furthermore, the increase in the antibiotic resistance patterns of the microbes has forced the scientific community to look into the source of such pathogens and their mechanisms of acquired resistance in different environmental compartments. Water bodies were also considered to be a reservoir for the antibiotic resistance, especially because it facilitates the interaction of pathogenic bacteria with the non-pathogenic microorganism. On the other hand, it is important to know the knowledge of water storage and delivery to outlet systems. Improper cleaning and not following regular disinfection policy may provide favorable conditions for microorganisms to grow. This may also favor the biofilm formations by potential pathogens which lead to transfer of antibiotic resistance mechanisms.

All samples were recollected after disinfectionmesures and were found to be sterile. However it is difficult to maintain regular disinfection in the big stepup hospital, but we would advice to follow and maintain own disinfection policy.

However, we would like to add the following limitations to our study:

1. Follow-up sampling not done.
2. Time and economics were also our constraints. In the future, we would like to undertake a study with a larger sample size.

CONCLUSION

The findings of this study showed that the hospital's water supply is a breeding ground for a variety of waterborne pathogens. The identification of these microbes as well as their resistance pattern, would be helpful in preparing strategy for dealing with hospital acquired microbes.Hygiene measures include routine cleaning, disinfection of sinks, proper sterilisation of equipment that is rinsed in tap water, and most importantly healthcare personnel hand hygiene to prevent transmission of pathogens.Health care staff should be trained and awareness should be raised on disinfection practices.

No single approach guarantees that hospital water will be safe for vulnerable patients, but a combination of engineering and hygiene measures and clinical strategies can minimize the risk. Their should be proper surveillance programs and guidelines for safe water supply and infection control practices are highly recommended to limit the spread of infection.

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