

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Assessment of microbiological quality and contamination level of Hospital water samples collected from storage points

Rajani Chowdary Akkina 1 , Payala Vijayalakshmi * 2, Vidyullatha Peddireddy 1

ABSTRACT

¹Department of Microbiology and Food Science and Technology, GITAM Institute of Science, GITAM (Deemed to be University) Visakhapatnam-530045, Andhra Pradesh, India ²Department of Microbiology, GITAM Institute of Medical sciences and Research (GIMSR), GITAM (Deemed to be University) Visakhapatnam-530045, Andhra Pradesh, India

Article History:

A

Received on: 22 May 2020 Revised on: 13 Jun 2020 Accepted on: 27 Jun 2020

Keywords:

Antibiotics, Hospital water, MPN, Multi Drug Resistant, Storage points

Drinking water contamination at the level of storage points poses a potential threat to the hospital environment as it can lodge some resistant pathogenic microorganisms that may cause hospital acquired infections. The study analyzed the physico-chemical and microbiological parameters of drinking water samples collected from the main water storage points from ten different local hospitals in and around Visakhapatnam using standard protocols. The samples were processed within 2 hours after collection and the identification of pathogenic bacteria was performed through Most Probable Number (MPN) method, cultural characteristics and biochemical reactions. Antimicrobial susceptibility test was carried out by Agar well diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. In the current study, all the tested ten water samples cross the permissible MPN count indicating that the water samples were not potable for drinking purpose and needs further and better disinfection procedures. Among the isolated pathogens, *Pseudomonas aeruginosa* exhibited highest sensitivity to antibiotics imipenem (50%) and tetracycline (50%) and resistance towards other tested antibiotics, whereas *E.coli* showed 100% susceptibility to imipenem and 100% resistance to ampicillin. Out of ten isolated strains of *Proteus* species, majority have exhibited 80% resistance to ampicillin and Tetracycline and 80% sensitivity to imipenem. All the four isolated strains of *Shigella* species expressed 100% resistance to ampicillin and 75% sensitivity to imipenem, meropenem, azithromycin, linezolid, vancomycin and chloramphenicol. The five isolated strains of *Vibrio* species showed 100% resistance to ampicillin and 80% sensitivity to imipenem, doxycycline and tetracycline.

*Corresponding Author Name: Payala Vijayalakshmi Phone: Email: vpayala@gitam.edu

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2667

Production and Hosted by

IJRPS | www.ijrps.com

© 2020 *|* [All rights reserved.](https://doi.org/10.26452/ijrps.v11i3.2667)

INTRODUCTION

Hospital water safety is the prime and constant concern for health care epidemiologists, safety officers, engineers and administrators. Water, sanitation and hygiene program from World Health Organization (WHO) has issued several rules for water quantity, quality and for the access required in healthcare facilities. Drinking water supplies should meet the WHO Guidelines for ensuring safe drinking-water quality (WHO, 2006).

Contamination in hospital water is broadly classi-

fied into three forms, which include microbiological, physical and chemical. In general, contamination may happen at the main source, between the source and the storage points and in tanks (Feachem, 1980). Defective joins in pipelines, back siphonage, rusted pipelines, air condition systems and the cooling towers operated in hospitals have also been reported as the significant contributors [of several](#page-10-0) [outbr](#page-10-0)eaks. Contamination of incubators and respirators of newborns with contaminated water acts as the main source of transmit (Ngwenya *et al.*, 2013; Graman *et al.*, 1997).

Water borne infections serve as the principal source of morbidity and mortality, [however majority of](#page-11-0) [these are preventa](#page-10-1)ble. Along with many health care associated infections, occurrence of nosocomial water borne diseases gradually destroys public confidence in health care facilities. The quality of water supplies in many healthcare facilities are often overlooked, however it is important for patient safety and a well-regulated source of infection (Franzin *et al.*, 2001).

It is mandatory to have trustworthy drinking water supply at all important points within the he[althcare](#page-10-2) [setting and](#page-10-2) in the service areas to ensure safe water supply to staff and patients. Drinking water supplies may often enclose a diverse microbial community, which include opportunistic pathogens (Anaissie *et al.*, 2002). The microbiological contamination of water is a potential health hazard causing several gastrointestinal disorders. Different opportunistic pathogens may enter into piped [potable](#page-10-3) [water thereb](#page-10-3)y colonizing the pipeline surfaces and inducing biofilm production. The common modes of transmission for waterborne infections include direct contact, ingestion of contaminated water, indirect contact, inhalation of aerosols disseminated from water sources, and aspiration of contaminated water (de Abreu *et al.*, 2014; Krishnan *et al.*, 2007).

Many bacteria resist the effects of antibiotics designed to kill them. Multidrug-resistant organisms a[re bacteria that exhi](#page-10-4)[bit resistance to man](#page-10-5)y antibiotics, and these antibiotics can no longer be used to control or kill these bacteria. Microbes can be intrinsically resistant to antimicrobial agents due to their inherent structural and functional components (Moges *et al.*, 2014). However, the increasing concern is about initially sensitive bacteria developing acquired resistance to antibiotics (Guardabassi *et al.*, 1998; Ekhaise and Omavwoya, 2008). This can b[e attributed to m](#page-11-1)utations in chromosome or by acquiring a resistant gene from another bacterium, or can also be produced be[cause of mis](#page-10-6)[use or over](#page-10-6)[use of the valuable therapeutic](#page-10-7) drugs.

Multidrug-resistant organisms are found mainly in hospital environment and long-term care facilities. They usually affect aged and immune compromised people (Chagas *et al.*, 2011; Roberts *et al.*, 2003).

The capability of microbes to live in hospital water reservoir was described long back and several studies have established hospital water as a source of nosoco[mial infections, hen](#page-10-8)c[e the present study](#page-11-2) was intended to analyse the quality of hospital drinking water sources in terms physical, chemical and microbiological aspects using standard procedures.

MATERIALS AND METHODS

Sample collection

The study was designed to analyze the physical, chemical and microbiological quality of drinking water samples collected from the water storage points of ten Government and private hospitals inand-around Visakhapatnam, Andhra Pradesh. The study was carried for a period of one month Jan to Feb 2020 in order to check and compare the level of contamination. Water samples were collected in sterile bottles as per the APHA (1992) standard protocol. Collected water samples were immediately stored in a chilled insulation container at a temperature between 2*◦*C and 4*◦*C.

Physico-Chemical Parameters

All the hospital water samples were analysed for physic-chemical parameters using standard methods of American Public Health Association (APHA) (APHA, 1995) and American Society for Testing and Materials (ASTM) (Dezuane, 1997). The pH of the water samples was measured by using a digital pH meter (Metsar technologies pvt. Ltd., Hyderaba[d, Model D](#page-10-9)PM-500-145000569). The pH values of water samples [varied betwee](#page-10-10)n 6.3 to 7.3 indicating near neutral pH and only minor fluctuation in pH was recorded which was found to be within the limit prescribed by WHO and the limits set for domestic use prescribed by APHA 1995. The Electrical conductivity of the samples was measured using a conductivity meter (Model 304 Systronics pvt. Ltd., Gujarat, India).

Total dissolved solids (TDS) of water samples were measured using a TDS Meter (Model WD-356044- 24, Oakton global technology Ltd., Hyderabad). Water containing more than 500 mg/L of TDS is not considered desirable for drinking water supplies. The TDS concentration considered as a secondary drinking water standard, and not considered as a risk to human health. The turbidity of the water samples was measured using a turbidity meter (model 2100P HACH, Colombia, USA).

Samples pH		Condu	TDS	Turb idity	Chloride Total	alka-	Carbo nate	Bicarbo nate	Total hard-		Calciun Magnesium
		ctivity			(mg/l)	linty			ness		
S ₁	7.18	2.09	337	0.21	210	200	30	170	290	55	24
S ₂	7.22	0.7	448	0.24	110	170	30	140	220	61	19
S ₃	7.29	2.84	187	0.22	200	360	100	260	260	72	21
S4	7.38	2.43	155	0.2	230	270	60	210	310	69	28
S ₅	7.25	1.59	117	0.2	210	200	40	160	210	43	18
S6	7.68	1.9	216	0.2	280	160	20	140	220	58	25
S7	6.93	2.68	315	0.23	220	240	50	190	230	73	29
S ₈	7.13	2.1	344	0.21	250	270	70	200	270	61	26
S9	7.16	1.88	203	0.24	240	160	40	120	250	70	23
S ₁₀	7.31	2.68	395	0.21	219	290	60	240	230	63	27

Table 1: Physico-chemical parameters of drinking water samples of present study

Table 2: Isolation and identification microorganisms in 0.1 ml of water samples

Sample 0.1 ml volume	Growth on EMB	Growth on TCBS	Growth on SS agar	Growth on Mac-Conkey agar	Interpretation
S ₁	Green metallic sheen colonies	No growth	N _o growth	Pink colour colonies	E.coli
S ₂	Colourless colonies	No growth	N _o growth	colourless colonies	Proteus species
S ₃	Colourless colonies	Yellow colour colonies	N _o growth	colourless colonies	Proteus species, Vibrio species
S ₄	Colourless colonies	No growth	N ₀ growth	colourless colonies	Proteus species
S ₅	Colourless colonies	No growth	N _o growth	colourless colonies	Proteus species
S ₆	Colourless colonies	Yellow colour colonies	N _o growth	Colourless colonies	Proteus species, Vibrio species
S7	Green metallic sheen colour colonies	Yellow colour colonies	N _o growth	Pink colour colonies	E.coli, Vibrio species
S ₈	Colourless colonies	Yellow colour colonies	Colour- less colonies	Colourless colonies	Shigella species, Vibrio species, Pseudomonas species
S ₉	No colonies	No Growth	Colour- less colonies	Colourless colonies	Shigella species
S10	Colourless colonies	No growth	N ₀ growth	colourless colonies	Proteus species, Pseudomonas species

Sample 1 ml vol- ume	Growth on EMB	Growth on TCBS	Growth on SS agar	Growth on Mac- Conkey agar	Interpretation	
S ₁	metallic Green sheen colonies	No growth	No growth	colour Pink colonies	E.coli	
S ₂	Colourless colonies	No growth	No growth	colourless colonies	Proteus species	
S ₃	Colourless colonies	Yellow colour colonies	No growth	colourless colonies	Proteus species, Vibrio species	
S4	Colourless colonies	No growth	No growth	colourless colonies	Proteus species	
S ₅	Colourless colonies	No growth	No growth	colourless colonies	Proteus species	
S ₆	Colourless colonies	Yellow colour colonies	No growth	Colourless colonies	Proteus species, Vibrio species	
S7	metallic Green colour sheen colonies	Yellow colour colonies	No growth	Pink colour colonies	E.coli, Vibrio species	
S ₈	Colourlesscolonies	Yellow colour colonies	Colourless colonies	Colourless colonies	Shigella species, Vibrio species, Pseudomonas species	
S ₉	No colonies	No Growth	Colourless colonies	Colourless colonies	Shigella species	
S10	Colourless colonies No growth		No growth	colourless colonies	Pseudomonas species	

Table 3: Isolation andidentification microorganisms in 1.0 ml of water samples

Figure 1: Total coliform counts from sampling points

The turbidity values ranged from 0.1 to 0.2 NTU for various samples and all are within the admissible limits. Alkalinity is measured by titrating a water sample with a strong acid like HCl and sulfuric acid and expressed by the calcium carbonate content (mg/L) equivalent to the amount of acid consumed until the pH value reaches the prescribed value. Alkalinity measured with the endpoint of pH4.8 called total alkalinity. Alkalinity, Total hardness, Calcium hardness, Magnesium hardness, Chloride, carbonate and bicarbonate levels etc., were carried out as per the methods described in APHA guidelines (Verma *et al.*, 2011). All the chemicals and reagents used were of analytical grade. The highest concentration of these ions in drinking water is an indication of pollution due to high organic waste of animal origin.

Microbiological analysis of water samples

Bacteriological analysis of drinking water samples was performed within 48 hours of collection. Drinking water samples were analyzed by Most Probable Number (MPN) method given by McCrady in 1915. The presence of various water borne pathogens and indicator organisms was examined by using specialized media to study the cultural characteristics and biochemical tests for identification of bacteria.

MPN test is the most desirable method to check the

Water sample	Microorganisms isolated	MPN index per 100ml	95% confidence Lower	95% confidence Upper
S ₁	E.coli, Proteus species	280	120	690
S ₂	Proteus species	22	9	56
S ₃	Proteus species, Vibrio species	30	10	110
S ₄	Proteus species	23	9	86
S ₅	Proteus species, Vibrio species	170	80	410
S ₆	Proteus species, Vibrio species, Shigella species	300	100	1300
S7	E.coli, Proteus species, Vibrio species, Shigella species	500	200	2000
S ₈	Shigella species, Vibrio species, Proteus species Pseudomonas aeruginosa	280	120	690
S ₉	Shigella species, Proteus species	17	7	40
S ₁₀	Proteus species Pseudomonas aeruginosa	220	100	580

Table 5: Microorganisms, MPN index of water samples collected from hospitals

Table 6: Grades of water Sample

 P -value = > 0.05

quality of water by means of coliform detection. The test includes three stages: presumptive, confirmed and completed test. Lactose broth tubes are inoculated with different water volumes, the tubes that are positive for gas production are inoculated into brilliant green lactose bile broth in the confirmed test, and positive tubes are used to calculate the most probable number (MPN) of coliforms in the water sample following the statistical table.

The completed test, involving the inoculations of EMB (Eosin Methylene blue) agar plate, SS agar (Salmonella Shigella agar) nutrient agar slant and brilliant green lactose bile broth, TCBS (Thiosulfatecitrate bile-salts sucrose agar medium) and preparation of a Gram-stain slide from Nutrient slant, is used to establish that coliform bacteria are present in the sample.

The complete process, including the confirmed and completed tests requires at least 4 days of incubation and transfers and later on colony morphology has been studied.

Antibiotic susceptibility testing: (According to CLSI guidelines)

Disc diffusion method

Disc diffusion or Antibiotic susceptibility testing was performed according to CLSI guidelines using Mueller-Hinton agar (MHA) plates using the concentration of antibiotics per discs, recommended by the WHO experts committee on biological standardization. The plates were incubated at 37*◦*C for 16-18 hrs. The inhibition zone was measured according to CLSI guidelines (CLSI Catalogue, 2016) (Collee *et al.*, 2006). The following antibiotics were used in the study (Piperacillin (PI), Amikacin (AK), Gentamicin (GEN), Ceftazidime (CAZ), Ciprofloxacin (CIP), Imipenem (IMP), Ampicillin/Sulbactu[m \(AS\),](#page-10-11) [Ampi](#page-10-11)c[illin \(](#page-10-11)AMP), Co-Trimoxazole (COT), Cefepime (CPM), Cefuroxime (CXM), Ceftazidime/Clavulanic acid (CEC), Nitrofurantoin (NIT), Tetracycline (TE), Colistin (COL), Cefotaxime (CTX), Cotrimaxazole (COT), Tetracycline (TE), Meropenem (MPM), Azithromycin (AZM), Linezolid (LZ), Vancomycin

Table 8: Antimicrobial susceptibility testing of *Shigella species*

P-value $= < 0.05$

 P -value = < 0.05

Figure 3: Growth of Microorganisms on Various Selective media

(VA), Nalidixic acid (NAL), Ciprofloxacin (CPL), Doxycycline (DOX))

Statistical Analysis

Obtained data was statistically analyzed using SPPS software version 24.0. Frequency and percentages were calculated for categorical and ordinal variables. Fischer's test was carried out and p value ≤0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Physico-chemical analysis

The results of physico-chemical parameters of water samples from various hospital water source systems was illustrated in Table 1. The pH values of water samples ranges from 6.93 to 7.68 and all are well within the permissible range (6.5-8.5) of drinking water standards. Electric conductivity values of the all samples observed we[re](#page-2-0) in a permissible range 2.0 to 2.84, except S2 water sample, which has low EC value. The TDS of water samples range within the permissible limit of 500 mg/lit. This indicates the presence of optimum levels of soluble solid matter making the water suitable for drinking. The samples showed chloride ion concentration within the permissible limit of 250 mg/lit. TA in the water samples varies from 160-290 mg/lit and lies within the permissible limit of 300 mg/lit. Total hardness of water samples lies within the permissible limit of 300mg/lit. Hence, these waters are suitable for

drinking purpose.

 Ca^{2+} ion levels in the water samples were in permissible limit (75mg/lit). Hence, the water is suitable for human consumption. Excessive Ca2+ can cause extrusion on water supply system. Hence, all these samples are fit for usage. Mg2+ion concentration in water samples is not excess to the permissible range (30mg/lit). Excessive Mg⁺² can cause gastrointestinal irritation if used by humans. In the present study, the turbidity slightly crosses the permissible range 0.2 NTU that indicates that water samples are slightly turbid due to sand particles and microbial presence. The concentration of carbonate and bicarbonate levels in the tested water samples were within the permissible range 500mg/lit which indicates that the water does not show hardness.

Microbiological analysis

The results of MPN test after 24hrs incubation in lactose broth (LB) was interpreted by identifying the presence of yellow color in tubes indicating the positive result for the presence of bacteria whereas no change in color of Lactose Broth medium shows no bacterial growth after 24hrs incubation. The samples collected from the ten different Government and private hospitals involving 10 sampling points were analyzed for total and fecal coliform counts. The total coliform counts and microbial pathogens from the sampling sites were shown in Tables 2, 3, 4 and 5. The grades of water samples were shown in Table 6. The presumptive coliform count was >10

which indicates that the tested water samples were not exactly fit for drinking purpose. The samples S7 showed the highest total and fecal coliform counts (MPN = 500) compared with the other sample sites. The samples S6, S8 and S1 also showed potential MPN count (MPN > 280). At the same time the sample S9 showed very less counts of fecal coliforms (MPN count 17) (Figure 1). Majority of the microorganisms isolated were *Proteus* species and *Vibrio* species. The organisms were identified based on the morphological, cultural [an](#page-3-1)d biochemical characteristics (Figures 2 and 3).

Determination of Antibiotic susceptibility

The antimicrobial susceptibility pattern of isolated microorganis[ms](#page-5-0) scr[ee](#page-8-0)ned from the ten sampling points were depicted in Tables 7, 8 and 9. *Pseudomonas aeruginosa* showed highest sensitivity to antibiotics imipenem (50%) and tetracycline (50%) and resistance to all other tested antibiotics whereas *E.coli* showed 100% suscepti[bil](#page-6-0)i[ty](#page-7-0) toi[m](#page-7-1)ipenem and 100% resistance to ampicillin. Out of ten isolated strains of *Proteus* species, majority of them showed 80% resistance to ampicillin and Tetracycline and 80% sensitivity to imipenem. All the four isolated strains of *Shigella* species showed 100% resistance to ampicillin and 75% sensitivity to imipenem, meropenem, azithromycin, linezolid, vancomycin and chloramphenicol. The five isolated strains of *Vibrio* species showed maximum resistance 100% to ampicillin and 80% sensitivity to imipenem, doxycycline and tetracycline.

The intake of drinking water contaminated with pathogenic microorganisms particularly of fecal origin poses significant risk to the health of humans. As a customary procedure, water proposed for human consumption is circulated to consumers only after treatment. On the other hand, the quality of treated water can worsen during the distribution due to added contaminants and improper storage facilities and hence create significant health problems in human beings. In the present study, all the water samples collected from the water storage points of various hospitals in and around Visakhapatnam showed the optimum permissible ranges of physico-chemical parameters but showed turbidity greater than the optimum ranges. However, the presumptive coliform count >10 indicates that the water samples of storage points were not potable for drinking purpose. Experimental outcomes strongly recommend the need of appropriate disinfection treatments for the water at the storage points to protect patient health. According to Moges *et al.* (2014) among 113 drug resistant bacterial isolates, 65 were from hospital environ-

ment and 48 were from non-hospital environments. The most common isolates were Klebsiella species (26.6%) followed by Pseudomonas species (16.8%), *E. coli*(11.5%) and Citrobacter species (11.5%), and *Staphylococcus aureus* (8.2%). Ekhaise and Omavwoya (2008) presented similar reports and they collected water samples from Benin hospital revealed the frequent presence of species of Klebsiella, Pseudomonas and Serratia. Similars[tudy by](#page-10-7) Chagas *et al.* [\(2011\) on w](#page-10-7)ater samples collected from hospital Rio de Janeiro, Brazil reported that the most common isolates from hospital water were *Klebsiella pneumoniae*, *Enterobacter cloacae* and *E. coli*[. In the](#page-10-8) [presen](#page-10-8)t study, majority of the microorganisms isolated were Proteus species and Vibrio species followed by Shigella species, Pseudomonas species and *E.coli*, which were in accordance to the results of earlier studies. *Pseudomonas aeruginosa* showed highest sensitivity to antibiotics like imipenem (50%) and tetracycline (50%) and resistant to all other tested antibiotics whereas *E.coli* showed 100% susceptibility to imipenem and 100% resistance to ampicillin. Out of ten isolated strains of *Proteus* species, majority of them showed 80% resistance to ampicillin and Tetracycline and 80% sensitivity to imipenem. All the four isolated strains of *Shigella* species showed 100% resistance to ampicillin and 75% sensitivity to imipenem, meropenem, azithromycin, linezolid, vancomycin and chloramphenicol. The five isolated strains of *Vibrio* species showed maximum resistance 100% to ampicillin and 80% sensitivity to imipenem, doxycycline and tetracycline. Moges *et al.*, (2014) found the similar pattern of multiple drug resistance in isolated microrganisms *E. coli*, Klebsiella species Citrobacter species and Enterobacter species, they were 100% resistant to ampicillin. Among all isolates of Gramnegative bacteria 97% of the isolates were resistant to ampicillin, followed by cephalotin 49%, Cotrimoxazole 38%, tetracycline 37%, nalidixic acid 36% cefotaxime 33%. Multi-drug resistance in hospital environment was 53/65 (81.5%) while in nonhospital environment was 26/48 (54.2%). Our study showed more than 80% of multidrug resistant isolates similar to Moges *et al.,* (2014). Similar finding demonstrate that, waste effluent from hospitals contains high numbers of resistant bacteria and antibiotic residues at concentrations able to inhibit the growth of susceptible bacteria (Zhan and Miller, 2003; Hall *et al.*, 2004). Distribution of MDR strains holding resistance genetic markers may impose high risk of spread of resistance genes (Halabi *et al.*, 2001; Merrer *et al.*, 2005).

[Hospi](#page-11-4)[tal water should](#page-10-12) be cultured routinely specifically at the water storage points, ho[wever should](#page-10-13) be considered during clusters of infection particularly in low-and middle-income countries (LMICs). Hand washing sinks should be placed in the corridors or at the entrance to the patient clinical areas to reduce spread of multi drug resistant bacteria. Use of alcohol-based hand rubs (ABHR) should be encouraged. Water should be sampled monthly, and bacteria must not be <200 bacteria/ml. Chloride levels in hospital water should be tested periodically and the points for testing should be established using national or local standards. Using safe drinking water which is either boiled of filtered for temporarily immunocompromised patients is recommended; in situation where environmental ventilation is mechanically controlled, cooling towers with drift reducers should be installed to sweep vapors away from the hospital's air-intake system. Regular usage of a successful biocide to sanitize the water storage tanks is strongly recommended (Chaberny and Gastmeier, 2004).

CONCLUSION

[Contamination of the](#page-10-14) water supplies in healthcare facilities should be better prevented than remediated. Fortunately, many waterborne diseases are preventable with observance of optimal healthcare hygiene practices. Hospitals must have potential water management program that are monitored and updated regularly. The current study demonstrated the contamination degree of studied water samples collected from water storage points and revealed the presence of multi drug resistant bacteria should not be overlooked. The study suggests the practice of novel decontamination methods based on novel disinfection technologies. Simple and relatively inexpensive technologies can show high impact on controlling hospital infections. Regular and thorough investigation of water quality is the best control measure in infection prevention.

Conflict of Interest

Nill.

Funding Support

Nill.

REFERENCES

- Anaissie, E. J., Penzak, S. R., Dignani, M. C. 2002. The Hospital Water Supply as a Source of Nosocomial Infections. *Archives of Internal Medicine*, 162(13):1483–1483.
- APHA 1995. Standard Methods: For the Examination of Water. *Wastewater*.

Chaberny, I. F., Gastmeier, P. 2004. Should Electronic

Faucets Be Recommended in Hospitals?

- Chagas, T. P. G., Seki, L. M., Cury, J. C., Oliveira, J. A. L., Dávila, A. M. R., Silva, D. M., Asensi, M. D. 2011. Multiresistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro, Brazil. *Journal of Applied Microbiology*, 111(3):572–581.
- Collee, A., Marmion, Bp, Simmons, A. 2006. Mackie & McCartney Practical Medical Microbiology. 14th Edition. *J.G publishers*, pages 609–700.
- de Abreu, P., Farias, P., Paiva, G., Almeida, A., Morais, P. 2014. Persistence of microbial communities including Pseudomonas aeruginosa in a hospital environment: a potential health hazard. *BMC Microbiology*, 14(1):118–118.
- Dezuane, J. 1997. Handbook of Drinking Water Quality. 592.
- Ekhaise, F. O., Omavwoya, B. P. 2008. Influence of hospital wastewater discharged from university of Benin teaching hospital (UBTH). *Benin City on its receiving environment. American-Eurasian J Agric Environ Sci*, 4(4):484–488.
- Feachem, R. 1980. Bacterial standards for drinking water quality in developing countries. *The Lancet*, 316(8188):255–256.
- Franzin, L., Scolfaro, C., Cabodi, D., Valera, M., Tovo, P. A. 2001. Legionella pneumophilaPneumonia in a Newborn after Water Birth: A New Mode of Transmission. *Clinical Infectious Diseases*, 33(9):e103–e104.
- Graman, P. S., Quinlan, G. A., Rank, J. A. 1997. Nosocomial Legionellosis Traced to a Contaminated Ice Machine. *Infection Control and Hospital Epidemiology*, 18(9):637–640.
- Guardabassi, L., Petersen, A., Olsen, J. E., Dalsgaard, A. 1998. Antibiotic Resistance in Acinetobacterspp. Isolated from Sewers Receiving Waste Effluent from a Hospital and a Pharmaceutical Plant. *Applied and Environmental Microbiology*, 64(9):3499–3502.
- Halabi, M., Wiesholzer-Pittl, M., Schöberl, J., Mittermayer, H. 2001. Non-touch fittings in hospitals: a possible source of Pseudomonas aeruginosa and Legionella spp. *Journal of Hospital Infection*, 49(2):117–121.
- Hall, J., Hodgson, G., Kerr, K. G. 2004. Provision of safe potable water for immunocompromised patients in hospital. *Journal of Hospital Infection*, 58(2):155–158.
- Krishnan, R., Dharmaraj, R., Kumari, K., Bsr 2007. A comparative study on the physicochemical and bacterial analysis of drinking, borewell and

sewage water in the three different places of Sivakasi. *Journal of Environmental Biology*, 28(1):105–108.

- Merrer, J., Girou, E., Ducellier, D., Clavreul, N., Cizeau, F., Legrand, P., Leneveu, M. 2005. Should electronic faucets be used in intensive care and hematology units? *Intensive Care Medicine*, 31(12):1715–1718.
- Moges, F., Endris, M., Belyhun, Y., Worku, W. 2014. Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia. *BMC Research Notes*, 7(1):215–215.
- Ngwenya, N., Ncube, E. J., Parsons, J. 2013. Recent Advances in Drinking Water Disinfection: Successes and Challenges. *Reviews of Environmental Contamination and Toxicology*, 222:111–170.
- Roberts, R. R., Scott, R. D., Cordell, R., Solomon, S. L., Steele, L., Kampe, L. M., Trick, W. E., Weinstein, R. A. 2003. The Use of Economic Modeling to Determine the Hospital Costs Associated with Nosocomial Infections. *Clinical Infectious Diseases*, 36(11):1424–1432.
- Verma, P. U., Chandawat, D. K., Solanki, H. A. 2011. Seasonal Variation In Physico-chemical and Phytoplankton. *Analysis of Kankaria Lake*, pages 842– 854.
- WHO 2006. Guidelines for drinking-water quality [electronic resource]: incorporating first addendum. *Incorporating first addendum*, 1.
- Zhan, C., Miller 2003. Excess Length of Stay, Charges, and Mortality Attributable to Medical Injuries During Hospitalization. *JAMA*, 290(14):1868–1868.