ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2024; 9(1): 95 - 103

SJMLS - 9(1) - 010

Comparative Analysis of Water Sources from Some Selected Healthcare Facilities in Calabar Metropolis, Cross River State, Southern Nigeria.

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Abstract
Hospital water contamination can cause morbidity and mortality especially to immunocompromised patients. This research work was carried out to determine the bacteriological, physiochemical state and quality of water in three health care facilities in Calabar metropolis. A total of 60 samples were collected for this study, 30mls each was collected into sterile bottles. Twenty samples each, were collected from the General Hospital Calabar (GHC), University of Calabar Teaching hospital (UCTH) and University of Calabar Medical Centre (UMC), following aseptic procedures. Eight bacteria species were isolated which include *Pseudomonas aeruginosa*, with a prevalence of 21.2%, followed by *Escherichia coli* 18.2%, *Klebsiella species* 13.6%, *Enterobacter species* and *Staphylococcus aureus* 12.1%, *Citrobacter species* 10.6%, *Bacillus species* 7.6% and *Streptococcus pyogenes* 4.6%. The GHC had the most contaminated water source with 16(80%). The physiochemical analysis of the samples indicated that all the water sources were acidic, with pH values ranging from 3.4 to 4.7. These values are more acidic than the WHO's recommended standard of 6.5-8.5 for hospital waters. Turbidity levels were all below the WHO limit of >5NTU, free chlorine range of 0.0-05mg/L which are below WHO standard of 0.2-0.5mg/L. The results for coliform count ranged from (1cfu/100ml -137cfu/100ml), suggesting that the samples were all contaminated with faeces. The results also indicated a higher total heterotrophic count, ranging between 1cfu/ml – 277cfu/ml. Hence, proper treatment of hospital waters should be made in line with the WHO's recommended standard.

Keywords: Analysis, Calabar, Facilities, Healthcare, Sources, Water.

Introduction

Water is a colorless and tasteless liquid, which is essential to all life forms (Russell, 2014). Water supplies to healthcare facilities are frequently overlooked, yet essential for patient's safety and can be the source of infections. Numerous outbreaks of healthcare-associated infections have been linked to contaminated water used for patients' care; particularly maternal, child health, hand washing and cleaning of medical devices. Good quality potable water is still an unmet need in many low to middle income countries (LMIC). Infrastructure is often damaged or inadequate, leading to erratic water supply and contamination with fecal matter through broken pipes and wastage of water (Mehtar 2018).

The purified water system that produces, stores and circulates water, under background conditions, is “susceptible to the establishment of adhesive biofilms or micro-organisms”, which can be of undesirable level of viable micro-organisms or endotoxins on the effluent water. Studies have shown that nearly all large water purification systems can cause the formation of biofilm on the pipes. This biofilm can spread micro-organisms within the system and contribute to an increase in microbial populations (Penna 2022). Water-borne infections cause significant morbidity and mortality, some of which are preventable. In addition to other health care associated infections, occurrence of nosocomial water-borne infections erodes public confidence in

Health care facilities (Decker & Palmore 2014). A common feature of water-based pathogens is their ability to grow problematic concentrations within biofilms on pipe walls and sediments, particularly during periods of water stagnation and warmer conditions (Ashbolt 2015).

A wide variety of opportunistic pathogens such as *Pseudomonas species, Escherichia coli, Klebsiella species, Enterobacter species* and *Staphylococcus aureus* can be incorporated into pipe water, colonizing the surface of pipelines with high numbers of biofilm forming bacteria. Tang *et al*. (2020) reported that *Legionella species, Mycobacterium species* and *Mycobacterium avium,* which are water-borne opportunistic pathogens had occurrence rate as high as 100%, and 76% respectively in water samples obtained from manual and automatic faucets in the consulting room, treatment room, dressing room, respiratory ward and non-medical rooms in the hospital building (Srivastava *et al*., 2018).

Routes of transmission of contaminated water to patients

Water-borne pathogens can be transmitted to patients in several ways. The following are examples of the diverse modes of transmission:

1. Direct aerosol transmission from water to patients: aerosol from a shower or room humidifier, or cooling tower, aspiration while drinking water (Osawa *et al.,* 2014).
2. Indirect transmission from fomites that had contact with contaminated water: bath supplies and lines (Higa *et al.,* 2015) inappropriate use of non-sterile water for tasks that warrant higher measures of caution, such as oral/tracheostomy care of ventilated patients (Williams *et al.,* 2021).
3. Exposure of implanted devices to water (e.g., bathing with a central venous catheter improperly covered) (Kline *et al.,* 2014).
4. Transmission on the hands of healthcare personnel: failure to perform hand hygiene after contact with a contaminated environment or patients colonized with water-borne organisms, hand washing with contaminated water, splash back from contaminated sink drains (Hota *et al.,* 2019).

Table 1: Diseases due to contaminated hospital water

Organisms	Reservoir	Diseases Caused
Pseudomonas aeruginosa.	Shower, sinks, Bath and tub immersion, Faucet outlet, Hospital water waste system.	Blood stream infection, Bacteraemia, Pneumonia, ventilator assisted pneumonia, Folliculitis. (Berrouane, 2000)
Legionella pneumophilia.	Shower (hot water supply), Potable water, hospital wastewater system.	Respiratory tract infection, Legionnaires disease, Pneumonia. (Dermirjian, 2015)
Stenotrophomonas maltophilia.	Aerator faucet, Potable water.	Bacteraemia, Peritonitis, Bacteraemia. (Guyot, 2013)
Alcaligenes spp.	Bathing and tub immersion.	Cholecystitis, meningitis. (Fujioka, 2008)
Exophiala jeanselmei	De-ionized water from hospital pharmacy.	Fungemia. (Nucci, 2002)
Burkholderia cepacian	Dialysis water supply, tap water.	Bacteraemia (Souza, 2004)

Klebsiella spp.	Hospital waste water system, sink.	Bacteraemia, ventilator assisted pneumonia, UTI, peritonitis, abdominal wall abscess. (Leitner, 2015)
Fusarium spp.	Hospital water system.	Invasive fusariosis. (Anaissie, 2001)
Pseudomonas fluorescens	Ice bath, potable water.	Bacteraemia (Wong, 2011)
Serratia marcescens	Sink, water humidifiers.	Pneumonia.
Enterobacter species	Sink	Respiratory tract infections, ventilator associated pneumonia, abscess, wound infection. (Wagenlehner, 2002)
Flavobacterium species	Tap-water	Meningitis with septicaemia, pneumonia (Hoque, 2001)
Sphingomonas spp.	Tap-water	Bacteremia. (Perola, 2002)
Klebsiella pneumoniae	Sink, shower	Bacteraemia, pneumonia. (Seara, 2015)
Mycobacterium spp.	Tap-water, Hospital water system, shower, faucet, sink, heater - cooler unit, contaminated equipment.	Disseminated infections, bacteraemia, nasal septumcellulitis, cutaneous infections, otitis, endocarditis. (Benito, 2012)
Norovirus	Toilet, shower.	Gastroenteritis.

Source: (Morter *et al.,* 2016).

Materials and Methods

Sampling Method

This research was a cross sectional study conducted between April 2022 and October 2023. A total of 60 samples of water were obtained from the General Hospital Calabar (GHC), University of Calabar Teaching Hospital (UCTH) and University Medical Centre (UMC). Water samples were collected into sterile 600ml plastic containers observing aseptic procedures by sterilizing the water outlet using cotton wool soaked in alcohol, the cotton wool was lit, and the flame was used to sterilize the water outlet, the outlet was turned on and the water was left to flow for a minute till it attained a stable temperature. Water sample was then collected, ensuring the bottle avoided contact with the outlet. After collection, the samples were properly labelled and transported within two hours to the laboratory for physiochemical and bacteriological analyses.

Membrane filtration technique was carried out for the analysis of total coliform count (TCC) using a sterile grid membrane filter with diameter of 47mm and pore size of 0.45µm and sterile 47mm diameter cellulose pads. Up to 100 ml of the samples were filtered through the sterile membrane filter which retains the contaminants, the filters were aseptically transferred into a petri dish containing absorbent pads soaked previously in lauryl sulphate broth using sterile forceps. Pour plate technique was used for Heterotrophic plate count (HPC). Up to 1ml of the original sample was poured into the different plates. The freshly prepared culture media (Nutrient agar and Blood agar) were each poured into the different plates, mixed gently, and left to dry.

All culture media used were prepared according to the manufacturer's instructions. Physiochemical analysis of the samples was carried out for pH, Chlorine, Conductivity, Temperature, Turbidity and Total dissolved salt (TDS). Samples were analysed for pH using a digital pH metre and results were recorded. The temperature of the water was determined by measuring the temperature of an aliquot of the sample in a beaker, using a digital thermometer.

The turbidity was measured using a digital turbidimeter. Each sample was left to stand for 1 minute and read using a spectrophotometer. The bacteria isolates were identified macroscopically based on colonial morphology, microscopically and biochemically.

Data Analysis

Data obtained from the study was analysed using SPSS software (Statistical Package for Social Sciences) version 21.0 for Windows. Comparisons were made to assess whether samples varied significantly based on facilities and type of outlets. A p value of $\leq$ 0.05 was considered significant for tested variables. The contamination rate of samples was interpreted following the World Health Organization (2014).

Results and Discussion

The mean aerobic bacterial counts (ABC) of the water samples collected from different Healthcare facilities in Calabar were enumerated following the World Health Organization (2014). Table 2 shows the distribution of bacterial colony counts based on facilities. For TCC of greater than 50cfu/100ml, GHC had the highest prevalence (50.0%) as 8 out of the 16 samples with total coliform count of greater than 50cfu/100ml were from GHC. For THC of greater than 50cfu/ml, GHC had the highest prevalence (42.9%) as 6 out of the 14 samples with total heterotrophic count of greater than 50cfu/ml were from GHC. The level of contamination of hospital water differs from hospital to hospital depending on the water sources, how often the storage tanks, and distribution pipes are cleaned and maintained and chlorination of the water before distribution. Also, contamination can also differ between the different water outlets and their ability to harbor different organisms. In this study *Pseudomonas aeruginosa* (21.2%) *was* the most encountered isolate. This may be as a result of its capacity to form capsules and subsequently biofilms. The organism can survive in water distribution system despite chlorination, the work is in line with World Health Organization (2020).

Table 2: Distribution of bacterial colony count.

Facilities	No examined	TCC (<20 cfu/100ml)	TCC (20–50 cfu/100ml)	TCC (>50 cfu/100ml)	THC (<20 cfu/ml)	THC (20-50 cfu/ml)	THC (>50 cfu/ml)
GHC	20	6(17.6)	6(60.0)	8(50.0)	12(37.5)	2(14.3)	6(42.9)
UCTH	20	14(41.2)	4(40.0)	2(12.5)	8(25.0)	8(57.1)	4(28.6)
UMC	20	14(41.2)	0(0.0)	6(37.5)	12(37.5)	4(28.6)	4(28.6)
Total	60	34(100.0)	10(100.0)	16(100.0)	32(100.0)	14(100.0)	14(100.0)

□2 = 18.436, P = 0.048. Where UCTH = University of Calabar Teaching Hospital, UMC = Unical Medical Centre, GHC = General Hospital Calabar, THC = Total heterotrophic count, TCC = Total coliform count.

Total coliform count obtained from the water samples ranged from 1cfu/100ml – 137cfu/100ml contrast to research findings in a similar study by (Kekeç *et al*., 2016). that had the most probable number coliform count from 0 – 16MPN/100ml. Total heterotrophic count (THC) in this study ranged from 1-277cfu/ml in comparison to the study carried out by (Agbabiaka *et al.,* 2014) with THC of 290cfu/ml. Table 3 shows the occurrence of bacterial growth based on the facilities or location. 20 samples each were collected from University of Calabar teaching hospital (UCTH), General hospital Calabar (GHC), and Unical medical center respectively. Out of 60 samples from the study 43(71.6%) had significant bacterial growth while 17(28.3) had insignificant bacterial growth. GHC had the most occurrence of bacterial growth with 16(80%) out of 20 samples, this was followed by UMC with 14(70%) out of 20 samples. The least occurrence of bacterial growth was from UCTH with 13(65%) out of 20 samples.

Table 3: The Occurrence of bacterial growth based on facilities/location.

Facilities	No (%) of samples examined	No (%) with significant. bacterial growth	No (%) with insignificant bacterial growth
UCTH	20	13(65.0)	7(35.9)
GHC	20	16(80.0)	4(20.0)
UMC	20	14(70.0)	6(30.0)
Total	60	43(71.7)	17(28.3)

□2 = 1.149, P = 0.563

Key

UCTH= University of Calabar Teaching Hospital

UMC= Unical Medical Center.

GHC= General Hospital Calabar.

Table 4 and figure 1 show the distribution of bacterial isolates based on the location of sample collection and the type of water outlet. The bacteria isolated were *Bacillus species, Citrobacter spp, Escherichia coli, Klebsiella species, Staphylococcus aureus, Streptococcus pyogenes, Enterobacter species* and *Pseudomonas aeruginosa. Pseudomonas aeruginosa* was the highest organism isolated with 14(21.2), followed by *Escherichia coli* 12(18.2), *Klebsiella species* 9(13.6), *Enterobacter* and *Staphylococcus aureus* 8(12.1), *Citrobacter* 7(10.6), *Bacillus* 5(7.6), lastly *Streptococcus pyogenes* 3(4.6) was the least isolated organism.

Table 4: Distribution of bacterial Isolates based on the different facilities/Locations.

Isolates	UCTH N (%)	GHC N (%)	UMC N (%)	Total N (%)
No of samples	20	20	20	60
examined.	3(15.8)	2(7.7)	0(0.0)	5(7.6)
Bacillus species				
Citrobacter species	2(10.5)	2(7.7)	3(15.3)	7(10.6)
E. coli	2(10.5)	4(15.4)	6(28.6)	12(18.2)
Enterobacter species	3(15.8)	5(19.2)	(0.0)	8(12.1)
Klebsiella species	0(0.0)	4(15.4)	5(23.8)	9(13.6)
P. aeruginosa	3(15.8)	7(26.9)	4(19.0)	14(21.2)
S. pyogenes	1(5.3)	0(0.0)	2(9.5)	3(4.6)
S. aureus	5(26.3)	2(7.7)	1(4.7)	8(12.1)
Total	19(100.0)	26(100.0)	21(100.0)	66(100.0)

Figure 1 shows the sample distribution from the various facilities based on water outlets, 57.0% were gotten from sinks, 33.0% from taps and 10.0% from shower faucets. Bacteriological identification of the isolates obtained from water samples indicates that the organisms present were *Streptococcus pyogenes* (4.6%), *Bacillus spp.* (7.6%), *Staphylococcus aureus* (12.1), *Citrobacter spp.* (10.6), *Escherichia coli* (18.2%), *Klebsiella species* (13.6%), *Enterobacter spp.* (12.1), *and Pseudomonas aeruginosa* (21.2) which differs slightly from research finding by Nwanya *et al.,* (2021), 8 isolates were identified which include *Bacillus spp., Chromobacterium spp., Pseudomonas spp., Corynebacterium spp., Micrococcus spp., Aerococcus spp., Flavobacterium spp., Staphylococcus spp.* Shamsi *et al.* (2015), in a related study isolated *Escherichia coli* (26%) and *Pseudomonas spp.* (20%).

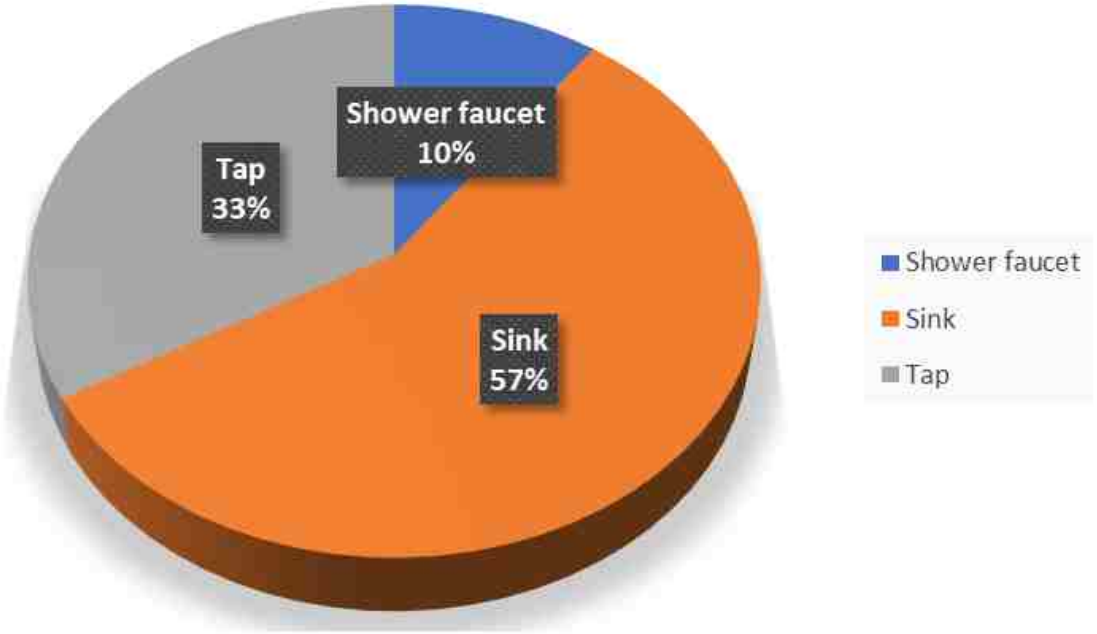

Fig. 1: Distribution of samples collected from the various facilities.

Table 5 shows the levels of chlorine, conductivity, temperature, turbidity, pH and total dissolved solids (TDS) of water obtained from the University of Calabar Teaching hospital (UCTH), University of Calabar Medical Centre (UMC) and General Hospital Calabar (GHC). University of Calabar Teaching Hospital had mean values of 3.72±0.36, 0.04±0.01mg/l, 38.40±1.58µs/cm, 29.50±0.53°C, 1.00±0.46NTU, and 23.04±0.95mg/l respectively, UMC had mean values of 3.98±0.27, 0.01±0.01mg/l, 198.60±93.24, 26.50±0.53°C, 0.71±0.36NTU and 119.16±55.95mg/l respectively while GHC had mean values of 3.87±0.35, 0.00±0.00mg/l, 113.70±8.46µs/cm, 28.30±0.48°C, 1.06±0.38NTU, and 67.62±4.77mg/l respectively. Chlorine, conductivity, temperature and TDS showed significant variations ($p < 0.05$) within and among the groups.

Table 5: Physicochemical analysis

Parameters	UCTH n = 20	UMC n = 20	GHC n = 20	F-ratio	P-value
pH	3.72±0.36	3.98±0.27	3.87±0.35	1.468	0.248
Chlorine(mg/l)	0.04±0.01a	0.01±0.01b	0.00±0.00	62.210	0.000*
Conductivity(µs/cm)	38.40±1.58a	198.60±93.24b	113.70±8.46	21.978	0.000*
Temperature(°C)	29.50±0.53c	26.50±0.53	28.30±0.48	86.704	0.000*
Turbidity (NTU)	1.00±0.46	0.71±0.36	1.06±0.38	2.111	0.141
TDS (mg/l)	23.04±0.95a	119.16±55.95b	67.62±4.77	22.011	0.000*

Values are expressed as mean ± STD. Where UCTH = University of Calabar Teaching Hospital, UMC = Unical Medical Center, GHC = General Hospital, Calabar, TDS = Total Dissolved Solids
* = significant at $p < 0.05$
Post hoc
a = significantly different from UMC and GHC
b = significantly different from GHC
c = significantly different from UCTH

All pH values were acidic and fell between 3.4 - 4.7 which are below the prescribed limit by World Health Organization (2014) 6.5-8.5 for hospital water. Temperature values of UCTH and GHC are within the limits (28-30^{0}C) while UMC temperature is slightly below the limit, ranging between 26 and 27^{0}C. Turbidity levels are all below the limit of >5NTU, this indicates that the water is more-clear, containing fewer particles. The free chlorine range as low as 0.0-0.05 and they all fall below the permissible limit prescribed by World Health Organization (2014) of 0.2-0.5mg/l, in comparison to Mansour *et al*., (2017) free chlorine range 0.01-0.03. Water gotten from GHC showed absence of free chlorine and higher levels of contamination. The chlorination lack explains water survival for long period in hospital tanks, low levels of free chlorine in the water and bacteria development in water in accordance with the work of World Health Organization (2014). respectively.

Total dissolved solids (TDS) and conductivity are directly proportional to each other, Conductivity is the ability of the water to conduct electricity and it is a function of the presence of inorganic solutes (ions), TDS is the weight of solute materials in the water. TDS samples from UCTH and GHC all fall below the prescribed permissible limit by World Health Organization (2014). and eight samples from UMC fall within the permissible limit by World Health Organization (2014). (150-1000mg/l). All conductivity values except four samples from UMC fall below the permissible prescribed limit by World Health Organization (2014). (300-1500us/cm)

Conclusion

This study showed significant bacterial growth and revealed the presence of bacteria in the various water sources. The pH of all samples was acidic, ranging between 3.4 and 4.7, with very low turbidity between 0.16 and 1.84NTU, which makes the water unsafe for consumption. All samples had low chlorine concentrations, ranging from 0.0 to 0.05 mg/L, which is not in line with the WHO standard. The temperature of all the water samples ranged between 26 ^{0}C and 30 ^{0}C. 8 samples fell within the WHO standard limit for total dissolved solids (150-1000mg/L) while others were below the standard and 4 samples fell within the WHO standard limit for conductivity (300- 1500µs/cm) while others fell below the limit. Hence, Hospital water should be chlorinated before distribution, presence of chlorine helps to kill microorganisms encountered within the distribution passages. Chlorine levels in hospital water should be tested periodically and the points for testing established using national or local standards. Storage tanks should be routinely washed and maintained. Distribution pipes should also be maintained appropriately to prevent biofilm formation. Faucets at water outlets should be cleaned regularly. Drift eliminators should be installed and use regularly and effective biocide to clean the water storage tanks, according to the manufacturer's recommendations.

Competing Interests

Authors have declared that no competing interests exist

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are very grateful to all academic members, technologists and technicians who were involved in, and contributed to the successful completion of, the study.

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Citation: Mbah, M., Akpan, S.S. and Njok, N.O. Comparative Analysis of Water Sources from Some Selected Healthcare Facilities in Calabar Metropolis, Cross River State, Southern Nigeria. ***Sokoto Journal of Medical Laboratory Science*; 9(1): 95 - 103.** DOI: 10.4314/sokjmls.v9i1.10