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ORIGINAL ARTICLE

Microbial Isolates in Augmented Water Receptacles and their Antimicrobial Susceptibility Pattern at the Tamale Teaching Hospital, Tamale, Ghana

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We set out to isolate microbes in augmented water receptacles and establish their antimicrobial susceptibility patterns. A cross-sectional study was conducted from 1st March to 31st May 2018. Samples were taken from the inner wall of each augmented water receptacle, transported immediately to the Microbiology Laboratory in a sterile tube containing 2-3ml of sterile physiological saline to prevent drying. Culture, isolation, identification of potential microorganisms and their susceptibility to the commonly used antibiotics in the hospital were done. Thirty-five (35) augmented water receptacles from 17 sites within the hospital were studied. All the containers were filled with tap water without further treatment after storage. The cleaning schedules of the receptacles varied among wards, with 7 (41.2%) being cleaned with detergents weekly, 3 (17.6%) twice weekly and 7 (41.2%) monthly. *Escheriachia coli* 13 (24.1%), *Pseudomonas aeruginosa* 13 (24.1%), *Staphylococcus aureus* 8 (14.8%), *Klebsiella* species 7 (12.9%), *Citrobacter* species 6 (11.1%), and *Legionella* species 5 (9.3%) were isolated. These isolates demonstrated multidrug resistance. Augmented water receptacles were found to be a reservoir for pathogenic microbes. These microorganisms are a potential source of water borne disease outbreaks.

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Annually, about 2 million healthcare-associated infections occur in the United States of America, causing significant morbidity, mortality, and financial burden (Centers for Disease Control (CDC), 1992). Healthcare-associated infection rates in low and middle income countries (LMIC) could be as high as 20 times that of developed countries (Zaidi *et al*., 2005). Immunocompromised patients are at increased risk of acquiring nosocomial infections which are reported to be resistant to the commonly used antibiotics (Depuydt *et al.,* 2006; Muntean *et al*., 2018; Nseir *et al*., 2007). The main source of cross infection in hospitals is from the hands of healthcare workers. Over three decades ago, keeping to personal hygiene such as hand washing was demonstrated as an effective method that interrupts the transmission of infectious agents between patients by healthcare workers (Boyce, 1999; Casewell & Phillips, 1977; Pittet, 1999; Sproat & Inglis, 1994). Among the numerous sources of pathogens that cause hospital-based infections, hospital water is perhaps an important but preventable source which seem to be overlooked (Anaissie *et al.,* 2002; Exner *et al.,* 2005; Williams e*t al*., 2013).

Augmented water containers/reservoirs almost always contain some water. Microorganisms prefer

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to attach to submerged surfaces when possible, as this provides several advantages over a planktonic/ free-floating existence (Kolari M, 2003; O'Toole *et al.,* 2000; Stoodley P et al., 2002). Free floating bacteria are less resistant to antimicrobial agents than bacteria growing in biofilms(Donlan & Costerton, 2002; Dunne, 2002).

Moist surfaces provide an optimum reservoir for biofilm-forming bacteria. Bacteria are known to colonize water mains, storage tanks and containers, and grow in biofilms when conditions are favourable (LeChevallier M. W, 1999). Mixed biofilms consist of a matrix-enclosed microcolonies of yeasts, hyphae and bacteria that exhibit interactions and communication between cells (Douglas, 2003). In general, biofilms raise the financial cost per year in health care systems due to apparatus damage, product infectivity, energy losses and medical infections (Kim *et al.,* 2014; Kurutkan *et al.,* 2015; Taylor J *et al.,* 2014).

Biofilms are of medical significance in microbial infections. The multi-species community of microorganisms that grow in biofilms associated with augmented water receptacles and its implications in health care delivery at a tertiary hospital in Northern Ghana are yet to be established. We set out to investigate augmented water receptacles as a potential source of healthcare-associated infection at the Tamale Teaching Hospital (TTH), Tamale, Ghana.

MATERIALS AND METHODS

Study design and site

A cross-sectional study was conducted from 1st March 2018 to 31st May 2018 at the TTH, an 802-bed capacity tertiary hospital. It is the main referral center for the five regions in Northern Ghana and beyond. Shortage of potable water supply to the hospital is a perennial challenge. The hospital receives potable water from the municipal water supply system and stores it in receptacles for future use whenever it experiences shortages in supply. These storage containers are of varied sizes and located at various vantage points within the hospital. The stored water receives no additional treatment.

Sample collection and sampling techniques

Using cotton swabs, samples were taken from the inner wall of each augmented water receptacle such as veronica bucket and plastic tank that had water stored in them. The samples were immediately transported to the laboratory in a sterile tube containing 2-3ml of sterile physiological saline to prevent drying. The veronica buckets and the plastic tank are made of opaque plastic material with maximum storage capacities of 65 liters and 500 liters respectively. The duration of storage of water in each receptacle varied as cleaning schedules varied among wards. All the containers were filled with tap water by connecting a water hose from a wall mounted tap to the receptacle. The water received no further treatment after storage. All the swabs were assigned unique identification numbers prior to transportation to the Microbiology Laboratory for culture, isolation and identification of potential organisms. Potable water from the hospital's main pipe system and other water dispensers were excluded. Data was collected from staff who were assigned to clean and maintain the augmented water receptacles at the TTH. We collected data on age of orderlies, duration of work experience, frequency of cleaning augmented water containers, type of detergent used for the cleaning, educational levels of the orderlies and the knowledge level on the proper ways of cleaning the containers. Knowledge was assessed by a pre-structured, interviewer-administered questionnaire and categorized using predefined scores of poor (<mean - 1 SD), average (mean ± 1 SD) and good (>mean + 1 SD).

Caretakers of the various augmented containers (veronica buckets and plastic tanks) were guaranteed confidentiality of all information that would be collected. Ethical clearance was obtained from the TTH Department of Research and Development with reference number TTH/R&D/ SR/58.

Laboratory procedures

A Standard Operating Procedure (SOP) was prepared for every procedure carried throughout the laboratory work. Each sample was streaked on disposable plastic petri dishes containing

MacConkey agar (Biomark, India), Blood agar (Biomark, India), Chocolate agar, (Biomark, India), ChromoCult coliform agar (Merck, Germany), Cetrimide agar (Oxoid, UK), Buffered Charcoal Yeast Extract agar (Oxoid, UK) and Mannitol Salt Agar (Oxoid, UK) by preparing a pool and extending the streak lines to obtain isolated colonies. The plates were incubated at 37°C for 24 hours after which Gram staining and the following biochemical tests were performed: Triple Sugar Iron Agar (TSI) Test, Citrate test, Urease test, Indole, Catalase test, Oxidase test and Motility Indole Ornithine (MIO) test were done to identify particular isolates.

Species identification

Gram Staining: An evenly spread smear of the specimen on a clean, grease-free slide was made. Smear was air-dried in a safe place. Specimen was heat fixed and stained by Gram staining technique. Smear was examined for the presence of bacteria using 100x objective lens and looked especially for: gram negative rods, gram positive cocci in pairs, chains or clusters, and gram-positive large rods with square ends.

Indole: Peptone water was used as the tryptophan broth. Isolated organism or test organism was inoculated in peptone water broth, incubated at 37° C for 24 hours in ambient air. About 0.5ml Kovac's reagent was added to the broth culture. Test was considered positive, that is, if pink-coloured ring was found after the addition of an appropriate reagent. This suggests the presence of *Escherichia coli* (*E. coli*).

Tube Catalase Test: 4 to 5 drops of 3% H_2O_2 was placed in a test tube. Using a wooden applicator, a small amount of organism from a well-isolated colony was collected and placed into the test tube and observed for immediate bubble formation (O_2 + water = bubbles) at the end of the wooden applicator stick against a dark background. Catalase positive reaction is suggestive of either *Staphylococcus* species or *Bacillus* species.

Citrate Utilization Test: Simmons citrate agar was inoculated lightly on the slant with a 24 hours old colony and incubated at 37°C. The development of blue colour, denotes alkalinization. Growth visible on the slant surface and medium change to an intense Prussian blue colour indicates the presence of citrate-utilizing bacteria such as *Citrobacter* species. Bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to above 7.6, causing the bromothymol blue to change from the original green colour to blue. *Citrobacter* spp. is citrate positive. Citrate negative: No growth was visible. No colour change occurred; the medium remains the deep forest green colour of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant e.g., *Escherichia coli.*

Christensen's Urea Agar: The entire slant surface was streaked with a heavy inoculum from a 24-hour culture (The butt was not stabbed as it will serve as a colour control). Tubes were incubated with loosened caps at 37°C. The slant was observed for a colour change at 6 hours and 24 hours. This test was used as part of the identification of several genera and species of Enterobacteriaceae including *Klebsiella.* If organism produces urease, the colour of the slant changes from light orange to magenta. If organism does not produce urease the agar slant and butt remain light orange (medium retains original colour).

Triple Sugar Iron Agar (TSI) Test: Using a sterilized straight inoculation needle, a well-isolated colony was picked, inoculated on TSI agar by first stabbing through the centre of the medium to the bottom of the tube and then streaking on the surface of the agar slant. The tube was capped loosely and incubated at 35°C in ambient air for 18 to 24 hours. Presumptive identification was done based on the biochemical reactions observed after the incubation period.

E. coli was the only enterobacteria encountered. The various media used and test process coupled with the condition of the test allowed us to isolate various enterobacteria but these were not available in the sample. *Legionella* is a fastidious organism and requires increased iron and cysteine for growth. Media containing iron and cysteine, that is buffered charcoal yeast extract agar (BCYE) was used. Culture plates were incubated at 37°C in a 5% CO2 environment for 48 hours. Colonies appear round

with entire edge, glistening, convex, green, or pink iridescent and have granular or speckled opalescence.

Susceptibility testing

Susceptibility testing was performed using single disc antibiotics on either Mueller Hinton Agar (MHA) or BCYE based on disc diffusion technique. Thirteen antibiotics namely: ampicillin, cotrimoxazole, gentamycin, cefuroxime, chloramphenicol, ceftriaxone, ciprofloxacin amoxicillin, augmentin, erythromycin, penicillin, tetracycline, and amikacin (ranging from 5-30mcg) were tested. For gram negative, eight antibiotics namely amikacin, ampicillin, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, cotrimoxazole and gentamicin. Also, for the gram positive a total of ten antibiotics were reported. The zone of inhibition of each antibiotic was measured in millimetres and compared to the standard in accordance to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute (CLSI), 2019). *Legionella* species were sub-cultured on BCYE plates and incubated for 48 hours at 37 °C in a humidified atmosphere. Colonies were suspended in peptone water, and the turbidity was adjusted to an optical density equivalent to 0.5 McFarland units. Approximately 107 colony-forming units (cfu)/mL were swabbed onto BCYE or MHA plates, and the surfaces of the plates were allowed to dry (15 min at room temperature). Then, antimicrobial discs were applied to each inoculated plate. The plates were incubated at 35 °C (without CO2) for 48 or 72 hours for *Legionella* species and 24 hours or 48 hours for the other isolates. The inhibition zones were read and the results interpreted according to the CLSI guidelines.

DATA ANALYSIS

Data collected was entered into Microsoft Excel 2016 (Microsoft corporation, Redmond, Washington, USA, www.mirosoft.com) compatible with Microsoft windows 8 version, edited to exclude errors and re-organized for efficient analysis. Data was transferred to Statistical Package for Social Science (SPSS) version 21 for analysis. Frequency distribution and percentages were calculated for quantitative variables.

RESULTS

General characteristics of augmented water containers

In all, 35 augmented water receptacles comprising 34 (97.1%) veronica buckets and 1 (2.9%) plastic tank from 14 wards and 3 operating theatres of the Tamale Teaching Hospital were included in the study. Potable water from the hospital main pipe system and other water dispensers were excluded. In all, 17 (48.6%) of the veronica buckets were stationed inside the wards/patient's bed area, and 10 (28.6%) were outside the ward/nurse's station. A total of 7 (20%) augmented veronica buckets were stationed in the operating theatres, whilst 1 (2.9%) plastic tank was stationed in the trauma and orthopedics ward. A total of 13 (76.5%) of the stations employed detergents in cleaning the containers whiles 4 (23.5%) of the stations did not use detergents in cleaning the containers. The remaining 17 veronica buckets, some of which were stationed outside the wards were not cleaned with detergents. Cleaning schedule of receptacles varied from ward to ward. Monthly cleaning was observed in 7 (41.2%) wards, whiles 7 (41.2%) wards cleaned the containers weekly. Only 3 (17.6%) wards practiced twice weekly cleaning of containers (Table 1).

Demographic characteristics of orderlies

There were 16 orderlies assigned to clean and maintain these augmented water receptacles. Twelve (75%) were females and 4 (25%) males. Their mean age was 42.7±5.7 years. The mean duration of work experience was 14.5 years, range 5-32 years. Their highest educational level was at the secondary school level in 6 (37.5%), with 9 (56.2%) having primary school education (Table 2).

Distribution of bacterial isolates in receptacles in the various wards and operation theatre

There were 54 bacterial isolates from 7 different genera. Of the total, 44 (81.5%) were Gram-negative and 10 (18.5%) Gram-positive isolates. E. coli, 13 (24.1%) and Pseudomonas aeruginosa, 13 (24.1%), accounted for 48.2% of the isolates.

These two Gram-negative bacteria were isolated from samples taken from augmented containers from all the wards and theatres except Ear Nose and Throat (ENT)/Urology, Gynaecology, Paediatrics and the Neurosurgery wards (Tables 3).
Of the 10 Gram-positive isolates, *Bacillus* species constituted 2 (3.7%). *Staphylococcus aureus* was the dominant Gram-positive organism isolated, 8 (14.8%). We observed that the highest number of bacterial isolates was found in containers stationed at the post-natal 7 (12.9%) and labour 6 (11.1%) wards (Table 3).
Table 4 reveals the antimicrobial susceptibility profile of bacterial isolates. The least effective antibiotics against Gram-negative bacterial isolates were Chloramphenicol and Cotrimoxazole. The most effective antibiotics against the isolates in this study were amikacin, ciprofloxacin and ceftriaxone. *E. coli* was the most susceptible to amikacin 11 (84.60%) among the Gram-negative isolates. None of the Gram-negative bacterial isolates was susceptible to tetracycline. *S. aureus* was highly susceptible to both Augmentin and Erythromycin but resistant to tetracycline. We observed that *Bacillus* species. were completely susceptible to all the antibiotics used, except that they demonstrated some resistance, 50% to cotrimoxazole. *Bacillus* species were not susceptible to ampicillin.

DISCUSSION

Augmented water receptacles as a source of healthcare-associated infection at the TTH were evaluated. The use of augmented water containers at the TTH became necessary due to periodic water shortages that hit the Tamale Metropolis and the hospital. Patients use the water for hand washing and cleaning of utensils. Whereas, other staff use the water for hand washing, cleaning of surfaces in the wards and theatres, the surgeons use the stored water for washing of hands before and after conducting surgical operations. In view of these invaluable uses, keeping the water in these receptacles clean and free of microbes is a necessity. This study sought to isolate

***Table 1*: Cleaning schedule of water receptacles**

Location of receptacles	Frequency of cleaning	Type of Detergent	Storage Type
Accident and Emergency (A &E) ward	Monthly	Omo(sodium sulfactant)	Opaque bucket
Antenatal clinic (ANC)	Weekly	Omo (sodium sulfactant)	Opaque bucket
Ear Nose and Throat/Urology ward	Weekly	Omo (sodium sulfactant)	Opaque bucket
Female medical ward	Monthly	Omo (sodium sulfactant)	Opaque bucket
Gynae theatre	Weekly	None	Opaque bucket
Gynaecology ward	Weekly	Omo (sodium sulfactant)	Opaque bucket
Intensive Care Unit	Weekly	Omo (sodium sulfactant)	Opaque bucket
Labour ward	Monthly	Omo (sodium sulfactant)	Opaque bucket
Main theatre	Twice weekly	None	Opaque bucket
Medical ward	Monthly	Omo(sodium sulfactant)	Opaque bucket
Neurosurgical ward	Weekly	Omo(sodium sulfactant)	Opaque bucket
Orthopaedic theatre	Weekly	Bleach (Hypochloride)	Opaque bucket
Paediatric ward	Monthly	None	Opaque bucket
Post-natal ward	Monthly	None	Opaque bucket
Septic ward	Twice weekly	Omo (sodium sulfactant)	Opaque bucket
Surgical ward	Monthly	Omo (sodium sulfactant)	Opaque bucket
Trauma orthopaedic ward	Twice weekly	Bleach (Hypochloride)	Opaque bucket and a plastic tank

Table 2: Demographic characteristics of orderlies

Variable	Frequency (%), N=16
Gender of Orderly	
Male	4 (25)
Female	12 (75)
Formal educational level	
None	1 (6.3)
Primary	9 (56.2)
Secondary	6 (37.5)
Level of knowledge on how to clean water receptacles	
Poor	4 (25)
Average	8 (50)
Good	4 (25)

micro-organisms in augmented water receptacles and demonstrated their susceptibility pattern. Data obtained should be of interest to the hospital authorities and could lead to change of policy on water storage.

Infection control entails cleaning and decontamination. Inadequate cleaning may enhance infection. To break the cycle of spread of infection in healthcare environments, daily and routine cleaning of touched surfaces is recommended (Health and Safety Executive, 2006; Hicks JD, 2020). The Public Health England guideline suggests that, water receptacles should be washed with hot water and detergents daily or twice daily. Such containers should be stored inverted when not in use and have their lids covered when in use (Public Health England, 2020). Dancer and Kramar also agreed that cleaning is essential so they described four steps required to clean a hospital and suggested that adoption of a systematic cleaning process could reduce healthcare-associated infections including outbreaks (Dancer & Kramer, 2019). Our study revealed that about three-quarters of the stations used detergents in cleaning the water receptacles whiles the remaining stations did not use detergents in cleaning the containers. Cleaning schedule of receptacles varied from ward to ward and ranged from weekly to monthly cleaning per receptacle. These varied and widely spaced cleaning schedules do not fit best practices as described in public health guidelines. This infrequent cleaning could leave these water receptacles heavily contaminated with pathogenic organisms and cause outbreak of water borne infections.

In this study, more than half the number of orderlies had primary education. This fairly low educational level of this category of workers could impact negatively on their level of knowledge, particularly their appreciation of infection prevention and control measures. When their level of knowledge on how to clean water receptacles was assessed, only about 25% of them had good knowledge. The remaining orderlies had average and poor knowledge on how to clean water receptacles. The higher the educational level, there is the possibility that one will be able to understand and carry out instructions concerning infection control. In our study, about 38% of the orderlies had secondary school education and are likely to be able to understand and carry out instructions. These same orderlies are likely to understand the in-service training programs on infection control carried out yearly in the hospital and will apply the knowledge learned diligently in cleaning the augmented containers. Staff education on various infection prevention control (IPC) measures in hospital environments are vital to meeting the requirement of limiting healthcare-related infections. The Asia Pacific Society of Infection Control (APSIC) guidelines for environmental cleaning and decontamination recommends that all aspects of environmental cleaning must be supervised and performed by knowledgeable, trained staff (Ling *et al*., 2015). Also, the guidelines recommended that healthcare facilities should include an environmental hygiene program as part of their infection control program.

Our study revealed that water receptacles at the TTH were heavily contaminated with pathogenic bacteria and not wholesome for the purpose of medical use. The highest number of bacteria isolates was found in containers stationed at the post-natal (12.9%) and labour wards (11.1%) respectively. The use of contaminated water from these containers for hand washing, cleaning of surfaces in the wards and in the case of the surgeons, for scrubbing, poses a risk of

Table 3: Distribution of bacterial isolates in receptacles in the various wards and operation theatre

Location of receptacles	BACTERIAL ISOLATES (Total N=54)						
	***Bacillus species* 2(3.7%) n(%)**	***Citrobacter species* 6(11.1%) n(%)**	***E. coli* 13 (24.1%) n (%)**	***Klebsiella species* 7(12.9%) n(%)**	***Legionella species* 5(9.3%) n (%)**	***Pseudomonas aeruginosa* 13(24.1%) n (%)**	***Staph. aureus* 8(14.8%) n(%)**
A&E ward	0(0.0)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	1(7.7)	0(0.0)
ANC	0(0.0)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	2(15.4)	0(0.0)
ENT/Urology ward	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(7.7)	1(12.5)
Female medical ward	0(0.0)	1(16.7)	1(7.7)	1(14.3)	0(0.0)	0(0.0)	0(0.0)
Gynaecology theatre	0(0.0)	0(0.0)	0(0.0)	1(14.3)	1(20.0)	0(0.0)	1(12.5)
Gynaecology ward	0(0.0)	0(0.0)	0(0.0)	1(14.3)	1(20.0)	0(0.0)	1(12.5)
ICU ward	1(50.0)	1(16.7)	1(7.7)	0(0.0)	0(0.0)	1(7.7)	1(12.5)
Labour ward	0(0.0)	0(0.0)	3(23.1)	2(28.5)	0(0.0)	0(0.0)	1(12.5)
Main theatre	0(0.0)	0(0.0)	1(7.7)	1(14.3)	0(0.0)	2(15.4)	1(12.5)
Male medical ward	0(0.0)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	1(7.7)	0(0.0)
Neurosurgical ward	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(7.7)	1(12.5)
Orthopaedic theatre	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(15.4)	0(0.0)
Paediatric ward	0(0.0)	3(50.0)	0(0.0)	1(14.3)	1(20)	1(7.7)	0(0.0)
Post-natal ward	0(0.0)	1(16.6)	2(15.3)	0(0.0)	2(40)	0(0.0)	0(0.0)
Septic ward	0(0.0)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Surgical ward	0(0.0)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Trauma & Orthopaedic ward	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(7.7)	1(12.5)

Key: ICU: Intensive Care Unit, ANC: Antenatal Clinic, ENT: Ear Nose and Throat, A&E: Accident and Emergency, SPP: Species

transmission of infection from staff to patients. Hand washing and cleaning of surfaces in the wards in particular would be compromised if done without detergents. In the setting of disruption of water supply, we recommend the use of sterile bottle water for hand washing to precede any sterile procedure. This is in line with best practices. The Center for Disease Control and Prevention (CDC) of United States of America. recognizes that potable water could be the source and reservoir of water borne pathogens such as *Pseudomonas* spp and *Legionella* spp. and therefore recommended that sterile bottled water should be used for surgical scrub, emergency surgical procedures, pharmaceutical procedures, and patient care equipment such as ventilators, when a situation of water supply disruption arises (Sehulster LM *et al*., 2003).

Supplemental treatment of hospital water systems with heat and/or a chemical is a proven strategy for controlling water borne microbial contamination in healthcare facilities. This study revealed that, the water receptacles were cleaned with detergents and water at room temperature at varied frequency. Following which they were filled with tap water from the main water supply system that had already been treated. Water hose and smaller buckets were used to transport water into the receptacles. This study did not establish if the process of transport into the receptacles was a potential source of contamination. There were however, no further demonstrable steps that were taken to either heat or chlorinate the stored water prior to use. Failure to institute supplemental treatment of stored water could leave these receptacles heavily contaminated with micro-organisms as several authors have suggested that supplemental treatment is a proven strategy. The main approaches to disinfection of potable water in healthcare facilities is heat flushing, using temperatures 71°– 77°C, hyperchlorination, and physical cleaning of hot-water tanks (American Society of Heating, Refrigerating, and Air-Conditioning Engineers, 2000; CDC, 1997; Mastro *et al*., 1991). As a consequence of recolonization, potable water require continuous intervention by raising water temperatures or chlorination (Ezzeddine *et al.,* 1989; Mastro *et al*., 1991). Sobsey and colleagues mentioned that chlorine disinfection and storage in an appropriate container significantly improved the microbiological quality of non-piped household drinking water (Sobsey *et al.,* 2003). Chlorination was aimed at achieving 1-2mg/L of free residual chlorine at the tap (Ezzeddine *et al*., 1989; Helms *et al*., 1988; Johnson *et al.,* 1985; T. J. Marrie *et al*., 1992; Thomas J. Marrie *et al*., 1991; Snyder *et al.,* 1990).

When distribution of bacterial isolates in these water receptacles were examined across the wards and theaters, *P. aeruginosa*, and *E. coli*, were the dominant bacteria isolates associated with augmented water receptacles at the TTH. *Pseudomonas aeruginosa*, a dominant bacterial isolate in our study with the potential of causing morbidity and mortality especially among immunosuppressed patients, has also been isolated in water supply systems of numerous hospitals across the world (Bert *et al.,* 1998; Eckmanns *et al.,* 2008; Ferroni *et al*., 1998; Loveday *et al*., 2014; Wong *et al*., 2011). *Pseudomonas aeruginosa* isolates are principally environmental organisms, and fresh surface water is an ideal reservoir of this bacteria (WHO, 2020). These organisms are not only found in hospitals but also in the domestic settings as well, according to de Victorica and colleagues. *Pseudomonas aeruginosa* was found in water distribution systems such as pipe lines and reservoirs in households in Mexico (de Victorica & Galván, 2001). Thus, in the domestic setting, *P. aeruginosa*, and *E. coli* found in the water supply system could account for varied illnesses such as pneumonia and gastroenteritis. *E. coli* is the most common member of faecal coliform, indigenous to the intestinal tract of humans and other warm-blooded animals (An *et al*., 2002). The detection of *E. coli* in hospital water shows a possible contamination from faecal matter (Lowry *et al*., 1991). *Legionella* spp known to cause pneumonia was isolated in both ice and heated water systems in hospitals, in the USA and Italy (Graman et al., 1997; Schuetz et al., 2009; Visca et al., 1999). We found *Legionella* spp was the least prevalent Gram-negative isolate in our study. Located in warm tropical climate, the TTH does not use ice and heated water systems, which are

known to promote the growth of *Legionella* spp. Hence the low prevalence of *Legionella* spp. recorded in our study.

A total of 54 isolates with different bacteria genera from 35 samples were obtained which demonstrated that one or more isolates were found in the augmented water containers in the current study. The heavy contamination of potable water could have happened in the course of filling up the receptacles using water hose or by means of smaller receptacles as has been the practice at the TTH. We found *S. aureus* in the water receptacles as well. It has been shown by several authors that unhygienic handling practices at the consumer points of chlorinated water may result in cross-contamination of the already disinfected water with pathogenic organisms such as *Staphylococcus aureus* (Ahiablame *et al*., 2012; Anyamene & Ojiagu, 2014; Onyango *et al*., 2018). In terms of distribution of bacterial isolates with respect to labelled water receptacles, we found *E. coli* was most prevalent in the Obstetrics and Gynaecological Department, specifically in the labour and postnatal wards. Since *E. coli* is of enteric origin, our finding suggests that there could be faecal contamination of the water receptacles at the labour and postnatal wards. Masoumeh and colleagues reported that *E. coli* contaminated warm water at a prevalence of 6.2% in a hospital in Gilan, Iran (Masoumeh *et al*., 2015). We also demonstrated in our study that *P. aeruginosa* was prevalent in the containers of the antenatal clinic, male medical and post-natal wards. This aspect of our work agrees with an already established fact that *Pseudomonas* species are a major cause of healthcare facility-based infection (Bert *et al*., 1998; Buttery *et al*., 1998; Grundmann *et al*., 1993; Kolmos *et al*., 1993; Richard *et al.,* 1994; Trautmann *et al*., 2001).

Citrobacter species was the highest implicated pathogenic organism in the augmented water containers in the paediatric ward of TTH representing 50%. *Citrobacter* species are of enteric origin (Kus & Burrows, 2007; Rogers *et al*., 2016). Hence, we attribute the heavy contamination of the receptacles at the paediatric ward to faecal contamination of the water receptacles and if this is not handled carefully it could result in an outbreak of water borne infection in the ward. An outbreak, caused by *Citrobacter* species in the paediatric wards, resulting in increased morbidity, prolonged hospital stay, with associated mortality has been reported (Crawford & Daum, 2008; Donnenberg, 2015; Palazzi *et al*., 2006; Powell & Marcon, 2012).

Of the fourteen (14) antibiotics tested, all the bacterial isolates demonstrated variable susceptibility. Isolated *Pseudomonas aeruginosa* from these receptacles demonstrated sensitivity to amikacin (76.9%), ciprofloxacin (69.2%), ceftriaxone (61.5%) and gentamicin (30.6%) respectively. The *Pseudomonas* isolates did not demonstrate sensitivity to ampicillin, cotrimoxazole, cefuroxime and chloramphenicol. It has already been demonstrated that *Pseudomonas aeruginosa* developed resistance to these antibiotics (Bert *et al*., 1998; Buttery *et al*., 1998; Delgado-Gardea *et al*., 2016). We also found that, *E. coli* showed complete sensitivity to ciprofloxacin, ceftriaxone and cefuroxime but varied susceptibility to amikacin, gentamicin, ampicillin, cotrimoxazole and chloramphenicol. The other bacterial isolates in this study namely *Citrobacter* species, *Legionella* species, *Klebsiella* species, *S. aureus* and *Bacillus* species all demonstrated varied susceptibility to antimicrobials. Should there be an outbreak of water borne infections caused by these organisms at the TTH, the drug may not be reliable in empirical treatment. Thus, newer and more expensive antibiotics such as meropenem, piperacillin/tazobactam may be recommended. Resistance to antimicrobial agents by microbes have been recognized as an emerging worldwide problem in both human and veterinary medicine (Cliford K *et al*., 2018; Woolhouse *et al*., 2015). The CDC of USA agrees that antibiotic resistance threatens everyone (CDC, 2020). The use of antimicrobial agents have been considered the most important factor for the emergence, selection, and dissemination of antimicrobial resistant bacteria (Sayah *et al.,* 2005). The evolving multidrug resistant characteristics of these bacterial isolates have been demonstrated at different jurisdictions across the globe (Breurec *et al.,* 2011; De Giglio *et al*., 2015; Howden *et al.,* 2010; Metri *et al*., 2011; Namaki *et al*., 2019; Osundiya *et al*., 2013)

There were some limitations to this study. In the first place, the findings cannot be generalized to include the entire water supply system of the

hospital because some sources of water such as those from the taps were excluded. In addition, not all wards were evaluated. Secondly, these findings cannot be extrapolated to include all hospitals in the Tamale Municipality, because the hospitals do not use the same methods of storing water and do not use the same orderlies with the same levels of education. Thirdly, the study was carried out within three months of the year, March to May, which mark the beginning of the raining season in Northern Ghana. Thus, the study could not account for seasonal variations particularly during the dry season. Fourthly, the exact duration of stored water preceding sample collection was not established. Fifthly, the sample we worked with was not water samples but rather a swabbed biofilm of the wall of water receptacles (containers) in the various wards of the hospital. The microbial load in each container was not established and hence this omission is considered a limitation. Finally, we did not analyze water from the main supply as control sample.

We recommend a prospective study involving the whole year, and all the receptacles and tanks in the hospital to get more information on the various variables in this study. Also, this follow up study should happen after the orderlies are educated on the proper way to maintain these receptacles. It will be interesting to see how education can influence the orderlies in maintaining safe water for clinical use. In addition, we recommend the use of sterile bottled water for use whenever there is disruption of potable water supply in critical areas such as: surgeon scrub areas of the operating theatres, all intensive care units of the hospital, and scrub areas in emergency ward where emergency procedures are performed. Also, sterile bottled water should be available for cleaning of patients and care for equipment such as ventilators, dialysis machines and others.

CONCLUSION

Augmented water receptacles at the TTH are potential reservoirs for bacterial transmission to users and therefore a potential source of healthcare-associated infections. The cleaning schedules for these containers varied from unit to unit and was carried out at irregular intervals. This, together with the multidrug-resistant nature of the bacteria isolates, should be a source of worry for the hospital authorities. Adherence to the principles of cleaning and disinfection outlined in public health guidelines could eliminate a potential outbreak of serious bacterial infections at the TTH.

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