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Antibiotic Resistance Patterns of Bacteria Isolated from Dechlorinated Water Samples

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Abstract

Chlorination has been the method of choice for disinfecting water used for drinking purposes. However, some stressed bacteria during chlorination are able to recover and alter the potability of water. This study assessed the recovery of stressed bacteria in dechlorinated water. Ten chlorinated water samples were collected from different points within Ilorin metropolis, Kwara, Nigeria. The samples (100ml) were dechlorinated with 0.1ml of 11.4mM sodium thiosulphate solution. The physicochemical characteristics of the chlorinated water samples were determined while bacteriological analyses were carried out on both chlorinated and dechlorinated water samples. The antibiotic susceptibility pattern of the isolates was determined using disc diffusion method. The physicochemical characteristics of the water samples ranged as follow: pH 7.3-8.4, chloride content 4.37-6.85 mg/l, suspended solids 0.004-0.017 g/100ml, and total hardness 30-72mg/l. The chlorinated water samples had bacterial, total, and faecal coliform counts ranging from $1.0 \times 10^1 - 1.9 \times 10^4$ cfu/ml, 0 – 480 MPN/100ml, and zero, respectively. The dechlorinated water sample had corresponding counts of $5.4 \times 10^2 - 7.36 \times 10^4$ cfu/ml, 6 - 1100 MPN/100ml, and 0 – 380 MPN/100ml. A total of eleven bacterial species belonging to the genera *Bacillus*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Staphylococcus*, *Serratia*, and *Streptococcus* were isolated. Not lower than 60% of the bacterial isolates were susceptible to ofloxacin and ciprofloxacin. All the isolates exhibited multiple antibiotic resistances. The antibiotic resistance pattern of an isolate of *Citrobacter freundii* to cefuroxime, cefixime, and gentamycin changed remarkably and was plasmid-mediated, while that of *E. coli* and *Enterobacter agglomerans* remained unchanged to all the antibiotics and was non-plasmid mediated. Chlorination of water at the point of use is recommended. It is concluded that chlorination is essential in order to prevent reactivation of stressed bacteria during distribution and prevent infection by bacteria with high multiple antibiotic resistance index.

Keywords: Stressed bacteria, dechlorination, recovery, water.

Introduction

The provision of safe drinking water relies on its prevention from contamination and disinfection from pathogens [1]. Ensuring the microbiological safety of water involves the use of multiple barriers from the treatment plant to the distribution network and the end users [2].

Disinfection of drinking water supplies is a critical requirement in the provision of safe drinking water [3]. Chlorination is commonly used for the disinfection of water meant for the pipeline distribution network because it is highly effective in destroying pathogens and has residual disinfection effects [4]. The degree of metabolic activities of microorganisms affects their susceptibility to chlorine disinfection [5].

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Assessment of the microbial quality of drinking water is mostly achieved by analyzing for faecal coliform or *E. coli* [2]. The quality of drinking water supply is expected to be maintained, starting from the water treatment plant to the point of consumption [5]. However, its quality may deteriorate during distribution due to recontamination or regrowth of stressed or injured bacteria [6-7]. It has been reported that the majority of indicator bacteria in water may not be detected due to chlorination stress, thereby leading to a false-negative result of bacteriological analysis [1].

With the emergence of bacterial regrowth in water distribution systems, it became necessary to validate the effectiveness of the disinfection process and, by extension, the possibility of regrowth occurring along the distribution network in the interest of public health.

The aims of this study were to determine the physicochemical characteristics of the chlorinated water; compare chlorinated and dechlorinated samples in terms of the bacterial, total coliform, and faecal coliform counts; and determine the antimicrobial susceptibility patterns as well as the plasmid curing profile of the resistant bacteria.

Materials and methods

Collection of chlorinated water samples

Chlorinated samples were collected from ten different taps within the distribution network in Ilorin metropolis using standard methods [2]. The samples were collected into sterile sampling bottles in an ice chest for immediate analysis [8].

Dechlorination of the water samples

Chlorinated water samples were dechlorinated by the addition of 0.1ml of sterile 11.4mM sodium thiosulphate solution to 100ml the sample. The water samples were then allowed to stand for 24 hours at room temperature. This served as dechlorinated water sample [9-10].

Determination of physicochemical characteristics of the water samples

The pH, total chloride, total hardness, and the suspended solid contents of the water samples were determined using standard methods [11].

Determination of bacteriological counts of the water samples

The counts of viable bacteria were enumerated using pour plate technique with nutrient agar as a medium for isolation. The total coliform count was estimated using the multiple tube fermentation technique with MacConkey broth as the selective medium. The faecal coliform count was isolated using the spread plate method and eosin methylene blue agar medium for cultivation [12].

Isolation and counting of pathogenic bacteria

The spread plate method was used for the selective isolation of pathogenic bacteria. *Escherichia coli* 0157:H7, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Salmonella* and *Shigella* species were isolated using Sorbitol MacConkey agar (SMAC) supplemented with cefixime-tellurite, cetrinide agar (CA), Slanetz and Bartley agar (SBA), and xylose lysine deoxycholate (XLD) agar, respectively. An aliquot (0.5ml) of the chlorinated and dechlorinated water samples were inoculated into the respective media and the inoculum was then spread evenly with sterile glass spreader. All the plates were incubated at 37°C for 24 hours. The population of colonies with typical growth characteristic for each medium was counted and expressed in cfu/ml [13-14].

Purification and preservation of bacterial isolates

The bacteria were subcultured on nutrient agar plates until a pure culture was obtained. The pure cultures were transferred into nutrient broth-glycerol mixture and stored at 4°C in a refrigerator until they were needed [12].

Characterization and identification of bacterial isolates

The cellular and biochemical characteristics of the isolates were determined. The Gram positive bacteria were identified based on a laboratory manual [14] as well as an online bacterial identification platform [15], while the Gram negative bacteria were identified using Oxoid Microbact™ 24E Gram negative bacilli kit [16].

Antimicrobial susceptibility test

The bacteria were standardized using 0.5 McFarland standard cultures [13] which typically contain 1.5×10^8 cfu/ml. The standardized cultures were spread on the sterile set plates of Mueller Hinton agar in order to obtain a confluent growth. The multiple antibiotic discs were placed on the agar and incubated at 37°C for 24 hours. Then, the diameter of the inhibition zones were measured and recorded. The results obtained were interpreted according to a previously described approach [17]. The rapid labs antibiotic discs which were used for the susceptibility tests were CM-12-PR100 and

CM-12-NR100. The sensitivity of Gram positive bacteria to vancomycin was determined using oxoid single vancomycin disc. Gram negative isolates with a Multiple Antibiotics Resistance (MAR) index value of 0.75 and above were selected for plasmid curing.

Multiple Antibiotics Resistance index

MAR index of each isolate was calculated by dividing the number of antibiotics to which the microorganism was resistant to by the total number of antibiotics to which the isolate was tested [18].

Plasmid curing of the bacterial isolates

Plasmid curing of the bacterial isolate was tested based on a previously described method [19]. Acridine orange solution (1mg/ml) was prepared by adding 0.38mM (0.1g) of acridine powder into 100ml of distilled water. It was then filtered using 0.45µm millipore filter. This served as a stock solution of acridine orange, which was sterilized and 1ml of it was added to 10 ml of cooled, sterile nutrient broth to give a concentration of 0.1mg/ml. The bacterial isolates (0.1ml) were inoculated into the acridine orange nutrient broths and placed on a rotary shaker at 120 rpm and 37°C. After 48 hours of incubation at 37°C, the organisms were freed from the chemical by subculturing onto sterile nutrient agar slants and incubated at 37°C. These isolates were tested for antimicrobial susceptibility pattern as described above.

Statistical analysis

The mean of the data obtained for each parameter was subjected to statistical analysis using SPSS 15.0 statistical package. The means of the data were separated using Duncan's multiple range tests. Furthermore, paired students' T-test was used to compare if there is any significant difference in the same sample after treatment [20].

Results

Physicochemical characteristic of the water samples

The physicochemical characteristics of the water samples ranged as follow: pH 7.3 – 8.4, total chloride 4.37 – 6.85 mg/l, total hardness 30 – 72 mg/l, and suspended solids 0.004 - 0.017 g/100ml (Table-1).

Table 1-Physicochemical properties of the chlorinated water samples

Samples	pH	Total chloride content (mg/l)	Total hardness (mg/l)	Suspended solids (g/100ml)
SP1	7.3 ^a ± 0.2	5.33 ^{cd} ± 0.3	58 ^b ± 3.0	0.004 ^a ± 0.001
SP2	7.6 ^{abcd} ± 0.2	6.85 ^e ± 0.35	59 ^b ± 4.0	0.008 ^{ab} ± 0.002
SP3	8.4 ^e ± 0.3	5.57 ^d ± 0.43	69 ^{cd} ± 1.0	0.008 ^{ab} ± 0.001
SP4	7.5 ^{abc} ± 0.0	4.62 ^b ± 0.28	65 ^c ± 4.0	0.017 ^c ± 0.005
SP5	7.8 ^{bcd} ± 0.3	4.86 ^{abc} ± 0.31	69 ^{cd} ± 4.0	0.015 ^c ± 0.003
SP6	7.9 ^{cd} ± 0.4	4.72 ^{ab} ± 0.34	72 ^d ± 2.0	0.008 ^{ab} ± 0.002
SP7	7.4 ^{ab} ± 0.2	5.54 ^d ± 0.34	30 ^a ± 3.0	0.013 ^{bc} ± 0.003
SP8	7.5 ^{abc} ± 0.0	5.25 ^{bcd} ± 0.25	32 ^a ± 1.0	0.013 ^{bc} ± 0.005
SP9	7.4 ^{ab} ± 0.1	4.37 ^a ± 0.32	33 ^a ± 2.0	0.013 ^{bc} ± 0.002
SP10	8.0 ^d ± 0.2	4.69 ^{ad} ± 0.28	32 ^a ± 2.0	0.008 ^{ab} ± 0.001

SP = Sampling points. Values are means of 3 replicates. Means with the same superscript along each column are not significantly different based on Duncan's multiple range test at $\alpha = 0.05$.

Bacteriological characteristics of the water samples

The bacterial count of dechlorinated water (7.36×10^4 cfu/ml) was higher than that of the chlorinated water (1.9×10^4 cfu/ml). The total coliform count of the chlorinated water samples ranged 0 – 480 MPN/100ml, while that of dechlorinated water ranged 6 – 1100 MPN/100ml. Although no faecal coliform was recovered from the chlorinated water samples, the faecal counts of the dechlorinated water samples ranged 0 – 380 MPN/100ml (Table-2).

Table 2-Bacteriological characteristics of chlorinated and dechlorinated water samples

Samples	Bacterial count (cfu/ml)		Faecal coliform count (cfu/ml)		Total coliform count (MPN/100ml)	
	Cl	Dc	Cl	Dc	Cl	Dc
SP1	$1.0^a \times 10^1$	$5.4^b \times 10^2$	0 ^a	0 ^a	0 ^a	7 ^b
SP2	$1.8^a \times 10^1$	$6.0^b \times 10^3$	0 ^a	22 ^b	0 ^a	6 ^b
SP3	$5.2^a \times 10^2$	$1.14^b \times 10^3$	0 ^a	0 ^a	0 ^a	6 ^b
SP4	$1.62^a \times 10^3$	$7.36^b \times 10^4$	0 ^a	0 ^a	4 ^a	21 ^b
SP5	$5.04^a \times 10^2$	$1.76^b \times 10^4$	0 ^a	106 ^b	9 ^a	21 ^b
SP6	$6.36^a \times 10^2$	$1.06^b \times 10^4$	0 ^a	186 ^b	6 ^a	15 ^b
SP7	$1.82^a \times 10^3$	$6.0^b \times 10^4$	0 ^a	380 ^b	0 ^a	7 ^b
SP8	$1.76^a \times 10^3$	$5.72^b \times 10^4$	0 ^a	0 ^a	0 ^a	15 ^b
SP9	$1.90^a \times 10^4$	$5.04^b \times 10^4$	0 ^a	40 ^b	0 ^a	6 ^b
SP10	$1.16^a \times 10^3$	$4.36^b \times 10^4$	0 ^a	24 ^b	480 ^a	1100 ^b

SP = sampling points, cfu = colony forming unit, Cl = chlorinated water sample, Dc = dechlorinated water sample. Mean values of each parameter followed by the different alphabets for the same sample are significantly different at $\alpha = 0.05$ using paired T-test.

Counts of pathogenic bacteria

Counts of pathogenic bacteria were determined for chlorinated (Cl) and dechlorinated (Dc) water samples. The results revealed that *E. coli* O157:H7, *Salmonella*, and *Shigella* sp. were not isolated in any of the samples. However, *Enterococcus faecalis* and *P. aeruginosa* were found in the dechlorinated water samples.

Table 3-Counts of pathogenic bacteria in the water samples collected within Ilorin metropolis, Nigeria.

Sampling points	Counts (cfu/ml)							
	<i>E. coli</i> O157:H7		<i>Enterococcus faecalis</i>		<i>Pseudomonas aeruginosa</i>		<i>Salmonella/Shigella</i> spp.	
	Cl	Dc	Cl	Dc	Cl	Dc	Cl	Dc
SP1	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
SP2	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
SP3	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
SP4	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
SP5	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
SP6	0 ^a	0 ^a	0 ^a	2 ^b	0 ^a	0 ^a	0 ^a	0 ^a
SP7	0 ^a	0 ^a	0 ^a	4 ^b	0 ^a	0 ^a	0 ^a	0 ^a
SP8	0 ^a	0 ^a	0 ^a	100 ^b	0 ^a	0 ^a	0 ^a	0 ^a
SP9	0 ^a	0 ^a	0 ^a	2 ^b	0 ^a	0 ^a	0 ^a	0 ^a
SP10	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	2 ^b	0 ^a	0 ^a

SP = Sampling Point, Cl = Chlorinated water sample, Dc = Dechlorinated water sample, Mean values of each parameter followed by the different alphabets for the same sample are significantly different at $\alpha = 0.05$ using paired T-test

Characterization and identification of bacterial isolates

A total of eleven bacterial species were identified, including *Bacillus* sp., *Bacillus pumilus*, *Burkholderia pseudomallei*, *Citrobacter freundii*, *Citrobacter gilleni*, *Enterobacter agglomerans*, *Enterococcus mundtii*, *Escherichia coli*, *Serratia marcescens*, *Staphylococcus xylosum*, and *Streptococcus rifensis* (Table- 4).

Table 4-Characterization and identification of bacterial isolates

Bacterial isolates	Gram reaction	Cell shape	Motility	Oxidase	Catalase	Starch hydrolysis	Citrate	Indole	Urease	Glucose	Lactose	Sucrose	Maltose	Mannitol	Raffinose	VP	Nitrate reduction	Cellobiose	Trehalose	MR	OF	Fructose	Galactose	D-Sorbitol	PD	Rhamnose	Glycerol	D-Xylose	Inositol	Adonitol	Arabinose	Probable identity
1	-	R	+	+	-	-	+	-	+	A	A	A	A	A	-	-	-	A	A	-	F	A	A	A	+	-	-	-	-	-	-	<i>Serratia marcescens</i>
2	-	R	+	+	+	-	+	-	-	A	A	A	A	A	-	-	-	-	-	+	F	A	A	A	-	-	A	A	A	-	-	<i>Burkholderia pseudomallei</i>
3	-	R	+	-	-	-	+	-	+	A	A	A	A	A	-	-	-	-	A	+	F	-	A	A	-	A	A	A	-	-	-	<i>Citrobacter freundii</i> n=1
4	+	C	-	-	-	-	-	+	+	A	A	A	A	A	-	-	-	-	-	-	F	-	A	A	-	A	-	A	-	-	-	<i>Enterococcus mundtii</i>
5	+	C	-	-	+	-	-	+	+	A	A	-	A	A	-	-	-	-	A	-	F	A	A	A	-	-	-	A	-	-	-	<i>Streptococcus rifensis</i>
6	-	R	+	-	+	-	+	-	+	A	A	A	A	A	-	-	-	-	A	-	F	A	A	A	-	A	-	A	-	-	-	<i>Citrobacter freundii</i> n=2
7	-	R	+	-	+	-	-	-	-	A	A	A	A	-	-	-	-	-	A	+	F	A	A	A	+	A	-	A	-	-	-	<i>Citrobacter gilleni</i>
8	-	R	-	-	+	-	+	-	+	A	A	A	A	A	-	-	-	-	A	+	F	A	A	A	+	A	-	A	-	A	A	<i>Citrobacter freundii</i> n=3
9	+	R	+	-	+	-	+	-	-	A	A	A	A	-	A	+	-	-	A	-	F	A	-	-	-	-	-	A	-	-	-	<i>Bacillus</i> sp.
10	+	C	-	+	+	-	+	-	+	A	A	A	A	A	-	-	-	-	A	+	F	A	-	-	-	A	A	A	-	-	-	<i>Staphylococcus xylosum</i>
11	+	R	+	-	-	-	+	-	-	A	A	A	A	A	-	+	-	-	A	-	F	A	-	A	-	-	-	A	-	-	-	<i>Bacillus pumilus</i>
12	-	R	-	-	-	-	+	+	-	A	A	A	A	A	-	-	-	-	-	+	F	A	A	A	-	A	-	A	-	-	-	<i>Citrobacter freundii</i> n=4
13	-	R	+	-	-	-	+	+	-	A	A	A	A	A	-	-	-	-	A	+	F	A	A	A	-	A	A	A	-	-	-	<i>Enterobacter agglomerans</i> n=1
14	-	R	+	-	+	-	-	+	+	A	A	-	A	A	-	-	-	-	A	+	F	A	A	A	-	A	-	A	-	-	-	<i>Escherichia coli</i>
15	-	R	+	-	+	-	-	-	+	A	A	-	A	A	-	+	-	-	A	+	F	A	A	A	-	A	A	A	-	A	-	<i>Enterobacter agglomerans</i> n=2

- = Negative reaction; + = Positive reaction; C = Cocci; R = Rod; F = Fermentative; OF = Oxidation-Fermentation; VP = Voges Proskauer; MR = Methyl red test; A= Acid production; AG = Acid and gas production; n= number of the same species isolated.

Antibiotics susceptibility profiles of the bacterial isolates

All the isolated Gram negative bacteria were resistant to Ceftazidime, Cefuroxime, Cefixime and Augmentin. Furthermore, all the Gram positive bacteria isolated were resistant to ceftazidime, Cefuroxime, Ceftriaxone, Cloxicillin, and Augmentin (Tables- 5 and 6).

Table 5-Antibiotics susceptibility pattern of Gram negative bacterial isolates before plasmid curing

Gram negative isolates	Zone of inhibition (mm)								MAR Index
	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	
<i>Citrobacter freundii</i> n=1	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	1.0
<i>Citrobacter freundii</i> n=2	0(R)	0(R)	12(R)	0(R)	22(S)	0(R)	0(R)	22(S)	0.75
<i>Citrobacter freundii</i> n=3	0(R)	0(R)	0(R)	0(R)	26(S)	0(R)	0(R)	26(S)	0.75
<i>Citrobacter freundii</i> n=4	0(R)	0(R)	6(R)	0(R)	24(S)	0(R)	0(R)	24(S)	0.75
<i>Enterobacter agglomerans</i> n=1	0(R)	0(R)	0(R)	0(R)	24(S)	0(R)	0(R)	32(S)	0.75
<i>Enterobacter agglomerans</i> n=2	0(R)	0(R)	0(R)	0(R)	22(S)	0(R)	0(R)	24(S)	0.75
<i>Serratia marcescens</i>	0(R)	0(R)	16(S)	0(R)	12(R)	0(R)	21(S)	22(S)	0.63
<i>Burkholderia pseudomallei</i>	0(R)	0(R)	15(S)	0(R)	24(S)	0(R)	20(S)	24(S)	0.5
<i>Escherichia coli</i>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	24(S)	0.88
<i>Citrobacter gilleni</i>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	1.0

R = Resistant, S= Susceptible, CAZ = Ceftazidime 30µg, CRX = Cefuroxime 30µg, GEN = Gentamycin 10µg, CXM = Cefixime 5µg, OFL = Ofloxacin 5µg, AUG = Amoxicillin/Clavulinate 30µg, CPR = Ciprofloxacin 5µg, NIT = Nitrofurantoin 300µg, n= number of the same species isolated.

Table 6-Antibiotics susceptibility patterns of Gram positive bacterial isolates

Gram positive isolates	Zone of inhibition (mm)									MAR index
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	VAN	
<i>Staphylococcus xylosus</i>	0(R)	0(R)	16(S)	17(I)	0(R)	0(R)	21(S)	0(R)	0(R)	0.67
<i>Bacillus</i> sp.	0(R)	0(R)	8(R)	0(R)	19(I)	0(R)	20(S)	0(R)	21(S)	0.67
<i>Bacillus pumilus</i>	0(R)	0(R)	0(R)	0(R)	13(R)	0(R)	20(S)	0(R)	11(R)	0.89

<i>Streptococcus rifensis</i>	0(R)	0(R)	18(S)	12(R)	0(R)	0(R)	20(S)	0(R)	0(R)	0.78
<i>Enterococcus mundtii</i>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	6(R)	0(R)	0(R)	1.0

R= Resistant; S= Susceptible, I= Intermediate, CAZ=Ceftazidime 30µg; CRX=Cefuroxime 30µg; GEN= Gentamycin 10µg; CTR=Ceftriaxone 30µg; ERY=Erythromycin 5µg, CXC= Cloxicillin 5µg; OFL=Ofloxacin 5µg; AUG= Amoxicillin/Clavulinate 30µg; VAN=Vancomycin 30 µg

Antibiotic resistance patterns after plasmid curing

The isolates were generally resistant to the antibiotics investigated, with all of them having MAR index values of >0.2. There was no change in the MAR index of *E. coli* and *Enterobacter agglomerans*. However, the resistance of the isolate of *Citrobacter freundii* to cefuroxime, cefixime and Gentamycin changed after plasmid curing. Furthermore, *Citrobacter gilleni* was susceptible to Augmentin and Nitrofuratoin after plasmid curing (Table-7).

Table 7-Antibiotics susceptibility patterns of Gram negative bacterial isolates after plasmid curing.

Gram negative isolates	Diameter of zone of inhibition (mm)								MAR Index
	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	
<i>Citrobacter freundii</i> n=3	10(R)	20(S)	20(S)	24(S)	10(R)	0(R)	0(R)	^{22(S)}	0.5
<i>Escherichia coli</i>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	24(S)	0.88
<i>Citrobacter freundii</i> n=1	22(S)	0(R)	11(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0.88
<i>Enterobacter agglomerans</i> n=1	0(R)	0(R)	0(R)	0(R)	20(S)	0(R)	0(R)	24(S)	0.75
<i>Citrobacter gilleni</i>	0(R)	0(R)	0(R)	0(R)	0(R)	20(S)	22(S)	0(R)	0.75

R = Resistant, CAZ = Ceftazidime 30µg CRX = Cefuroxime 30µg, GEN = Gentamycin 10µg, CXM = Cefixime 5µg, OFL = Ofloxacin 5µg, AUG = Amoxicillin/Clavulinate 30µg, CPR = Ciprofloxacin 5µg.

Discussion

The pH of water affects the growth and survival of microorganisms as well as the effectiveness of chlorine disinfection. The range of pH obtained in the chlorinated water samples in this study was within the limit of 6.5 – 8.5, which is suitable for the proliferation of bacteria if microbial contamination occurs along the distribution network [2]. A similar study [6] also obtained pH values of 7.0 - 7.9 during testing treated water samples in Ilorin metropolis.

The presence of suspended solids in water could shield microorganisms from the inhibitory activity of chemical disinfectants during water treatment and in the distribution network [1]. The suspended solids of the water samples in this study ranged 0.004 - 0.017g/100ml (equivalent to 40 – 170 mg/l), which is within the standard limit of 500mg/l [21].

Chloride in drinking water is mostly derived from natural sources, industrial effluents, urban runoff containing salty components, dissolution of soluble chlorides of rock minerals, and disinfectants [5].

Excess chloride in water distribution channels may lead to the precipitation of metals in the pipelines [3]. Microorganisms can attach to these precipitated metals and are thus protected from the action of disinfectants [1]. These precipitated metals may impart taste to the water [2]. Nevertheless, the chloride content of the water samples found in this study is lower than the allowed limit of 250 mg/l [21].

The total hardness of the drinking water affects its organoleptic and aesthetic acceptability [22]. The water samples were found to be 60% soft (≤ 60 mg/l) and 40% moderately hard (61 - 120 mg/l). The hardness of waters is due to the effects of the dissolved salts, primarily calcium and magnesium.

Only two of the water samples met the standard of a bacterial count lower than 100 cfu/ml [23]. The high count of bacteria in the dechlorinated water suggests the recovery of bacteria stressed by the presence of chlorine. It also suggests failure of disinfection and/or recontamination of the treated water as the distance from the treatment plant is increased [7]. A previous study [24] showed a bacterial count in the range of 1.0×10^2 - 2.6×10^4 cfu/ml.

Counts of total coliform bacteria detected in the dechlorinated water samples were higher than those in the chlorinated water samples in all the water samples. Total coliform was not found in 60% of the chlorinated water samples. Furthermore, faecal coliforms were not found in all the chlorinated water samples. It is concluded that dechlorination might result in the reactivation of the stressed bacteria in 60% of the water samples. In a previous study, total coliform and faecal coliform ranges of 0 - 248 MPN/100ml and 0 - 128 cfu/100ml, respectively, were obtained upon the investigation of the effect of chlorination on the bacteriological quality of water in the distribution system [25].

The bacterial species found in this study were *Bacillus* sp., *B. pumilus*, *Burkholderia pseudomallei*, *Citrobacter freundii*, *Citrobacter gilleni*, *Enterobacter agglomerans*, *Enterococcus mundtii*, *Escherichia coli*, *Staphylococcus xylosum*, *Serratia marcescens* and *Streptococcus rifensis*. This result is in consistence with those reported by previous studies [8, 10], where *Bacillus*, *Citrobacter*, *Enterobacter*, *Staphylococcus*, and *Serratia* were part of the bacteria isolated.

All the isolates were resistant to multiple antibiotics (MAR >0.2). At least 60% of the bacteria were susceptible to inhibition by Ofloxacin and Ciprofloxacin. The MAR index of the two *Citrobacter freundii* isolates changed from 0.75 to 0.50 and 1.0 to 0.88, respectively, after plasmid curing. This showed that the resistant factors to some of the antibiotics could be plasmid-mediated. The resistance of *E. coli* and *Enterobacter agglomerans* remains unchanged after plasmid curing. This indicates that their resistance to these antibiotics was non-plasmid mediated. Furthermore, the resistance of *Citrobacter gilleni* to augmentin and nitrofurantoin appeared to be plasmid borne. It was earlier observed that many Gram negative bacilli, such as *Burkholderia* sp., *Citrobacter* sp., and *Serratia marcescens*, have an extended spectrum of antibiotic resistance [22]. The emergence of multiple antibiotic resistant microorganisms in water is an important public health challenge, as it may result in infections that could be difficult to manage.

The recovery of stressed organisms in water poses a serious danger to health of consumers, as the organisms often retain their ability to cause disease, which may be expressed in susceptible individuals.

Conclusions

Most of the chlorinated water samples used in this study (80%) was found to have lower quality than that specified by the microbiological standards required for potability. In spite of this, chlorination may have effects in preventing the increase in the total coliform and faecal coliform content. Dechlorination may have impacts on the reactivation of the chlorine-stressed bacteria, as evident by their high counts. The resistance factors of *E. coli* and *Enterobacter agglomerans* to most of the antibiotics were non-plasmid mediated, while the resistance of an isolate of *Citrobacter freundii* to Cefuroxime, cefixime, and gentamycin could be plasmid mediated.

There is need for regular testing of water treatment plants and distribution conditions for the levels of certain materials, such as organic matter, in order to prevent the regrowth of bacteria in the system. The use of pretreatments, such as sand filtration, will reduce the amount of materials that may interfere with chlorine disinfection. The use of disinfectants, such as WaterGuard, at the point of use will prevent the regrowth of stressed bacteria. There is a need to use adequate amounts of chlorine during disinfection in order to maintain residual disinfection effects.

References

1. Rajnochova, M., Tuhovcak, L., and Rucka, J. **2018**. Drinking water supply without use of a disinfectant. E3S Web of Conferences 30, 01003. <https://doi.org/10.1051/e3sconf/20183001003>
2. WHO **2017**. World Health Organization Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum. Geneva, 541pp.
3. van Bel, N., Hornstra, L.M., van der Veen, A., and Medema G. **2019**. Efficacy of flushing and chlorination in removing microorganisms from a pilot drinking water distribution system. *Water* **11**(903): 2-18. Doi:10.3390/w11050903
4. Bertelli, C., Courtois, S., Rosikiewicz, M., Piriou P., Aeby, S., Robert, S., Loret, J-F, Greub, G. **2018**. Reduced chlorine in drinking water distribution systems impacts bacterial biodiversity in biofilms. *Frontiers in Microbiology*, **9**: 2520. Doi:10.3389/fmicb.2018.02520
5. Prest, E.I., Weissbrodt, D.G., Hammes, F., van Loosdrecht, M.C.M., and Vrouwenvelder, J.S. **2016**. Long term bacterial dynamics in a full scale drinking water distribution system. *Plos One* **11**(10): e0164445. <https://doi.org/10.1371/journal.pone.0164445>
6. Agbabiaka, T.O., Sule, I.O. and Oyeyiola, G.P. **2014**. Spatial Assessment of Public Water Supplies in Densely Populated Areas of Ilorin Metropolis, Kwara State, Nigeria. *Fountain Journal of Natural and Applied Sciences*, **3**(1): 20-28.
7. Eniola, K.I.T., Odaibo, A.D., Olayemi, A.B. and Ajiboye, T.O. **2015**. Bacteriological Assessment of Treated Piped Waters in Parts of Ilorin Metropolis. *Nigerian Journal of Microbiology*, **28**:2797-2803.
8. Kolawole, O.M., Olayemi, A., and Jimoh, F. **2016**. Distribution profile of extended spectrum Beta lactamase (ESBL) producing *Escherichia coli* isolates from Asa River Nigeria. *J. Appl. Sci. Environ. Manag.* **20**(4): 973 - 977
9. Sule, I.O., Oyeyiola, G.P. and Agbabiaka, T.O. **2009**. Comparative Bacteriological Analysis of Chlorinated and Dechlorinated Pipeborne Water. *Int. J. Bio. Sci.*, **1**(1):93-98.
10. Murray, A.L., Kumpel, E., Peletz, R., Khush, R.S., Lantagne, D.S. **2018**. The effect of sodium thiosulfate dichlorination on faecal indicator bacteria enumeration: laboratory and field data. *Journal of Water and Health*, **16**(1):70-77
11. Sule, I.O., Agbabiaka, T.O., Saliu, B.K., Adewumi, O.S. and Bello, A.B. **2017**. Bacteriological and Physicochemical Analysis of Sachet Water Sold in Ilorin Metropolis, Nigeria. *Nig. J. Pure & Appl. Sci.* **30**(1): 2978-2986.
12. Onyeadi, D.J., and Agbagwa, O.E. **2019**. Plasmid curing in multi-drug resistant hospital and community uropathogenic *Escherichia coli*. *J. Appl. Sci. Environ. Manag.* **23**(1): 29 -34
13. Cheesbrough M.C. **2006**. *District laboratory practice in Tropical Countries*, Cambridge University Press, 2nd edition Part 2. P.133 -153
14. Aneja K.R. **2014**. *Laboratory Manual of Microbiology and Biotechnology*. 1st edition, Scientific International PVT. Ltd., New Delhi, India pp. 51 – 162
15. ABIS **2018**. online Advanced bacterial identification software. [www.tgw1916.net> bacteria_logare_desktop.html](http://www.tgw1916.net/bacteria_logare_desktop.html)
16. Oxoid **2016**. Microbact Biochemical Identification kits [online] available from <http://www.oxoid.com/UK/blue/prod_detail.asp?pr=MB1130> accessed (14-06-2016).
17. CLSI **2013**. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty third informational supplement M 100-S23, Wayne, PA, USA.
18. Joseph, A.A., Odimayo, M.S., Olokoba, L.B., Olokoba, A.B., Popoola, G.O. **2017**. Multiple antibiotic resistance index of *Escherichia coli* isolates in a tertiary hospital in South-West Nigeria. *Medical Journal of Zambia*, **44**(4): 225-232
19. Churchill, O., and Romanus, A.A. **2019**. Plasmid curing of antibiotic resistant *Escherichia coli* isolates from urine and stool samples. *Journal of Microbiology and Antimicrobial*, **11**(1): 1-4.
20. SPSS **2010**. Statistical package for social scientists 15.0 for window evaluation.
21. SON **2007**. Standards Organization of Nigeria. Nigerian Standard for Drinking Water Quality (NSDWQ). Industrial Standards, Abuja, Nigeria. pp. 15 - 21.
22. Sengupta P. **2013**. Potential Health Impacts of Hard Water. *International Journal of Preventive Medicine*. **4**(8): 866 – 875.

23. Bartram, J., Cotruvo, J., Exner, M., Fricker, C. R., and Glasmacher, A. **2003**. Expert consensus. *Heterotrophic Plate Counts and Drinking-water Safety: The Significance of HPCs for Water Quality and Human Health*. IWA Publishing, London, pp. 1-12.
24. Umezuruike, O.I., Damilola, A.O., Adeola, O.T., Enobong, A., Shittu, O.B. **2008**. Microbiological and Physicochemical Analysis of Different Water Samples used for Domestic Purposes in Abeokuta and Ojota, Lagos State, Nigeria. *African Journal of Biotechnology*, **7**(5): 617-621.
25. Karikari, A.Y., and Ampofo, J.A. **2013**. Chlorine Treatment Effectiveness and Physico-Chemical and Bacteriological Characteristics of Treated Water Supplies in Distribution Networks of Accra-Tema Metropolis, Ghana. *Appl. Water Sci.* **3**: 535–543.