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Antimicrobial Resistance Profile of Bacteria Isolated from Boreholes and Hand Dug Wells Water in Ngaoundere Municipality of Adamawa Region in Cameroon

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Abstract

Uncontrolled uses of antibiotics have led to rapid evolution of antibiotic-resistance bacteria and antibiotic resistance gene transfer, especially in a pool of aquatic system where resistance, intermediate and susceptible bacteria to some antibiotics strive together. Consequently, there is a transfer of resistance genes. In this study, bacteria of the *Enterobacteriaceae* family and some gram positive bacteria isolated from some boreholes and hand dug wells water of public use were tested on 19 antibiotics of different classes. This was achieved through a disk diffusion technique to determine the antimicrobial resistance profile of the said bacteria, microbial resistance index of the drugs used (and their ability to produce Beta-lactamase). These isolates were shown to demonstrate a very high resistance to the drugs used in the area. The resistance was highest in *Escherichia coli* 1 (73.68%) and lowest in *Streptococcus pneumoniae* (47.82%). These isolates also indicated very high levels of multi-drug resistance. The minimum resistance index was 0.47, indicating that bacteria isolates were of fecal origin. It is evident from the present study that multiple antibiotic-resistant bacteria can thrive in water as an environmental reservoir, and can therefore provide a route to multidrug-resistant pathogens to enter human and animal population.

Keywords

Boreholes, Wells, Drug Resistance Bacteria, Microbial Resistance Index

1. Introduction

The global community of water, sanitation, hygiene researchers, practitioners and policy makers have to date inadequately addressed the challenge of the quality in relation to access to water and sanitation in development and humanitarian emergency contexts. Freshwater comprises 3% of the total water on earth, but only a small percentage (0.01%) of this freshwater is available for human use [1]. The burden of water-related diseases curtails efforts to improve public health in the developing world. Diarrhea most often related to unsafe drinking water, poor sanitation and inadequate hygiene is one of the leading causes of death among children under the age of five. The main risk for public health in water systems is that resistant genes are transferred from environmental bacteria to human pathogens. The potential of drinking water to transport microbial pathogens to a greater number of people, causing subsequent illness, is well documented in several countries at all levels of economic development [2]. The aquatic ecosystem plays a major role in the contamination and spread of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG). Water acts as the most important mode for bacterial propagation and distribution of antibiotic resistance between man and environment. For the evolution of antibacterial resistance, urban water system, animal husbandry operations and pharmaceutical industry effluents are considered as the major hotspots [3]. The controlled process and engineered water cycle, which is operated in a local environment otherwise known as "urban cycle" is performed in two stages: firstly, the abstraction of surface water and groundwater for consumption; secondly, the treatment and disinfection of sewage before it's discharged into the environment. This serves as a path for the transfer of antibiotic resistance from the environment to human and back to the environment, as a large number of resistant bacteria enter the sewage through faeces, which is further carried into water bodies. Resistant bacteria and other pathogens in wastewater remain in close contact with sewage sludge bacteria and other microorganisms during biological treatment at wastewater treatment units, where the horizontal transfer resistant genes takes place [4]. Therefore, wastewater treatment plant becomes a hub of gene transfer and evolution of ARB. Resistant bacteria can also reach the ground water in an agricultural field due to manure spreading and animal grazing. New forms of resistance can be acquired by the bacterial strains in the environments. Pathogens that last longer and are dispersed in the environment cause greater hazard, than those that can be transmitted from one person to another. The spread of these resistant bacteria in the environment poses a serious threat to public health [5]. Hence, there is a growing concern regarding the occurrence of antibiotic resistance bacteria and antibiotic resistance genes in aquatic environments [6]. Direct or indirect contact with water (for drinking, or recreational use) contaminated by ARB could harm and infect the human population with antibiotic resistant pathogens, and/or ARG carried by bacteria may be transferred from microorganisms into humans, as a consequence of horizontal gene transfer [7]. Such

events would undermine our ability to prevent and control disease, and thus expose a great threat to public health. Little is known about the fate of ARG in drinking water systems, and it was recently proposed that ARG are emerging contaminants [8]. It is estimated that about half of the patients occupying African hospital beds suffer from water-borne illnesses due to lack of access to clean water and sanitation [9]. This holds true in Cameroon and in Vina division in particular where most of the population live below poverty level and cannot afford to have good antibiotics in pharmacies since most of the drugs sold in their vicinity are resistance to many infections. As such they turn to traditional medicine. As such it was necessary to carry out this exercise to determine the anti-microbial resistance profile of bacteria isolated from potable water source since it acts as a source transmission.

2. Material and Methods

2.1. Study Site

The study was carried out in Ngaoundere urban council in Vina Division in the months of April and October 2017 and 2018. It covers a surface area of 17.196 km^2 and as from 2001 had a population of 247,420 [10] inhabitants. The capital is Ngaoundere, which had in 2005 a population of 152.700 inhabitants. It has one general hospital, integrated health centers in each council area and 3 private hospitals, with number pharmaceuticals shops to take care of the citizens. The city is divided into Ngaoundere I, II and III councils, which are rapidly growing. The main activities in the locality are cattle rearing and agriculture [10]. The monthly rainfall varies between 0 to 1500 mm [10]. The municipality is highly cosmopolite. With a good number of people from different parts of Cameroon and more especially from the far north who have escaped from the activities of Boko haram and a good number of refugees from Central African Republic. The Ngaoundere municipality has 97 officially known **Figure 1** boreholes and a good number of wells not known all meant for public consumption.

2.2. Sample Collection

Sampling took place in the dry and rainy season during the months of April and October from fourteen (14) boreholes, (nine) 9 hand dug wells meant for public consumption were chosen based on population agglomeration. 500 mL sampling bottles were washed and rinsed with distilled water. Bottles were then sterilized under the laminar hood using UV light. For boreholes, water was pumped out for 3 - 4 minutes to cool the metal pipe to eliminate the influence of water temperature with that of the metal pipe. Sampling bottles were rinsed with some of the boreholes water and then completely filled and covered. For wells, water was drawn using the rope and bucked found in the sampling points. **Table 1** Sampling bottles were rinsed with well water, and then filled completely and covered. Water Samples were stored in a flask at −4˚C, and aseptically transported to the microbiology laboratory unit of the Institute of Agricultural Research for Development for analysis.

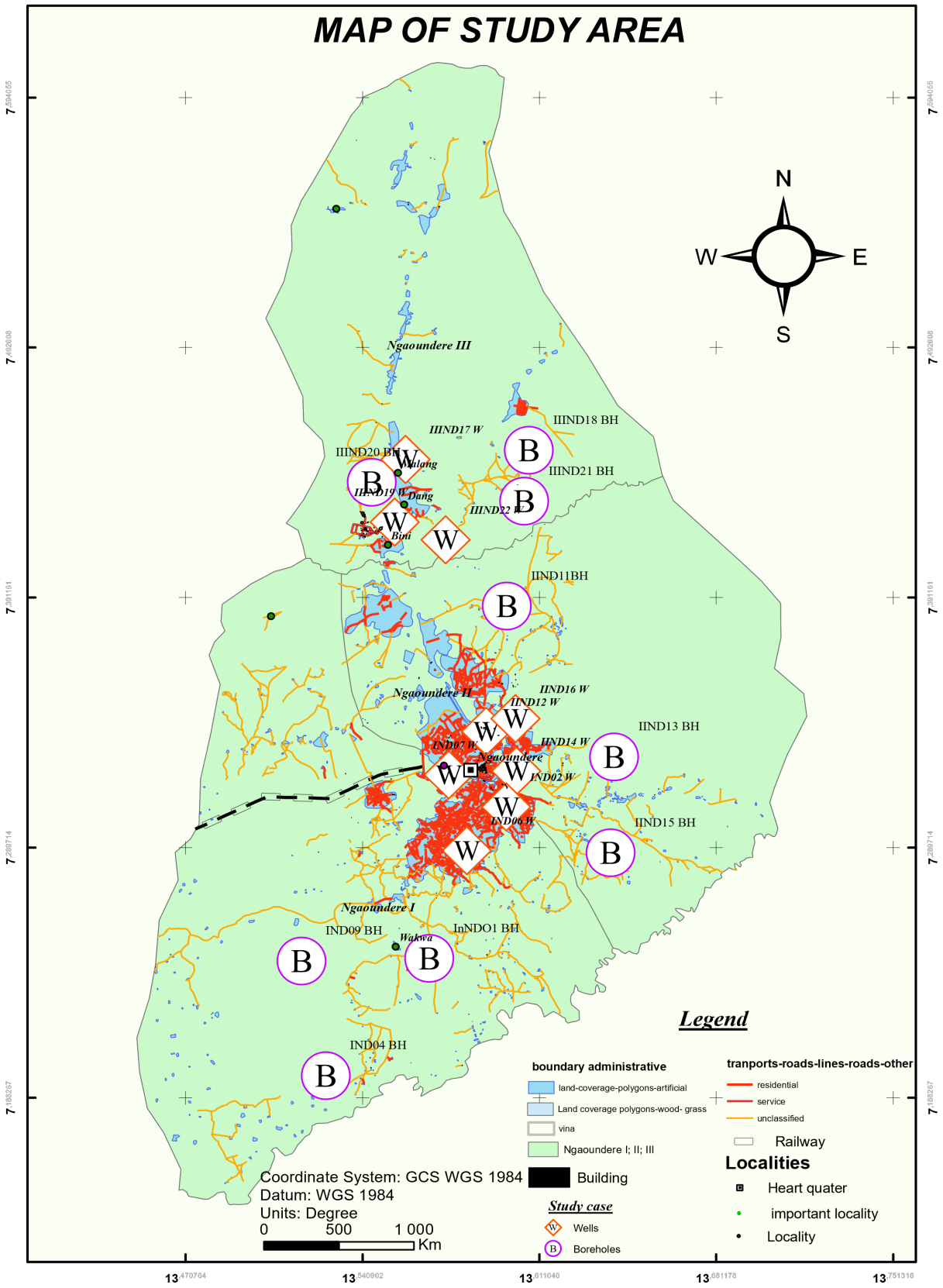

Figure 1. Map of the study site showing some boreholes and wells in Ngaoundere councils (Source: open street map Sogefi, 2018) [11].

Table 1. Indicates the characteristics of sample points.

Samples	Sample points	Geographic coordinates				Water source
		North	East	Height above sea level (m)	depth (m)	
1	Cathedral	7˚19'271"	13˚34'464"	1142	42	borehole
2	Novegian	**07˚18'699"**	**013˚35'850"**	**1135**	**42**	borehole
3	Boukina	07˚18'334"	013˚35'870"	1123	**4**	well
4	Socaret	07˚19'132"	013˚35'568"	1112	**42**	borehole
5	Maza	07˚16'686"	013˚34'703"	1109	**45**	borehole
6	Maza	07˚16'788"	13˚34'561"	1104	**1**	well
7	Bintu	07˚17'845"	013˚33'742"	1159	**45**	borehole
8	Sabongari America	07˚18'994"	013˚35'310"	1113	**41**	borehole
9	Onaref	7˚18'694"	13˚34'301"	1114	**3**	well
10	Bambari II	07˚18'071"	013˚35'652"	1138	**45**	borehole
11	Burkina west	07˚18'397"	013˚36'067"	1118	**5**	well
12	Quartier Bamun	07˚373"	013˚34'518"E	1136	**43**	borehole
13	Jolie Soir	07˚19'726"	013˚34'836"	1106	**12**	well
14	Sbongari gar	07˚19'647"	013˚35'400"	1107	**45**	borehole
15	Carrefour Auodi	07˚19'251"	013˚35'575"	1111	**41**	Borehole
16	Bantai	07˚19'146"	013˚35'667"	1102	**10**	well
17	Grand Marche	07˚19'529"	013˚35'077"	1129	**5**	well
18	N. Cifan	07˚19'477"	013˚35'915"E	1115	**42**	borehole
19	Gada	07˚19'914"	013˚36'057"	1099	**12**	well
20	Carrefour Borongo	07˚26'504"	013˚33'284"	1091	**10**	well
21	Carrefour Borongo	07˚26'388"	013˚33'336"	1091	**40**	borehole
22	Dang	07˚25'186"	013˚33'131"	1088	**10**	well
23	Dang	07˚24'788"	013˚32'944"	1081	**40**	borehole
24	Mmawi	07˚23'617"	013˚33'152"	1071	**42**	borehole

3. Isolation of Bacteria

3.1. Isolation by Membrane Filtration

For all the boreholes and wells samples, three volumes of 400 µl and 200 µl respectively were filtered through 0.45 µm pore-sized filter (cellulose nitrate membranes, Whatman sterile membrane filter) using a high vacuum pump (model E2, M5). These membranes were aseptically placed up on plates with appropriate selective media ensuring that air bubbles were not trapped. The selective media used were as follows: M-FC agar enriched with rosolic acid used as a selective medium used for faecal coliforms, m-Endo LES agar for total coliforms, nutrient agar used for heterotrophic plate count. For *Staphyilococcus* spp., mannitol salt agar was used, while Hektoen enteric agar for *Enterobacteriaceae*. For

Streptococcus spp., nutrient agar was used. All the media were prepared according to the manufacturers' instructions (Biolab).

Plates were incubated at 37˚C except for m-FC agar which were incubated at 45˚C for 18 - 24 hours in a water bath. During the isolation of *streptococcus,* the inoculated nutrient agar plates with the filtrate were incubated for 72 hours at 37˚C. The colonies were enumerated, characterized, and recorded. The results were expressed as the number of fecal coliforms, total coliforms in 100 mL of water. Blue colonies from m-FC agar (presumptive coliforms), metallic-sheen colonies from m-Endo agar (presumptive total coliforms) as indicated by Eaton, Rice and Baird (2005) [12]. For sub-culturing, suspected bacteria were inoculated separately onto different bacteriological agar media under aseptic condition and incubated at 37˚C for 24 hours. Pure cultures were achieved as per procedures described by Cowan (1985) [13]. Colony morphology, hemolytic characteristics, Gram staining, catalase test, motility test, triple sugar iron reaction, Citrate), and cytochrome oxidase tests were conducted to identify the isolates according to the procedures adopted by Quinn *et al.* (2002) [14]. Furthermore, biochemical identifications by commercial kits were carried out (Integral System Enterobacteria, Integral System *Staphylococci*, Integral System *Streptococci*, and Liofilchem®).

3.2. Assessment of Antimicrobial Susceptibility

Pure cultures of selected bacterial isolates were tested for susceptibility to different antimicrobial agents using in vitro disk diffusion (Kirby-Bauer) method as described by Quinn *et al.* (2002) [14]. Cultured broth was cross-checked with 0.5 McFarland standard before applying on Mueller Hinton agar and disk application.

Nineteen different antimicrobial disks obtained from commercial sources (Becton Dickson and company and Liofilchem®, Bioanalyse) were selected for the testing and they included: Ceftazidime (CAZ) 30 mcg Amoxicillin (10 µg), Erythromycin (E) (15 µg), Doxycycline (30 µg), Penecillin G (P) 10 µg, Amoxicillin/Clavulanic acid (20/10 µg), (Genentamycin (CN) 120 MCG, Pefloxacin (PEF) 5 MCG, Tetracycline (TE) 30 MCG) (TZP) 100 ug, Ceftriazone (CRO) 30 MCG, Etilimicin (NET) 30 MCG, Ciprofloxacin (CIP) 30 MCG, Impenen (IPM) 10 µg, Levofloxcin (LVX) 5 µg, Cefepime (FEP) (30 µg), Ofloxacin (OFX) 10 µg, Amikacin (Ak) 10 µg, Citixime (CFM). Susceptibility test **Table 2** plates were incubated at 37˚C for 18 - 24 hours; and the ensuinginhibition zone diameters (IZDs) were measured to the nearest millimeter using a Mitutoyovernier caliper, this was repeated three times and the average readings of the zone of inhibition was calculated. The interpretation was done according to the guidelines of Clinical and Laboratory Standard Institute [15].

3.3. Detection of ESBL Producers

All 39 bacteria that showed reduced susceptibility to either ceftazidime were tested ESBL production using the standard double disk synergy tests. In this test,

Table 2. Antimicrobial susceptibility test.

N°	Bacteria tested	Susceptible antibiotics	Intermediate antibiotics	Resistance antibiotics
1	***E. coli* 1**	TZP, CRO, OFX, AK, CN	00	CAZ, AML, E, P, DO, PEF, TE, NET, CIP, IPM, FEP, CFM
2	*Klebsiella pneumoniae*	DO, CN, TZP, CRO, NET, CIP, IPM, OFX	NA	CAZ, AML, AMC, E, P, PEF, TE, FEP, CFM
3	*Bulkholderia cepaceae*	P, DO, CN, TZP, CRO, NET, CIP, LVX, OFX, AK	AML	CAZ, E, NA, PEF, TE, IPM, FEP, CFM
4	*Pseudomonas pneumonae*	CN, TZP, CRO, CIP, IPM, OFX, CFM	***E***	CAZ, AML, NA, P, DO, PEF, TE, LVX, FEP, CFM
5	*Salmonella* spp.	CN, TZP, CRO, LVX, OFX, AK	CIP, NET	CAZ, AML, AMC, E, NA, P, DO, PEF, TE, IPM, FEP, CFM
6	*Streptococcus pneumoniae*	DO, CN, TZP, CRO, NET, CIP, IPM, LVX, OFX, AK	AML, E	CAZ, AML, NA, PEF, TE, FEP, CFM
7	*Staphilococcusaureuse*	DO, CN, TZP, CRO, IPM, OFX, AK	-	CAZ, AML, E, NA, P, PEF, TE, NET, CIP, LVX, FEP, CFM

Ceftazidime (CAZ), Amoxicyline (AML), Amoxicylineclauvulunicacid (AMC), Erythromycine (E), Pénicilline G (p), Doxycicline (DO), Gentamycine (CN), Pefloxacine (PEF), Tetracycline (TE), Piperacin-TAZBACTAM (TZP), Ceftriazone (CRO), Nethilimicin (NET), Ciproflolaxin (CIP), Impenen (IPM), Levoflolaxin (LVX), Cefepime (FEP), Ofloxacin (OFX), Amikacin (AK), Citixime (CFM).

disks containing amoxicillin-claviculanic acid was placed at the center of inoculated Mueller-Hinton agar plates, surrounded by ceftazidime ((30 μg/ml) and ceftriaxone (30 MCG) placed 20 mm equidistant from the amoxicillin-claviculanic disks. Any distortion or increase in the zone towards the disc of amoxicillin-clavulanate was considered as positive for the ESBL production [16].

3.4. Statistical Analysis

SPSS version 16 was used to calculate the percentages and Microsoft excel 2013 was used to draw the histograms.

4. Results

4.1. Bacterial Isolates Recovered from Freshwater in Boreholes and Wells in Ngaoundere Municipality

A total of 14 bore holes and 9 hand dug wells were sampled based on population agglomeration and the selection process was aided by the councilors of each municipality. Among the fourteen sampled bore holes **Figure 2** five had fecal contaminates giving a percentage of 35.7%. This is contrary to the WHO policies which stipulate that any potable water in which fecal contamination is seen is completely out of use [17]. Nine hand dug wells of public use were equally selected randomly and from the analysis made 8 of the wells had fecal coliforms

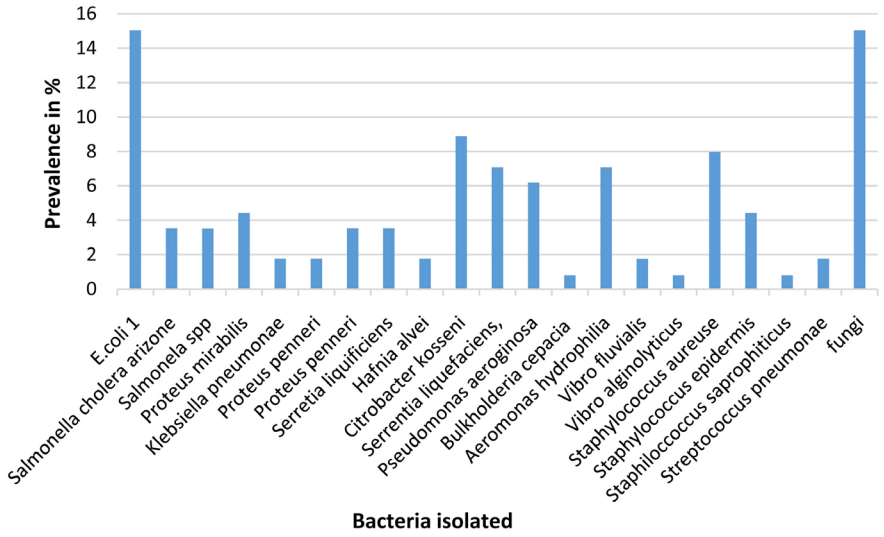

Figure 2. Prevalence of bacteria species in boreholes and wells.

both in the dry and the rainy season giving a percentage contamination of 88.89% highly against the recommendations of the world health organization. Fungi was also seen to play a dominant factor in both boreholes and wells sampled. Fungi was detected in 9 of the 14 bore holes giving a fungal contamination percentage of 64.29%. Equally, fungi was detected in 8 of the 9 wells sampled giving a contamination rate of 88.88%. Fungi has been known to play an organoleptic property in water quality (**Figure 3**) [18].

Bacteria isolated were placed in classes and prevalence per specie isolated in the dry and rainy season were placed together.

For convenient, those antibiotics that were intermediate were considered as resistance, hence **Table 3** was obtained. High susceptibility observed Netilimicin (87.5%) Levofloxacin (71.4%), amikacine and ofloxacine (71.4%) and Piperacillin (100%).

4.2. Identification of Multidrug Resistance (MDR) Strains

The antibiotic susceptibility analysis to detect multidrug-resistant (MDR) bacteria among enterobacteriaceae and some gram positive bacteria isolates was performed using 19 different antibiotics disks. The number of antibiotics to which each bacterium was resistant to in the disk diffusion test was noted for identification of multidrug resistant strains. Multidrug resistance (MDR) was understood as resistant to four or more antibiotics tested [19]. Multiple Antibiotic Resistance (MAR) Index **Table 4** was calculated as a/b where: "a" represents the number of antibiotics to which the isolates were resistant to, while "b" represents the total number of antibiotics to which the isolate was exposed (MAR index for isolates = (a/b) [20]. Bacteria may exhibit intrinsic (primary) resistance to certain antimicrobial agents. Intrinsic resistance was based on either the lack or the inaccessibility of the antimicrobial target site among the bacteria in question. In other cases, intrinsically resistant bacteria are known to produce

Table 3. Susceptibility of bacteria isolated from water to antibiotics (%).

antibiotic	AMC	OFX	AK	FEP	p	DO	CN	PEF	TE	TZP	NET	CIP	IPM	LVX
E. coli 1 (17)	00	100	100	00	00	100	100	00	00	100	100	00	00	100
Salmonella spp. (10)	nt	100	100	00	00	00	70	00	00	100	100	26	100	100
Bulkholderia cepaceae (1)	nt	100	100	00	100	100	100	00	00	100	100	100	00	100
Klebsiella pneumoniae (2)	00	00	00	00	00	100	100	00	00	100	00	00	100	100
Pseudomonas aeroginosa (4)	00	00	100	00	00	00	100	00	00	100	100	100	100	00
Streptococcus pneumoniae (5)	00	100	100	00	100	100	nt	00	100	100	100	100	100	100
Staphilococcusaureuse (5)	100	100	100	100	00	100	nt	80	100	100	100	00	80	00

Key: nt: not tested.

Table 4. Multiple Antibiotic Resistance (MAR) Index of some bacteria isolated from water samples.

Bacteria isolated	MARI	antimicrobial agents
E. coli	0.7342	(CAZ) (AML), (AMC) (E), (NA) (AMB), (p), (DO) (PEF) (TE) (TE) (NET), (CIP), (IPM), (FEP), (CFM))
Klebsiella pneumoniae	0.5263	(CAZ) (AML), (AMC) (E) (NA), (AMB), (p), (PEF) (TE), (TE), (NET), (IPM), (FEP) (CFM))
Bulkholderia cepaceae	0.5263	(CAZ) (AML) Acid (AMC) (E), (NA), (AMB) (PEF) (TE), (TE) (TZP) (CRO), (IPM), (FEP), (CFM)
Pseudomonas aeroginosa	0.6332	(CAZ), (AML), (AMC) (E) (AMB), (p), (DO), (PEF), (TE), (TE) 30 MCG, (TZP), (IPM), (LVX), (FEP), (CFM))
Salmonella spp.	0.6842	(CAZ), (AML), (AMC), (E), (NA), (AMB), (PEF), (TE), (TE) (TZP), (NET), (CIP), (IPM), (FEP), (CFM)
Streptococcus pneumoniae	0.4736	(CAZ), (AML), (AMC), (E), (NA), (AMB) (PEF), (TE) NET), (FEP) (CFM)
Staphilococcusaureus	0.6842	(CAZ), (AML), (AMC) (E), (NA), (AMB), (p), (PEF) (TE) (TE) (NET), (CIP), (FEP), (CFM)

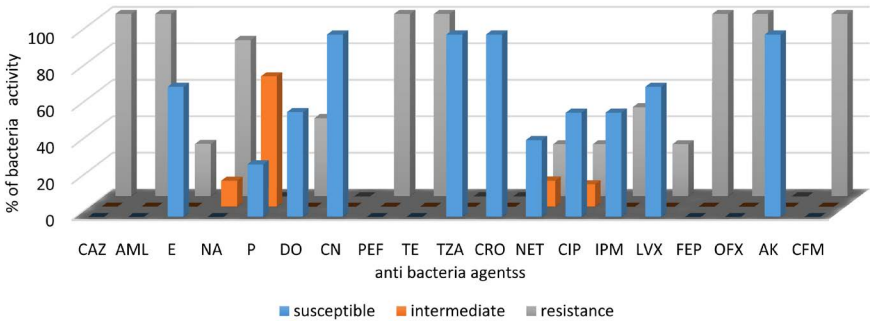

Figure 3. Susceptibility of antibiotics to bacteria isolated from water samples.

inactivating enzymes, such as species-specific β-lactamases, contain multidrug transporters and/or exhibit permeability barriers [21].

Multi drug resistance was observed in all the isolates used in this study with the highest index found in *E. coli* 1.

4.3. Detection of ESBL in Isolates

See Figure 4 and Table 5.

5. Discussion

A bacterium undergoes massive selection pressure due to the excessive use of antibiotics on it. The genetically adopted bacterium continues to reproduce and survive in the presence of antibiotic and transfers resistance to its following generations. The antibiotic slowly becomes ineffective towards these resistant bacteria [22]. There are specific targeted molecular structures in the bacterial cell which are attacked by the antibiotics. In case of spontaneous mutation, the antibiotic fails to locate these targets, hence the bacterium develops resistance. Moreover, the antibiotic gets thrown out of the bacteria cell in a process called efflux**.** The rise and spread of AMR threatens the effective control and treatment of various bacterial diseases worldwide [23]. Availability of routine and research data on pathogen susceptibilities is an important step towards designing targeted strategies to tackle the global AMR crisis. The lack of consistency in the measurement and reporting of susceptibility data makes it difficult to compare findings among different countries and laboratories, sometimes even within one country.

Figure 4. White arrows indicating the absence of synergy between Amoxicillin-clavulunic acid and ceftriaxone on *E. coli* 1 culture.

Table 5. *In vitro* susceptibility of ESBLs to β-lactam antibiotics.

antibiotic	*E. coli* 1 (17)	*Pseudomonas aeroginosa* (2)	*Klebsiella Pneumoneae* (2)	*Bulkholderia cepaceae* (1)	*Salmonella* spp (10)	*Staph. aureuse* (4)	*Total*
Ceftazidime	0	00	00	00	00	00	**0%**
Cefotaxime	00	00	00	00	00	00	**0%**
Ceftriaxone	00	00	00	00	000	00	**0%**
Ccefepime	00	00	00	00	00	00	**0%**

In the present study, it was generally observed that the level of contamination of both bore holes and wells was very high, since fecal coliforms were seen in 35.7% of the bore holes and in 88.89% of the wells. This goes ahead to confirm the world health organization statistic which says that about two third of the patients occupying hospital beds have infections which are transmitted by water. Mold (fungi) had a high prevalence rate both in wells and bore holes of 15.04%. Cases of dermatophilic reactions have been reported in some women with fragile skin due to the presence of fungi in water in this municipality. This allergic reactions have been reported by Wioletta *et al*., (2013) [24] who estimated that approximately 2% - 6% of the general population in developed countries is allergic to fungi which manifest itself as asthma, rhinitis or conjunctivitis, atopic dermatitis, this is not only particular to developed countries.

Among all the bacteria isolated, five gram negative and two gram positive bacteria isolates from natural sources of portable water were tested against 19 different antibiotics of different categories or classes. The results showed *E. coli* 1, *Salmonella* spp., *Klebsiella pneumoniae* and *Pseudomonas aeroginosa* had very high level of resistance to amoxicyline, ceftriazone, amoxicylineclauvulunic acid, erythromycin cifixime, pefloxacine, cefepime, ceftriazonetetracycline and Penicillin G and Nalidic acid. Oluyege (2009) [25] reported the very high resistance of *E. coli* spp. and *Salmonella* spp. *Pseudo monasaeroginosa*, *Klebsiella pneumoniae* to Penicillin G, Amoxicillin, nalidic acid and tetracycline. Similarly Carmen *et al.* (2016) [4] reported very high level resistance of *E.coli1*, *Klebssiella pneumoeae* and *Salmonella* spp. against Ceftriazone, Cefepime (Cephalosporin) and Piperacilline (Antipseudomonal penicillin + β-lactamase inhibitor). In contrast, Piperacilline was susceptible to all the bacteria species isolated he indicated also resistance to gentamycin and ofloxacin which to me were susceptible. Resistance of bacteria to antibiotics ranges from 100% in pefloxacine, ceftazidime, Amoxyllin, citizime Erythromycine to 14.2% in Amoxylineclauvulunic acid. *E. coli* 1 was also resistance to ciprolaxine (fluoroquinolones) and imepenem (carbapenems) as indicated by Carmen *et al*., (2016) [4]. It was also noticed that *Samonella* spp. were resistance to Doxicycliene (tetracycline), Ciproflolaxine (Floroquinones) and Netilimcin (Aminoglocosides). Ahmed (2011) [20] reported a 40% resistance of *Salmonella* spp. isolated from chickens to doxycline. Ciproflolaxine was 74% resistance to *Salmonella* spp. in this study. According to Hossain (2017) [26] 100% resistance was reported to ciproflolaxicine to bacteria isolated from urinary tract infections in India. *Bulkholderia cepaceae* of the family *Pseudo monadaceae* gave a resistance perctange of 47.6%. The *Burkholderia cepacia* organisms are opportunistic nosocomial pathogens capable of causing severe disease in immunocompromised individuals, especially those with cystic fibrosis [27]. Intrinsic resistance of *Bulkholderia cepaceae* has been reported in many other catergories of antibiotics except, ceftazidime and other extended-spectrum cephalosporins, But in this study we discovered that Bulkholderia was resistance to ceftazidme and cefepime all of the extended spectrum cepha-

losporine. It was discovered that *Burkholderia cepacia* was 52.3% susceptible to antibiotics used in this study. These include damikacin, gentamycin netilicin of the aminoglocosides group, Levofloxacine and ciproflolaxcine of the quinolone group and doxycycline of the tetracycline group.

Klebsiella pneumoniae has become the most common pathogenic bacterium accountable for nosocomial infections due to its high virulence factor and general occurrence of resistance to most antibiotics [28]. Of the 19 antibiotics used in this study *Klebsiella pneumoniae* was resistant to 14 of them giving a resistance perctange of 73.68% as confirmed by Gajul *et al*., (2015) [28] *Klebsiella pneumoniae* showed very high resistance to different categorize of antibiotics such as *B*-lactam; ceftazidime (CAZ); Penecillin G(P); Cephalosporines; cefepime (FEP); aminoglocosides; netilimcine (NET); fluoroquinones; cefepime, but it was 26.5% susceptible to other antobiotics like Amikacin, gentamycine (glycopeptides), Doxycycline (Tetracycline), Piperacillin and imepenem (β-lactam). High level of resistance observed within these gram-negative bacteria may be due to the non-respect of pharmaceutical norms and vendors of these pharmaceutical products who have not undergone any training of any sort equally the exposure of these product in fluctuating temperatures is a cause for concern for these products are degraded under high temperatures. The sources of supply to most of these fake pharmaceutical products are not often known in third world countries.

Streptococcus pneumoniae was seen to be 47.8% resistant to antibiotics tested, including Piperacillin, erythromycin, amoxicillin, Amoxylineclauvulunic acid ceftriaxone and Pefloxacine, and 52% susceptible the bacteria tested in this study. This is similar to the results obtained by Kandakai and Dido (2009) [5] who demonstrated that *Streptococcus pneumoniae* isolated from the pharynx was highly resistance to erythromycine and tetracycline, but susceptible to ciproflolaxcine. These results are also similar to those obtained by Emina *et al*., (2015) [29], when studying the antimicrobial susceptibility/resistance of *S. pneumoniae* isolated from the eyes, resistance was highest to erythromycine and lowest at ciproflolaxcine. Multi drug resistance was also noted in *Streptococcus pneumoniae* (tetracycline; aminoglycosides; netilimcin; *B*-lactam; ceftazidime; Cephalosporine; cefepime). This may be due to the fact that people do not buy drugs from the right source and do not respect the dosage prescribed by the medical personnel.

Staphilococcus aureus was seen to have a high resistance index 0.68 of as seen in **Figure 5**. This is similar to the results obtained by Akanbi *et al*., (2017) [30] where they showed *Staphilococcus aureus* was resistance to a host of antibiotics like imipenem (96.76%), ciproflolaxcine (66.7%) and a host of others.

With an increase in the antibiotic load, the prevalence of acquiring resistance increases within a bacterial community. The MAR index is an excellent tool that permits the analysis of the relative prevalence of resistant bacteria in the environment. For all the bacteria isolated, MAR indices varied from 0.47 (*Streptococcus*

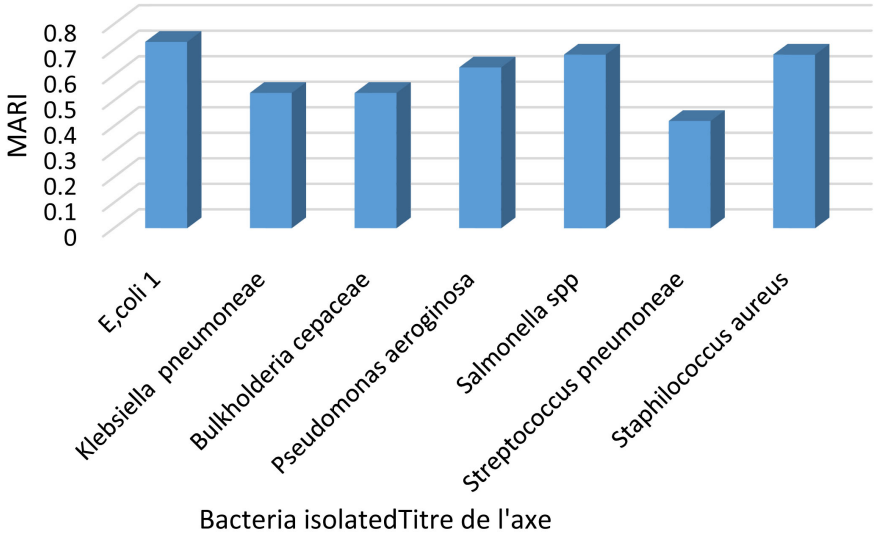

Figure 5. Multi antibacterial resistance index of the bacteria isolated Multi drug resistance was observed in all the isolates used in this study with the highest index found in *E. coli* 1.

pneumoniae) to 0.73 (*E. coli* 1) **Figure 5**. According to Krumperman (1983) [31] and Tambekar (2011) [32], MAR index more than 0.4 is usually obtained from human fecal origin, while MAR indices less than 0.4 are from non-human fecal contamination. This confirms the fact that all the isolates were of fecal origin, considering the level of human activities and waste disposal in this community, it is obvious that all the bacteria isolated were of fecal origin. When antibiotics are seldom or never used, a MAR index value less than or equal to ≤0.2 is observed [33]. In this study, 100% of the bacterial isolates showed MAR value more than 0.4, which is a possible indication that the bacterial isolates have been exposed to several antibiotics. The high resistance seen may be due to the selective pressure exerted by the use of antibiotics in the management of bacterial infections in animals, humans, waste disposals, unhygienic nature of the surrounding of the boreholes and wells and poor sewage disposal systems.

It is unfortunate that enteropathogens like *E. coli* 1, *Salmonella* spp., *Klebsiella pneumoniae, Streptococcus pneumoniae* were resistant to most common antibiotics (erythromycine, Penicillin Amoxicillin) found in this zone (**Table 3**), but susceptible to antibiotics that are not assessable to common man such as Piperacillin, imepenem and levofloxacine except *E. coli* 1 and *Bulkholderia cepaceae* that were not susceptible to all the three mentioned antibiotics. This was confirmed by Shubra *et al.* (2014) [34]. Who demonstrated that bacterial isolates from water sources were found sensitive to imipenem and piperacillin/tazobactam, with maximum resistance found to ampicillin (57.5%).

According to Pontes *et al.* (2009) [35], Multiple Drug Resistant (MDR) bacteria with the susceptible ones increases the chances of transfer of antibiotic resistance to the sensitive ones. This maintains a pool of resistant bacteria with a pool of resistance genes in the population that further contributes to the general increase

and dissemination of bacterial resistance, and can be a source of resistance genes for pathogens and this was observed in this study. As such, there are many ways of transmission of resistance to bacteria such as municipal, agricultural sewage, and human and animal excrement on the open ground surface and, known as the sources of spreading antibiotics-resistant bacteria in the aquatic environment.

Wherever *Enterobacteriaceae* occur, ESBL resistance mechanism can be observed [36]. This resistance mechanism is currently the one whose dynamic spread causes the most difficulties. The water environment is conducive to transfer between species, where ESBL producing bacteria from various sources get in contact with a broad range of potential recipients. In this study the detection of ESBL was done using standard double disc diffusion technic and it was observed that there was synergy reaction between the Amoxicillin clavulunate and the ceftazidime, ceftriaxone and cefotaxime. This is contrary to the results obtained by Adelowo, (2018) [37] who detected very high presence of ESBL in hospital waste water and aquatic sources (**Figure 4** and **Table 5**).

6. Conclusion

The effectiveness of antibiotic treatment of diseases will decline due to the development of antibiotic resistance in the environment. It is evident from the present results that multiple antibiotic-resistant bacteria can thrive in water as an environmental reservoir, and can therefore provide a route to multidrug-resistant pathogens to enter human and animal population. The study of resistance in the environmental bacteria can help in guiding the development of strategies to counteract this resistance. From this study, it can also be said that there is a need to monitor antibiotic sensitivity at regular intervals. Treatment of water is essential not only to kill the pathogenic bacteria, but also to stop the transfer of antibiotic. Finally, legislation and education should be enforced to control the pharmaceutical products in our markets, the respect of dose prescribed by the medical officials and accessibility to these drugs both for humans and animals.

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Conflicts of Interest

The author declares no conflict regarding the publication of this research work.

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