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**Antibiotic Resistance pattern/ Multiple drug Resistance Index of
Water-borne Bacteria isolated from Underground well Water of some
Wards in Gusau Metropolis Zamfara State**

Nusaiba Ibrahim Danmaliki^{1,2} A. A. Haroun¹, P. A. Vantsawa¹, A. I. Alhaji¹

¹Department of Biological Sciences, Nigerian Defense Academy, Kaduna, PMB 2109, Nigeria.

²Department of Biotechnology, Nigerian Defense Academy, Kaduna, PMB 2109, Nigeria.

Corresponding Author's Email: danmalikinusaiba@gmail.com

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ABSTRACT

Resistance of bacteria to many antibiotics is now a challenge that has become a source of mortality in many countries. Pathogens that are previously susceptible are now becoming resistant and are spreading among water bodies. This study will determine the Antibiotic Resistance pattern and Multiple drug Resistance Index of Water-borne bacteria isolated from Underground well Water of some Wards in Gusau metropolis. Eighteen water samples were collected from 6 locations (three from each) in Gusau. These samples were plated, cultured and emerging strain of bacteria were isolated and characterized using standard biochemical method. 24 bacterial isolates were found and characterized into eight genera. The bacteria with the highest frequency was *Pseudomonas spp.* (29.2%) followed by *Shigella spp.* and *Alicycigenes* with (16.7%) then (12.5%) *Citrobacter freundii*, *Salmonella spp* and *Proteus mirabilis* with (8.3%) followed finally by *Serratia* and *Yersinia* (4.2%). Antibiotic resistance patterns were determined using disk-diffusion method. Twelve antibiotics belonging to the class B-lactams were tested. The isolates show 96 % resistance to Cefotaxime, 92% Ampiclox, 87.5% Cefuroxime, 75% Amoxicillin and Imipenem, 50% Nalidixic acid, 33.3% Gentamycin, 8.3% Ofloxacin and 4.2 % Cefexime. It was observed that the isolates were 100% susceptible to Lovofloxacin, 96% Cefexime, 92% Ofloxacin, 75% Ceftriaxone sulbactam, 66.7% Gentamycin, 58% Nitrofurantoin, 50% Nalidixic acid, 25% Amoxicillin, 12.5% Cefuroxime, 8.3% Ampiclox, and 4.2% Cefotaxime. A MAR index > 0.2 was observed for the isolates, indicating frequent antibiotic use in the area.

Keywords: Antibiotics, Water, Bacteria, Resistance

1.0 INTRODUCTION

The presence of antibiotics in different backgrounds is due to their usage by humans and in veterinary medicine. Inappropriate and illogical uses of antimicrobial drugs provide favourable conditions for resistant microorganisms to emerge, spread and persist (WHO, 2011). Antimicrobials are any kind of agent with inhibitory or killing effect to a microorganism. In a more confining term, Antibiotics refer to the natural source of the antimicrobial agent. Antibiotics were primarily considered small molecular weight organic molecules or metabolites used in response of some microorganisms against others that inhabit the same 'habitat' and compete for the same nutrients (Wander, 2018).

In the 21st century, Antimicrobial resistance (AMR) is globally considered as a threat to

human

health and no single or straightforward strategy will serve to contain the emergence fully and spread of infectious organisms that become resistant to the available antimicrobial drugs (Liu *et al.*, 2016). Increased outbreaks of enteric illnesses have invariably led to increased antibiotics for managing infections. The prevention and effective treatment of many diseases caused by infectious pathogens such as bacteria, parasites, viruses and fungi are threatened by Antimicrobial resistance. The development of AMR is a natural phenomenon in microorganisms and is hastened by the selective pressure exerted by the use and misuse of antimicrobial agents in humans and animals. Antimicrobial agents are divided into two groups based on their mode of action. The main groups are agents that depolarize the cell membrane, inhibit cell wall synthesis, Inhibit protein

synthesis, nucleic acid synthesis, and metabolic pathways in bacteria.

There is a wide range of bacterial pathogens and other devious pathogens found in water and wastewaters. Human enteric pathogens (viruses, bacteria, and protozoa) cause significant water-borne diseases with bacteria dominating as the causative agent of many enteric illnesses (Desouky *et al.*, 2003). *Pseudomonas aeruginosa*, *Campylobacter jejuni*, enterotoxigenic *Escherichia coli* (*E. coli*), *Shigella* spp, *Vibrio cholerae* O1, *Salmonella typhi*, and enteropathogenic *E. coli* are the commonly detected bacterial pathogens in water sources (Fricker, 2003). Bacterial pathogens found in water can be enteric and non-enteric, although non-enteric pathogen e.g. *Legionella* spp. and *Leptospira* causes non-enteric illnesses. Gram-negative bacteria have a broad family, including many pathogens such as *Klebsiella*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Escherichiacoli*, *Shigella*, *Proteus*, *Serratia* and other species which are known as *Enterobacteriaceae*. These pathogens are found in humans and are the leading cause of urinary tract infections (UTIs), and some species can also cause diarrhea. These pathogens can diffuse into blood vessels, compromising circulation and resulting in deadly complications. Antibiotic resistance developed by all bacteria is also employed by *Enterobacteriaceae*, they can be resistance to antibiotics including the carbapenem group of antibiotics, which are sometimes referred to as the last line of antibiotic treatment against resistant organisms. To treat infections caused by *Enterobacteriaceae*, Beta-lactam drugs such as extended-spectrum penicillins, cephalosporins, monobactams, carbapenems, fluoroquinolones (e.g. ciprofloxacin), and aminoglycosides (e.g. gentamicin) are among the most prescribed antibiotics (David, 2005). The widespread use of beta-lactam antibiotics has driven the expansion of resistant *Enterobacteriaceae*. The *E. coli* in water can be used as a microbiological indicator for fecal contamination and as a measurement for sanitary quality (Geldreich, 1983). Most outbreaks caused by *E. coli* O157:H7 have been water or food related. *Salmonella* represents the most frequent agent responsible for bacterial gastroenteritis and typhoid when transmitted via water to humans. *Salmonella* remains viable for

several hours upon isolation from water samples (Malorny *et al.*, 2008).

2.0 MATERIALS AND METHODS

2.1 Study Area

The samples were obtained from six wards (three location from each) in Gusau metropolis (Sabon gari, Mayanawa, Galadima, Rijjiya, Madawaki and Tudun Wada area) Zamfara State. Zamfara State is situated in the northwestern part of Nigeria comprises fourteen local governments and shares borders with Kebbi, Kaduna, Sokoto and Katsina State. It has a landmark of 39,762km² with a population of 3,278,873 as of 2006 (Source: Zamfara State Government).

2.2 Sample Collection/Preparation and Isolation of Bacteria

Eighteen (18) water samples were collected from underground wells three from each of the six different wards in Gusau metropolis (Sabon gari, Mayanawa, Galadima, Rijjiya, Madawaki and Tudun wada area) Zamfara State. After sampling, microorganisms in water samples were harvested by filtering 200ml of water on a sandwich filter of a 0.4µm pore size nitrocellulose membrane (Millipore Corp., Bedford, MA, USA). After filtration, the membrane containing the bacteria was placed on a selective and differential medium (MacConkey agar, Mannitol salt agar, SSA) and incubated at 37°C for 18 hrs. Standard methods were used for the enrichment, isolation, identification, and biochemical confirmation of water-borne pathogens (Lemarchand *et al.*, 1996).

2.3 Antibacterial Susceptibility Testing

Antimicrobial susceptibility of the isolates was tested as described by Dalsgaard *et al.* (1996) and Scarscia *et al.* (2003). Commercially prepared single antibiotic discs was used containing about twelve antibiotics including Gentamycin (10µg), Nalidixic (30 µg), Cefexime (5µg), Levofloxacin (5µg), Nitrofurantoin (30µg), Cefuroxime (30µg), Ceftriaxone sulbactam (45 µg), Imipenem/cilastatin (10/10µg), Ofloxacin (5µg), Ampiclox(10µg), amoxicillin-clavulanic acid (30µg), Cefotaxime (25µg). A colony of the test organism was picked using a sterile wire loop and emulsified in a clean bijoux bottle using normal saline before streaking on Muller Hinton agar plate. A sterile cotton swab was dipped into the inoculums, and excess liquid was drained. The surface of the agar plate was streaked with the

swab, and the inoculums were allowed to dry for 5-10 minutes with the aid of sterile forceps. The single antibacterial disc was then placed on the agar two millimetres away from each other. The plates were incubated at 37°C for 24 hours and examined for growth. Plates without colonies were further incubated for 24 hours, and the radius of each inhibition zone was measured in millimeters. The diameter of the zone is related to the susceptibility of the isolate. Results were interpreted using standardized thresholds as defined by NCCLS (2011).

2.4 Determination of Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance index % was calculated where 'a' represent the number of antibiotics to which the isolates were resistant, and 'b' represent the total number of antibiotics to which the isolates were exposed.

3.0 RESULTS

3.1 Frequency of Bacterial Isolates from well Water Samples

The frequency of bacterial isolation in well water samples is shown in Table 1. *Pseudomonas spp.* had a frequency of (29.2%) followed by *Shigella* and *Aliccaligenes* with (16.7%). *Citrobacter* had a frequency of 12.5%, while *Salmonella spp.* and *Proteus mirabilis* had isolation frequencies of (8.3%). Lastly, *Serratia* and *Yersinia* had the lowest frequency of 4.2%.

Table 1. Frequency of Bacterial Isolates from Well Water Samples

S/N	ISOLATES	NO OF ISOLATES	FREQUENCY (%)
1	<i>Pseudomonas spp.</i>	7	29.2
2	<i>Shigella spp.</i>	4	16.7
3	<i>Aliccaligenes</i>	4	16.7
4	<i>Citrobacter spp.</i>	3	12.5
5	<i>Salmonella spp.</i>	2	8.3
6	<i>Proteus Mirabilis</i>	2	8.3
7	<i>Yersinia</i>	1	4.2
8	<i>Serratia</i>	1	4.2

Key: %= Percentage

3.2 Distribution of Bacterial Isolates in Well Water Samples in Six Wards of Gusau Metropolis

The bacteria found in well water samples in six wards of Gusau is presented in the Table 2. which shows that well two of Tudun wada ward, well one and two of Galadima ward as well as well

three of Madawaki no growth of gram negative bacteria was found after isolation, this was the same in well three of Rijjiya and well two and three of Sabon gari ward. While Some bacterial isolates were found in other wells in the wards as shown below.

Table 2. Distribution of Bacterial Isolates in Well Water Samples in Six Wards of Gusau Metropolis

S/N	WARD	LOCATION	ISOLATE PRESENT
1	Tudun Wada	Yar kwakwa	<i>Proteus spp, Shigella spp</i>
		Awala	NIL
		Shaiskawa	<i>Pseudomonas spp, Alcaligenes spp, Citrobacter spp</i>
2	Galadima	Tsohon kara	NIL
		Barakallahu	NIL
		Bayan mega	<i>Salmonella spp, Psedomonas spp.</i>
3	Madawaki	Kofar jange	NIL
		Kwararon dangiwa	<i>Psedomonas spp, Shigella spp</i>
		Lugun malamai	<i>Proteus spp, Pseudomonas spp</i>

4	Rijiya	Geba	<i>Citrobacter spp, Serratia</i>
		Tashan mai lena	<i>Alcaligenes spp, Shigella spp</i>
		Yar dantsi	<i>NILL</i>
5	Mayanawa	Gada biyu	<i>Citrobacter spp, Alcaligenes spp</i>
		Gidan dutsi	<i>Yersinia, Alcaligenes spp, Pseudomonas</i>
		Taqama	<i>Shigella spp, Pseudomonas</i>
6	Sabon Gari	Mortogage	<i>Salmonella spp, Pseudomonas spp.</i>
		Salisa School	<i>NIL</i>
		Daraja cassette	<i>NIL</i>

Key: NIL- no growth of gram-negative bacteria found

3.3 Antibiotic Resistance Pattern of Bacteria Isolated from Well Water Samples

The resistance patterns of the isolated bacteria to individual antibiotics shows that *Pseudomonas spp.* isolate had 71% resistance to Gentamycin, 85% to Amoxicillin and Nalidixic acid, 71% to Imipenem and Cefuroxime, 14% to Ofloxacin and Ceftriaxone sulbactam, and 100% resistance to Cefotaxime and Ampiclox. *Shigella spp.* was 75% resistant to Amoxicillin, Cefotaxime, Imipenem, Cefuroxime, Ceftriaxone sulbactam, 25% to Ofloxacin, 50% resistance to Nalidixic acid and Nitrofurantoin, and 100% to Ampiclox. *Aliccaligenes* isolates were 75% resistance to Amoxicillin, Imipenem and Ampiclox, and 100% resistant to Cefotaxime and Cefuroxime. *Citrobacter* was 66.7% resistant to Gentamycin,

Nalidixic acid and Nitrofurantoin, and 100% resistant to Amoxicillin, Cefotaxime, Imipenem, Gentamycin, Nalidixic acid, Nitrofurantoin, Ampiclox and Ceftriaxone. The resistance patterns showed by *Salmonella spp.* were Gentamycin (50%), Nalidixic acid (50%), Nitrofurantoin (50%), and Ceftriaxone sulbactam (50%), and 100% for Cefotaxime, Imipenem, Ampiclox, and Cefuroxime. The *Proteus Mirabilis* isolates were 100% resistant to Amoxicillin, Cefotaxime, Ampiclox and Cefuroxime. While *Yersinia* was 100% resistant to Cefotaxime, Imipenem, Nalidixic acids, Ampiclox and Cefuroxime. The final isolates from *Serratia* were 100% resistant to Amoxicillin, Cefotaxime, Imipenem, Ampiclox and Cefuroxime which is shown in Table 3.

Table 3. Antibiotic Resistance Pattern of Bacteria Isolated from Well Water Samples

Isolates	Antibiotic Resistance Pattern of Bacterial Isolates (%)							
	<i>Pseudomonas</i>	<i>Shigella</i>	<i>Aliccaligenes</i>	<i>Citrobacter</i>	<i>Salmonella</i>	<i>Proteus</i>	<i>Yersinia</i>	<i>Serratia</i>
No. of Isolates	7	4	4	3	2	2	1	1
Antibiotics(µg)								
Gentamycin (10 µg)	71	-	-	66.7	50	-	-	-
Amoxicillin (30 µg)	85	75	75	100	-	100	-	100
Cefotaxime (25 µg)	100	75	100	100	100	100	100	100
Imipenem (10/10 µg)	71	75	75	100	100	-	100	100
Ofloxacin (5 µg)	14	25	-	-	-	-	-	-
Nalidixic acid (30 µg)	85	50	-	66.7	50	-	100	-
Ampiclox(10 µg)	100	100	75	100	100	100	100	100
Cefexime (5 µg)	-	-	-	-	-	-	-	-
Levofloxacin (5 µg)	-	-	-	-	-	-	-	-
Nitrofurantoin (300 µg)	57	50	-	66.7	50	-	-	-
Cefuroxime (30 µg)	71	75	100	100	100	100	100	100

Ceftriaxone Sulbactarm (45 µg)	14	75	-	-	50	50	-	-
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Key: (-) susceptible

3.4 Susceptibility Pattern of Bacteria Isolated from Well Water samples

The percentage susceptibility of the bacterial isolates to antibiotics was as follows: 100% Levofloxacin, 96% Cefexime, 92% Ofloxacin, 75% Ceftriaxonesulbactarm, 67% Gentamycin, 58% Nitrofurantoin, 50% Nalidixic, 25% Amoxicillin and Imipenem, 12.5% Cefuroxime,

8.3% Ampiclox, and 4.2% Cefotaxime. The corresponding resistance patterns of the isolates to antibiotics is shown in table 2. Levofloxacin shows the highest susceptibility 100% while Cefotaxime shows the highest Resistance 96% and on the other hand cefotaxime shows the lowest susceptibility 4.2%.

Table 4. Susceptibility Pattern of Bacteria Isolated from Well Water samples

Antibiotics(µg)	%RESISTANCE	%SUSCEPTIBILITY
Gentamycin (10 µg)	33.3	66.7
Amoxicillin (30 µg)	75	25
Cefotaxime (25)	96	4.2
Imipenem (10/10 µg)	75	25
Ofloxacin (5 µg)	8.3	92
Nalidixic acid (30 µg)	50	50
Ampiclox (10 µg)	92	8.3
Cefexime (5 µg)	4.2	96
Levofloxacin (5 µg)	-	100
Nitrofurantoin (300 µg)	33.3	58
Cefuroxime (30 µg)	87.5	12.5
Ceftriaxonesulbactarm (45 µg)	25	75

Key: %= Percentage, µg= Microgram

3.5 Multiple Antibiotic Resistance Pattern of Bacteria Isolated from Well Water Samples

The MAR index of the isolates shown in (Table 3) revealed that *Pseudomonas spp.*, *Shigella*, and *Salmonella spp.* isolates had similar MAR index of 0.7. *Yersinia*, *Serratia*, an isolate of

Aliccaligenes had 0.4 MAR index. *Citrobacter* and *Pseudomonas* isolates had 0.6 a MAR index of 0.6. *Aliccaligenes*, *Proteus Mirabilis*, and *Salmonella* isolates had a MAR index of 0.3. In summary, all the isolates had a MAR index > 0.2, indicating frequent use of antibiotics in the area.

Table 5. Multiple Antibiotic Resistance Pattern of Bacteria Isolated from Well Water Samples

BACTERIAL ISOLATE	No. of Antibiotics		MAR index (a/b)
	Resistant (a)	Tested (b)	
<i>Pseudomonas spp.</i>	6	12	0.5
	7	12	0.6
	8	12	0.7
<i>Shigella spp.</i>	6	12	0.5
	8	12	0.7
	3	12	0.3
<i>Alcaligenes</i>	4	12	0.3
	5	12	0.4
<i>Citrobacter spp.</i>	7	12	0.6

	6	12	0.5
<i>Salmonella spp</i>	4	12	0.3
	8	12	0.7
<i>Proteus spp</i>	2	12	0.2
	4	12	0.3
<i>Yersinia spp.</i>	5	12	0.4
<i>Serratia</i>	5	12	0.4

4.0 DISCUSSION

In the result obtained in this study, pathogenic bacteria (gram-negative) have successfully been isolated from well water samples which yielded 24 bacterial isolates and were characterized into eight genera (*Pseudomonas spp*, *Shigella*, *Salmonella spp*, *Alcaligenes*, *Citrobacter*, *Proteus*, *Yersinia* and *Serratia spp*). The presence of *Salmonella* in the sample might be due to environmental contaminants or lack of proper sanitation around the area, this was in line with a research carried out by Hayder and Abdul 2019, where they found out that about 18.66% of their isolates were *Salmonella* and was the predominant agent responsible for foodborne and waterborne diseases. Nazzal *et al.*, 2012 also reported that *Salmonellae* are constantly found in environmental samples as a result of excretion by humans, pets, farm animals and wildlife. The presence of *Salmonella spp.* shows a possibly serious public health risk to waste handlers, scavengers and people living in the vicinity. The presence of *Shigella spp* in the sample in this research, was also in agreement with the work of Xuanxian *et al.*, 2002 who reported that *Shigella* species are one of the most etiological agents that cause many infectious diseases such as dysentery. They found about 65% of their isolates from environmental sample to be *Shigella spp.* The isolation of water-borne pathogen (Gram negative Bacteria) in this study revealed the presence of *Pseudomonas spp.* (29.2%) which displays the highest frequency followed by *Shigella spp.* and *Alicaligenes* with (16.7%) then (12.5%) *Citrobacter*, *Salmonella spp.* and *Proteus mirabilis* with (8.3%) and finally *Serratia* and *Yersinia* (4.2%) have the lowest frequency, the prevalent of these bacteria might be attributed to poorly constructed wells or uncased well that may result in the contamination of water by these pathogens. This was similar to a study carried out by Teklu *et al.*, 2019 where they reported the prevalence of ESBL-producing *Enterobacteriaceae* in different clinical specimens.

In this research, also high Antibiotic resistance was observed to Cefotaxime 96% followed by Ampiclox 92%, 87.5% to Cefuroxime, Amoxicillin and Imipenem 75%, Nalidixic acid 50%, Gentamycin 33.3%, Ofloxacin 8.3% and Cefexime 4.2% which may be due to overuse of these antibiotics as well as poor infection prevention and control in the area. This was similar to the Study conducted by Sales *et al.*, 2019 which reported highest resistance was observed to the antibiotics of amikacin (81.81%), nalidixic acid (89.09%) and ceftriaxone (75.45%) which they believed the reason for the increased antibiotic resistance of this bacteria is due to inappropriate prescription of antibiotics. In this Study, *Pseudomonas spp.* isolate showed 71% resistance to Gentamycin, 85% resistance to Amoxicillin and Nalidixic acid, 71% resistance to Imipenem and Cefuroxime, then 14% to Ofloxacin and Ceftriaxone sulbactarm and the isolate also display 100% resistance to Cefotaxime and Ampiclox. *Shigella* was observed to have 75% resistance to Amoxicillin, Cefotaxime, Imipenem, Cefuroxime, and Ceftriaxone sulbactarm, 25% to Ofloxacin, 50% resistance to Nalidixic acid and Nitrofurantoin and 100% to Ampiclox. *Alicaligenes* isolates showed 75% resistance to Amoxicillin, Imipenem and Ampiclox, resistance to Cefotaxime and Cefuroxime observed to be 100%. *Citrobacter* shows 66.7% resistance to Gentamycin, Nalidixic acid and Nitrofurantoin, and 100% resistance to Amoxicillin, Cefotaxime, Imipenem, Gentamycin, Nalidixic acid, Nitrofurantoin, Ampiclox and Ceftriaxone. *Salmonella spp.* shows 50% resistance to Gentamycin, Nalidixic acid, Nitrofurantoin, and Ceftriaxone sulbactarm, and also the isolate shows 100% resistance Cefotaxime, Imipenem, Ampiclox, and Cefuroxime. It was also observed that *Proteus* isolate showed 100% resistance to Amoxicillin, Cefotaxime, Ampiclox and Cefuroxime. *Yersinia* showed 100% resistance to Cefotaxime, Imipenem, Nalidixic acid, Ampiclox

and Cefuroxime. And lastly *Serratia* isolates shows while 100% resistance to Amoxicilin, Cefotaxime, Imipenem, Ampiclox and Cefuroxime.

The Susceptibility pattern of the bacteria isolated from well water samples in this Study shows 100% susceptibility to Lovofloxacin, followed by 96% to Cefexime, 92% Ofloxacin, 75% Ceftriaxone sulbactam, 66.7% to Gentamycin, 58% are subceptible to Nitrofurantoin, 50% to Nalidixic acid, 25% to Amoxicillin and 12.5% to Cefuroxime, 8.3% are subceptible to Ampiclox and only 4.2% of the isolates are subceptible to Cefotaxime which was similar to a study conducted by Teklu *et al.*, 2019 which they concluded that the antibiotics are active treatment of choice against these pathogens.

From this Study, almost all the isolates are revealed to have a MAR index >0.2 which indicates that the isolates are from an environment where antibiotic is frequently used, or the isolates are from high risk sources of antibiotic resistance which is line with the Study conducted by Nyandjou *et al.*, 2019 in which about 50% of their isolates (*Salmonella*) tested exhibited multidrug resistance, suggesting the existence of higher frequency of MDR species in our study area.

5.0 CONCLUSION

This Study shows prevalence of water-borne bacteria (gram negative bacteria) in the samples which may be due to poor water treatment or lack of proper sanitation of the environment. A higher level of resistance to multiple classes of antibiotics was observed among the isolates. The better options for the treatment of these isolates is Levofloxacin, Cefexime and Ofloxacin. And the isolates showed a high rate of resistance to Cefotaxime, Ampiclox, Cefuroxime, Amoxicillin and Imipenem due to the presence of resistance genes that were detected after amplification. The rise of MDR necessitates the strengthening of clinical bacteriology research and the diagnostic capacity of laboratory professionals for the detection and surveillance of antibiotic resistance.

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