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Distribution and Antibiotic Resistance Profile of Enterobacteriaceae Isolates from Well Water in the Rural Communities of Ezza South Local Government Area of Ebonyi State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Enterobacteriaceae as an indicator of water sanitary quality and their frequency of occurrence as antibiotic-resistant microbial pathogens in water remain a threat to humans and the environment. This research work aimed to determine the distribution and antibiotic resistance profile of Enterobacteriaceae isolates from different well water sources in Ezza South Local Government Area of Ebonyi State, Nigeria. A total of 100 well water samples were collected and subjected to bacteriological analysis using Standard Microbiological protocol for isolation and identification. Antimicrobial resistance studies of Enterobacteriaceae were determined using the Kirby–Bauer disk diffusion method and, the results were analyzed and compared with the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. The result of isolation revealed a high colony enumeration of bacterial isolates from well water, revealing high bacterial counts of 1.0x104-8.1 x104 cfu/ml from Nsukara. The distribution of bacteria in well water revealed a high proportion of *E. coli* 42 (42.0%) followed by *Salmonella* species 12 (12.0%) and *Shigella* species 7(7.0%). The isolates exhibited a high percentage of resistance to ceftriaxone 100%, Sulfamethoxazole/ trimethoprim 100% tetracycline 66.7%, 66.7% but were susceptible to Ciprofloxacin 100 %, and Imipenem 100%. Our findings indicate the presence of antibiotic-resistant bacteria in well water. Within these communities, awareness should be given to the populace on the implication of antibiotic residues in the environment as well as the importance of maintaining a clean and hygienic environment around the wells to ensure the safety of water and also to prevent the spread of resistant determinant in the environment and human. The Government should make provision for a portable water source that will be accessible and well-sustained in the communities.

Keywords: Well water; E. coli; Salmonella species; Shigella species; antibiotic resistance; Enterobacteriaceae.

1. INTRODUCTION

In most rural locations, well water is the most frequent source of drinking water for residential usage and human consumption. It has grown more challenging to meet all of the water requirements in the rural settlements in the Ezza South local government region due to the mostly nonexistent public water supply, which is also inaccessible when it does exist. This has prompted many homes to resort to unsafe and non-potable water sources, resulting in many digging of wells.

Most Nigerian rural areas do not have access to improved water supplies [1,2,3,4]. Their primary sources of free water are usually rivers, perennial streams, ponds, and unprotected wells, all of which are reservoirs of virulence and drug-resistant bacteria.

One of the main environmental problems caused by the inappropriate and negligent disposal of sewage, industrial, and chemical waste is groundwater pollution.

Based on multiple research, it has been determined that groundwater is easily contaminated by rainstorm overflows, runoff from farming areas and areas with septic systems and latrines that are improperly situated which makes the water clinically unsafe for human consumption [5,6,7,8].

The majority of human diseases are associated with unsanitary drinking water supplies, which cause infections such as dysentery, diarrhea, cholera, and typhoid. Earlier reports have shown that over 20% of the world's population has a scarcity of safe drinking water, and over a million people die every year from illnesses related to drinking water due to inadequate sanitation and the convergence of antibiotic-resistant bacteria [9,10,11]. The most thriving bacteria family in water is the Enterobacteriaceae [12,13]. Antibiotic resistance develops naturally, but misuse of antibiotics in humans and veterinary medicine accelerates the process. As a result, water acts not only as a vehicle for the rapid spread of antibiotic-resistant organisms among humans and animals but also as a reservoir for introducing Enterobacteriaceae harboring resistance genes into natural bacterial ecosystems. The rate of increasing cases of antimicrobial resistance has become a worldwide problem in different ecosystems [14].

Although the present guidelines for water quality are established to decrease the number of cases of fecal contamination, but do not consider the tendency that water could serve as a reservoir for antimicrobial resistance determinants that confer resistance against clinically important antimicrobials. However, determining whether antimicrobial-resistant Enterobacteriaceae are

present in well-sourced drinking water would reveal details about the spread and durability of these bacteria, which can live and even become mobilized within the human population and pose a health risk.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at Ezza South local government area, located at latitude 6.1322°N and longitude 8.0216°E in Ebonyi state in south-eastern Nigeria. Its headquarters is Onueke, which also serves as a central unification town for the Ezza nation as well as the headquarters of Ebonyi Central senatorial zone. It was created on October 1, 1996, amongst other local government areas in the then-new Ebonyi state by the military government of late General Sani Abacha. Ezza South before its creation was part of the old Ezza Local government area. The people are predominantly of Igbo stock. They speak the Ezza dialect and the central Igbo language. Their major occupations are farming and trading as well as the emerging civil servant class. It has an area of 324 km^2 and a population of 133,625 at the 2006 census.

2.2 Sample Collection

Following multiple initial visits to villages around the study area, nine communities—Onueke Urban, Amuzu, Ezzama, Echara, Umunwagu Idenbia, Ikwuato Idembia, Amagu Amaezekwe, Amana, Nsukara, and Amudo/Okoffia—were subsequently identified and selected for sampling.

Well water was drawn using a fetcher found in the sampling points. A total of one hundred (100) well water samples, each containing approximately 500 ml of well water, were taken from ten wells in each of the selected communities. Water Samples were stored in a flask at −4˚C, and aseptically transported to the laboratory within 2 hours for bacteriological analysis.

2.3 Isolation, Colony Enumeration of Bacterial Strains and Identification

Ten folds serial dilutions were carried out following standard microbiological procedures, by using 1ml of each water sample in 9ml of sterile water [15]. After the dilution, 0.5 ml from dilution factor five (10^5)was transferred to each sterile petri dish before pouring a sterilized plate count agar (Hi Media, Mumbai, India). The plates were incubated at room temperature for 24 hours. After 24 hrs of incubation, colonies were counted using Colony counter (Reichert, Inc. Quebec®), and a loopful of each colonies were aseptically streaked on solidified eosin methylene blue agar plate, *Salmonella*/*Shigella* agar (Hi Media, Mumbai, India). The plates were incubated for 18-24 hrs at 37℃. Bacterial colonies with a greenish-metallic sheen on the eosin methylene blue agar plate, black-centered colonies on *Salmonella/Shigella* agar, and smooth and opaque or colorless on *Salmonella*/*Shigella* agar were inferred as the presence *of Escherichia coli, Salmonella species*, and *Shigella* species respectively. An API 20E kit (BioMérieux, Marcyl'Etoile, France) was used to identify and differentiate the Gram-negative bacteria of the family Enterobacteriaceae following the manufacturer's instructions.

2.4 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller Hinton agar based on the Clinical Laboratory Standard Institute (CLSI) standards [16]. The suspension of standard inoculums from the pure culture was adjusted to achieve turbidity equivalent to 0.5 McFarland standard solutions, the suspension was emulsified using sterile cotton swabs onto Mueller Hinton agar plate. The antibiotics to be tested are; Meropenem (10 µg), Gentamicin (30 µg), Imipenem (10 µg), Ciprofloxacin (30 µg), Sulthamethoxazole/trimethoprim (12.5 µg), Ceftriaxone (30 µg), Amikacin (30 µg), Tetracycline (30 µg), Azithromycin (30 µg), Cefepime (30 µg) was placed on the inoculated agar and incubated at 37 ^{O}C for 16 to 18 h. The inhibition zone of each antimicrobial agent were interpreted according using CLSI standards [16,17].

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Colony enumeration of bacteria isolated from different well water sources in Ezza south LGA

Colony enumeration of bacteria isolated from well water revealed a high Bacteria Count of $1.0x10^4$-$8.1\ x10^4$ from Nsukara followed by Onueke Urban $1.0x10^3$-$1.6x10^3$, Amuzu $1.0x10^3$-$1.0\ x10^4$, Ezzama $0.9x10^3$-$2.0x10^3$, Echara $1.0x10^3$-$1.0x10^4$, Umunwagu Idenbia $1.3x10^3$-$1.4x10^3$, Ikwuato Idembia $1.3x10^3$-$1.0x10^4$, Amagu/Amaezekwe $1.3x10^3$-$1.0x10^4$,

Amana1.3x10^3-2.0x10^2 and Amudo/Okoffia 1.2x10^3-1.6 x10^3as shown in Table 1.

3.1.2 Distribution of bacteria isolated from different well water sources in Ezza South LGA

The distribution of bacteria in well water revealed a high proportion of E*. coli* 42 (42.0%) followed by *Salmonella* species 12(12.0%) and *Shigella* species 7(7.0 %). *E. coli* was highly predominant in samples from Onueke Urban 60.0 % over Amagu Amaezekwe 50.0%, and Nsukara 40.0 % while *Salmonella* species comprising 50.0%, 30.0%, and 20.0% from samples in Amuzu, Amana and Ezzama respectively. *Shigella* species accounted for 30.0%, 20.0%, and 20.0% of Onueke Urban, Amuzu, and Ezzama respectively as shown in Table 2.

3.1.3 Antibiotic Susceptibility profile of *Shigella* species isolated from different well Water sources in Onueke Urban Ward, Ezzama Ward, Amuzu Ward in Ezza South LGA

In Onueke Urban ward, *Shigella* species from well water samples were 100 % resistant to tetracycline Trimethoprim-Sulfamethoxazole, Azithromycin, Cefepime, and ceftriaxone but were sensitive to meropenem 100%, Ciprofloxacin 100%, Imipenem 100%, Gentamicin 100%. *Shigella* species from the Ezzama Ward well water sample were resistant meropenem 100%, Amikacin 100%, Tetracycline 100%, Trimethoprim-Sulfamethoxazole 100%, Azithromycin 100%, Cefotaxime 100 %but were susceptible to ciprofloxacin 100%, Imipenem 100% and Gentamicin 100%. The majority of the *Shigella* species from well water in Amuzu Ezza were susceptible to meropenem 50.0%, Ciprofloxacin 100%, Imipenem 100%, Gentamicin 100% but were resistant to Tetracycline 100%, Trimethoprim-Sulfamethoxazole 100%, Cefepime 100%, Ceftriaxone 100% and Azithromycin 100% as presented in Table 3.

3.1.4 Antibiotic Susceptibility profile of *Salmonella* species isolated from different well Water sources in Onueke Urban Ward, Amuzu Ward, Ezzama Ward and in Ezza South LGA

The antibiotic susceptibility profile of *Salmonella* species shows that in Onueke Urban Ward, *Salmonella* species from well water samples were 100% resistant to tetracycline Trimethoprim-Sulfamethoxazole, Azithromycin, Ceftriaxone, Cefepime, and meropenem but were sensitive to Ciprofloxacin 100% Imipenem 100%, Gentamicin 100% while in Well Water from Amuzu Ward, majority of the isolates were susceptible to Imipenem 100%, Gentamicin 100%, ciprofloxacin 100%, but were resistant to meropenem 40.0%, Amikacin 60.0%, Tetracycline 100%, Trimethoprim-Sulfamethoxazole 100%, Azithromycin 100%, Ceftriaxone 100% and Cefepime 100 %. In the Ezzama community, isolate from well water sample demonstrated resistance to Amikacin 66.7%, Tetracycline 100%, Trimethoprim-Sulfamethoxazole 100%, Ceftriaxone 100%, Azithromycin 100%, Cefepime 100% but were susceptible to ciprofloxacin 100%, Imipenem 100% and Gentamicin 66.7% while in well water from Amana Ward the isolate was susceptible to meropenem 66.7%, Gentamicin 100%, Imipenem 100% and ciprofloxacin 100% respectively but were resistant tetracycline, Cefepime, Ceftriaxone, Trimethoprim-Sulfamethoxazole, Azithromycin recording 100 % respectively as presented in Table 4.

3.1.5 Antibiotic susceptibility profile of *E. coli* isolates from different well water sources in Onueke, Ezzama, Amuzu, Echara and Amudo/Okoffia communities in Ezza South, Ebonyi State

Escherichia coli isolates from well water Onueke were susceptible to Amikacin 50.0%, Ciprofloxacin 100%, Imipenem 100%, and Gentamicin 100% respectively but were resistant to meropenem 66.7%, Azithromycin 100%, tetracycline 100%. In the Amuzu community, *E. coli* from well water were resistant to Azithromycin 75.0%, Trimethoprim-Sulfamethoxazole 100%, and Ceftriaxone100 % but were sensitive to Amikacin 50.0%, meropenem 75.0%, Imipenem 100%, Gentamicin 100 % and Ciprofloxacin 100%.

E. coli isolate from well water samples in Ezzama ward were susceptible to Meropenem 66.7%, Amikacin 66.7%, Ciprofloxacin 100 %, Imipenem 100%, Gentamicin 100% but were 100% resistant to Tetracycline 100%, Trimethoprim-Sulfamethoxazole, Azithromycin, Ceftriaxone 100% and Cefepime 100%. Isolate from well water samples in Echara were susceptible to meropenem 75.0%, Imipenem 100%,

Table 1. Colony enumeration of bacteria isolated from different well water sources in Ezza south L.G.A

S/No.	Onueke Urban		Amuzu		Ezzama		Echara		Umunwagu Idenbia		Ikwuato Idembia	
	Colonies	Cfu/ml	Colonies	Cfu/ml	Colonies	Cfu/ml	Colonies	Cfu/ml	Colonies	Cfu/ml	Colonies	Cfu/ml
1	59	$1.2x10^4$	58	$1.2x10^3$	45	$1.0x10^3$	65	$1.3x10^3$	66	$1.3x10^3$	67	$1.3x10^3$
2	48	$1.0x10^4$	54	$1.0x10^3$	81	$1.6x10^3$	61	$1.2x10^3$	71	$1.4x10^3$	81	$1.6x10^3$
3	47	$1.0x10^4$	70	$1.4x10^3$	58	$1.2x10^3$	51	$1.0x10^3$	75	$1.5x10^3$	77	$1.5x10^3$
4	81	$1.6x10^3$	72	$1.4x10^3$	39	$0.8x10^3$	91	$1.8x10^3$	80	$1.6x10^3$	66	$1.3x10^3$
5	55	$1.1x10^3$	48	$1.0x10^4$	101	$2.0x10^3$	72	$1.4x10^3$	61	$1.2x10^3$	81	$1.6 x10^3$
6	56	$1.1x10^3$	45	$1.0x10^4$	60	$1.2x10^3$	65	$1.3x10^3$	58	$1.2x10^3$	70	$1.4x10^3$
7	70	$1.0x10^3$	47	$1.0x10^4$	42	$0.8x10^3$	61	$1.2x10^3$	70	$1.4x10^3$	67	$1.3x10^3$
8	40	$1.0x10^3$	40	$1.0x10^3$	46	$0.9 x10^3$	70	$1.4x10^3$	81	$1.6x10^3$	58	$1.2x10^3$
9	50	$1.0x10^3$	79	$1.6x10^3$	50	$1.0x10^2$	48	$1.0x10^4$	67	$1.3x10^3$	60	$1.2x10^3$
10	62	$1.2x10^3$	55	$1.1x10^3$	47	$0.9x10^3$	49	$1.0x10^4$	72	$1.4x10^3$	49	$1.0x10^4$

Table 1 contd: Colony enumeration of bacteria isolated from different well water sources in Ezza south L.G.A

S/No.	Amagu Amaezekwe		Amana		Nsukara		Amudo/Okoffia	
	Colonies	Cfu/ml	Colonies	Cfu/ml	Colonies	Cfu/ml	Colonies	Cfu/ml
1	67	$1.3x10^3$	71	$1.4x10^3$	39	$1.0x10^4$	80	$1.6x10^3$
2	80	$1.6x10^3$	80	$1.6x10^3$	81	$1.6x10^3$	72	$1.4x10^3$
3	77	$1.5x10^3$	100	$2..0x10^2$	66	$1.3x10^3$	60	$1.2x10^3$
4	61	$1.2x10^3$	102	$2.0x10^2$	70	$1.4x10^3$	58	$1.2x10^3$
5	58	$1.2x10^3$	66	$1.3x10^3$	81	$8.1 x10^4$	60	$1.2 x10^3$
6	70	$1.4x10^3$	58	$1.2x10^3$	59	$1.2x10^3$	78	$1.4x10^3$
7	48	$1.0x10^4$	96	$1.9x10^3$	48	$1.0x10^4$	80	$1.6x10^3$
8	49	$1.0x10^4$	72	$1.4x10^3$	50	$1.0x10^3$	78	$1.4x10^3$
9	60	$1.2x10^3$	81	$1.6x10^3$	82	$1.6x10^3$	69	$1.4x10^4$
10	71	$1.4x10^3$	68	$1.3x10^3$	73	$1.5x10^3$	58	$1.2x10^3$

***Key**: **Cfu-**Colony Forming Unit, **ml-**Milligram*

Table 2. Distribution of bacteria isolated from different well water sources in Ezza South LGA

Location	No. sampled	*E. coli* (%)	*Salmonella* species (%)	*Shigella* species (%)
Onueke Urban	10	6(60.0)	2(20.0)	3(30.0)
Amuzu	10	4(40.0)	5(50.0)	2(20.0)
Ezzama	10	3(30.0)	2(20.0)	2(20.0)
Echara	10	4(40.0)	0(0.0)	0(0.0)
Umunwagu Idenbia	10	5(50.0)	0(0.0)	0(0.0)
Ikwuato Idembia	10	4(40.0)	0(0.0)	0(0.0)
Amagu Amaezekwe	10	5(50.0)	0(0.0)	0(0.0)
Amana	10	3(30.0)	3(30.0)	0(0.0)
Nsukara	10	4(40.0)	0(0.0)	0(0.0)
Amudo/Okoffia	10	4(40.0)	0(0.0)	0(0.0)
Total	**100**	**42(42.0)**	**12(12.0)**	**7(7.0)**

Table 3. Antibiotic Susceptibility profile of *Shigella* species isolated from different well Water sources in Onueke, Ezzama, Amuzu communities in Ezza South, Ebonyi State

	Ezza South Communities of Ebonyi State					
	Onueke Urban Ward		Ezzama Ward		Amuzu Ezza	
	Well Water (n=3)		Well Water (n=2)		Well Water(n=2)	
Antibiotic (µg)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
MEM (10)	3(100)	0(0.0)	0(0.0)	2(100)	1(50)	1(50)
CN (30)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)
IPM (10)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)
CIP (30)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)	0(0.0)
SXT (12.5)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)
CRO (30)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)
AK (30)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)
TE (30)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)
AT (30)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)
FEP (30)	0(0.0)	3(100)	0(0.0)	2(100)	0(0.0)	2(100)

Key: *Mem: Meropenem (10), CN = Gentamicin 30, IPM = Imipenem 10, CIP = Ciprofloxacin 30, SXT = Sulthamethoxazole/trimethoprim 12.5, CRO = Ceftriaxone 30, Ak = Amikacin 30, TE = Tetracycline 30, ATM = Azithromycin 30, FEP = Cefepime 30 and % = percentage, S=Susceptible, R=Resistance, n=number of isolate*

Table 4. Antibiotic Susceptibility profile of *Salmonella* species isolated from different well Water sources in Onueke Urban Ward, Amuzu Ward, Ezzama Ward and in Ezza South LGA

	Onueke Urban Ward		Amuzu Ward		Ezzama Ward		Amana Ward	
	Well Water (n=2)		Well Water (n=5)		Well Water(n=2)		Well Water (n=3)	
Antibiotic (µg)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
MEM (10)	0(0.0)	2(100)	3(60)	2(40)	0(0.0)	(100)	2(66.7)	1(33.3)
CN (30)	2(100)	0(0.0)	5(100)	0(0.0)	2(66.7)	(33.3)	3(100)	0(0.0)
IPM (10)	2(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	3(100)	0(0.0)
CIP (30)	2(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	3(100)	0(0.0)
SXT (12.5)	0(0.0)	2(100)	0(0.0)	5(100)	0(0.0)	(100)	0(0.0)	3(100)
CRO (30)	0(0.0)	2(100)	0(0.0)	5(100)	0(0.0)	(100)	0(0.0)	3(100)
AK (30)	0(0.0)	2(100)	2(40)	3(60)	1(33.3)	2(66.7)	1(33.3)	2(66.7)
TE (30)	0(0.0)	2(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	3(100)
AT (30)	0(0.0)	2(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	3(100)
FEP (30)	0(0.0)	2(100)	0(0.0)	5(100)	0(0.0)	(100)	0(0.0)	3(100)

Key: *Mem: Meropenem, CN = Gentamicin, IPM = Imipenem, CIP = Ciprofloxacin, SXT = Sulthamethoxazole/trimethoprim, CRO = Ceftriaxone, Ak = Amikacin, TE = Tetracycline, ATM = Azithromycin, FEP = Cefepime and % = percentage, S=Susceptible, R=Resistance n=number of isolate*

Table 5. Antibiotic Susceptibility profile of *E. coli* isolates from different well Water sources in Onueke, Ezzama, Amuzu, Echara and Amudo/Okoffia communities in Ezza South, Ebonyi State

	Onueke		Ezzama		Amuzu		Echara		Amudo/Okoffia	
	Well water (n=6)		Well Water (n=3)		Well water (n=4)		Well Water (n=4)		Well Water (n=4)	
Antibiotic (µg)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
MEM (10)	2(33.3)	4(66.7)	2(66.7)	1(33.3)	3(75)	1(25)	3(75)	1(25)	0(0.0)	4(100)
CN (30)	6(100)	0(0.0)	3(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)
IPM (10)	6(100)	0(0.0)	3(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)
CIP (30)	5(83.3)	1(16.7)	3(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)
SXT (12.5)	0(0.0)	6(100)	0(0.0)	3(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)	4(100)
CRO (30)	0(0.0)	6(100)	0(0.0)	3(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)	4(100)
AK (30)	3(50)	3(50)	2(66.7)	1(33.3)	2(50)	2(50)	0(0.0)	4(100)	0(0.0)	4(100)
TE (30)	0(0.0)	6(100)	0(0.0)	3(100)	0(0.0)	4(100)	0(0.0)	4(100)	0(0.0)	4(100)
AT (30)	0(0.0)	6(100)	0(0.0)	3(100)	1(25)	3(75)	0(0.0)	4(100)	0(0.0)	4(100)
FEP (30)	0(0.0)	6(100)	0(0.0)	3(100)	0(0.0)	4(0.0)	0(0.0)	4(100)	0(0.0)	4(100)

Key: Mem: Meropenem, CN = Gentamicin, IPM = Imipenem, CIP = Ciprofloxacin, SXT = Sulthamethoxazole/trimethoprim, CRO = Ceftriaxone, Ak = Amikacin, TE = Tetracycline, ATM = Azithromycin, FEP = Cefepime and % = percentage, S=Susceptible, R=Resistance, n=number of isolate

Table 6. Antibiotic Susceptibility profile of *E. coli* isolates from different well Water sources in Umunwagu Idenbia, Ikwuato, Amagu/amezekwe, Amana and Nsukara communities in Ezza South, Ebonyi State

	Umunwagu Idenbia		Ikwuato		Amagu/amezekwe		Amana		Nsukara	
	Well water (n=5)		Well Water (n=4)		Well water (n=5)		Well Water (n=3)		Well Water (n=6)	
Antibiotic (µg)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**
MEM (10)	2(40)	3(60)	3(75)	1(25)	3(60)	2(40)	2(66.7)	1(33.3)	4(66.7)	2(33.3)
CN (30)	5(100)	0(0.0)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)	0(0.0)
IPM (10)	5(100)	0(0.0)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)	0(0.0)
CIP (30)	4(80)	2(40)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)	0(0.0)
SXT (12.5)	0(0.0)	5(100)	0(0.0)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)
CRO (30)	0(0.0)	5(100)	0(0.0)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)
AK (30)	2(40)	3(60)	0(0.0)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)
TE (30)	0(0.0)	5(100)	0(0.0)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)
AT (30)	0(0.0)	5(100)	0(0.0)	4(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)
FEP (30)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	5(100)	0(0.0)	3(100)	0(0.0)	6(100)

Gentamicin 100% and Ciprofloxacin 100% but were resistant to Trimethoprim-Sulfamethoxazole 100 %, Cefepime 100 %, tetracycline 100% and Ceftriaxone 100% while in well water from Amudo/Okoffia, the *E. coli were* susceptible to Imipenem 100%, Gentamicin 100%, ciprofloxacin 100%, but were resistant to Amikacin 100%, Tetracycline 100%, Azithromycin 100%, Trimethoprim-Sulfamethoxazole 100%, meropenem 100%, Ceftriaxone 100 % and Cefepime100 % as presented in Table 5.

3.1.6 Antibiotic Susceptibility profile of *E. coli* isolated from well Water sources in Umunwagu Idenbia and Ikwuato Idenbia in Ezza South LGA

Isolate from Umunwagu Idenbia well water samples were resistant to meropenem 60.0%, Amikacin 60.0%, Tetracycline 100%, Trimethoprim-Sulfamethoxazole 100%, Cefotaxime 100% but were susceptible to ciprofloxacin 100%, Imipenem 100% and Gentamicin 100%, while the isolate from Well Water was sensitive to meropenem 75.0%, Imipenem 100%, ciprofloxacin 100%, Gentamicin 10 % but were resistant tetracycline Trimethoprim-Sulfamethoxazole, Azithromycin, Ceftriaxone and cefepime recorded 100 % while in Well Water from Amagu/amezekwe, the majority of the isolates were susceptible to Imipenem 100%, Gentamicin 100%, ciprofloxacin 100%, but were resistant to Tetracycline 100%, Trimethoprim-Sulfamethoxazole 100%, Azithromycin 100%, Ceftriaxone 100% and Cefepime 100 %.and in well water from Amana Ward the isolate was susceptible to meropenem 66.7%, ciprofloxacin 100%, Imipenem 100%, Gentamicin 100% respectively but were resistant tetracycline, Cefepime, Ceftriaxone, Trimethoprim-Sulfamethoxazole, Azithromycin recording 100 % and Antibiotic susceptibility profile of *Escherichia coli* shows that in Nsukara, *E. coli* from well water samples were 100% resistant to Ceftriaxone, Cefepime, tetracycline Trimethoprim-Sulfamethoxazole, Azithromycin but were sensitive to meropenem 66.7%, Ciprofloxacin 100%, Imipenem 100%, Gentamicin 100% respectively as presented in Table 6.

3.2 Discussion

The failure of the government to provide safe drinking water led to people sourcing potable water by themselves by digging wells for household use. In most cases the standard quality of the dug well may not be achieved, and the depth may directly impact successful proliferation of different bacteria species. A total bacterial counts in all well water samples analyzed in this study indicated a high microbial load in the water. Colony enumeration of bacteria isolated from well water revealed a high Bacteria Count of $1.0x10^4$-$8.1 x10^4$ from Nsukara followed by Onueke Urban $1.6x10^3$ which comparatively exceeds the WHO's standard limits. The standard limit of 1.0×10^3 CFU/ml for heterotrophic bacteria and coliforms was set by WHO and USEPA [18,19]. This could be due to the wells continually receiving dirty water from surface runoff and seepage from contaminated groundwater. Some of the wells are located in densely populated areas and receive doses of feces from the septic tank, water from an abattoir, sewage water, and pit latrines; also, some of the wells have been exposed or kept open. The open exposure of most of the wells increases the rate of airborne spread of pathogenic bacteria. Also, the unhygienic nature of storing and placing of the fetcher may facilitate the spread of numerous bacteria into the well. This result aligns with findings from earlier research on the microbial assessment of drinking-water sources in Bokkos, Plateaus State, Abakaliki Metropolis, Ebonyi State and Ekosodin Community, Edo state, where a high prevalence of heterotrophic bacteria, and coliforms were found, many of which exceeded the acceptable water quality limits [20,21,22].

Many different species of bacteria were isolated from well water in our study. These include *Salmonella* species, which can cause typhoid fever and acute diarrheal infections, *Shigella* species, which can cause dysentery, and *E. coli*, which can cause gastroenteritis, urinary tract disease, neonatal meningitis, hemorrhagic colitis, and Crohn's disease.

Kalu *et al.* [23] stated that research by the WHO indicates that about 80% of diseases globally are linked to the consumption and use of contaminated water.

The presence of coliform bacteria such as *E. coli*, in these well water samples makes them unsafe for drinking for human consumption. In a similar studies, members of the coliform group were found in drinking water in rural Peshawar, India [24], in well water in Shagamu and Iwo Nigeria [25,26], and also in stream water [20]. Although no differentiation was made between pathogenic

and non-pathogenic *E. coli* when this species is isolated from well water in our study, therefore water that contains *E. coli* is unsafe for consumption due to the strong association between *E. coli* and fecal contamination.

The high percentage of *Salmonella* species and *E. coli* in this study implies that the well may be contaminated with feces due to human and animal activity. This finding supports previous report by Ekelozie *et al*. [27] and Tangwa *et al*. [28], who found *Salmonella* spp. and *E. coli* as the most prevalent bacteria in hand-dug wells and boreholes in Ngaoundere municipality of Adamawa region in Cameroon, as well as water sources in two local government areas of Anambra State, Nigeria.

According to Gugu *et al*. [20], the most often isolated bacterium was Salmonella spp. [18(22.8%)], followed by *E. coli* [16(20.3%)]. In a related study, Geta and Kibret. [29] found that *E. coli, Streptococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Citrobacter* spp., and *Salmonella* spp. were the most commonly found bacteria from waste sources in Hotspot Environments in Bahir Dar City, Northwestern Ethiopia.

The presence of intestinal bacteria species such as *Salmonella, Shigella,* and *E. coli* indicates that the water was likely contaminated with feces. Enterobacteria species, especially *E. coli*, are commonly found organisms in various water sources such as rivers, streams, rainwater, well water, groundwater, and even tap water [30].

Resistance to third- and fourth-generation cephalosporins was also frequent. A similar pattern of resistance has been documented in environmental water sources from Southern Chile and water supplies used in poultry production in the Ashanti region of Ghana [31,32]. Atobatele, and Owosen. [26] reported Multi-antibiotic-resistant bacteria were present in all (30/30) of the well water samples, and a high number of the identified bacteria (80%) were resistant to all antibiotics in the cephalosporins group. Cephalosporin resistance may attributed to the presence of ESBL-coding genes (*bla*TEM and *bla*CTX-M) in some strains and have been previously found in water sources [31,33,34,35] indicating potential horizontal gene transfer between environmental and pathogenic bacteria [36,37]. In addition, due to increased anthropogenic activities and incessant intake of antibiotics, many enterobacteria have acquired antibiotic-resistant genes (ARGs) and become pathogenic ARB.

E. coli demonstrated MDR to Azithromycin, sulphamethoxazole/trimethoprim, and tetracycline. The findings of this study confirm the pattern of antibiotic resistance in *E. coli* isolates obtained from water similar to the study conducted by Sayah et al*.* [38] revealed that *E. coli* isolates obtained from surface water samples exhibited resistance to drugs such as tetracycline and sulphamethoxazole/trimethoprim. The results align with a study conducted by Wose-Kinge et al. [39] that examined the antibiotic resistance profiles of *E. coli* isolates obtained from several water sources in the Mmabatho location of South Africa. The isolates were shown to possess resistance against antibiotics like tetracycline. The frequency of multi-drug resistant *E. coli* is consistent with a report by Ogunleye et al. [40], who revealed that all isolates of *E. coli* from poultry in Abeokuta, Southwestern Nigeria, were multi-drug resistant. According to Bueno et al. [41], these results emphasize the potential for these common water-borne bacteria to spread, as well as the existence of ARGs such as β-lactamase genes (bla*SHV*, bla*CTX-M*, bla*KPC*, and bla*TEM*) and other markers of antibiotic resistance that are frequently used in both human and animal medicine (quinolones, tetracyclines, and sulfonamides, among others). Such critically important pathogens, demand the urgent development of novel therapeutic strategies.

Between the drug class aminoglycoside and carbapenems; most bacteria were resistant to amikacin but susceptible to gentamicin. A similar pattern was found along with some resistance to carbapenems (meropenem) over strain susceptible to imipenem. However, no alleles associated with mobile resistance were detected. Therefore, amikacin and meropenem resistance would be attributed to the presence of some intrinsic or acquired resistance mechanisms encoded at the chromosome level in these isolates.

There is a red flag in drug prescription due to the observed pattern of resistance against most of the tested antibiotics, which could be reduced as the majority of the isolates were susceptible with the range of 80-100% to gentamicin, ciprofloxacin, and imipenem and will likely make treatment of bacterial infections less difficult and reduces the severity of life-threatening infection.

4. CONCLUSION

Our report presence of ARB in the well water, enterobacteria in the water sample presented a resistance phenotype to at least one antibiotic. There is a need for constant monitoring of the changes in their abundance and diversity at the different well-drinking water sources. Amongst the communities, awareness should be given to the populace on the implication of antibiotic residues in the environment as well as the importance of maintaining a clean and hygienic environment around the wells to ensure the safety of water. It is also advisable that every individual should embark on in-house water treatment to avoid water-borne diseases. A recommended distance of 50–100 feet from potential sources of groundwater contamination like soakaways, pit latrines, etc., by health authorities should be maintained. Finally, it should be noted that, although the samples obtained are not representative of all well water in Ebonyi State, Nigeria, it would be interesting to increase the sampling of well water and the identification of GNB not only in the state but also in the regions of the country, to correlate the presence of ARG-encoding species with different environmental factors and also relate them to genomic transfer process.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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