CME LIVE OPEN ACCESS

Journal of Biomedical and Engineering Research

Research Article

ISSN: 3065-8780

Prevalence, Characterization and Drug Resistance of Gram-Negative Bacteria Isolated from Domestic and Environmental Water Sources in Gusau, Zamfara State

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Received: 2026 Jan 12 **Accepted:** 2026 Feb 03 **Published:** 2026 Feb 13

Abstract

Access to clean and safe water is essential for human health, yet water contamination with pathogenic microorganisms, particularly Gram-negative bacteria, remains a major public health challenge, especially in developing regions. This study investigated the prevalence, phenotypic characterization, and antibiotic resistance patterns of Gram-negative bacteria isolated from various domestic and environmental water sources in Gusau Metropolis, Zamfara State, Nigeria. A total of 32 water samples were randomly collected from wells, taps, streams, dams and pond. Standard microbiological and biochemical methods were employed for the isolation and identification of Gram-negative bacterial species. Antibiotic susceptibility testing was conducted using the disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. The total bacterial count ranged from 1.10 ×10-4 to 9.0 ×10-4cfu/ml. The results revealed the presence of multiple Gram-negative bacteria, including Escherichia coli, Klebsiella spp., Salmonella spp, Enterobacter spp, Shigella spp and Proteus spp. Notably, a significant proportion (46%) of isolates exhibited resistance to commonly used antibiotics such as Gentamycin, Ampicillin, Ceporex, Nalidixic acid, Augmentin and Septrin while higher susceptibility was observed for Ciproflox, Tarivid, Streptomycin, and Reflacine. The detection of multidrug-resistant strains in both domestic and environmental water samples underscores the potential health risks posed by untreated or poorly managed water sources. These findings highlight the urgent need for improved water sanitation infrastructure, regular monitoring of water quality, and strategic public health interventions aimed at controlling the spread of antimicrobial resistance through environmental pathways.

Key words: Gram-negative, water, Antibiotics, Bacteria

1. Introduction

Access to clean and safe water is a fundamental human necessity, yet many communities in developing regions, including Gusau, Nigeria, continue to rely on untreated or poorly treated water sources for their daily domestic and environmental use. These water sources are often contaminated with a variety of microorganisms, including pathogenic Gram-negative bacteria, which are responsible for a wide range of waterborne diseases, such as diarrhea, cholera, and typhoid fever [1,2]. These pathogens can also harbor antimicrobial resistance (AMR) genes, which further complicate treatment options and pose significant public health risks [3]. Gram-negative bacteria, such as *Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa*, and *Proteus spp*., are frequently detected in contaminated water. These organisms are not only indicators of fecal contamination but are also capable of causing serious infections, particularly in immunocompromised individuals [4]. Moreover, the spread of antimicrobial resistance in these bacteria is an emerging global concern, with studies showing that water environments often serve as reservoirs for multidrug-resistant (MDR) strains [5]. The increasing prevalence of MDR pathogens poses a significant challenge to public health systems in developing countries, where access to advanced medical care and antibiotics may be limited [6]. In Gusau, the capital of Zamfara State in northwestern Nigeria, access to treated water remains a major challenge due to poor infrastructure and sanitation practices. Many residents rely on untreated water sources, including wells, boreholes, rivers, and household storage tanks. These water sources are highly susceptible to contamination from poor sanitation, open defecation, and agricultural runoff, which may introduce both pathogenic bacteria and AMR genes into the water system [5,7]. Despite these risks, there is a lack of detailed studies on the prevalence of Gram-negative bacteria in Gusau water sources and their associated antibiotic resistance patterns. This study aims to fill this gap by investigating the prevalence, characterization, and antibiotic resistance profiles of Gram-negative bacteria isolated from domestic and environmental water sources in Gusau. By identifying the predominant bacterial species and their resistance profiles, this study seeks to provide evidence that can inform future water safety interventions and antimicrobial resistance control strategies in the region.

2. Materials and Methods

2.1 Materials Petri dish, Swab stick, Foil paper, Wire loop, glass spreader, Incubator, Oven, Cotton wool, specimen bottle, Autoclave, test tube, Glass slide, conical flask, Micro centrifuge tube, Vortex machine, Binding column tube, Micropipette, Microscope, Nutrient Agar, Eosin methylene blue, Mueller-Hinton agar, Safranin, Iodine, Distilled water, Crystal violet, Ethanol, Triple sugar Iron, Citrate, trypton broth, Kovac's reagent, Urease, Methyl red broth, Hydrogen peroxide, Phosphate Buffer Saline, Lysis buffer, Ethanol, Wash buffer 1 and 2, Elution solution, Agarose powder, Tri's acetate EDTA, Ethidium bromide.

2.2 Sample Collection

Thirty-two water samples were collected from various water sources across Gusau Metropolis. The samples were transported to the laboratory for analysis. Upon arrival, the samples were prepared for analysis.

2.3 Culture Media Preparation

The following culture media were used; Nutrient agar, EMB, Nutrient broth, Triple Sugar Iron agar (TSI), Simmon Citrate agar, Mueller-Hinton agar. All the media were prepared as directed by the manufacturers.

2.4 Bacteriological Analysis of Samples

Samples were immediately taken to Microbiology Laboratory. Four serial dilutions was done for each sample. 1ml of water sample was transferred into the first test tube containing 9mls of distilled water, 1ml was then withdrawn from the first test tube and transferred into the next test tube, and this was maintained for the whole test tubes aseptically. This procedure was repeated to all samples. 0.1ml was then withdrawn from first test tube and then inoculated in to the plate and labelled, glass spreader was then used to spread the sample into the plate, this maintained for all the serial dilutions and respected plates. The plates were then allowed for some minutes. The plates were incubated at 37OC for 24 hours [8].

2.5 Sub-culture

A loop of a clear and well isolated colony from the mixed culture was aseptically picked and streaked on to a nutrient agar plate and incubated at 37°C for 24hrs to get pure culture.

2.6 Gram staining

Gram staining is a differential staining procedure that separates bacteria to either, Gram positive or Gram negative. It was done by using a sterile wire loop to drop a loop full of sterile distilled water on a clean, grease free glass slide. A colony of the bacteria was smeared on the slide and allowed to air dry. The smear was heat fixed by gently passing over flame thrice. The smear was flooded with crystal violet for 60 seconds and washed off immediately with water, it was then flooded with Lugols iodine for 60seconds and washed off with water. It was decolorized with acetone for few seconds and washed off. The smear was counterstained with neutral red for 60 seconds, then washed off with water. The slide was allowed to air dry. It was viewed under the X100 objectives with oil immersion. Gram positive cells picked up the colour of the primary stain while Gram negative cells pick up the colour of the secondary stain [9].

2.7 Biochemical Analysis

The subculture isolates were biochemically tested to identify the specific enteric bacteria isolates by Urease, Catalase, H2S, Motility, Indole, Methyl red, Triple sugar iron test.

2.7.1 Catalase Test

A drop of 3% hydrogen peroxide was made on a clean glass slide. With a wooden applicator, a colony of the test bacteria was brought in contact with the hydrogen peroxide and observed for bubble. The presence of bubble was a positive catalase test and no bubbles showed a negative catalase test [10].

2.7.2 Urease Test

The urease test was performed by inoculating a urea broth with a sample of the test organism and incubating the mixture at an appropriate temperature of 35°C, for 24 hours [4]. The presence of urease, an enzyme that hydrolyzes urea into ammonia and carbon dioxide, was indicated by a change in the color of the broth from yellow to pink due to the increase in pH caused by the release of ammonia.

2.7.3 Citrate utilization

Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrate permease. Simmons citrate agar slants of 2 ml in each vial was prepared by autoclaving at 15 psi 121°C. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the vials by means of a streak inoculation method with an inoculating needle and the vials were incubated for 48 hours at 37°C. Following incubation, citrate positive culture was recognized by the presence of growth on the surface of the slant of Simmons citrate agar and deep Prussian blue coloration of the medium [11].

2.7.4 Indole Test

The isolate was inoculated in 5ml peptone water (tryptophan broth) and incubated at 37°C for 24 hours. After 24 hours, 3 drops of Kovac's reagent was added to the inoculum, shaken and observed for reaction. A positive reaction is indicated by the 246development of a red colour in the reagent layer above the broth within one minute and a negative reaction is indicated by the reagent retaining its yellow colour [12].

2.7.5 Methyl Red

Methyl red broth was prepared in a test tubes, then inoculated the broth aseptically with 2 loop full of respective bacterial culture, then the test tube was incubated at 37ºC for 24hours, the methyl red indicator few drops was added in the incubated test tubes [4].

2.7.6 Triple Sugar test

The Triple Sugar Iron (TSI) test was performed by inoculating a TSI agar slant with the test organism using a straight inoculation needle. The organism was streaked on the surface of the agar slant and then stabbed into the butt of the agar to promote anaerobic growth. This procedure allows for the detection of glucose fermentation, lactose fermentation, sucrose fermentation, and gas production, as well as hydrogen sulfide production. After inoculation, the agar was incubated at 35°C for 24 hours. A yellow color change in the agar indicated acid production from the fermentation of the sugars, while blackening of the medium suggested hydrogen sulfide production. Gas production was evident if cracks or lifting of the agar occurred. This test is commonly used to differentiate Enterobacteriaceae and other gram-negative rods based on carbohydrate fermentation and sulfur reduction [4].

2.7.7 Voges-proskauer (VP) Test

The Voges-proskauer media was prepared in a test tubes, then inoculated the broth aseptically with 2 loop full of the respective bacterial cultured, then the test tube was incubated at 37ºC for 24hours.

2.8 Susceptibility Test

An antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method. The following antibiotic discs (Mast Diagnostics, UK) at the final concentrations that are indicated were used: ampicillin (PN) 10 ug, streptomycin (S) 10 ug, ciprofloxacin (CPX) 10 ug, ofloxacin (OFX) 10 ug, Gentamycin (CN) 10ug, Nalidixic acid (NA) 10 ug. These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them. Three colonies were picked from each sample and each colony was transferred in to 3 mL of sterile distilled water to prepare bacterial suspension. Aliquots of 100 L from each suspension were spread-plated on Mueller-Hinton agar plates. Antibiotic discs were applied on to the plates using sterile needles and the plates were incubated at 37°C for 24 hours. After incubation, the antibiotic inhibition zone diameters (IZD) were measured. Results obtained were used to classify isolates as being resistant, intermediate resistant, or susceptible to a particular antibiotic using standard reference values according to National Committee for Clinical Laboratory Standards, now Clinical and Laboratory Standards Institute (CLSI). Multiple antibiotic resistance (MAR) phenotypes were generated for isolates that showed resistance to 3 or more antibiotics.

3. Results

S/N	SAMPLES	Bacterial counts (×10^4 CFU/mL)
1	A1	7.0
2	A2	2.50
3	A3	6.9
4	A4	1.92
5	A5	1.92
6	A6	1.80
7	A7	1.10
8	A8	3.0
9	A9	1.3
10	A10	2.0
11	A11	2.3
12	A12	9.0
13	A13	9.2
14	A14	6.0
15	A15	7.0
16	A16	2.16
Keys: CFU- Colony forming units, A1-Gusau dam, A2-Rijiyar g/baga, A3-Labun g/baga, A4-Gulbin g/baga, A5-Ruwan g/yardantsi, A6-gadar water board, A7-Damba dam, A8-Tabkin damba, A9-Rijiyar damba, A10-Rijiyar samaru, A11-Koramar bayan Uthaimin, A12-Rijiyar damba quarters, A13-Rijiyar cinema, A14-Tabkin daza, A15-Koramar janyau, A16-Tabkin jauri.		

Table 1: Total Bacterial Count (CFU/ml) of Water Samples

S/N	SAMPLES	Bacterial counts (×10⁴ CFU/ mL)
17	A17	3.0
18	A18	1.8
19	A19	2.3
20	A20	2.5
21	A21	5.9
22	A22	4.0
23	A23	1.23
24	A24	9.0
25	A25	1.2
26	A26	1.4
27	A27	1.9
28	A28	3.9
29	A29	2.8
30	A30	7.0
31	A31	2.7
32	A32	8.3

Keys: A17-Gulbin gadina, A18- Fanfon bakin cinema, 19-Rijiyar hizburrahim, A20-Rijiyar dan dutsi, A21-Rijiyar Malan akashe, A22-Rijiyar sardauna, A23-Gulbin mareri, A24-Fanfon Sabon gari, A25-Rijiyar dan hili, A26-Rijiyar yar mangwarora, A27-Rijiyar birnin ruwa, A28-Rijiyar Hayin Malan Sani, A29-Fanfon birnin ruwa, A30-Fanfon kwata, A31-Fanfon Kanwuri, A32-Tabkin Alasawa.

Table 2: Total Bacterial Count (CFU/ml) of Water Samples

S/N	SAM	Gram	Shape	Cit	Cat	TSI	H2S	KCN	GAS	MR	VP	IND	ORGANISM
1	A1	-	Rod	+	+	+	+	-	+	-	+	+	proteus vulgaris
2	A3	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
3	A41	-	Rod	+	+	+	-	-	+	-	+	-	Klebsiella
4	A42	-	Rod C	+	+	+	+	-	+	-	+	+	proteus vulgaris
5	A51	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
6	A52	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella
7	A53	-	Rod	+	+	+	-	-	+	-	+	-	Klebsiella
8	A61	-	Rod	+	+	+	-	-	-	-	+	-	Enterobacter
9	A62	-	Rod C	+	+	+	+	-	+	-	+	+	proteus vulgaris
10	A71	-	Rod	+	+	+	+	-	+	-	+	+	proteus vulgaris
11	A72	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
12	A81	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
13	A82	-	Rod	+	+	+	+	-	+	+	-	_	Klebsiella

Keys: Cat. - Catalase test; Cit. – Citrate test; Ind. – Indole test; M.R. – Methyl red test; V.P. – Voges-proskauer test; TSI- Tripple sugar iron test, KCN- Potassium cyanide, H^2 S-Dihydrogen Sulphide.

Table 3: Morphological and Biochemical Identification

Citation: Shehu, H., Hamza, A. B., Suleiman, L., Mohammed, Z., Moriki, B. I., Sadisu, A. H. (2026). Prevalence, Characterization and Drug Resistance of Gram-Negative Bacteria Isolated from Domestic and Environmental Water Sources in Gusau, Zamfara State. J Biomed Eng Res, 4(1), 1-9.

S/N	SAM	Gram	Shape	Cit	Cat	TSI	H2S	KCN	GAS	MR	VP	IND	ORGANISM
14	A83	-	Rod	+	+	+	-	-	+	+	-	-	Ecoli
15	A91	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
16	A92	-	Rod	+	+	+	+	-	+	-	+	+	proteus vulgaris
17	A101	-	Rod C	-	+	+	-	-	+	+	-	-	Ecoli
18	A102	-	Rod	+	+	+	-	-	+	-	+	-	Klebsiella
19	A111	-	Rod	+	+	+	-	-	+	-	+	-	Klebsiella
20	A112	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
21	A121	-	Rod	+	+	+	+	-	+	-	+	+	proteus vulgaris
22	A122	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
23	A131	-	Rod	-	+	+	+	-	+	+	-	-	Shigella
24	A132	-	Rod	+	+	+	-	-	-	-	+	-	Enterobacter
25	A133	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella
26	A151	-	Rod C	-	+	+	-	-	+	+	-	-	Ecoli

Keys: Cat. - Catalase test; Cit. – Citrate test; Ind. – Indole test; M.R. – Methyl red test; V.P. – Voges-proskauer test; TSI- Tripple sugar iron test, KCN- Potassium cyanide, H^2 S-Dihydrogen Sulphide.

Table 4: Morphological and Biochemical Identification

S/N	SAM	Gram	Shape	Cit	Cat	TSI	H2S	KCN	GAS	MR	VP	IND	ORGANISM
27	A152	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella
28	A161	-	Rod	+	+	+	-	-	-	+	_	+	vibrio cholera
29	A171	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella
30	A172	-	Rod C	+	+	+	-	-	+	-	+	-	Pneumoniae
31	191	-	Rod	-	+	+	+	-	+	+	-	-	Shigella
32	A192	-	Rod	+	+	+	-	-	-	+	_	+	vibrio cholera
33	A201	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella
34	A202	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
35	A221	-	Rod	+	+	+	-	-	+	-	+	-	Pneumoniae
36	A222	-	Rod C	-	+	+	+	-	+	+	-	-	Shigella
37	A231	-	Rod	+	+	+	-	-	+	-	+	-	Kleb
38	A232	-	Rod	+	+	+	-	-	-	+	_	+	Pneumoniae

Keys: Cat. - Catalase test; Cit. – Citrate test; Ind. – Indole test; M.R. – Methyl red test; V.P. – Voges-proskauer test; TSI- Tripple sugar iron test, KCN- Potassium cyanide, H_2 S-Dihydrogen Sulphide.

Table 5: Morphological and Biochemical Identification

S/N	SAM	Gram	Shape	Cit	Cat	TSI	H2S	KCN	GAS	MR	VP	IND	ORGANISM
39	A241	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella
40	A242	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
41	A251	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella
42	A261	-	Rod	+	+	+	-	-	+	-	+	-	Pneumoniae
43	A262	-	Rod	-	+	+	+	-	+	+	-	-	Shigella
44	A271	-	Rod	+	+	+	-	-	-	+	_	+	vibrio cholera
45	A281	-	Rod C	+	+	+	+	-	+	-	-	+	Salmonella
46	A291	-	Rod	-	+	+	+	-	+	+	-	-	Shigella
47	A301	-	Rod	+	+	+	-	-	+	-	+	-	Kleb
48	A302	-	Rod	-	+	+	+	-	+	+	-	-	Shigella

49	A321	-	Rod	-	+	+	-	-	+	+	-	-	Ecoli
50	A322	-	Rod	+	+	+	+	-	+	-	-	+	Salmonella

Keys: Cat. - Catalase test; Cit. – Citrate test; Ind. – Indole test; M.R. – Methyl red test; V.P. – Voges-proskauer test; TSI- Tripple sugar iron test, KCN- Potassium cyanide, H_2 S-Dihydrogen Sulphide.

Table 6: Morphological and Biochemical Identification

Organisms	Domestic water (n=26) f(%)	Environmental water (n=24) f(%)
Pneumonie (n=4)	2(50)	2(50)
Enterobacter (n=2)	1(50)	1(50)
Ecoli (n=13)	4(30.77)	7(69.23)
Klebsiella (n=7)	3(42.86)	4(57.14)
Salmonella (n=8)	5(62.5)	3(37.5)
Shigella (n=7)	7(100)	0(0)
Vibrio cholarae (n=3)	2(66.67)	1(33.33)
Proteus vulgaris (n=6)	2(33.33)	4(66.67)
Total (n=50)	26(52)	24(48)

Table 7: Bacteria Isolated from Both Domestic and Environmental Water Sample

SAM	ORGANISMS	OFX	PEP	CPX	AU	CN	S	CEP	NA	SXT	PN
A4	Klebsiella	R	R	S	R	R	S	R	R	R	R
A4	Proteus vulgaris	R	R	S	R	R	S	R	R	R	R
A10	Ecoli	S	R	R	R	R	S	R	R	R	R
A10	Klebsiella	S	S	R	R	S	I	R	R	R	R
A12	Proteus vulgaris	I	S	S	R	S	I	R	R	I	R
A12	Ecoli	S	S	S	R	R	R	R	R	R	R
A13	Shigella	S	S	S	R	R	S	R	R	R	I
A13	Enterobacter	S	S	S	R	R	S	R	R	R	R
A13	Salmonella	R	R	R	R	R	S	R	R	R	R
A19	Shigella	S	S	S	R	S	S	R	R	R	R
A20	Salmonella	R	R	S	R	R	S	R	R	R	R
A20	Ecoli	S	R	R	R	S	I	R	R	R	R

Keys: Sam-Sample, OFX-Tarivid (10mcg), PEF- Reflacine (10mcg), CPX- Ciproflox (10mcg), AU- Augmentin (30mcy), CN- Gentamycin (10mcg), S- Streptomycin (30mcg), CEP- Ceporex (10mcg), NA- Nalidixic Acid (30mcg), SXT- Septrin (30mcg), PN- Amplicin (30mcg).

Table 8: Antibiotic Susceptibility Test Result for Bacterial Isolate from Domestic Water Samples

SAM	ORGANISMS	OFX	PEP	CPX	AU	CN	S	CEP	NA	SXT	PN
A22	Pneumoniae	S	R	R	R	I	R	S	R	S	S
A22	Shigella	S	S	S	R	I	R	R	R	R	R
A24	Salmonella	S	S	S	S	R	R	R	R	S	S
A24	Ecoli	R	R	S	S	R	R	I	R	R	R
A25	Salmonella	R	R	R	R	R	R	S	R	R	R
A26	Pneumoniae	R	S	S	R	R	I	I	R	R	S
A26	Shigella	S	S	S	R	R	S	R	R	I	R
A27	Vibrio cholarae	R	S	S	R	S	S	S	S	S	R
A28	Salmonella	R	S	S	S	S	S	R	R	S	S
A29	Shigella	R	R	R	R	R	R	R	R	R	I
A30	Klebsiella	S	S	R	R	S	I	S	R	R	R

A30	Shigella	I	R	R	S	R	R	R	R	R	R

Keys: Sam-Sample, OFX-Tarivid (10mcg), PEF- Reflacine (10mcg), CPX- Ciproflox (10mcg), AU- Augmentin (30mcy), CN- Gentamycin (10mcg), S- Streptomycin (30mcg), CEP- Ceporex (10mcg), NA- Nalidixic Acid (30mcg), SXT- Septrin (30mcg), PN- Amplicin (30mcg).

Table 9: Antibiotic Susceptibility Test Result for Bacterial Isolate from Domestic Water Samples

SAM	ORGANISMS	OFX	PEP	CPX	AU	CN	S	CEP	NA	SXT	PN
A1	Proteus vulgaris	R	R	S	R	R	I	R	R	R	R
A3	Ecoli	R	S	S	R	R	I	R	R	R	R
A5	Ecoli	R	R	S	R	R	S	R	R	R	R
A5	Salmonella	S	R	R	R	S	S	R	R	S	S
A5	Klebsiella	S	S	S	R	R	S	R	R	R	I
A6	Enterobacter	S	S	S	R	R	S	R	R	R	R
A7	Proteus vulgaris	S	R	S	R	S	S	R	R	R	R
A7	Ecoli	S	S	S	R	R	R	R	R	R	R
A8	Ecoli	R	R	R	R	R	R	R	R	R	R
A8	Klebsiella	S	S	S	R	S	I	R	S	R	R
A8	Ecoli	R	R	R	R	R	I	R	R	R	R
A9	Ecoli	R	R	R	R	R	S	R	R	R	R

Keys: Sam-Sample, OFX-Tarivid (10mcg), PEF- Reflacine (10mcg), CPX- Ciproflox (10mcg), AU- Augmentin (30mcy), CN- Gentamycin (10mcg), S- Streptomycin (30mcg), CEP- Ceporex (10mcg), NA- Nalidixic Acid (30mcg), SXT- Septrin (30mcg), PN- Amplicin (30mcg).

Table 10: Antibiotic Susceptibility Test Result for Bacterial Isolate from Environmental Water Samples

SAM	ORGANISMS	OFX	PEP	CPX	AU	CN	S	CEP	NA	SXT	PN
A9	Proteus vulgaris	S	S	S	R	R	R	R	R	R	R
A11	Kleb	R	S	S	R	R	S	R	R	R	R
A11	Ecoli	S	S	S	R	S	S	R	R	I	R
A15	Ecoli	R	R	R	R	R	S	I	R	R	R
A15	Salmonella	S	R	R	S	R	I	R	R	S	R
A16	Vibrio cholarae	S	R	R	S	S	I	R	R	R	S
A17	Salmonella	S	S	I	R	R	S	R	R	S	S
A17	Pneumoniae	R	R	S	R	S	I	R	R	R	R
A23	Klebsiella	S	R	R	S	S	S	S	I	S	R
A23	Pneumonie	R	R	R	S	R	I	R	R	S	S
A32	Ecoli	R	R	S	I	R	R	R	R	R	R
A32	Salmonella	S	S	R	R	S	S	R	I	R	R

Keys: Sam-Sample, OFX-Tarivid (10mcg), PEF- Reflacine (10mcg), CPX- Ciproflox (10mcg), AU- Augmentin (30mcy), CN- Gentamycin (10mcg), S- Streptomycin (30mcg), CEP- Ceporex (10mcg), NA- Nalidixic Acid (30mcg), SXT- Septrin (30mcg), PN- Amplicin (30mcg).

Table 11: Antibiotic Susceptibility Test Result for Bacterial Isolate from Environmental Water Samples

ORG	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN	PN
Pneu 2	1(50)	1(50)	1(50)	2(100)	1(50)	1(50)	0(0)	2(100)	1(50)	0(0)	R
Ente 1	0(0)	0(0)	0(0)	1(100)	1(100)	0(100)	1(100)	1(100)	1(100)	1(100)	R
Ecol 4	1(25)	3(75)	2(50)	3(75)	3(75)	2(50)	3(75)	4(100	4(100	4(100	R
Prot 2	1(50)	1(50)	0(0)	2(100)	1(50)	0(0)	2(100)	2(100)	1(50)	2(100)	R
Salm 5	4(80)	3(60)	2(40)	3(60)	4(80)	2(40)	4(80)	5(100)	3(60)	3(60)	R
Shig 7	2(28.6)	3(42.9)	2(28.6)	6(85.7)	4(57.1)	3(42.9)	6(85.7)	6(85.7)	5(71,4)	4(57.1)	S
Kleb 3	1(33.3)	1(33.3)	2(66.7)	3(100)	1(33.3)	0(0)	2(66.7)	3(100)	3(100)	3(100)	S

Vibr 2	2(100)	0(0)	1(50)	2(100)	1(50)	0(0)	1(50)	1(50)	1(50)	2(100)	R
T=26	12(46.2)	12(46.2)	10(38.5)	22(84.6)	16(61.5)	8(30.8)	19(73.1)	24(92.3)	19(73.1)	19(73.1)	R
Keys: Org-Organism, OFX-Tarivid (10mcg), PEF- Reflacine (10mcg), CPX- Ciproflox (10mcg), AU- Augmentin (30mcy), CN- Gentamycin (10mcg), S- Streptomycin (30mcg), CEP- Ceporex (10mcg), NA- Nalidixic Acid (30mcg), SXT- Septrin (30mcg), PN- Amplicin (30mcg), pneu-Pneumonie, Ente- Enterobacter, Ecol-Escherichia coli, Prot- Proteus vulgaris, Salm-Salmonella, Shig-Shigella, Kleb-Klebsiella, Vibr-Vibrio cholarae.											

Table 12: Antimicrobial Susceptibility Pattern for Gram Negative Bacteria from Domestic Water Samples

ORG	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
Pneu 2	2(100)	2(100)	1(50)	1(50)	1(50)	0(0)	2(100)	2(100)	1(50)	1(50)
Ente 1	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)
Ecol 9	7(77.8)	6(66.9)	4(44.4)	8(88.9)	8(88.9)	3(33.3)	8(88.9)	7(77.8)	8(88.9)	8(88.9)
Prot 4	2(50)	3(75)	1(25)	3(75)	3(75)	2(50)	4(100)	4(100)	4(100)	3(75)
Salm 3	0(0)	2(66.7)	3(100)	3(100)	2(66.7)	0(0)	3(100)	2(66.7)	1(33.3)	2(66.7)
Shig 0	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Kleb 4	1(25)	1(25)	1(25)	3(75)	2(50)	0(0)	3(75)	2(50)	3(75)	3(75)
Vibr 1	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	1(100)	1(100)	0(0)
T=24	12(50)	15(62.5)	11(45.8)	19(79.2)	17(70.8)	5(20.8)	22(91.7)	19(79.2)	19(79.2)	18(75)
Keys: Org-Organism, OFX-Tarivid (10mcg), PEF- Reflacine (10mcg), CPX- Ciproflox (10mcg), AU- Augmentin (30mcy), CN-Gentamycin (10mcg), S- Streptomycin (30mcg), CEP- Ceporex (10mcg), NA- Nalidixic Acid (30mcg), SXT- Septrin (30mcg), PN- Amplicin (30mcg), pneu-Pneumonie, Ente- Enterobacter, Ecol-Escherichia coli, Prot- Proteus vulgaris, Salm-Salmonella, Shig-Shigella, Kleb-Klebsiella, Vibr-Vibrio cholarae.										

Table 13: Antimicrobial Susceptibility Pattern for Gram Negative Bacteria from Environmental Water Samples

4. Discussion

The bacterial counts from the water samples ranged from **1.10×10^4 to 9.2×10^4 CFU/ml** These values exceed the World Health Organization (WHO) recommended limits for safe drinking water, which states that potable water should be free from coliforms and contain no detectable bacterial growth. This finding shows that both domestic and environmental water sources in Gusau are heavily contaminated and unsafe for direct consumption. Similar high bacterial counts in untreated water sources have been reported in other Nigerian studies [2,5]. The bacterial isolates obtained included *Escherichia coli, Klebsiella spp., Salmonella spp., Shigella spp., Proteus spp., Enterobacter spp., Vibrio cholarae, and Pneumoniae spp*. E. coli was the most frequently detected organism (26%), followed by Salmonella (16%), Klebsiella (14%), and Shigella (14%). The presence of E. coli is particularly significant, as it is a reliable indicator of fecal contamination, suggesting that the water sources are contaminated by human or animal waste. Vibrio cholarae was also detected, which raises serious public health concerns, as cholera outbreaks are commonly linked to contaminated water. Comparable results have been documented in South Africa and Ethiopia, where enteric pathogens were frequently isolated from community water sources [6,13]. Antibiotic susceptibility testing revealed widespread resistance to commonly used antibiotics. High levels of resistance were observed against ampicillin, septrin, augmentin, ceporex, and nalidixic acid, while moderate to high susceptibility was recorded for ciprofloxacin, ofloxacin (tarivid), streptomycin, and gentamycin. This pattern indicates that many of the bacterial isolates are multidrug-resistant (MDR), which aligns with global concerns about environmental reservoirs of antimicrobial resistance [3]. The high resistance to beta-lactam antibiotics (ampicillin, augmentin) suggests the possible presence of extended spectrum beta-lactamase (ESBL)-producing strains, while resistance to nalidixic acid points to mutations affecting quinolone resistance genes. The results also showed that isolates from both domestic and environmental water exhibited similar resistance patterns, although some variations existed. For example, Shigella isolates from domestic water samples showed 100% resistance to augmentin and high resistance to other antibiotics, whereas isolates from environmental water sources showed slightly better sensitivity to fluoroquinolones. This difference may reflect differences in contamination sources and antibiotic exposure. Overall, the study confirms that water sources in Gusau are reservoirs of pathogenic and MDR Gram-negative bacteria. These findings are consistent with other studies in Nigeria and elsewhere in Africa, highlighting the environmental spread of antimicrobial resistance [5,6]. The detection of multiple resistant strains, especially E. coli and Salmonella, is a critical public health issue because these organisms are associated with outbreaks of diarrhea, typhoid, and cholera.

5. Conclusion

This study revealed that water sources in Gusau metropolis are contaminated with Gram-negative bacteria, with bacterial counts far exceeding WHO permissible limits. A wide range of Gram-negative bacteria were isolated, including *Escherichia coli, Klebsiella spp., Salmonella spp., Shigella spp., Proteus spp., Enterobacter spp., Vibrio cholarae,* and *Pneumoniae spp.,* with *E. coli* being the most prevalent, indicating fecal contamination. Antibiotic susceptibility testing showed high resistance to commonly used antibiotics such as ampicillin, septrin, nalidixic acid, and augmentin, while moderate sensitivity was observed to ciprofloxacin, ofloxacin, streptomycin, and gentamycin. The detection of multidrug resistance among several isolates suggests that these water sources serve as reservoirs for antimicrobial resistance. The presence of potential pathogens such as *Salmonella, Shigella, and Vibrio cholarae* further highlights the public health risks associated with the consumption or use of untreated water in the study area. These findings emphasize the urgent need for improved water treatment, continuous monitoring, stricter regulation of antibiotic use, and public health education to limit the spread of resistant bacteria and safeguard community health [14,15].

Author's contributions: All authors contributed equally to this research work and have read and agreed to the published version of the ma**nuscript.**

Funding sources: The Authors received no financial support for the research, Author-ship and publication of this article.

Conflict of interest: The authors declare no conflicts of interest.

Acknowledgement: The Authors thank the Head of Biochemistry Department Federal University Gusau Dr. A.B. Hamza for all his support

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