UMYU Scientifica, Vol. 2 NO. 3, September 2023, Pp 009 – 014
https://doi.org/10.56919/usci.2323.002
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Antibiotic Resistance Profile of Bacteria Isolated from Stream Water in Bokkos Local Government Area of Plateau State, Nigeria.

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ABSTRACT

Water from selected streams in Bokkos local government area of Plateau State Nigeria was obtained and analysed for the presence of antibiotic resistant bacteria and the resistance pattern of the isolates against several antibiotics. A total of 25 stream water samples were collected for testing from five (5) different study sites (Tudu, Mabel, Dairy Farm, Maihakorin Gold and Kuba). Heterotrophic and coliform bacteria were isolated from the samples using 10-fold serial dilution and the pour plate method. The isolated bacteria were *Proteus vulgaris, Salmonella* spp*., Escherichia coli, Pseudomonas* spp., *Shigella* spp*., Bacillus* spp., *Streptococcus spp., Staphylococcus aureus.* Antibiotic susceptibility of isolates was determined using the disc diffusion method. Among the eight bacterial isolates, *Salmonella* spp. was the most frequently occurring bacterium 18(22.8%), followed by *E. coli* 16(20.3%), while the least occurring bacterium was *Staphylococcus aureus* 5(6.3%). The highest antibiotic resistance was recorded for *Salmonella* spp., *E. coli*, and *Pseudomonas* spp. against ceftazidime 12(67%), Ampicillin 12 (75%), and ceftazidime 11 (92%), respectively. However, *E. coli*, *Proteus* spp., *Staphylococcus* spp., and *Shigella* spp. showed high antibiotic susceptibility against cefuroxime 0(0%) and Gentamicin 0(0%). The results suggests that there are antibiotic resistance bacteria in stream water in Bokkos local government area of Plateau State. Educational awareness regarding the proper use of safe drinking water and the treatment of water before use should be encouraged.

ARTICLE HISTORY

Received February 12, 2023
Accepted March 24, 2023
Published September 20, 2023

KEYWORDS

Antibiotics resistance, Bacteria, Stream water, Bokkos.

INTRODUCTION

Water is one of the most abundant natural resources on earth. Although essential for the survival of all organisms, it has been found to harbour infectious agents that can cause multiple diseases in different countries (Hoekstra & Hung, 2005; Osei *et al.*, 2021). Fresh water is essential for agriculture, industry, and even human existence. Without sufficient quantity and quality of freshwater, sustainable development is impossible (Sumathi & Manonmani, 2016).

The potential for microbial pathogens that cause serial illness in drinking-water and spread to multiple individuals is well documented in several countries at all levels of economic development (Pan & Chu, 2018; Tangwa *et al.*, 2019). More than 90% of child deaths from diarrheal diseases are directly linked to contaminated water, and India alone accounts for 24% of all deaths under 5-years old (Reliefweb, 2013). Among the pathogens disseminated in water sources, enteric pathogens such as enterotoxigenic *Escherichia coli*, Shigella spp., *Salmonella* spp., and so forth are the ones most frequently encountered that are responsible for a variety of diseases like diarrhea, dysentery, and enteric fever. Approximately 1.7 billion cases of diarrheal disease occur each year (Poonia *et al.*, 2014). According to Tangwa *et al.* (2019), diarrhoea is one of the leading causes of death in children under 5 years of age and is usually associated with dirty water, poor sanitation, and poor hygiene.

Pathogens released into water sources include enteric pathogens such as enterotoxigenic *Escherichia coli, Shigella spp.* and *Salmonella spp.* They are the most common and cause various illnesses such as diarrhoea, dysentery, and enteric fever (Pooniaet *al.*, 2014). To further complicate the problem, numerous reports have shown that enteric bacterial pathogens are resistant to multiple antibiotics enteric bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics. Some bacteria pathogens with highest level of resistance were isolated from environment, surface water which serves as the main reservoir of antibiotics and antibiotic-resistant

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How to cite: Gugu M. F., Vomagai K. N., Mwanja P. Y., Ombugadu A., Adejoh V. A. and Attah S. A. (2023). Antibiotic Resistance Profile of Bacteria Isolated from Stream Water in Bokkos Local Government Area of Plateau State, Nigeria. *UMYU Scientifica*, 2(3), 009 – 014. https://doi.org/10.56919/usci.2323.002

bacteria in the environment (Chaitanand *et al.*, 2010; Verma *et al.*, 2011; Sharma & Rai, 2012; Geta and Kibret, 2022). Antimicrobial resistance remains a global problem (Poonia *et al.*, 2014), and it has been 20 years of increased antimicrobial resistance since it was reported (Kapil, 2004). Determining the efficacy of antimicrobial agents against specific human or animal pathogens is essential for appropriate treatment.

Rural communities like Bokkos rely on surface water from streams for drinking and domestic use. The use of untreated water sources for drinking and domestic purposes remains a major public health threat as they can act as reservoirs for the transmission of antibiotic-resistant pathogens. To this end, the present study was conducted to assess the antibiotic resistance profile of bacteria isolated from stream water in the Bokkos Local Government Area (LGA) of Nigeria's Plateau State.

MATERIALS AND METHODS

Study Location

Water samples were taken during the middle of the rainy season (June-July) from the five different locations in Bokkos LGA, Plateau State. These were Tudu, Mabel, Dairy Farm, Maihakorin Gold, and Kuba. These stations were about several kilometers apart. From each stream, three replicate samples were collected and immediately sent to the laboratory for analysis.

Sample collection

A total of 25 samples were collected for the assay, 5 samples from each of 5 different study sites. Samples were collected in sterile sealed bottles using the standard technique described by Collee (1996). Bottles were labelled with details of water source, time, and date of removal. Bottles were stored in an icebox and shipped to the National Veterinary Research Institute (NVRI) Microbiology Laboratory in Vom for analysis.

Isolation and identification of bacterial isolates

All heterotrophic bacteria and coliforms were isolated from the samples using serial 10-fold serial dilutions and the pour plate method as described by Oluyege (2014) and Babalola *et al.* (2021). Pure cultures were plated on nutrient agar slants and stored at 4°C. The isolates underwent morphological and biochemical tests such as Gram staining, catalase test, indole test, urease test, trisaccharide iron agar (TSIA) test, citric acid utilization test, antibiotic susceptibility test, and their identities determined according to Holt *et al.* (1994).

Antibiotic susceptibility testing of isolates

Antibiotic susceptibility of isolates was determined using the disc diffusion method on Mueller-Hilton agar according to Cheesbrough (2006). The isolates were tested against 10 ABTEK antibiotic discs containing cefuroxime (30 µg), ceftazidime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), amoxicillin (30 µg), nitrofurantoin (300 µg) and ampicillin (10 µg) (CLSI, 2012). The inoculum was standardized by adjusting the density to barium sulphate (BaSO4) turbidity (McFarland turbidity standard 0.5). One millilitre each of the standardized broth cultures of test isolates were swabbed onto Mueller-Hinton agar plates, antibiotic discs were placed firmly on solidified plates and incubated at 37°C for 24 hours. Non-inoculated agar plates containing antibiotics were used as controls. Growth inhibition zone diameters were measured to the nearest millimetre and interpreted based on Clinical and Laboratory Standard Institute (CLSI, 2012) guidelines.

Statistical analysis

The obtained data were analysed using R Console version 4.0.2. Pearson's Chi-square test was used to compare differences in total plate numbers per site. The significance level was set at $P<0.05$.

RESULTS

Bacteria Isolates from selected Streams in Bokkos Local Government Area, Plateau State

A total of 25 water samples were processed for the presence of antibiotic-resistant bacteria. Ninety two percent (92%) of the isolated samples were positive for one or more bacterial isolates (Table 1). Among the total samples, 79 bacterial isolates were recovered, with Tudu stream water sample having the highest number of bacterial isolates [24 (30.4%)], followed by Mabel [17 (21.5%)], while Maihakorin Gold had the lowest number of isolates [11 (13.9%)] (Table 2). *Salmonella spp.* was the highest isolate recorded from the combined study locations [18 (22.8%)], followed by *Escherichia coli* [16 (20.3%)], while *Staphylococcus aureus* was the least isolated organism [5 (6.3%)] (Table 2).

Total Bacterial Count from the Study Sites

Stream water samples from Tudu recorded the highest bacterial count 6.1×10^{-5}, followed by dairy farm **(**3.3×10^{-5}), Maihakorin Gold **(**3.1×10^{-5}) and Mabel (2.5×10^{-5}), while the least total bacterial count was recorded from Kuba (2.2×10^{-5}) (Table 3). The differences in total bacterial counts in relation to location did not show a significant difference ($\chi^2 = 2.8 \times 10^{-5}$, df = 4, P = 1).

Antibiotic Resistance Profiles of Bacteria Isolated from streams in Bokkos LGA, Plateau State

All the isolated bacteria are either resistant to one or more of the antibiotics used (Table 4). *Staphylococcus* spp. showed the highest resistance pattern to ceftazidime 3(60%), ofloxacin 3(60%), Nitrofurantoin 3(60%), Amoxicillin 3(60%) and Ampicillin 3(60%) (Table 4). The highest resistance was recorded for *E. coli, Salmonella spp.,* and *Pseudomonas spp.* against Ampicillin 12 (75%), ceftazidime 12(67%), and ceftazidime 11 (92%), respectively (Table 4). A lower resistance pattern was observed in *Salmonella* spp.

against cefuroxime 1(6%) and ciprofloxacin 2(11%), *Pseudomonas* spp. against gentamicin 1(8%), *Bacillus spp.* against cefuroxime 1(14%), *Shigella* spp. against ciprofloxacin 1(13) and *Streptococcus spp.* against cefuroxime 1 (17%) and Ampicillin 1(17%). However, *E. coli*, *Proteus spp.*, *Staphylococcus spp.*, and *Shigella* spp. showed high susceptibility against cefuroxime 0(0%) and Gentamicin 0(0%). The resistance of bacteria against ceftazidime 53(513%) was higher than other antibiotics, and the results showed that there was a statistically significant difference among bacterial isolates for the resistance to all antibiotics except in Nitrofurantoin (p>0.05) (Table 4).

Table 1: Positive and Negative water samples taken from Bokkos LGA, Plateau State

Sample location	Total samples N (%)	Positive samples N (%)	Negative samples N (%)
Mabel	5	5	0
Dairy Farm	5	4	1
Maihakorin Gold	5	5	0
Tudu	5	4	1
Kuba	5	5	0
Total	**25 (100)**	**23 (92)**	**2 (8)**

Table 2: Proportion and types of bacterial isolates across the sampled locations in Bokkos LGA

	Locations					**Total N (%)**
Bacterial isolates	**Mabel**	**Dairy Farm**	**Maihakorin Gold**	**Tudu**	**Kuba**	
Proteus vulgaris	3(17.6)	0(0.0)	2(11.8)	0(0.0)	2(13.3)	**7 (8.9)**
Salmonella spp.	5(29.4)	2(16.7)	2(11.8)	5(20.8)	4(26.7)	**18 (22.8)**
Escherichia coli	4(23.5)	3(25.0)	3(27.3)	3(12.5)	3(20.0)	**16 (20.3)**
pseudomonas spp.	2(11.8)	3(25.0)	2(11.8)	4(16.7)	1(6.7)	**12 (15.2)**
Shigella spp.	1(5.9)	1(11.3)	0(0.0)	4(16.7)	2(13.3)	**8 (10.1)**
Bacillus pp.	0(0.0)	2(16.7)	1(5.9)	4(16.7)	0(0.0)	**7 (8.9)**
Streptococcus spp.	2(11.8)	1(8.3)	0(0.0)	2(8.3)	1(6.7)	**6 (7.6)**
Staphylococcus aureus	0(0.0)	0(0.0)	1(5.9)	2(8.3)	2(13.3)	**5 (6.3)**
Total	**17(21.5)**	**12(15.2)**	**11(13.9)**	**24(30.4)**	**15(19.0)**	**79(100)**

Table 3: Total Bacterial Count across the sample locations in Bokkos LGA

Sample Location	**Total Bacteria Count** (CFU/ml)
Mabel	2.5×10^{-5}
Dairy Farm	3.3×10^{-5}
Maihakorin Gold	3.1×10^{-5}
Tudu	6.1×10^{-5}
Kuba	2.2×10^{-5}

Table 4: Antibiotic resistance pattern of the bacterial isolates

Bacteria Isolation	CAZ (%)	CRX (%)	OFL (%)	NIT (%)	AMX (%)	AMP (%)	CPR (%)	GEN (%)
E. coli (16)	11(69)	0(0)	8(50)	9(56)	11(69)	12(75)	4(25)	0(0)
Bacillus spp. (7)	5(71)	1(14)	3(43)	4(57)	0(0)	4(57)	3(43)	0(0)
Streptococcus spp. (6)	3(50)	1(17)	4(67)	3(50)	3(50)	1(17)	0(0)	2(33)
Proteus spp. (7)	2(29)	0(0)	4(57)	4(57)	4(57)	2(29)	2(29)	0(0)
Staphylococcus spp. (5)	3(60)	0(0)	3(60)	3(60)	3(60)	3(60)	1(20)	0(0)
Shigella spp. (8)	6(75)	0(0)	6(75)	6(75)	3(38)	2(25)	1(13)	0(0)
Pseudomonas spp. (12)	11(92)	0(0)	9(75)	8(67)	7(58)	5(42)	3(25)	1(8)
Salmonella spp. (18)	12(67)	1(6)	6(33)	10(56)	9(50)	8(44)	2(11)	0(0)
Total	53(64.1)	3(4.6)	43(57.5)	47(59.8)	40(47.8)	37(43.6)	16(20.8)	3(5.1)
p-value	0.0001	0.0001	0.0003	0.4	0.0001	0.0001	0.0001	0.0001

Key: CAZ =ceftazidime, CRX = cefuroxime, OFL = ofloxacin, NIT = Nitrofurantoin, AMX = amoxicillin, AMP =ampicillin, CRP =ciprofloxacin, GEN = Gentamicin

DISCUSSION

Results from this study shows that 92% of the total stream water samples processed and examined for bacteria isolates in Bokkos LGA, were highly contaminated with pathogenic antibiotic resistant bacteria of public health importance. The streams in Bokkos LGA of Plateau state are usually open water bodies which are sometimes stagnant. The indiscriminate dumping of waste in the environment causes rain water run offs from erosion which usually released contaminated water alongside large number of antibiotics and antibiotic-resistant bacteria from the environment into the streams through flooding which could be the main factor for the accumulation of antibiotic-resistant bacteria. This is in agreement with studies by Osei *et al.* (2019) and Geta and Kibret (2022) who reported that 91% and 92.1% of the water used on poultry farms in the Ashanti region of Ghana and waste water in Bahir Dar City, Northwestern Ethiopia were contaminated with various levels of bacteria contaminants. A total of 79 bacterial isolates were recorded from this study, where stream water samples from Tudu recorded the highest frequency of bacterial isolates [24 (30.4%)], while stream water samples from Maihakorin Gold recorded the least bacterial isolates [11(13.9%)]. Result from the study also showed that *Salmonella* spp was the most frequently isolated bacterium [18(22.8%)], followed by *E. coli* [16 (20.3%)], while the least was *Staphylococcus aureus* [5(6.3%)]. The high percentage of *Salmonella* spp. and *E. coli* in this study suggests that the streams in Bokkos LGA could be contaminated with fecal matter due to humans and animal activities. This result supports the findings of Ekelozie *et al.* (2018) and Tangwa *et al.* (2019) who reported *Salmonella spp.* and *E. coli* as the most dominant bacteria from hand dug wells and boreholes water samples in Ngaoundere municipality of Adamawa region in Cameroon and Water Sources in Two Local Government Areas of Anambra State, Nigeria. Similarly, the result agrees with the findings from the studies by Momtaz *et al.* (2014) who reported high prevalence of *E. coli* and *Salmonella spp.* isolates in tap water and bottled drinking water in Isfahan, Iran. In a similar study conducted by Geta and Kibret (2022) it was observed that the most frequently identified bacteria from waste sources in Hotspot Environments in Bahir Dar City, Northwestern Ethiopia were, *E. coli*, *Streptococcus* spp. *Klebsiella* spp, *Pseudomonas* spp, *Citrobacter* spp. and *Salmonella* spp. All the eight (8) bacteria isolates recorded in this study are of enterobacterial genera. The presence of intestinal bacteria species such as *Salmonella, Proteus, 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 (Environmental Protection Agency, 2002).

The total bacterial counts and total coliform counts in all water samples analysed in this study indicated a high microbial load in the water. The result from the study recorded the highest total bacteria count in Tudu 6.1 x 10^{-5} CFU/ml, while the lowest was recorded in Kuba (2.2 x 10^{-5} CFU/ml) which comparatively exceeds the WHO's standard limits. The standard limit of 1.0 x 10^{3} CFU/ml for heterotrophic bacteria and coliforms in bottled water was set by WHO, USEPA and ISI 25.18 (WHO, 2006). This could be due to environmental pollution caused by human and animal activities (e.g., human waste disposal, excretion, fishing, swimming, etc.). Increasing climate change and other ecological disturbances such as flooding are all potential sources of pollution (Nevondo and Cloete, 1999; Obi *et al.*, 2002; Mbah *et al.*, 2016). This result corresponds with findings from previous studies on the microbial assessment of drinking-water sources in Ado-Ekiti, Ekiti State Nigeria, Ekosodin Community, Benin City, Edo state Nigeria and Abakaliki Metropolis of Ebonyi state Nigeria, where high prevalence of heterotrophic bacteria and coliforms were recorded, many of which exceeded the acceptable water quality limits (Oluyege *et al.*, 2014; Ekhosuehi *et al.*, 2018; Onwa *et al.*, 2019).

Result from the study indicates that *Staphylococcus* spp. showed the highest resistance pattern to ceftazidime 3(60%), ofloxacin 3(60%), Nitrofurantoin 3(60%), Amoxicillin 3(60%) and Ampicillin 3(60%). Such resistance was also seen in *Salmonella, Pseudomonas* and *E. coli*. Hleba *et al.* (2011) reported a high resistance of *Salmonella* spp. to ampicillin (57.14%), streptomycin and tetracycline (14.28%), and to chloramphenicol (9.52%). This is also supported by Osei *et al.* (2020) whose findings on antibiotic resistance of bacteria isolated from water supplies used in poultry production in Ashanti region of Ghana reported resistance in *Salmonella* isolates to cephalexin, ampicillin, ceftazidime, cefoxitin, cefpodoxime, aztreonam, cefotaxime and chloramphenicol. The highest resistance was recorded for *Salmonella* spp., *E. coli*, and *Pseudomonas* spp. against ceftazidime 12(67%), Ampicillin 12 (75%), and ceftazidime 11 (92%), respectively. A study conducted in eastern Ethiopia showed that the highest frequency of resistance was recorded against ampicillin (94.7%) among *E. coli* isolates from hospital wastewater (Teshome *et al.*, 2020). Osei *et al.* (2020) reported that 76.6%, 75%, 48.4%, and 6.3% of *S. aureus* isolates were resistant to erythromycin, tetracycline, chloramphenicol, and tobramycin, respectively. Citrobacter isolates were resistant to ampicillin, amoxicillin, and cefuroxime but susceptible to gentamicin, ciprofloxacin, and amikacin (Borquaye *et al.*, 2020). The resistance of bacteria against ceftazidime 53(64.1%) was higher than other antibiotics, and the results showed that there was a statistically significant difference among bacterial isolates for the resistance to all antibiotics except Nitrofurantoin ($p>0.05$).

The isolated bacterial species were resistant to at least one antibiotic, and the highest antibiotic resistance was observed in *Pseudomonas* spp. (92%), followed by *E. coli* and *Shigella* spp. (75%), which is higher than the result reported by Moges *et al.*, (2014) who reported that among 113 isolates, 0.9% were susceptible to all antibiotics tested.

CONCLUSION

Results from the study indicates that the stream water sources in Bokkos LGA, Plateaus State are contaminated with medically important bacteria, which suggest that these water sources are a potential source of resistant bacteria. Resistance profiles of bacteria varied from isolate to isolate and the highest antibiotic resistance of 11 (92%) was observed in *Pseudomonas* spp. Environmental pollution through human activities could be responsible for the contamination. Therefore, raising awareness and education regarding proper waste management strategies are needed to avoid environmental pollution. Water from the streams should be well treated before being used. Future work will focus on the physicochemical characterization of water samples and the molecular identification of resistant isolates and genes.

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