OCCURRENCE AND ANTIBIOTICS PROFILES OF *ESCHERICHIA COLI* ISOLATED FROM STREAMS IN AKOKO METROPOLIS

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ABSTRACT
Stream water can serve as sources of drinking water and other domestic use. Hence, this study focused on the occurrence and antibiotic resistance of *Escherichia coli (E. coli)* found in streams located in Akoko Metropolis, Ondo State, Nigeria. Multiple water samples were taken from different streams in Iwaro-Oka, Akungba, Etioro, and Ayegunle. These were cultured on EMB for the presence of *E. coli* strains. The identity of the *E. coli* isolates were confirmed by microscopy and biochemical test. Antibiotic susceptibility testing (AST) was performed on the isolates by disc diffusion method using commonly used antibiotics. *E. coli* was isolated from all the water samples with coliform count ranging from 3.0×10^2 to 9.5×10^3 CFU/mL AST showed that 47% of the *E. coli* isolates were resistant to cefixime and 40% resistant to ceftriaxone. However, 80% of the isolates were susceptible to imipenem and 60% susceptible to Augmentin and nalidixic acid. The findings of this study revealed that streams can serve as potential reservoir for antibiotic-resistant *E. coli* which is of public health concern. In addition, it highlights the importance of monitoring and managing water quality in these areas to mitigate potential public health risks associated with antibiotic-resistant bacteria.

Keywords: Antibiotic resistance, *Escherichia coli*, streams, water quality, antibiotic susceptibility testing

INTRODUCTION
The increase in antimicrobial resistance of microbial populations is an issue of global concern (Salam *et al*., 2023). This increase is foster by the increased utilization of antibiotics for medical treatments and as growth enhancers in animal farming, resulting in an increased selective pressure within bacterial populations (Albernaz-Gonçalves *et al*., 2022). The dissemination of bacterial resistance is primarily facilitated by human settlements and animal farms, where pharmaceutical residues are released into wastewater and subsequently, either directly or indirectly, infiltrate water ecosystems (Manaia *et al*., 2024).

Streams and water bodies serve as crucial components of ecosystems, supporting diverse flora and fauna (Biggs *et al*., 2017). They also provide essential resources for communities worldwide ranging from domestic and recreational purposes (Allan *et al*., 2021). However, these water sources face persistent challenges due to contamination, notably from bacterial pollution from both humans and animal (Cho *et al*., 2018). Human sources of contamination may include the discharge of wastewater, sewage leaks, and malfunctioning septic tanks, along with activities related to municipal, residential, medical, and industrial waste facilities (Cho *et al*., 2018; Oduah & Ogunye, 2023).

Escherichia coli, typically found in the intestinal flora of warm-blooded animals, including humans, is widespread in the environment and is employed as an indicator of fecal contamination for evaluating the safety and quality of water (Cho *et al*., 2018; Jang *et al*., 2017). Although, the majority of *E. coli* strains are benign, specific strains are pathogenic and can induce illnesses like watery diarrhea, bloody diarrhea, urinary tract infections, meningitis, and sepsis, which may result in fatalities (Hassan *et al*., 2021; Pokharel *et al.*, 2023).

Since surface streams are often used for recreational and domestic purposes, the presence of resistant *E. coli* in streams may increase the likelihood of human infections after exposure to these water sources. Hence, the study aims to determine the occurrence and distribution of resistant *E. coli* from streams in Akoko metropolis.

MATERIALS AND METHODS
Sample collection
Samples were collected from four (4) Akoko towns, three (3) streams from each town. The samples were collected from representative location that reflects the water quality of the stream, avoiding areas with obvious contamination sources, such as near industrial discharges or sewage outfalls. The containers were rinsed three times with the stream water before they were submerged to avoid surface scum and sediments and were filled up to minimize the headspace, which can introduce air and alter the sample. The containers were properly labelled and transported to Microbiology Laboratory of the Adekunle Ajasin University Akungba-Akoko for analysis and examination.

Isolation of *E. coli* from the stream samples
Isolation of *E. coli* was performed using serial dilution and pour plate method as adapted from (Fawole and Oso, 2007). One milliliter of the water sample was dispensed into 9.0 mL of sterile distilled water in a test tube to give 10^1. The mixture was shaken and 1.0 mL was dispensed into another test tube containing 9.0 mL of sterile distilled water to give 10^2. This protocol was repeated until 10^4 dilution was achieved. Aliquot of 1.0 mL was taken from the 10^2 and the 10^4 dilutions and dispensed into sterile Petri dishes. Molten EMB agar was added to the inoculum on the plate. The plates were swirled and left to solidify. The plates were incubated at 37°C for 24 hours. Colonies of *E. coli* with green metallic sheen characteristics found after 24 hours were counted using a colony counter and the results were multiplied using appropriate dilution factor, counts were expressed in colony-forming units per mL of sample. The identity of the *E. coli* isolates was confirmed by viewing under the microscope with X100 oil immersion objective lens and carrying out of biochemical tests (Indole test, Oxidase test, Methyl red test and Voges-Proskauer test**)**.

Antimicrobial susceptibility testing
An overnight broth culture was used for the antibiotic susceptibility

test as described by (Makinde, 2023). The broth culture was diluted to 0.5 McFarland turbidity standard using normal saline. The diluted broth was inoculated on the plates by dipping sterile swab into the culture and using it to swab the entire surface of freshly prepared Mueller Hinton agar. The plates were left to dry and antibiotics discs (Celtech, Belgium) were applied to the agar using sterile forceps. The plates were incubated at 37°C for 24 hours. The antibiotics used for this study include; Augmentin (30μg), Cefotaxime (30μg), Gentamicin (30μg), Ceftriaxone (30μg), Cefixime (30μg), Ofloxacin (5μg) and Nalidixic Acid (30μg), Imipenem (10μg). The diameter of the zone of inhibition around each antibiotic disc was measured and isolates were classified either as resistant, intermediate or susceptible using CLSI guideline (CLSI, 2023).

RESULTS
E. coli was isolated from the entire stream water sample (100% occurrence). The counts ranges from 3.0×10^2 to 9.5×10^3 CFU/mL (Table 1). The highest population of *E. coli* was recorded in Etioro-3 stream water sample with a count of 9.5×10^3 CFU/mL while the lowest count (3.0×10^2 CFU/mL) was observed in Iwaro-1 sample. The *E. coli* isolates recovered from the water samples showed a smooth, raised, and green metallic sheen colonies on EMB Agar. All the isolates stained pink to Gram staining. The cells were rod-shaped and some were arranged in pairs while others were arranged in singles when observed under the microscope, indicating that the organism was Gram negative.
The antibiotic susceptibility test of the *Escherichia coli* isolates revealed that 47% were resistant to cefixime and 40% resistant to ceftriaxone (Figure 1). However, 80% of the isolates were susceptible to imipenem and 60% susceptible to Augmentin and nalidixic acid.

Table 1: The *Escherichia coli* count

S/N	Sample	Count (CFU/mL)
1	Akungba-1	7.0×10^2
2	Akungba-2	8.0×10^2
3	Akungba-3	8.0×10^2
4	Iwaro-1	3.0×10^2
5	Iwaro-2	1.3×10^3
6	Iwaro-3	8.9×10^3
7	Ajegunle-1	2.3×10^3
8	Ajegunle-2	3.1×10^3
9	Ajegunle-3	1.3×10^3
10	Etioro-1	1.0×10^3
11	Etioro-2	8.0×10^3
12	Etioro-3	9.5×10^3

Table 2: Cellular Morphology of *Escherichia coli* Isolated from the samples

Isolates	Gram stain	Shape	Arrangement
AJ1	Pink	Short rod	In pairs
AJ2	Pink	Rod	In singles
AJ3	Pink	Rod	In singles
AJ4	Pink	Short rod	In pairs
AJ5	Pink	Rod	In singles
AJ6	Pink	Rod	In pairs
AJ7	Pink	Rod	In singles
AJ8	Pink	Rod	In pairs
AJ9	Pink	Short rod	In singles
AJ10	Pink	Rod	In singles
AJ11	Pink	Short rod	In pairs
AJ12	Pink	Rod	In singles
AJ13	Pink	Short rod	In singles
AJ14	Pink	Rod	In pairs
AJ15	Pink	Rod	In pairs

Table 3: Antibiogram profile of the *Escherichia coli* isolates.

Isolates	CTX 30µg	CXM 30µg	ZEM 30µg	GN 10µg	IMP 10µg	OFX 5µg	CRO 30µg	AUG 30µg	NA 30µg	NF 300µg	ACX 10µg	LBX 5µg
AJ1	S	I	R	S	S	I	R	S	I	R	I	I
AJ2	I	S	S	S	S	I	R	S	S	I	S	S
AJ3	I	I	I	I	S	S	R	I	S	S	S	I
AJ4	I	S	R	S	S	I	S	S	S	R	I	S
AJ5	R	I	I	S	S	I	R	S	S	S	R	S
AJ6	S	S	I	I	S	S	S	I	I	I	S	I
AJ7	S	S	S	R	I	S	S	S	S	I	S	I
AJ8	I	I	R	I	S	S	S	S	S	S	I	S
AJ9	S	I	R	R	S	S	S	I	I	I	S	I
AJ10	I	S	I	I	S	I	S	I	R	S	I	I
AJ11	R	S	R	S	S	S	R	S	R	R	R	S
AJ12	I	S	R	I	I	S	R	S	S	S	S	I
AJ13	S	S	I	S	S	S	S	I	S	I	S	I
AJ14	I	I	I	I	I	I	S	S	S	S	S	S
AJ15	S	I	R	R	S	I	S	I	I	I	I	S

Keywords
S= Susceptible, R= Resistance, I= Intermediate according to CLSI standard 2023.
CTX= Cefotaxime, CXM= Cefuroxime, ZEM= Cefixime, GN = Gentamycin, IMP= Imipenem
OFX= Ofloxacin, CRO= Ceftriaxone, AUG= Augmentin, NA= Nalidixic Acid, NF= Nitrofurantoin, ACX= Ampicillin-Cloxacillin, LBX= Levofloxacin.
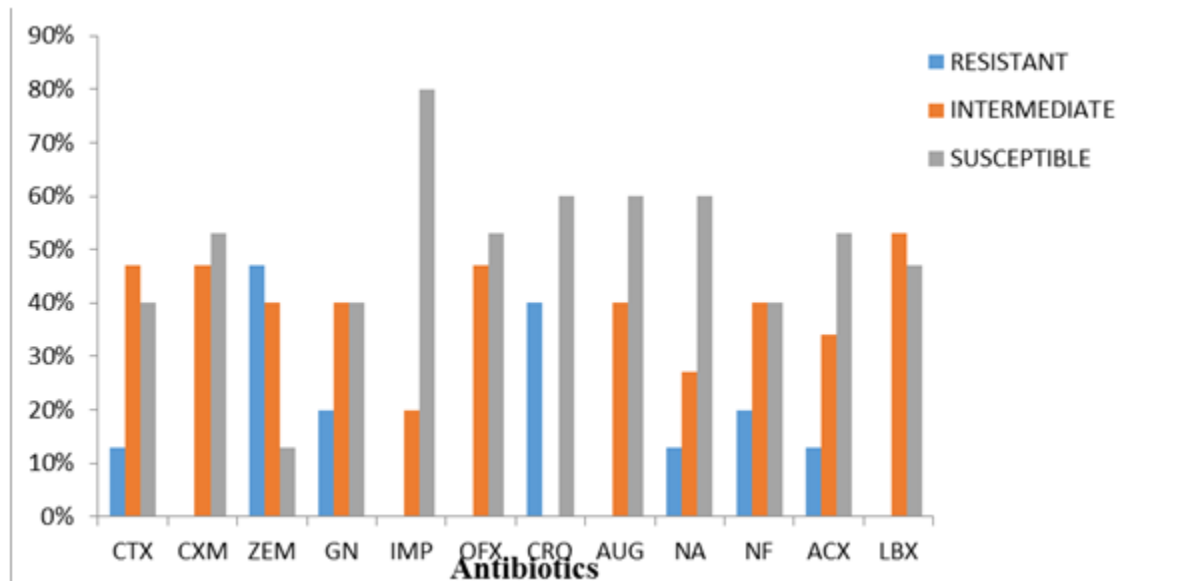

Figure 1: Percentage resistance, intermediate and susceptibility of the *E. coli*

DISCUSSION

The natural flora of the intestines of humans and other warm-blooded animals includes the Gram-negative, rod-shaped bacterium *Escherichia coli* (Liu, 2017; Basavaraju & Gunashree, 2022), which aids in the breakdown of food and the synthesis of certain vitamins (Sarowska *et al*., 2019). It is considered a commensal bacterium, which means that it usually coexists harmlessly with the host (Leimbach *et al*., 2013; Peng *et al*., 2024). While most strains of *E. coli* are safe and serve important functions in the digestive system, some strains can cause illness, such as gastrointestinal infections (Vila *et al*., 2016). *E. coli* pollution in streams therefore can cause waterborne diseases, especially gastrointestinal infections.

When water is consumed or comes into contact with the mouth, pathogenic strains of *E. coli* can induce symptoms such diarrhea, cramping in the abdomen, nausea, and vomiting (Peng *et al*., 2024).

Children, the elderly, and people with weakened immune systems are among the most vulnerable population and they easily succumbed to the negative health effects of waterborne infections. These populations are more susceptible to serious sickness from contaminated water. Contaminated water sources, including streams, can serve as a vehicle for the transmission of enterotoxigenic *E. coli* (ETEC) (Smith & Fratamico, 2017), Enteropathogenic *E. coli* (EPEC) (Luna-Guevara et al., 2019) and the infamous O157:H7 serotype of the enterohemorrhagic *E. coli* (EHEC) strain (Shen *et al.*, 2022), which can have serious health consequences.

Stream water contamination is a serious environmental and public health hazard because it can affect aquatic life, ecosystems, and the availability of clean drinking water. Because *E. coli* can survive in the environment for a long time, it is used as an indicator to determine whether other bacteria or pathogens, especially those with gastrointestinal origins, may be present (Odonkor and Ampofo, 2013). A study carried out on surface water in Australia suggested that more than 50% of *E. coli* isolates in surface water environment are likely to have originated from wastewater effluents deposited in streams (Peng *et al*., 2024), underlining the importance of wastewater treatment plants as potential point sources of pathogens into surface water.

The result of this study shows the presence of *E. coli* isolates in the stream samples. Although the vast majority of the water sample were clouded and appeared brown in color, even the clean looking ones were also contaminated with strains that were resistant to antibiotics. Antibiotic susceptibility test revealed that there was no 100% susceptibility to all the antibiotics used and exhibited resistance as high as 40% to ceftriaxone, an antibiotic in the group of cephalosporins, a large group of bactericidal antimicrobials that works via cell wall synthesis inhibition by binding to penicillin-binding protein and inhibit its normal activity by inhibiting the synthesis of cell wall, leading to the death of bacteria (Zhou *et al*., 2022). The highest resistance was observed in Cefixime with 47%, an antibiotic in the group of cephalosporins.

The highest percentage susceptibility was observed in Imipenem with 80%, Imipenem is a carbapenem that, like other β-lactam antibacterials, inhibits cross-linking of peptidoglycan during cell wall synthesis by inactivating penicillin binding proteins, leading to bacterial cell lysis and ultimately death (Heo, 2021). Ceftriaxone, Augmentin and nalidixic acid showed susceptibility of 60%. Hence, the Bacteria harboring antibiotic resistance genes can be found in streams and other bodies of water. With the increasing use of antibiotics in veterinary care, agriculture, and other fields, the revelation that streams contain microorganisms resistant to antibiotics is alarming.

Conclusion
According to the findings of this study, stream water is a possible point source of antibiotic-resistant genes that might spread to other pathogens in the receiving watersheds and act as a reservoir for pathotypes of antibiotic-resistant *E. coli* and possibly other infections. This poses a great public health risk in the environment. The worrying scenario of potentially running out of treatment options for some serious bacterial infections in the near future is brought about by the catastrophic occurrence of antibiotic resistance and growing virulence in pathogenic bacteria. This phenomenon coupled with high poverty level in the Akoko community calls for priority attention to bring under control the spread of antibiotic resistance in order to safeguard the health of the general public. In the face of underdevelopment, inadequate water supply and sanitation practices, lack of access to proper health care system, low levels of education, a greater proportion of the populace particularly the poor rely on the streams as their main source of water for household use hence becoming highly susceptible to infections and diseases. Efforts to address stream water contamination require a holistic and collaborative approach involving governments, communities, industries, and scientific communities.

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