

Occurrence of *Escherichia coli* from Environmental Effluents during Rainy and Dry Season in Keffi Metropolis, Nasarawa State

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ABSTRACT

Environmental issues have escalated over the past four decades, largely due to poor management practices leading to severe aquatic pollution and increased waterborne diseases. This study investigated the occurrence of *Escherichia coli* in environmental effluents during the rainy and dry seasons of 2019 in Keffi Metropolis. A total of 192 effluent samples were collected, and *E. coli* was isolated using standard microbiological methods. Antibiotic susceptibility was tested and interpreted following CLSI guidelines. Out of the 192 samples collected during the rainy season 2019 the highest occurrences from abattoirs effluent was in the 2nd month (22.9%) and the lowest were in 4th month (16.6%). From hotels the highest occurrence was in 1st month (10.4 %) and the lowest were in 3rd month (2.0 %). From Hospitals, the highest occurrence was in 2nd month (10.4 %) and the lowest were in 4th month (4.1%). From homes the highest was in 1st month (16.6%) and lowest were in 3rd month (6.2 %). During the dry season 2019 from effluent the highest *E. coli* isolated was in 4th month (35.4%) and the lowest were from 1st month (27.0%). From hotels the highest was in 3rd month (22.9%) and the lowest were in 2nd month (10.4 %).

From Hospitals, the highest was in 4th month (18.7 %) and lowest was from 1st month (10.4%). From homes the highest *E. coli* isolated was in 4th month (25.0 %) and lowest were from 1st month (16.6%) The *E. Coli* isolated from environmental effluents during rainy season in 2019 were highly resistant to Suphamethoxazole/Trimethopim (89.7%) and but less resistant to imipenem (20.4 %). While *E. coli* isolated during dry season in 2019 were highly resistant to streptomycin (86.8%) but less resistant to gentamicin (18.1%). and imipenem (12.1%). The occurrence of *E. coli* form environmental effluents shows different levels of *E. coli* contamination in the environmental effluents that flows into farm land and river bodies in Keffi

Keywords: Environmental, *E. coli*, rainy and dry season, effluents, antibiotics

1. Introduction

It is widely acknowledged that conventional wastewater treatment processes often result in the persistence, and in some cases an increase, of enteric bacteria. The degree to which these bacteria remain in treated wastewater varies significantly depending on the treatment method employed. Biological treatment processes, though effective to some extent, frequently leave behind fecal bacteria and other pathogens in the final effluent [1,2]. To mitigate the environmental and public health risks associated with these microorganisms, disinfection steps—primarily involving oxidative processes—are employed to eliminate or inactivate harmful microbes [3]. Among these, chlorine remains the most commonly used disinfectant, though alternative methods such as ozonation and ultraviolet (UV) irradiation are increasingly adopted or undergoing evaluation in many countries [4].

Inadequately designed or poorly managed disinfection systems can fail to eliminate all microbial contaminants, allowing pathogenic microorganisms to survive and enter the environment through treated effluents [5].

The release of such pathogens into surface waters is a significant concern for public health and environmental safety. Effluent discharges from sewage treatment plants (STPs), along with surface runoff and agricultural waste, serve as the primary contributors to microbial contamination in aquatic ecosystems [6,7]. To monitor the microbiological safety of water bodies, fecal indicator bacteria such as *Escherichia coli* and enterococci are commonly used to assess levels of fecal contamination and infer the presence of potential pathogens. However, it is important to note that certain enteric pathogens—such as viruses, protozoa, and some bacterial strains—exhibit different survival dynamics compared to these indicator organisms [8]. Particularly concerning are *E. coli* strains that carry virulence genes (VGs), which have not been extensively studied in the context of their survival in STPs and subsequent release into the environment.

E. coli is not only a reliable indicator of fecal pollution but also a significant pathogen in its own right, capable of causing gastrointestinal infections such as diarrhea. Monitoring its presence in surface waters is therefore crucial for both

environmental assessment and public health surveillance. The present study aims to assess the occurrence and seasonal variation of *E. coli* in environmental effluents during the rainy and dry seasons of 2019 in Keffi Metropolis, Nasarawa State, Nigeria. The findings are expected to contribute to improved understanding of effluent contamination trends and the effectiveness of existing treatment protocols.

2. Material and Methods

2.1 Study Area

The study was carried out in Keffi, which is geographically located on longitude 7°8 E and latitude 8°5 N, North West of Lafia (the Capital of Nasarawa State, Nigeria). Keffi is situated on an altitude of 850m above sea level. Samples of effluents from four areas in Keffi Metropolis.

2.2 Sample collection

Sampling was carried out in three successive months in the; dry season of 2019 (January to April, 2019). Sampling was repeated during season (June to September). A total of three (3) samples was collected in triplicates from each sampling area (hospital, homes, hotels and abattoirs) making a total of thirty six (36) samples a month. Samples was collected in the morning during the peak activities between 8.00am and 9.00am using the grab sampling method using a wide-mouthed 20mL sterilized Pyrex glass bottles with tight screw dust proof stoppers, which was filled leaving a top space of about 2.5cm. Waste water samples were collected from three points (3); First at the point where it is thoroughly mixed and close to the discharging point (outlet), 100mm below the surface. The second sample was taken at a distance of 100m after the first one (measured with a meter measuring tape). The third sampling point would be at the point of release into receiving water body [8]. Data from each sample was collected and recorded in the data book. Samples were stored on ice for transportation to the laboratory and was processed and incubated within five (5) hours of sampling.

2.3 Sample processing and isolation

The isolation *E. coli* was carried out as describe by Farasat et al. [9]. A solution of the effluent was made by pipetting 1 ml of sample into a test tube containing 9 ml Nutrient Broth (NB), vortexes for one minute and left for thirty; minutes at room temperature. Then the supernatant (1ml) was taken from this test tube and transferred into the first test tube containing 9 ml of sterile water and mixed thoroughly, after which another 1ml was taken from first test tube to second test tube containing 9ml of sterile water, this step was repeated five times; obtaining dilution rate of up to 10^{-5} . After this, 0.1 aliquot portion of the 10^{-5} dilution was spread onto duplicate sterile plates containing prepared MacConkey medium and Eosin methylene blue medium. The Petri dishes was kept in the incubator for 24 hours at 37°C. After 24 hours, plates were studied for the colonies of *E. coli* growing on the media

2.4 Identification of the *E. coli* isolate

The *E. coli* growth was identified using cultural and morphological characteristics such as Gram staining reaction test and biochemical tests as described below:

2.5 Gram Staining Examination

Gram staining was performed following the method described by [10]. A small amount of the cultured organism was transferred onto a clean, grease-free glass slide and emulsified in a drop of distilled water to form a thin, uniform smear.

The smear was allowed to air-dry and then heat-fixed by briefly passing the slide through a flame. The slide was flooded with crystal violet for 1 minute, then rinsed with distilled water. Lugol's iodine was applied for 1 minute as a mordant, followed by another rinse. Decolorization was done using acetone-alcohol until the runoff was clear, and the slide was again rinsed with distilled water. Finally, the smear was counterstained with neutral red for 1 minute, rinsed, air-dried, and examined under a microscope using the oil immersion objective (100x magnification).

2.6 Biochemical Tests

A series of biochemical tests were conducted to confirm the identity of suspected *Escherichia coli* isolates. These included: Catalase test, Indole production, Methyl Red (MR) test, Voges-Proskauer (VP) test, Nitrate reduction test, Urease production, Citrate utilization, and Glucose fermentation test. Each test was performed using standard microbiological procedures to evaluate the metabolic and enzymatic characteristics of the isolates.

2.7 Indole Test

The Indole test was performed following the method described by [11]. A single colony from the culture plate was inoculated into 5 mL of tryptone broth and incubated at 37°C for 24 hours. After incubation, a few drops of Kovac's reagent were added to the broth and gently shaken. A positive result was indicated by the appearance of a red ring in the reagent layer within 10 minutes.

2.8 Methyl Red and Voges-Proskauer Tests

The Methyl Red and Voges-Proskauer (MR-VP) tests were conducted using MR-VP broth, as described by [11]. A pure culture of the test organism was inoculated into the medium and incubated at 37°C for 72 hours. After incubation, the culture was divided into two equal portions:

- **Methyl Red Test:** Three drops of methyl red indicator were added to one portion. The appearance of a red color indicated a positive result.

- **Voges-Proskauer Test:** To the second portion, 10 drops of 40% potassium hydroxide (KOH) and 4 drops of alpha-naphthol were added. The mixture was gently shaken and observed for 30 minutes. A pink to red color indicated a positive result, while a yellow color indicated a negative result.

2.9 Citrate Utilization Test

The citrate utilization test was conducted as described by [11]. A pure culture of the suspected organism was inoculated in a single streak along the slant surface of citrate agar and incubated at 37°C for 24 hours. A change in the medium color from green to blue indicated a positive result, signifying the production of alkaline metabolites. A green color indicated a negative result.

2.10 Catalase Test

The catalase test was performed following the procedure described by [11]. A pure colony of the organism was aseptically streaked on a nutrient agar slant and incubated at 37°C for 24 hours. Three drops of hydrogen peroxide (H_2O_2) were added to the slant. The immediate formation of oxygen bubbles indicated a positive catalase reaction.

2.11 Nitrate Reduction Test

This test was carried out as per the method described by [11]. A pure colony was inoculated aseptically into nitrate broth and incubated at 37°C for 24 hours. After incubation, five drops each of nitrate reagent A (sulfanilic acid) and nitrate reagent B (dimethyl- α -naphthylamine) were added. The appearance of a red color indicated a positive result. If no color developed, a pinch of zinc powder was added as a confirmatory step. A red color after zinc addition indicated a true negative, while no color change confirmed complete nitrate reduction to ammonia or nitrogen gas, indicating a positive result.

2.12 Urease Test

The urease test was performed as described by [11]. The test organism was inoculated into urea broth and incubated at 37°C for 24 hours. A positive result was indicated by a bright pink color, while no color change indicated a negative result.

2.13 Sugar Fermentation Test

The sugar fermentation test was conducted according to [11], using phenol red broth containing different test sugars and an inverted Durham tube to detect gas production. A loopful of the test organism was inoculated into the sugar broth and incubated at 37°C for 18 hours. A bright yellow color indicated acid production from sugar fermentation, while the presence of a gas bubble in the Durham tube indicated gas production.

2.14 Susceptibility Testing

Antibiotic sensitivity patterns of *E. coli* isolates were determined on Muller Hinton agar plates by Kirby-Bauer disc diffusion. The criteria for inferring whether the isolate is resistant or susceptible (R or S) were based on the measured zone of inhibition [12]. Colony of the *E. coli* was inoculated into 5 ml of Mueller Hinton broth and incubated at 37°C for 24 hours. After which, the overnight culture was adjusted to the turbidity equivalent 0.5 McFarland standard. The adjusted overnight inoculum was flooded on the surface of Mueller Hinton agar and allowed to settle. The antibiotic disc (Kirby's disc) was aseptically placed at the center of the plates and incubated at 37°C for 24h. The zone of inhibition in millimeter was recorded.

3. Results

3.1 Isolation and Identification of *Escherichia coli*

The cultural, morphological, and biochemical characteristics of *Escherichia coli* isolated from environmental effluents are presented in Table 1. The isolates produced pink colonies on MacConkey Agar (MCA) and exhibited a greenish metallic sheen on Eosin Methylene Blue (EMB) agar. Microscopically, the isolates were identified as Gram-negative rods.

Table 1: Cultural, Morphological, Biochemical characteristics of *Escherichia coli* isolated from environmental effluents during different seasons in Keffi

Cultural characteristics	Morphological characteristics		Biochemical Characteristics													Inference
	Gram reaction	Morphology	IND	MR	VP	CT	TDA	ONPG	LYS	ORN	UR	NT	H ₂ S	MAL		
Pinkish colonies on MCA and Greenish metallic sheen on EMB agar	-	Rod	+	+	-	P	-	+	+	+	-	+	-	-	<i>E. coli</i>	

+ = Positive, - = negative, IND = Indole; MR = Methyl red; VP = Voges-Proskauer, CT = Citrate, LYS = Lysine, ORN = Ornithine; ONPG = Ortho-Nitrophenyl- β -galactosidase, UR = Urease, NT = Nitrate, H₂S = Hydrogen Sulphide, Mal = Malonate, TDA = Phenylalanine deaminase

Biochemical tests revealed the following profile: Indole-positive, Methyl Red-positive, Voges-Proskauer-negative, Citrate-negative, and ONPG-positive. These characteristics are consistent with the identification of *Escherichia coli*.

3.2 Occurrence of *Escherichia coli* during rainy and dry season in 2019

The occurrence of *E. coli* isolated from the effluent rainy season in Keffi is as shown in Table 2. The highest *E. coli* isolated from abattoirs effluent was from the 2nd month (22.9%) followed by 1st month (33.3 %), 3rd month (18.7%) and the lowest were from 4th month (16.6%). From Hotels the highest occurrence was from the 1st month (10.4 %) followed by 2nd month (8.3%) and the lowest were in 3rd month (2.0 %). From Hospitals the highest occurrence was from 2nd month (10.4 %) followed by 1st month and (8.3 %), 3rd month (6.2 %) and the lowest were from 4th month (4.1%). From Homes the highest occurrence was from 1st month (16.6%) followed by 2nd month (14.5%), 4th month (10.4%) and the lowest were from 3rd month (6.2 %) as given in Table 2.

During the dry season of 2019 from a battoir effluent the highest *E. coli* isolated was from the 4th month (35.4%) followed by 2nd month and 3rd month (31.2 %), and the lowest were from 1st month (27.0%). From hotels the highest occurrence of *E. coli* was from the 3rd month (22.9%) followed by 4th month (16.6%), 1st month (12.5%) and the lowest were in 2nd month (10.4 %). From Hospitals the highest occurrence was from 4th month (18.7 %) followed by 2nd month (14.8%), 3rd month (12.5 %) and lowest was from 1st month (10.4%). From homes the highest *E. coli* isolated was from 4th month (25.0 %) followed by 3rd month (25.0%), 2nd month (18.7 %) and lowest were from 1st month (16.6%) as given in Table 3.

3.3 Antibiotic Resistance of *E. coli* isolated from environmental effluents

The antibiotic resistance of *E. coli* isolated from environmental effluents during rainy and dry seasons in 2019 in Keffi is as shown in Table 4. The *E. coli* isolated from environmental effluents during rainy season in 2019 were highly resistant to Suphamethoxazole/Trimethoprim (89.7%) followed by streptomycin (87.5%); amoxycillin (62.5 %); Cefexime (79.5%); Ceftriaxone (77.2%); ampicillin (71.5%), and but less resistant to imipenem (20.4 %). While *E. coli* isolated from environmental effluents during dry season in 2019 were highly resistant to streptomycin (86.8%) followed by sulphamethoxazole/trimethoprim (81.8 %), ceftriaxone (76.7%), ampicillin (66.6 %), nitrofurantoin (65.6 %), but less resistant to gentamicin (18.1%). and imipenem (12.1%)

Table 2: Occurrence of *E. coli* isolated from environmental effluents during rainy season 2019 in Keffi

Location	No. Sample	No. (%) isolated in rainy season				
		1 st month	2 nd month	3 rd month	4 th month	
Abattoirs	48	10 (20.8)	11 (22.9)	9 (18.7)	8 (16.6)	
Hotels	48	5 (10.4)	4 (8.3)	1 (2.0)	0 (0.0)	
Hospitals	48	4 (8.3)	5 (10.4)	3 (6.2)	2 (4.1)	
Homes	48	8 (16.6)	7 (14.5)	3 (6.2)	5 (10.4)	
Total	192	27 (14.0)	27 (14.0)	16 (8.3)	15 (7.8)	

Key: 1st month= June, 2nd month= July, 3rd month August and 4th month September

Table 3: Occurrence of *E. coli* isolated from environmental effluents during dry season 2019 in Keffi

Location	No. Sample	No. (%) isolated in dry season				
		1 st month	2 nd month	3 rd month	4 th month	
Abattoirs	48	13 (27.0)	15 (31.2)	15 (31.2)	17 (35.4)	
Hotels	48	6 (12.5)	5 (10.4)	11 (22.9)	8 (16.6)	
Hospitals	48	5 (10.4)	4 (14.8)	6 (12.5)	9 (18.7)	
Homes	48	8 (16.6)	9 (18.7)	10 (25.0)	12 (25.0)	
Total	192	32 (18.6)	33 (19.1)	42 (24.4)	46 (26.7)	

Key: 1st month= June, 2nd month= July, 3rd month August and 4th month September

Table 4 Antibiotics Resistance of *E. coli* isolated from environmental effluents for rainy and dry season 2019

Antibiotics	Disc content (µg)	No (%) of Resistance <i>E. coli</i>	
		Raining season (n= 88)	Dry season (n= 99)
Amoxicillin(AML)	10	55(62.5)	62(62.6)
Ampicillin (AMP)	10	63(71.5)	66(66.6)
Cefexime (CFM)	5	70(79.5)	61(61.6)
Ceftriaxone (CRO)	30	68(77.2)	76(76.7)
Ciprofloxacin (CIP)	5	51(57.9)	49(49.4)
Gentamicin (CN)	30	24(27.2)	18(18.1)
Imipenem (IMP)	10	18(20.4)	12(12.1)
Nitrofurantoin (F)	30	54(61.3)	65(65.6)
Streptomycin (S)	30	77(87.5)	86(86.8)
Sulphamethoxazole/Trimethoprim(SXT)	25	79(89.7)	81(81.8)

4. Discussion of the findings

The presence or isolation of *Escherichia coli* in natural waters has long been used as an indicator of fecal contamination. However, emerging evidence suggests the existence of specialized subgroups of *E. coli* strains capable of reproducing and persisting in secondary environments, such as effluents, rather than being strictly confined to the intestinal tracts of warm-blooded animals. This phenomenon has been observed in both tropical [4] and temperate climates [13]. The present study focused on the antimicrobial resistance patterns of *E. coli* strains isolated from environmental effluents in Keffi during the rainy and dry seasons of 2019. The isolation rate of *E. coli* from homes, hotels, hospitals and abattoirs effluent in the study area during the rainy season was lower than during the dry season but similar to study reported by [14]. The low isolation of *E. coli* during rainy season may; be due to the constant rain that flash or carries way these effluent from hospital, and abattoirs into farm lands or nearby streams, but the high occurrence of *E. coli* during dry season shows the level of *E. coli* the were release from effluent into the environment which may lead to outbreak of *E. coli* infection in the society. In this study it was observed that occurrence of *E. coli* was more from abattoirs and homes both in rainy and dry this suggest that the two places lack the necessary amenities which are used for disposal of effluents such as septic tank or sewages but were allowed to flow in the environment this findings were similar to studies earlier reported by [15] and [16]. The findings also suggested that the effluents are highly contaminated with human or animal faeces as *E. coli* are known to normal flora of the intestine.

The high resistance of commonly used antibiotics observed in this study is something of great interest due to its public health effect. It was recorded that *E. coli* isolated from different environmental effluent during raining season were highly resistant to streptomycin, Sulphamethoxazole/Trimethoprim and amoxicillin. This finding is consistent with the study reported by [8], where *E. coli* isolates from various environmental effluents during the dry season also exhibited high resistance to streptomycin. The presence of multidrug-resistant *E. coli* suggests that these isolates from effluents pose a potential public health risk. However, it was also observed that the isolates were highly susceptible to gentamicin, indicating that this antibiotic may still be effective against such strains. Despite this, the resistance of *E. coli* to several other antibiotics highlights the potential for horizontal gene transfer of antimicrobial resistance genes to other bacteria present in the effluent. Antimicrobial resistance genes are widely found in environmental settings [17] and play a crucial role in bacterial adaptation and survival. The high prevalence of multidrug-resistant *E. coli* in effluents may be attributed to a combination of biological and ecological factors. Notably, the ability of these organisms to harbor plasmids carrying multiple resistance genes enables them to withstand a broad range of antibiotics.

5. CONCLUSION

In this study, *E. coli* were isolated from different environmental effluent sampled from different abattoirs in Keffi, homes, hotels and hospitals effluent during dry and raining season 2019. The occurrence of *E. coli* during rainy season 2019 from abattoir effluent was highest from the 2nd month and the lowest were

from 4th month. From Hotels the highest occurrence was from the 1st month and the lowest were in 3rd month. From Hospitals the highest occurrence was from 2nd month and the lowest were from 4th month. During the dry season of 2019 from abattoir effluent the highest *E. coli* isolated was from the 4th month and the lowest were from 1st month. From hotels, the highest occurrence of *E. coli* was from the 3rd month and the lowest were in 2nd month. The antibiotic resistance of the *E. coli* isolated were highly resistant to streptomycin but less resistant to Imipenem.

Conflict of Interest statement

The authors declare no conflicts of interest

4. Discussion of the findings

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