



Prevalence Of *Escherichia coli* isolated from Environmental Effluents During Rainy And Dry Season In Keffi Metropolis, Nigeria

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Abstract

Environmental problems have increased over the last four decades with improper management practices being largely responsible for the gross pollution of aquatic environment with concomitant increase in water-borne disease. This study aimed at Prevalence of *E. coli* isolated from environmental effluents during rainy and dry season in Keffi Metropolis was carried out. A total of 192 of environmental effluents samples were collected and *E. coli* were isolated and identified using standard microbiological method. The antibiotic susceptibility test was carried out and interpreted in accordance with clinical and laboratory standard institute (CLSI) Protocol. Out of the 192 samples collected during rainy season 2018 the total occurrence of *E. coli* in the 1st month was 11.9%, 2nd month was 10.4%, 3rd month was 7.8% and the 4th month was 6.2%. From the dry season of 2018 the total occurrence of *E. coli* in the 1st month was 13.0 %, 2nd month was 14.0%, 3rd month was 13.5% and the 4th month was 17.1%. The antibiotics resistances of the *E. coli* during rainy season in 2018 were highly resistant to Suphamethoxazole/Trimethopin (87.1 %) and but less resistant to ciprofloxacin (30.0%), gentamicin (20.0 %), and imipenem(11.4%) While *E. coli* isolated from environmental effluents during dry season in 2018 was highly resistant to streptomycin(90.6 %) but less resistant to ciprofloxacin(38.6 %), imipenem(17.3%) and gentamicin (14.6%). The occurrence of *E. coli* from environmental effluents shows different level of *E. coli* contamination in the environmental effluents that flows into farm land and river bodies in Keffi

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

Introduction

Human health and environmental quality are constantly undergoing degradation by the increasing amount of wastes being produced (Ogedengbe and Akinbile, 2010). Groundwater is a valuable resource often used for industry, commerce, agriculture and most importantly for drinking. Often the raw water used for domestic purposes is vulnerable to contamination due to the human influence resulting in pollution from progressive industrialization and urbanization (Akinbile, 2012). Industries are involved with the total environment from the farm to the customer and water is absolutely necessary for many steps in the processing industry. At present, there is no economical substitute of water, consequently water conservation and water reuse are necessary. By practicing conservation and reuse, the amount of liquid waste and pollution potential is reduced (Farasat *et al.*, 2012).

On a global scale, contamination of drinking water by pathogenic bacteria causes the most significant health risk to humans, and there have been countless numbers of disease outbreaks and poisonings resulting from exposure to untreated or poorly treated drinking water. However, significant risks to human health may also result from exposure to toxic contaminants that are often globally ubiquitous in waters from which drinking water is derived. The presence of *E. coli* is a definite indication of fecal contamination (WHO, 2004).

Escherichia coli (*E. coli*) a Gram-negative, non-sporulating, facultative anaerobic bacterium, belonging to the family Enterobacteriaceae of the class Gamma Proteobacteria. *E. coli* are the normal inhabitant of gastrointestinal microflora of various birds and animals and a versatile enteric microorganism, acting both as commensal and pathogenic organism (Tenaillon, *et al.*, 2010). *E. coli* are one of the most extensively studied bacteria worldwide. They may inhabit a host as harmless symbioses, or depending on their panoply of virulence traits and/or certain predisposing conditions cause disease (Anastasi, *et al.*, 2012). The magnitude of the problem of

bacterial contamination deserves more elaborative studies from the point of production of waste effluents to the point of disposal. The aim of this research aimed at Prevalence of *E. coli* isolated from environmental effluents during rainy and dry season in Keffi Metropolis, Nasarawa State.

Materials and Methods

2.1 Study Area

The study will be carried out in Nasarawa State University Keffi, Nasarawa State Nigeria. Keffi is approximately 68km away from the Federal Capital Territory (FCT), Abuja and 128km away from Lafia, the Capital town of Nasarawa State. Keffi is located at longitude 7° 52' E and latitude 8°50'E along the Greenwich Meridian and at the equator and altitude 850m above sea level (Akwa, *et al.*, 2007).

2.2 Sample collection

Sampling was carried out in four successive months in the rainy season of 2018 (June, July, August and September). Sampling was repeated in the dry season of 2018 (December, January February and March). A total of three (3) samples were collected from each sampling area (hospital, homes, hotels and abattoirs) making a total of forty eight (48) samples a month. Samples were 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.

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 (Nafarnda *et al.*, 2010).

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 Isolation of *E. coli*

The isolation *E. coli* was carried out as describe by Farasat *et al.* (2012). 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 portions 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

The Gram staining technique was carried out as described by Akhtar *et al.* (2016). A small portion of cultural organism was transferred onto a clean grease-free glass slide, and emulsified in a drop of distilled water until a thin homogeneous film is obtained, then the wire loop was re-sterilized and the thin homogeneous film was allowed to air-dry, and heat-fixed by passing through the flame. The slide was then flooded with crystal violet for 1 minute, and then rinsed with distilled water. The stain was again flooded with Lugol's iodine for 1 minute, and rinsed with

distilled water. It was then decolourised, rapidly with acetone alcohol until no more colour appears to flow from preparation and rinsed appropriately with distilled water. The stain was then counter-stained with neutral red for 1 minute, and rinsed with distilled water, allowed to air dry and viewed microscopically using x100 oil immersion objective.

2.6 Biochemical Tests

The following biochemical tests were carried out on the suspected *E. coli* isolates: Catalase test, Indole, Methyl red, Vorges-Proskauer tests, Nitrate reduction, Urease production, Citrate utilization, and glucose fermentation tests.

Indole Test

The Indole test for the suspected organism was carried out as described by Akhtaret *al.* (2016).A colony of the organism from culture plate was inoculated unto 5ml tryptone broth and incubated at 37°C for 24 h. After which a few drops of Kovac's reagent was added to the overnight tryptone broth culture, and shaken. A positive reaction is indicated by the development of red ring colour in the reagent layer above the broth within 10 minutes observation time.

Methyl Red/Vorges-Proskauer Test

The Methyl Red Test for the suspected organism was carried out as described by Akhtar *et al.* (2016). A pure culture of test organism was inoculated in to MR-VP medium and incubated at 37°C for 72h after which the culture was divided in to two portions. To the first portion, three drops of methyl red was added and formation of red colour was indicative of methyl red positive. To the second portion 10 drops of 40% KOH (Potassium hydroxide) was added, followed by four drops of alpha-naphthol was added and observed for 30 min. Formation of pink/red colour indicates Voges-Proskauer positive and formation of yellow colour indicates Voges-Proskauer negative.

2.9 Citrate Utilisation Test

The citrate utilization test for the suspected organism was carried out as described by Akhtar *et al.* (2016). A pure culture of the organism was inoculated as a single streak on the slant surface of citrate agar and was incubated at 37°C for 24 hours, blue colour on the medium indicates the presence of alkaline products and it is therefore positive, while green colour is negative.

2.10 Catalase Test

Catalase test was performed as described by Akhtar *et al.* (2016). A pure colony of the organism was streaked aseptically on Nutrient agar slant and incubated at 37°C for 24 hours. Three drops of Hydrogen peroxide (H₂O₂) was added to the slant and observed for bubbling gas.

2.11 Nitrate Reduction Test

This test was carried out as described by Akhtar *et al.* (2016). A pure colony of the isolate was inoculated aseptically into Nitrate broth and incubated at 37°C for 24h. Five drops of nitrate reagent A (Sulfanilic acid) and five drops of nitrate reagent B (dimethyl-alpha-naphthalamine) was added to the medium. Red colour indicates positive reaction, otherwise, it is negative. A confirmatory test for nitrate reduction was performed by adding Zinc powder to the negative tubes. If the colour changes then the result is confirmed negative. If there is no change then, it is positive because the organism have reduced nitrate completely into ammonia and nitrogen gas.

2.12 Urease Test

This test was carried out as described by Cheesbrough, (2006). This was performed by inoculating the organism into Urea broth and incubated at 37°C for 24h. Intense pink colour indicates positive outcome, otherwise, it is negative.

2.13 Sugar Fermentation Test

This test was carried out as described by Cheesbrough, (2006). This test uses Phenol Red

Broth to test for the fermentation of different sugars. Phenol Red Broth is a general purpose fermentation media that includes the pH indicator Phenol Red and a series of tubes each with a different sugar. A wire loop was aseptically use to inoculate the test organism into the broth containing the test sugar and inverted Durham tubes. This was incubated at 37°C for 18 hours. A bright yellow colour indicates the production of enough acid products from fermentation of the sugar. Production of gas will be determined with a Durham tube, a small inverted vial filled with the broth. Gas production during fermentation of the sugar, was seen trapped at the top of the Durham tube and appear as a bubble.

2.14 Susceptibility Testing

Antibiotic sensitivity pattern 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, intermediate or sensitive (R, I or S) will be based on the measured zone of inhibition (CLSI, 2018). 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 Mcferland 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 (Sabir *et al.*, 2014).

Results

3.1 Isolation and Identification of *Escherichia coli*

The cultural, morphological, and biochemical characteristics *Escherichia coli* from isolated from environmental effluents is as given in Table 1. Pinkish colony on MCA which grew with greenish metallic sheen on EMB agar, was Gram negative rod and biochemical reactions namely: Indole positive, methyl red positive, Voges-Proskauer negative, Citrate negative, ONPG-positive indicated *Escherichia coli*

3.2 Occurrence of *Escherichia coli* during rainy and dry season in 2018

From abattoirs the highest *E. coli* isolated was from the 1st month(33.3 %) followed by 2nd month(25.9 %) and the lowest were from 3rd month and 4th month(22.2 %). From Hotels the highest occurrence was from the 1st month (14.8 %) followed by 2nd month (7.4 %) and the lowest were in 4th month (3.7 %). From Hospitals the highest occurrence was from 3rd month(14.8 %) followed by 1st month and 2nd month (11.1 %) and the lowest were from 4th month (7.4 %). From Homes the highest occurrence was from 2nd month (29.6 %) followed by 1st month (25.9 %), 3rd month (18.5 %) and lowest were from 4th month (11.1 %) as given in Table 2.

During the dry season 2018 from abattoir waste water the highest *E. coli* isolated was from the 4th month (27.0 %) followed by 1st month (25.0 %), 3rd month (22.9 %), and the lowest were from 2nd month (20.8 %). From hotels the highest occurrence of *E. coli* was from the 4th month (12.5 %) followed by 3rd month (10.4 %), 2nd month (8.3 %) and the lowest were in 1st month (4.1 %). From Hospitals the highest occurrence was from 2nd and 4th month (10.4%) followed by 1st and 3rd

month (6.2 %). From homes the highest occurrence was from 4th month (18.7 %) followed by 1st (16.6 %), 3rd month (14.5%) and lowest were from 2nd month (12.5 %). 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 2018 in Keffi as shown in Table 4. The *E. coli* isolated from environmental effluents during rainy season in 2018 were highly resistant to Suphamethoxazole/Trimethopim (87.1 %) followed by streptomycin (81.4%), amoxycillin (50.0%), ampicillin (47.1 %), Cefexime (42.8%), Ceftriaxone (40.0 %) and but less resistant to ciprofloxacin (30.0%), gentamicin (20.0 %), and imipenem(11.4%). While *E. coli* isolated from environmental effluents during dry season in 2018 was highly resistant to streptomycin(90.6 %) followed by sulphamethoxazole/trimethoprim (86.6 %), cefexime (68.0%), ceftriaxone (61.3%), nitrofurantoin (58.6%), amoxicillin(56.0%) but less resistant to ciprofloxacin(38.6 %), imipenem(17.3%) and gentamicin (14.6%).

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

Table 2 Percentage occurrence of *E. coli* isolated from environmental effluents during rainy season 2018 in Keffi

Location	No. Sample	Rainy season No. (%) isolated			
		1 st month	2 nd month	3 rd month	4 th month
Abattoirs	48	9 (18.7)	7 (14.5)	6 (27.2)	6 (27.2)
Hotels	48	4 (8.3)	2 (4.1)	0 (0.0)	1 (2.0)
Hospitals	48	3 (6.2)	3 (6.2)	4 (8.3)	2 (4.1)
Homes	48	7 (14.5)	8 (16.6)	5 (10.4)	3 (6.2)
Total	192	23 (11.9)	20 (10.4)	15 (7.8)	12 (6.2)

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

Table 3 Percentage occurrence of *E. coli* isolated from environmental effluents during dry season 2018 in Keffi

Location	No. Sample	Dry season No. (%) isolated			
		1 st month	2 nd month	3 rd month	4 th month
Abattoirs	48	12 (25.0)	10 (20.8)	11 (22.9)	13 (27.0)
Hotels	48	2 (4.1)	4 (8.3)	5 (10.4)	6 (12.5)
Hospitals	48	3 (6.2)	5 (10.4)	3 (6.2)	5 (10.4)
Homes	48	8 (16.6)	6 (12.5)	7 (14.5)	9 (18.7)
Total	192	25 (13.0)	27 (14.0)	26 (13.5)	33 (17.1)

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 2018

Antibiotics	Disc content (µg)	No (%) of Resistance <i>E. coli</i>	
		Rainy season (n= 70)	Dry season (n= 75)
Amoxicillin(AML)	10	35(50.0)	42(56.0)
Ampicillin (AMP)	10	33(47.1)	40(53.3)
Cefexime (CFM)	5	30(42.8)	51(68.0)
Ceftriaxone (CRO)	30	28(40.0)	46(61.3)
Ciprofloxacin (CIP)	5	21(30.0)	29(38.6)
Gentamicin (CN)	30	14(20.0)	11(14.6)
Imipenem (IMP)	10	8(11.4)	13(17.3)
Nitrofurantoin (F)	30	24(34.2)	44(58.6)
Streptomycin (S)	30	57(81.4)	68(90.6)
Sulphamethoxazole/Trimethoprim(SXT)	25	61(87.1)	65(86.6)

Discussion

Bacteria from effluents either from abattoir or homes that is discharged into water or soil environment can subsequently be absorbed to sediments, and when the bottom stream is disturbed, the sediment releases the bacteria back into the water columns or air presenting long term health hazard (Nafarnda, *et al.*, 2012). This study aimed evaluation of Prevalence of *E. coli* isolated from environmental effluents during rainy and dried in 2018 season in Keffi Metropolis. In this study it was recorded that the highest prevalence was at 1st month being June (11.9 %) and the lowest was at 4th month being September (6.2 %) this is similar to study earlier reported by Prayoga *et al.*(2021). The occurrence of *E. coli* in raining season was lower than the dry season but in agreement with study earlier reported by Claudious *et al.* (2021) in Bulawayo, Zimbabwe. But also lower than 30.0 % and 32.3% reported by Odo *et al.* (2022) in Makurdi, Nigeria and Onuoha *et al.*(2022) in Abuja, Nigeria. The highest occurrence of *E. coli* from the environmental effluents recorded in this study was from abattoirs effluents which is similar to studies reported by Elemile *et al.*(2019) but in disagreement with Adebami *et al.*(2021) who reported high occurrence of *E. coli* from effluent from fish ponds than abattoirs effluents and other effluents from homes this may due to the fish pond effluents is contaminated with more faecal matter that originate from different animal.

The occurrence of *E. coli* in dry season was high, it is in agreement with studies reported by Mezrioui and Baleux(2004). The highest *E. coli* isolated during the dry season in this study was from abattoir and the lowest was hotels effluent this is in disagreement with study reported Kraupner *et al.* (2021) who reported higher occurrence of *E. coli* from hospital effluents than hotels and homes. The high occurrence of *E. coli* from the abattoir and other sources of effluents sampled in this study were not surprising because is known that *E. coli* is normal flora of warm blooded animal. The high percentage of biological nutrients and growth factors in abattoir, home, hospital and hotel effluents in the study area

provide *E. coli* isolated with the appropriate nutrition to proliferate rapidly (Dankaka *et al.*, 2018). According to Onuoha *et al.* 2016), other factors adding to high *E. coli* loads in home effluent are poor sanitary and hygienic practices of individuals as well as high faecal contamination from intestinal contents.

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 (88.0%), Sufamethoxazole/Trimethopim (85.7%) and amoxicillin (83.3%). This is similar to study reported by Nfongeh *et al.*(2012), *E. coli* isolated from different environmental effluent during dry season were also highly resistant to streptomycin(87.8%), the multidrug-resistant *E. coli*, which suggests that *E. coli* isolated from effluents was dangerous It also observed that the isolates were highly susceptible to Gentamicin, making it less and less of a public health threat; however, resistance to some antibiotics observed in the *E. coli* makes it a potential source of resistance horizontal gene transfer to other bacteria in the effluent. Antimicrobial-resistant genes are common in the environment (Fonseca *et al.*, 2018) and play an essential role in bacterial survival. The high prevalence of multidrug resistant *E. coli* in effluent is probably due to biological and ecological factors. The fact that these organisms are multidrug-resistant implies that these *E. coli* can harbour plasmids with several genes conferring resistance to a broad array of antibiotics.

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. The occurrence of *E. coli* during raining season 2018 highest from 1st month (13.3 %) and the lowest was from 4th month (6.9 %). During the dry season 2018 the highest *E. coli* was isolated from 4th month (19.1%) and the lowest was from 1st month (14.5 %).

The antibiotic resistance of the *E. coli* isolated were highly resistant to streptomycin but less resistant to Imipenem.

Conflict of Interest statement

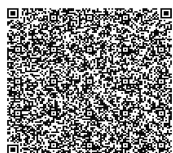
The authors declare no conflicts of interest

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