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Antibiotic Resistance Pattern and Plasmid Curing of *Escherichia coli* Isolated from Soil Samples in Girei, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this study, seventy five (75) soil samples were collected from farmlands, riverbanks and dumpsites and screened for the presence of *Escherichia coli* via inoculation on EMB, Gram's staining and some biochemical tests. Overall, 15 *E. coli* isolates were identified. Dumpsite has the highest number of isolates with 9(60%), followed by farmland with four 4(26.7%) isolates, and lastly riverbank with two 2(13.3%) isolates. Subculture on Sorbitol-MacConkey agar revealed that 2 isolates from dumpsite were EHEC O157:H7. Most of the isolates were resistant to Amoxicillin and Augmentin (66.7% and 73.3% respectively). One of the EHEC strains was found to be resistant to Pefloxacin. Multidrug resistant isolates later became susceptible to previously resisted antibiotics after plasmid curing using 10% sodium dodecyl sulphate (SDS). The outcome of this study suggests that EHEC O157:H7 is not common in the soil environment in comparison with other *E. coli* strains and it is found to be associated with dumpsites. Resistance to some of the antibiotics was plasmid-borne; therefore, indiscriminate use of antibiotics should be avoided to minimize rapid development of resistant bacterial strains. Dumping of refuse close to households should also be avoided so as to minimize the risk of infection with EHEC O157:H7.

Keywords: Antimicrobial resistance; Escherichia coli; plasmid curing; soil environment.

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1. INTRODUCTION

Escherichia coli is a Gram-negative gammaproteobacterium which occurs in diverse forms in nature ranging from commensal strain to pathogenic on humans or animal host. Most strains are harmless natural inhabitants commonly found in the lower intestine of warm blooded organisms including humans. In this regard, the bacterium has been considered as an indicator of fecal pollution in the environment. However, some strains such as enterotoxigenic *E. coli* (ETEC) and enterohaemorrhagic *E. coli* (EHEC) are naturally pathogens and are found to cause a number of ailments such as food poisoning, hemorrhagic bloody diarrhea and haemolytic uremic syndrome [1].

Several methods have been employed to track the potential source of *E. coli* in the soil environment and many have suggested that application of animal manure as fertilizers on farmlands, contaminated waste sources, including feces from humans and other mammals are considered as the major sources that add *E. coli* to the soil environment [2,3,4].

Studies have revealed that *E. coli,* including the EHEC strains which can cause severe haemorrhagic colitis and haemolytic uraemia in humans can persist for days to more than one year following its application to soil environment [4]. Similarly, it was also reported that *E. coli* can grow in river bank soils and move back into water by erosion [5]. Specific strains of *E. coli* have become naturalized or indigenous to the soil environment and these strains may contribute to the elevated *E. coli* counts through the inoculation of water by run-off of *E. coli* from soils and sands [6]. It was also suggested that the existence of *E. coli* and its ability to persist for long in the soil environment confounds its usage as reliable indicator of recent fecal contamination [7].

E. coli strains isolated from both clinical and soil samples have been reported to exhibit resistance to multiple drugs, and an increasing number of resistance pattern are reported each year [8]. It has also been observed that about 30 to 80% of the antibiotics given to livestock may be egested as part of feces or dropping owing to poor absorption. When manure that has a considerable quantity of antibiotics is applied to farmlands, these antibiotics could disturb the important biological works in the soil. This could lead to the development of antibiotic resistant bacteria that could affect human beings, animals, wildlife, fish and other aquatic animals [9].

The presence and survival of antibiotic resistant *E. coli* alongside other pathogenic bacteria in the soil environment is a global challenge as non-pathogenic strains continuously acquire resistance genes through horizontal gene transfer. This study was conducted to determine the distribution of *E. coli* in soil samples and their resistance pattern to conventional antibiotics. This is important as the outcome would help medical practitioners in making informed decisions regarding appropriate therapy against infection with multidrug *E. coli*.

2. MATERIALS AND METHODS

2.1 Sample Collection

Seventy five (75) soil samples were collected (25 each) from river banks, farmlands, and dumpsites around Girei Local Government of Adamawa State, Nigeria in 2016 Using standard method [10]. The soil was collected in depth of 15 to 20 cm using a clean spade and transferred into small sterile polyethene bag. The samples were then placed into an ice-packed cooler and were transported to the laboratory for further processing.

2.2 Sample Processing

Upon arrival to the laboratory, the samples were brought out of the cooler until they attained room temperature. Tenfold serial dilution was carried out on each soil sample using sterile normal saline and then inoculated unto EMB agar plates by spread plate method [11].

2.3 Isolation and Identification of *E. coli*

After incubation overnight at 37^{o}C on EMB, presumptive identification of *E. coli* was based on the selection of colonies characterized by the production of distinctive metallic green sheen, which were sub-cultured on fresh EMB plates. Pure isolates were obtained and subjected to Gram's stain and Biochemical tests, viz; Methyl red - Vorges-Proskauer (MR-VP), Indole, Hydrogen sulphite test, Motility, Gas production, Urease and Citrate utilization test.

2.4 Identification of EHEC O157:H7 on Sorbitol-MacConkey Agar (SMAC)

Each of the confirmed *E. coli* isolates was streaked unto a freshly prepared plate of

Sorbitol-MacConkey agar and incubated at 37^{o}C for 24 hours. EHEC being non-sorbitol fermenters among *E. coli* isolates appeared as colorless colonies and were sub-cultured.

2.5 Antimicrobial Susceptibility Test

Karby-Buer disk diffusion method was carried out on Mueller-Hinton agar for all *E. coli* isolates. List of tested antibiotics is given in Table 3.

2.6 Plasmid Curing

A total number of 15 *E. coli* isolates were identified out of which 6 most resistant isolates were cured of plasmids using 10% sodium dodecyl sulphate [12]. Overnight culture of each isolate was incubated in nutrient broth in separate vials at 37˚C for 24 hours. 10% (w/v) SDS was prepared by diluting 3g of SDS in 27ml nutrient broth such that 1/10 of the required volume is needed to give the final concentration.

Overnight culture of each isolate was diluted to a turbidity equivalent to 0.5 McFarland standard and subjected to 10 fold dilution using sterile nutrient broth as diluent until an approximate suspension of 10^4cells/ml was attained. From each of the microbial suspension, 0.5ml was added to a test tube of 4.5ml nutrient broth containing SDS to give 10% (w/v) concentration and a final microbial suspension of 10^3cells/ml.

The tubes were incubated at 37˚C for 48 hours. The turbidity of each cured broth culture was adjusted to 0.5 McFarland standard and spread using sterile cotton swab unto Mueller- Hinton agar plate and a nutrient agar plate (which served as control). Disks of the initially resisted antibiotics were aseptically placed on the Mueller-Hinton agar plates. For each of the cured isolates, the two plates were incubated at 37˚C for 24hours and observed for cured cells.

All results were tabulated and presented in percentages where necessary.

3. RESULTS

Growths were observed on all 75 EMB agar plates with 15 of them confirmed to contain *Escherichia coli* using the identification standards provided in Table 1.

The highest occurrence of *E. coli* was obtained from dumpsite soils with 9(60%), followed by farmlands with 4(26.7%) and river bank soils had the least number of isolates with 2(13.3%) Further identification on Sorbitol-MacConkey agar revealed that 2(13.3%) of the 15 isolates were EHEC both of which were from dumpsite soils (Table 2).

The isolates showed considerable resistance towards Amoxicillin 10(66.7%) and Augmentin 11(73.3%) and only 1(6.7%) showed resistance to pefloxacin (Table 3).

Six isolates were treated with 10% SDS to cure them of plasmid. Result revealed that initial resistance to Augmentin, Amoxicillin and Pefloxacin were plasmid borne as the isolates later became sensitive to the antibiotics (Table 4).

Table 1. Identification of *E. coli*

S/N	Test	Reaction for *E. coli* Identification
1	EMB Agar	Colonies with Metallic Green Sheen
2	Gram Staining	Gram-ve Rods
3	Methyl Red	+ve
4	Voges-Proskauer	-ve
5	Indole	+ve
6	H_2S	-ve
7	Motility	+ve
8	Gas	+ve
9	Urease	-ve
10	Citrate	-ve

Key; +ve, positive; -ve, negative

Table 2. Distribution of *E. coli* in various soil samples

S/N	Soil Source	No. of Samples Collected	No. of Samples Positive for *E. coli*	Percentage (%)
1	Dumpsites	25	7 and 2 EHEC O157:H7	60.0
2	Farmlands	25	4	26.7
3	River banks	25	2	13.3
	Total	75	15	100

Table 3. Antibiotic resistance pattern of *E. coli* Isolates

S/N	Antibiotics	Resistant		Intermediate		Susceptible	
		No.	(%)	No.	(%)	No.	(%)
1	SXT; Septrin (30ug)	0	0.0	0	0.0	15	100
2	CH; Chloramphenicol (30ug)	0	0.0	0	0.0	15	100
3	SP; Sparfloxacin (10ug)	0	0.0	0	0.0	15	100
4	CPX; Ciprofloxacin (10ug)	0	0.0	0	0.0	15	100
5	AM; Amoxicillin (30ug)	10	66.7	4	26.7	1	6.7
6	AU; Augmentin (30ug)	11	73.3	3	20.0	1	6.7
7	CN; Gentamycin (10ug)	0	0.0	0	0.0	15	100
8	PEF; Pefloxacin (30ug)	1	6.7	-	-	14	93.3
9	OFX; Tarivid (10ug)	0	0.0	0	0.0	15	100
10	S; Streptomycin (30ug)	0	0.0	0	0.0	15	100

Table 4. Antibiogram of cured *E. coli* isolates on resisted antibiotics before and after plasmid curing

S/N	Source / *E. coli* Isolate		Antibiotics / Zone of Inhibition (mm)		
			AM	AU	PEF
1	DS-1	Before	0 (R)	0 (R)	-
		After	19 (S)	21 (S)	
2	DS-8 (EHEC)	Before	0 (R)	0 (R)	8 (R)
		After	20 (S)	22 (S)	21 (S)
3	DS-9 (EHEC)	Before	0 (R)	6 (R)	-
		After	21(S)	23(S)	
4	FM-1	Before	7 (R)	9 (R)	-
		After	22 (S)	23 (S)	
5	FM-3	Before	0 (R)	0 (R)	-
		After	18 (S)	21 (S)	
6	RB-1	Before	4 (R)	14 (I)	-
		After	22 (S)	23 (S)	

Key: AM, Amoxicillin; AU, Augmentin; PEF, Pefloxacin; DS, Dumpsite; FM, Farmland; RB, Riverbank; S, Susceptible; I, Intermediate; R, Resistant

4. DISCUSSION

Result from this study showed that out of the different sampling sites, dumpsite contains the highest frequency of *Escherichia coli* with 9(60%) out of 15 isolates. This may be attributed to the fact that dumpsites are associated with different waste sources including feces from humans and domestic animals which can add to their high microbial numbers. A consistent finding also reported that there is abundant population of *E. coli* in soils from landfills particularly those located close to the people environment which is influenced by human activities [13]. Similarly, soil around landfills can be defiled bacteriologically through permeation of public residual channels such as septic tanks [14].

Four (26.7%) of the 15 isolates were from farmland soils. The survival of *E. coli* in agricultural soils has overtime been reported. Some reports suggested that the presence of *E.*

coli in farmland soils is attributed to the use of animal dung manure and contaminated irrigation water on the farmlands [2,15]. However, on monitoring the concentration of *E. coli* in agricultural soil in relation to manure application, some reports indicated that the frequency of *E. coli* detection was higher before manure was applied than after [16]. This made them arrive at a conclusion that the presence and abundance of *E. coli* was not strongly related to manure application. They therefore suggested that a consistent source of *E. coli* exists within the field which may include naturalized strains of *E. coli*.

Only 2(13.3%) of the isolates were from riverbank soils. This low number of *E. coli* in the riverbank soils may be due to its sandy nature and hence its inability to retain enough organic matter to serve as a factor that support the replication of *E. coli* and other soil microbiota in the soil environment. A laboratory experiment indicated that organic matter is one of the important factors that influence the survival of *E. coli* in soil environment [5]. Similarly, another study suggested that *E. coli* can survive two times longer in soil collected from farmland than in soils from riparian area [16].

Enterohaemorrhagic *Escherichia coli* were found to be associated with dumpsite soils only. This agrees with other report which confirmed the presence of EHEC O157:H7 and other pathogenic Gram-negative bacteria from dumpsite soils [17]. Ruminants are widely known to be the primary host of EHEC O157:H7 and several outbreaks have been associated with consumption of their products [18]. The communities in the study area do not have a proper drainage channels and as such, during rainy season, the contents of these dumpsites may contaminate run-off water that goes down the street lanes, which may serve as vehicle to disseminate parasites harboured by these dumpsites across the street. Children usually play around dumpsites and are at risk of acquiring infection with EHEC and other enteric pathogens which may result into outbreak within these communities. Dumping refuse close to households should therefore be avoided as they can harbour pathogens and serve as means of their transmission to immediate residents.

The presence of EHEC O157:H7 might have resulted from fecal matter from ruminants that usually go about dumpsites harbouring the EHEC O157:H7 in their intestinal tracts. Soil samples from farmlands and riverbank did not contain any EHEC O157:H7. A study on the survival pattern of *E. coli* O157:H7 and non-pathogenic ones in different soils revealed that the pathogen, EHEC O157:H7 was unable to survive for long period in the soil [19]. The inability of EHEC O157:H7 to survive for long periods in soil is possibly the major factor that attributed to their low number in the soil samples.

Bacterial resistance to conventional antibiotics has now become a threat to modern chemotherapy globally with increase resistance being reported each year. Antimicrobial susceptibility test revealed that 66.7% and 73.3% of the *E. coli* isolates were resistant to Amoxicillin and Augmentin respectively. This is in line with the work conducted by other researchers [20,21,22], where they all reported *E. coli* isolates to have high resistance to Amoxicillin and other antibiotics. All the *E. coli* were significantly sensitive to Septrin (100%), Chloramphenicol (100%), Sparfloxacin (100%), Ciprofloxacin (100%), Tarivid (100%) Streptomycin (100%) Pefloxacin (93.3%) and Gentamycin (100%). Other reports showed that *E. coli* are significantly sensitive to the above stated antibiotics [20,23,21]. The only *E. coli* isolate that showed resistance to pefloxacin was one of the EHEC O157:H7 obtained from dumpsite. Resistance of EHEC O157:H7 to pefloxacin was also reported in another study [24].

The susceptibility of the isolates to initially resisted antibiotics after plasmid curing is an indication that the resistance was plasmid borne. Plasmid mediated resistance to Augmentin, Amoxicillin and Pefloxacin in *E. coli* was also reported by other researchers [25,26]. The presence of resistant plasmids among bacteria is considered to be the major factor in rapid and uncontrolled increase in antibiotic resistance as they can be easily transmitted to other bacteria through horizontal and lateral gene transfer. There is therefore need for further studies on development of new antimicrobial strategies aimed at targeting and elimination of resistant plasmids and other transposable genetic elements.

5. CONCLUSION

Results confirmed the presence of *Escherichia coli*, including the Enterohaemorrhagic *E. coli* O157:H7 in different soil samples tested. The EHEC O157:H7 strain is not common in the soil environment in comparison with other *E. coli* strains. Findings also indicated that *Escherichia*

coli isolated from soil samples exhibited considerable resistance to Augmentin and Amoxicillin, and this resistance was confirmed to be plasmid borne as the isolates displayed sensitivity to the antibiotics they were previously resistant to after plasmid curing. Enterohaemorrhagic *E. coli* O157:H7 is found to associate mainly with dumpsite soils. Therefore, locating dumpsite close to households should be avoided to minimize the risk of *E. coli* O157:H7 transmission as well as other enteric pathogens. Proper care and hygiene should be taken by workers handling refuse to reduce the risk of infections. Soils from dumpsites should be treated before use as source of organic manure on farmlands. And lastly, indiscriminate use of antibiotics should be avoided so as to reduce the risk of developing resistance by microorganisms.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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