Incidence Of *Escherichia Coli* Associated With Effluent From Wupa Sewage Treatment Plant On The Surrounding Water Body.

Adayi, Florence Iyaji

Department of Microbiology, Uniersity of Abuja, P.M.B. 117, Abuja, Nigeria.
Email: florenceadayi@yahoo.com

ABSTRACT: Incidence of *Escherichia coli* associated with effluent from wupa sewage treatment plant on the surrounding water body was conducted. Ten (15) water samples were collected from Wupa river, with five (5) each from the upstream, downstream and point of effluent discharge into the river and screened for the incidence of *Escherichia coli*. One milliliter of sample was dissolved in 10 ml sterilized distilled water. Ten-fold dilutions of the samples were made up to 10^{-6} and some 0.1 ml was inoculated on Nutrient agar (10^{-6}) and MacConkey agar (10^{-3}) as well as Eosin Methylene Blue agar (10^{-3}) using the spread plate method. The inoculated plates were incubated at 37 ^{0}C for 24 hours and were subsequently sub cultured to obtain pure isolates. The resulting total aerobic bacterial loads in upstream station of Wupa River before discharge point showed that, the resulting colonies range from $1.06\times10^9\pm0.20$ Cfu/ml to $1.23\times10^9\pm0.21$ Cfu/ml while the coliform ranges from $2.65\times10^8\pm0.21$ Cfu/ml to $2.9\times10^8\pm0.28$ Cfu/ml. Similarly, the total aerobic bacterial loads in downstream of Wupa river after effluent discharge point showed that, the resulting colonies range from $1.40\times10^9\pm0.30$ Cfu/ml to $1.80\times10^9\pm0.21$ Cfu/ml while the coliform ranges from $2.60\times10^8\pm0.22$ Cfu/ml to $2.80\times10^8\pm0.28$ Cfu/ml. However, the total aerobic bacterial loads at the point of effluent discharge to the River showed that, the resulting colonies range from $8.20\times10^8\pm0.28$ Cfu/ml to $9.40\times10^8\pm0.22$ Cfu/ml while the coliform ranges from $2.10\times10^7\pm0.11$ Cfu/ml to $2.40\times10^7\pm0.14$ Cfu/ml. A total of ten (10) *Escherichia coli* were isolated from this study and, maximum of six (6) were isolated from the upstream, and three (3) from the downstream of the Wupa river after effluent discharge point while point of effluent discharge to the river had only one (1) incidence of *E. coli*. This indicator organism shown there feacal contamination in the River. Hence, the need for need for proper management and purification of the effluent before discharging it to the stream. [Adayi, Florence Iyaji. **Incidence Of *Escherichia Coli* Associated With Effluent From Wupa Sewage Treatment Plant On The Surrounding Water Body.** *Biomedicine and Nursing* 2025;11(4):11-16]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 02. doi:10.7537/marsbnj110425.02

1.0 Introduction

E. coli is commonly regarded as one of first microorganisms of choice in water quality monitoring programs and serves as the primary indicator for water contaminated with faecal matter due to their prevalence in the gut of warm-blooded animals as well as high numbers excreted in both human and animal faeces (Leclerc *et al*., 2011). Six major pathogenic classes that have been identified namely, enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic or shiga-toxin poducing *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enterpathogenic E. coli (EPEC), enteroadherent-aggregative *E. coli* (EA-AggEC) and diffuse adherent *E. coli* (DAEC) (Nataro and Kaper, 2010). EPEC have been primarily associated with outbreaks of infantile gastroenteritis whilst EIEC are known to produce dysentery by a mechanism similar to Shigella sp. causing severe bloody diarrhoea whilst ETEC are known to possess a heat-labile enterotoxin similar to the cholera toxin (UNICEF/WHO, 2012). The most important is EHEC which includes the -O111 and -O157 serogroups all of which produce a shiga-like toxin resulting in mild diarrhoea to haemorrhagic colitis (Rice *et al*., 2016). EHEC has been implicated in a range of foodborne-related outbreaks since 1983 with one of the largest European outbreaks occurring in 2011, caused by the Shiga toxin-producing *E. coli* (STEC) O104:H4. Numerous studies revealed a unique combination of virulence factors from two distinct *E. coli* classes, namely, enteroaggregative *E coli* (EAEC) and STEC which contributed to its pathogenic nature (Wu *et al*., 2011; Soon *et al*., 2013). Despite being a foodborne pathogen, numerous studies have implicated contamination from infected individuals (Rice *et al.*, 2016). Therefore, this study was undertaken to assess the incidence of *Escherichia coli* associated with effluent from wupa sewage treatment plant on the surrounding water body

2.0   Materials and Method

2.1   Study Area

This study was carried out at Wupa Abuja sewage treatment plant and the Microbiology laboratory of

University of Abuja, Gwagwalada Federal Capital Territory, Abuja.

2.2　　Sample Collection

A total of 15 effluent samples were collected from Wupa Abuja sewage treatment plant with five (5) random samples each from three (3) different points. The samples were collected from the point of discharge into Wupa River, upstream of Wupa River (20 meter from the point of discharge) and downstream of Wupa River (50 meters from the upstream). The samples were collected aseptically, using sterile universal bottles and transported in an ice-cold container to the Microbiology Laboratory of the University of Abuja for the assessment. The samples were analyzed on the day of collection as described by Kulikov *et al*. (2015) with some modifications.

2.3　　Preparation and Sterilization of Media

The sterilization of glass ware such as conical flasks, beaker and test tubes after washing with detergent were carried out in hot air oven at 160 ℃ for 2 hours. The media used in this study include: Nutrient agar (Oxoid), MacConkey agar (Oxoid), Salmonella-Shigella agar (Himedia) and Eosin Methylene Blue (EMB) agar (Himedia). The media were prepared according to their manufacturers' instructions.

2.4　　Assessment of Enteropathogens in Effluent from WSTP on the Surrounding Water Body

The isolation of enteropathogens associated with effluent from wupa sewage treatment plant samples was determined using the spread plate technique according to Tassadaq *et al*. (2013). One milliliter (1 ml) of the sewage effluent and Wupa river samples were aseptically transferred into separate 10 ml of sterile distilled water as the stock culture. Ten fold serial dilutions of the stock sample were made using sterile water as diluents. Then 1.0 ml of the dilution sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterile distilled water. The content was mixed thoroughly. Other ten-fold dilutions were similarly made up to 10^{-6}, and some 0.1 ml were inoculated on the Nutrient agar (10^{-6}) and Mac Conkey Agar (10^{-3}) respectively using the spread plate method according to Cheesbrough (2006). The plates were allowed to stand undisturbed for about 15 minutes and then incubated at 37 ^{0}C for 24 hours. The numbers of colony forming units were counted using a colony counter and the colonial density was calculated as the colony forming unit (CFU) multiplied by the dilution factor. The mean total count obtained were recorded and expressed in colony forming units per milliliter (Cfu/ml) of the sample.

2.5 Preparation of Pure Cultures of Isolated Bacteria

Representatives of each colony type (that is discrete colonies) on Mac Conkey Agar were aseptically transferred to freshly prepared sterile Salmonella-Shigella Agar and Eosine Methylene Blue Agar respectively to obtain pure cultures. The pure cultures weree maintained on nutrient agar slants and stored at 4 ℃ for biochemical test (Cheesbrough, 2006). Purification was done by repeated subculturing.

2.6 Identification of Bacteria Isolates

Identifications were done on the basis of microscopy, gram-staining, biochemical tests, and morphological characteristics through macroscopic features (Cheesbrough, 2006; Ravea *et al*., 2019). The biochemical characteristics used were catalase test, oxidase test, urease test as well as IMViC test (citrate utilization test, indole test, methyl red and voges-proskauer test).

2.8 Determination of frequencies of occurrence

The frequency of occurrence of isolated bacteria associated with the Wupa Abuja sewage treatment effluent were determined using descriptive statistics. The sum of all the numbers of Cfu/ml of the organisms in each sample and the percentage were calculated thus:

$$\frac{\text{Number of each Isolates}}{\text{Total number of Isolates}} \times 100$$

2.9　　Statistical Analysis

Data obtained in this study were analyzed using Analysis of Variance (ANOVA) from Ms Excel Statistics and the test applied were F-test statistic at $p < 0.05$.

3.0　　Results

3.1　　Microbial Density of Effluent from WSTP on the Surrounding Water Body

Table 3.1 showed the total aerobic bacteria loads and the coliforms of effluent from Wupa sewage treatment plant on the surrounding water body. The total aerobic bacterial loads in upstream station of Wupa River before discharge point showed that, the resulting colonies range from $1.06 \times 10^9 \pm 0.20$ Cfu/ml to $1.23 \times 10^9 \pm 0.21$ Cfu/ml while the coliform ranges from $2.65 \times 10^8 \pm 0.21$ Cfu/ml to $2.9 \times 10^8 \pm 0.28$ Cfu/ml as seen in Table 4.2. Similarly, the total aerobic bacterial loads in downstream of Wupa river after effluent discharge point showed that, the resulting colonies range from $1.40 \times 10^9 \pm 0.30$ Cfu/ml to $1.80 \times 10^9 \pm 0.21$ Cfu/ml while the coliform ranges from $2.60 \times 10^8 \pm 0.22$ Cfu/ml to $2.80 \times 10^8 \pm 0.28$ Cfu/ml. However, the total aerobic bacterial loads at the point of effluent discharge to the River showed that, the resulting colonies range from $8.20 \times 10^8 \pm 0.28$ Cfu/ml to $9.40 \times 10^8 \pm 0.22$ Cfu/ml while the coliform ranges from $2.10 \times 10^7 \pm 0.11$ Cfu/ml to $2.40 \times 10^7 \pm 0.14$ Cfu/ml as shown in Table 3.1

Table 3.1: Total Aerobic Bacteria Loads and Coliforms of Effluent from WSTP on the Surrounding Water Body

Sample locations	Microbial Density (CFu/mL)	
	Total aerobic bioloads	Coliform loads
UPS		
1	$1.06 \times 10^9 \pm 0.20^a$	$2.65 \times 10^8 \pm 0.21^a$
2	$1.15 \times 10^9 \pm 0.14^a$	$2.8 \times 10^8 \pm 0.14^a$
3	$1.23 \times 10^9 \pm 0.21^b$	$2.9 \times 10^8 \pm 0.28^a$
4	$1.10 \times 10^9 \pm 0.20^b$	$2.7 \pm \times 10^8 0.14^b$
5	$1.11 \times 10^9 \pm 0.14^a$	$2.85 \times 10^8 \pm 0.07^b$
DSS		
1	$1.02 \times 10^9 \pm 0.28^b$	$2.70 \times 10^8 \pm 0.04^a$
2	$1.40 \times 10^9 \pm 0.30^b$	$2.80 \times 10^8 \pm 0.28^a$
3	$1.10 \times 10^9 \pm 0.14^a$	$2.60 \times 10^8 \pm 0.22^b$
4	$1.06 \times 10^9 \pm 0.22^b$	$2.75 \times 10^8 \pm 0.10^a$
5	$1.80 \times 10^9 \pm 0.21^a$	$2.70 \times 10^8 \pm 0.22^b$
PED		
1	$8.30 \times 10^8 \pm 0.14^a$	$2.10 \times 10^7 \pm 0.11^a$
2	$8.60 \times 10^8 \pm 0.28^a$	$2.20 \times 10^7 \pm 0.16^b$
3	$9.40 \times 10^8 \pm 0.22^a$	$2.40 \times 10^7 \pm 0.14^b$
4	$9.10 \times 10^8 \pm 0.14^b$	$2.30 \times 10^7 \pm 0.00^a$
5	$8.20 \times 10^8 \pm 0.28^b$	$2.20 \times 10^7 \pm 0.21^a$

Values are means ± standard deviation of triplicate values.

Keys: UPS= Upstream station of Wupa River before discharge point,

DSS=Downstream of Wupa river after effluent discharge point,

PED= Point of Effluent discharge to the River

[a] = superscript

[b]= superscript. Mean with the same superscript are not significantly different ($P > 0.05$).

3.2 Identification of Isolated *E. coli*

Isolates obtained were identified on the basis of microscopy, biochemical tests, and morphological characteristics through macroscopic features. Among the characteristics used are: colonial characteristics such as size, surface appearance, texture and colour of the colonies. Table 3.2 showed the molecular characterization results of the bacteria isolates with their accession numbers respectively.

3.3   Identification of Isolated *Escherichia coli*

Table 4.3 showed the morphological characteristics and biochemical features of the isolated *Escherichia coli* from Wupa sewage treatment plant effluent on the surrounding water body. Isolates obtained were identified on the basis of microscopy, biochemical tests, and morphological characteristics through macroscopic features. Among the characteristics used are: colonial characteristics such as size, surface appearance, texture and colour of the colonies.

Table 4.3: Biochemical Characteristics of Isolated *Escherichia coli* from Wupa Sewage Treatment Plant Effluent and the Surrounding Water Body

| Isolates	Shape	Surface	GR	IN	CI	OX	CA	UR	MR	VP	Probable Organisms
UPS											
G1 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
G2 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
G3 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
G4 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
G5 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
G6 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
DSS											
G7 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
G8 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
G9 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	
PED											
G10 *Escherichia coli*	Rod	Smooth	-	+	-	-	+	-	+	-	

Key: GR=Gram reaction, IN= Indole, CI= Citrate, OX= Oxidase, CA= Catalase test, MR=Methyl red, VP=Voges-Proskauer, G1-G10= Isolate 1-10.

Keys: UPS= Upstream station of Wupa River before discharge point, DSS=Downstream of Wupa river after effluent discharge point, PED= Point of Effluent discharge to the River

3.4 Frequency of Occurrence of *Escherichia coli*
Figure 1 shows that a total of ten (10) *Escherichia coli* were isolated from this study and, maximum of six (6) were isolated from the upstream, and three (3) from the downstream of the Wupa river after effluent discharge point while point of effluent discharge to the river had only one (1) incidence of *E. coli*.
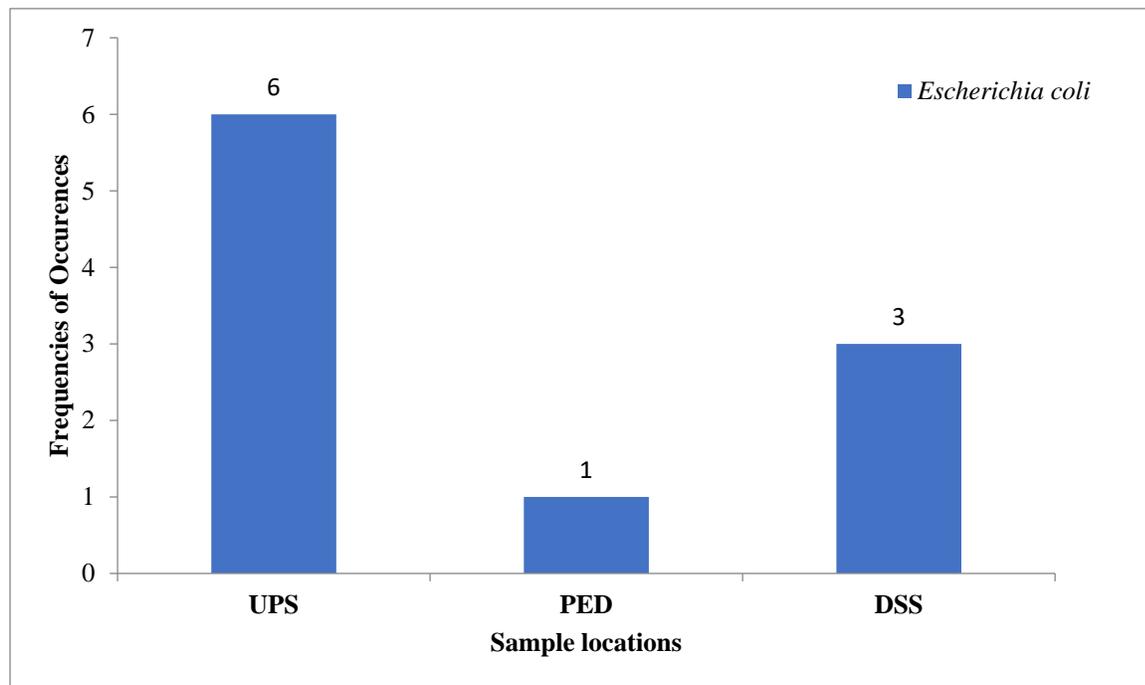
Figure 1: Frequency of Occurrence of *Escherichia coli* Associated With Effluent from Wupa Sewage Treatment Plant On The Surrounding Water Body
Keys: UPS= Upstream station of Wupa River before discharge point,
DSS=Downstream of Wupa river after effluent discharge point
PED= Point of Effluent discharge to the River.

4.0   Discussion

In this study, ten (10) *Escherichia coli* were isolated from this study and, maximum of six (6) were isolated from the upstream, and three (3) from the downstream of the Wupa river after effluent discharge point while point of effluent discharge to the river had only one (1) incidence of *E. coli*. This is also in agreement with Garba *et al*. (2009) who reported similar result of prevalence of *Escherichia coli* in some public water. The presence of coliforms is an index of bacteriological quality of water, the isolation of coliforms especially *Escherichia coli*, from the Wupa River is attributable to contamination by material of human and animal origin and this is of health significance as these organism have generally been reported as causative agent of gastroenteritis in humans. This is in conformity with Ahmed *et al*. (2005) report that Coliform bacteria such as *E. coli* have been widely used as indicator of the microbiological quality of surface and ground waters. Also from this study, the study analyzed total aerobic bacterial loads in upstream, downstream, and effluent discharge points of the Wupa River, revealing varying colony sizes and coliform levels. The coliform levels varied from 2.65×10^8 to 2.9×10^9 Cfu/ml. From the coliform count results, none of the water simples met the WHO standard for drinking water which states that the coliform count in drinking water both piped and unpiped should be zero/100ml. All the water sampled had very high counts and this indicates that they are unfit for human consumption. Equally the high coliform count observed is indicative of the likely presence of other pathogenic organism in the water sample analysed. This indicator organism shown there feacal contamination in the River. Hence, the need for need for proper management and purification of the effluent before discharging it to the stream.

4.1   Conclusion

This study revealed that there high coliform load in the Wupa surrounding water and presence of *Escherichia coli* which show that there are fecal contamination on the surrounding water body.

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