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Assessment Of Bacterial Enteropathogens Of Effluent From Wupa Sewage Treatment Plant On the Surrounding Water Body

[1]Adayi, Florence Iyaji and [2]Ijigbade Bamidele

Department of Microbiology, University of Abuja, P.M.B. 117, Abuja, Nigeria
Email: florenceadayi@yahoo.com[1] deleijigbade@yahoo.com[2]

Abstract: Fifteen (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 presence of enteropathogens. Results of the total aerobic bacterial loads upstream ranged 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. However, the total aerobic bacterial loads at the point of effluent discharge to the River 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 thirty-nine (39) enteropathogens belonging to six bacterial genera and six species were isolated from this study and they are *Escherichia coli*, *Salmonella enterica*, *Salmonella typhimurium, Proteus mirabilis*, *Klebsiella pneumonia*, *Enterobacter cloacae* and *Oblitimonas alkaliphila*. Ten bacterial isolates belonging to five strains were isolated from the upstream station of Wupa River which was nine (9) enteropathogens belonging to five (5) strains were isolated from the point of effluent discharge to the river while, the downstream of wupa river after effluent discharge point recorded the highest number of enteropathogens of twenty (20) with eight (8) strains of bacteria isolates. *Escherichia coli* was the most frequently isolated bacteria which represented 25.64%, followed by *Klebsiella pneumoniae* which represented 15.38% of the total isolates. *Salmonella enterica* serovars Eko EQAS2016S1 was 12.81% while *Proteus mirabilis* RCFS3, *Salmonella* Typhimurium FDAARGOS_319, *Oblitimonas alkaliphila* E1148 and *Enterobacter cloacae* EMP 13-3 recorded 10.26% each, whereas *Proteus mirabilis* ALK044 recorded 5.13 % being the least number of isolated bacteria. Although the bacteria isolated from the downstream was significantly high ($P\geq 0.05$), yet there was no significantly difference ($P\leq 0.05$) between the bacteria load isolated from the upstream and downstream as well as that of the effluent discharged into the river. However, there is an urgent need for for proper treatment, management, monitoring and sanitation of the effluent to avoid the transfer of enteropathogenic bacteria into the receiving water body.
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1.0 INTRODUCTION

Water is a crucial part of every living organism that is essential for maintenance of all forms of life. Freshwater availability is one of the major problems facing the world, and approximately, one third of drinking water requirement of the world is obtained from surface sources like rivers, dams, lakes, and canals (APHA, 2017). Water from surface sources especially in rural areas and developing countries are used for household needs such as drinking, cooking, washing, bathing, waste removal and also serve as best sinks for the discharge of domestic and industrial effluent (Momba *et al*., 2010; Kulikov *et al*., 2015). Surface water has been exploited for several purposes by humans. It has been used for irrigation purposes by farmers, and fishermen get their occupation from harvesting fish in so many freshwater sources. It is used for swimming and also serves as centers for tourist attraction. Surface water, therefore, should be protected from pollution and possible infections (APHA, 2017).

Major point sources of freshwater pollution are raw effluent from domestic and industrial origin and partially treated effluent from wastewater treatment plants. The release of domestic and industrial effluent into water bodies has led to the increase in freshwater pollution and depletion of clean water resources (Edokpayi *et al*., 2015). Most quantities of wastewater generated in developing countries undergo little or no treatment. Igbinosa and Okoh (2009) in a study, reported that in few urban centers, various forms of wastewater treatment facilities (WWTFs) exist but most of them are producing ill treated effluents, which are disposed off onto freshwater courses such as rivers and lakes. The release of poorly treated effluent into rivers has both short and long term effect on the environment and public health. Freshwater sources have been negatively impacted by effluent. Such

impacts are dependent on the composition and concentration of the effluent contaminants as well as the volume and frequency of wastewater effluents entering surface water source (Igbinosa and Okoh 2009; Akpor and Muchie 2011). Typhoid fever remains endemic to many parts of Africa, including Nigeria with outbreak occurring in mostly in West African countries. This study therefore aimed to assess the bacterial enteropathogens of effluent from Wupa Sewage Treatment Plant (WSTP) 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 ºC 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 Cheesebrough (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 ºC for biochemical test (Cheesebrough, 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 (Cheesebrough, 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).

2.7 Molecular Characterization of Bacterial Isolates

Genomic DNA extraction from enteropathogenic bacteria isolated from the upstream and downstream of Wupa River as well as the treated effluent discharge to the River was carried out using the DNeasy Blood and Tissue Extraction Kit (Qiagen, USA) following the protocol provided by the manufacturer (Theves *et al*., 2011).

2.7.1 Extraction of DNA

Overnight cultures grown in tryptone-soy broth (TSB) were centrifuged for 10 min at 5000 x g, to harvest cells. The pellet was washed 3 times in TE buffer. Two (2) mg/ml lysozyme, 25 Mm Tris HCl pH 8, 10 Mm EDTA, 25 % sucrose) and incubated at 37 °C for 30 min in an incubator (Uniscope SM9052, Surgifriend Medicals, England). Proteinase K and extraction buffer were added, mixed by vortexing and incubated at 56 °C in a water-bath (Uniscope SM101 Shaking Water bath, Surgifriend Medicals, England) for 30 min. The DNA was precipitated with ethanol (96 – 100 %, v/v) and transferred into the DNeasy Mini spin column for binding of DNA to the column, washed with two different 500 µl washing buffers and eluted with 200

µl elution buffer. The resulting DNA was stored at -20 °C.

2.7.2 Amplification of the 16S rRNA Genes

The 16S rRNA gene from genomic DNA was amplified by Polymerase Chain Reaction (PCR) using bacteria universal primers (27F–AGAGTTTGATCCTGGCTCAG and 1492R–GGTTACCTTGTTACGACTT). The PCR amplification was carried out in a Techne TC-412 Thermal Cycler (Model FTC41H2D, Bibby Scientific Ltd, UK) in a 50 µl reactions containing 25 µl of 2 X PCR Master Mix (Norgen Biotek, Canada), 1.5 µl of template DNA (0.5 µg), 1 µl of both forward and reverse primers (2.5 µM of each) and 21.5 µl of nuclease in a PCR tube added in that order. PCR was carried out at an initial denaturation step at 94°C for 2 min, followed by 30 cycles at 94 °C for 30 sec, 52 °C for 30 sec and 72 °C for 2 min, and a final extension step at 72 °C for 5 min. PCR products (amplicons) were separated by electrophoresis on a 1 % agarose TAE gel containing ethidium bromide and visualized by UV transillumination (Foto/UV 15, Model 3-3017, Fotodyne, USA).

2.7.3 DNA Sequencing and Analysis

PCR products from the genomic DNAs were sequenced with 518F and 800R primers using ABI PRISM Big Dye Terminator cycle sequencer (Macrogen, USA). The gene sequences obtained were compared by aligning the result with the sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) search program at the National Centre for Biotech Information (NCBI).

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 $1.06\times10^9\pm0.20$ 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 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 $1.40\times10^9\pm0.30$ 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 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	$1.06\ x10^9\pm0.20^a$	$2.65x10^8\pm0.21^a$
2	$1.15\ x10^9\pm0.14^a$	$2.8\ x10^8\pm0.14^a$
3	$1.23\ x10^9\pm0.21^b$	$2.9\ x10^8\pm0.28^a$
4	$1.10\ x10^9\pm0.20^b$	$2.7\pm\ x10^80.14^b$
5	$1.11\ x10^9\pm0.14^a$	$2.85x10^8\pm0.07^b$
DSS		
1	$1.02\ x10^9\pm0.28^b$	$2.70x10^8\pm0.04^a$
2	$1.40\ x10^9\pm0.30^b$	$2.80x10^8\pm0.28^a$
3	$1.10\ x10^9\pm0.14^a$	$2.60x10^8\pm0.22^b$
4	$1.06x10^9\pm0.22^b$	$2.75\ x10^8\pm0.10^a$
5	$1.80\ x10^9\pm0.21^a$	$2.70\ x10^8\pm0.22^b$
PED		
1	$8.30\ x10^8\pm0.14^a$	$2.10\ x10^7\pm0.11^a$
2	$8.60\ x10^8\pm0.28^a$	$2.20x10^7\pm0.16^b$
3	$9.40\ x10^8\pm0.22^a$	$2.40x10^7\pm0.14^b$

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 Enteropathogens

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.

Table 3.2: Molecular Characterization of Isolated Enteropathogens from WSTP Effluent and the Surrounding Water Body

Isolates	BLAST identification	Strain	DNA sequence (%) identity	Accession Number
A	*Klebsiella pneumoniae*	KP-1/yak-2014	(99)	KP866814.1
B	*Proteus mirabilis*	RCFS3	(94)	MN124173.1
C	*Oblitimonas alkaliphila*	E1148	(94)	CP012364.1
D	*Proteus mirabilis*	ALK044	(99)	KC456539.1
E	*Salmonella enterica* serovars Eko	EQAS2016S1	(99)	CP017232.1
F	*Salmonella* Typhimurium	FDAARGOS_319	(99)	CP027412.1
G	*Escherichia coli*	0157:H7	(99.1)	CP015845
H	*Enterobacter cloacae*	EMP 13-3	(97.2)	JQ308592.1

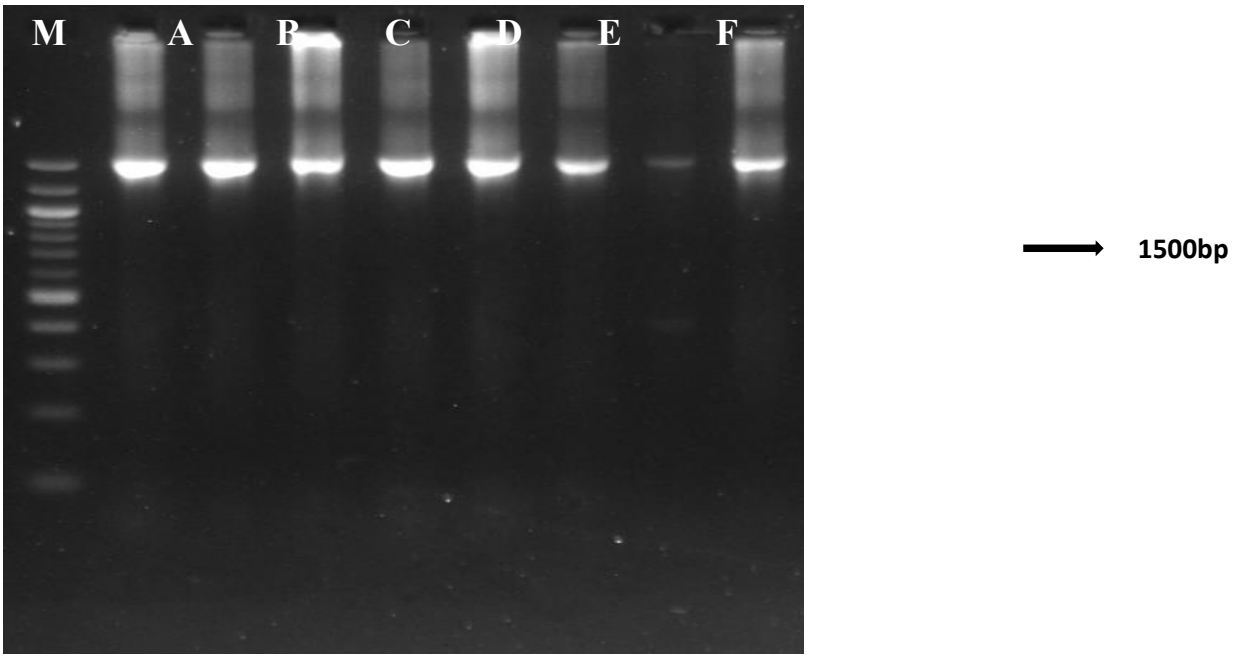

Plate 3.1: Agarose gel electrophoresis showing PCR result with the bond point.
From left to right, Legend: M = 1kb – Ladder, A = isolate A (*Klebsiella pneumoniae*), B = isolate B (*Proteus mirabilis*), C = isolate C (*Oblitimonas alkaliphila*), D = isolate D (*Proteus mirabilis*), E = isolate E (*Salmonella enterica* serovars Eko), F = isolates F (*Salmonella* Typhimurium), G = isolate G (*Escherichia coli*), H = isolate H (*Enterobacter cloacae*).

3.3 Enteropathogens Associated with Effluent and Surrounding Water Body

Table 1 shows that a total of thirty-nine (39) enteropathogens belonging to six bacteria genera and six species were isolated from this study and they include *Escherichia coli, Salmonella enterica, Salmonella typhimurium, Proteus mirabilis, Klebsiella pneumonia, Enterobacter cloacae* and *Oblitimonas alkaliphila*. *Escherichia coli* was the most frequently isolated bacteria which represented 25.64%, followed by *Klebsiella pneumoniae* which represented 15.38% of the total isolates. *Salmonella enterica* serovars Eko EQAS2016S1 was 12.81% while *Proteus mirabilis* RCFS3, *Salmonella* Typhimurium FDAARGOS_319, *Oblitimonas alkaliphila* E1148 and *Enterobacter cloacae* EMP 13-3 recorded 10.26% each, whereas *Proteus mirabilis* ALK044 recorded 5.13 % being the least number of isolated bacteria as seen in Figure 1. Although the bacteria isolated from the downstream was significantly high ($P \geq 0.05$), but there was no significantly different ($P \leq 0.05$) between the bacteria isolated from the upstream and downstream as well as that of the effluent discharged into the river.

Table 1: Frequencies of Occurrences of Enteropathogens of Wupa Sewage Treatment Effluent and Surrounding Water Body

Sample locations	Isolates	Strain	Frequencies
UPS	*Klebsiella pneumoniae*	KP-1/yak-2014	2
	Proteus mirabilis	RCFS3	1
	Salmonella enterica serovars Eko	EQAS2016S1	2
	Escherichia coli	0157:H7	3
	Enterobacter cloacae	EMP 13-3	2
PED	*Klebsiella pneumoniae*	KP-1/yak-2014	2
	Proteus mirabilis	ALK044	2
	Salmonella Typhimurium	FDAARGOS_319	2
	Oblitimonas alkaliphila	E1148	1
	Escherichia coli	0157:H7	2
DSS	*Klebsiella pneumoniae*	KP-1/yak-2014	2
	Proteus mirabilis	ALK044	3
	Salmonella enterica serovars Eko	EQAS2016S1	3
	Escherichia coli	0157:H7	5
	Proteus mirabilis	RCFS3	2
	Oblitimonas alkaliphila	E1148	1
	Salmonella Typhimurium	FDAARGOS_319	2
	Enterobacter cloacae	EMP 13-3	2
Total			**39**

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.

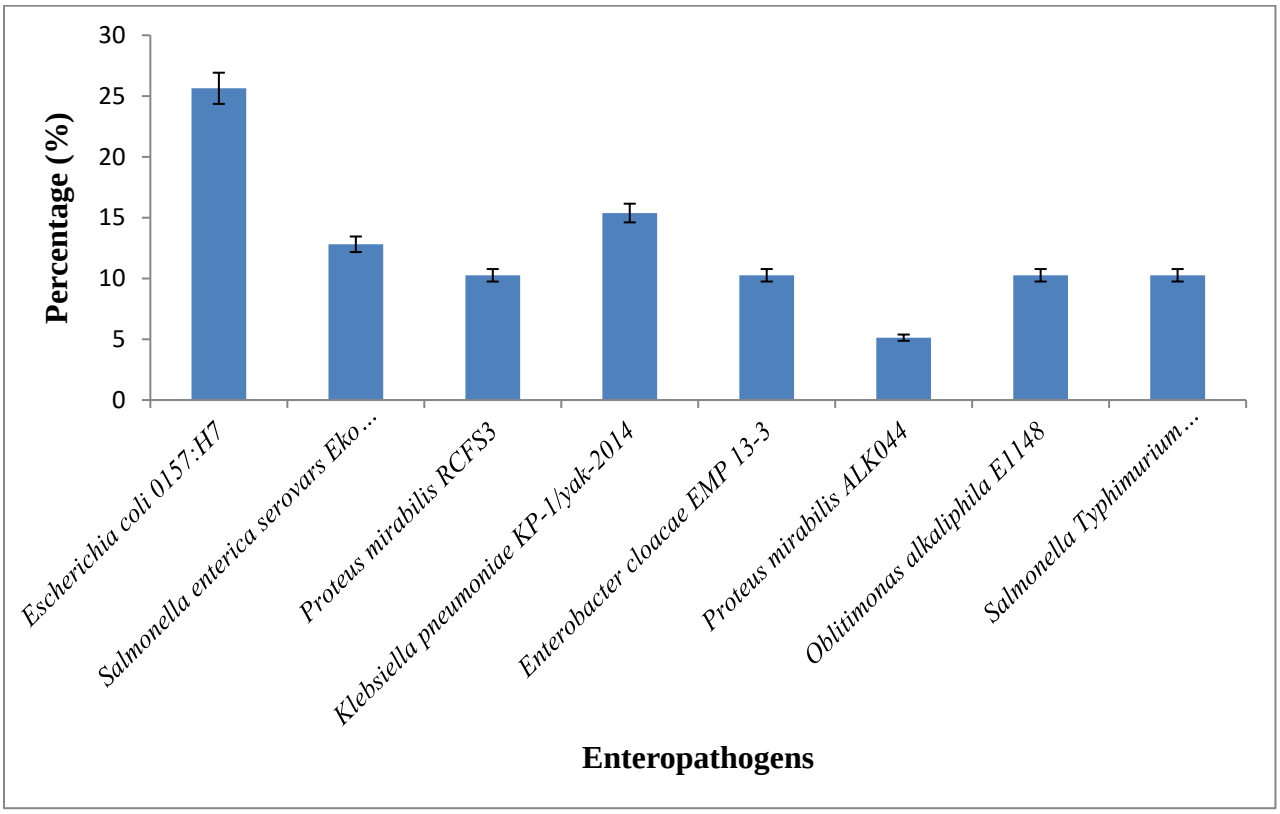

Figure 1: Percentage of Occurrences of Isolated Bacteria Enteropathogens From Wupa Sewage Treatment Plant Effluent and Surrounding Water Body

Discussion

The bacteria isolates from this study belong to the genera of potential pathogenic bacteria, and the microorganisms isolated were *Escherichia coli*, *Salmonella enterica*, *Salmonella* Typhimurium, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterobacter cloacae* and *Oblitimonas alkaliphila*. The isolation of these organisms is of great health concern because this domestic wastewater effluent was collected at the point of discharge into a nearby river, which may not only serve as a source of drinking water to the immediate community but also as a source of food (that is, through fishing) and its used for other domestic purposes.. According to Ugoh *et al*. (2013), *Escherichia coli* and *Salmonella* spp are associated with water borne diseases and reports from available health outposts in the areas in which this study was carried out revealed typhoid fever, dysentery, cholera and hepatitis to be the most prevalent (Ashbolt, 2014). Also, Cabral (2010) reported the existence of *Escherichia coli*, *Salmonella enterica*, *Salmonella* Typhimurium, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterobacter cloacae* environmental bacteria, enteric bacteria, and bacterial species being transmitted through wastewater. Among the bacterial pathogens isolated from the effluent in this study was *Oblitimonas alkaliphila*. *Oblitimonas alkaliphila* which belong to the family of *Pseudomonadaceae* has the potential to grow on the surface of any plastic pipe within the plant, thereby leading to infections in people with low immunity.

The isolation of *Escherichia coli* O157:H7 from both upstream and downstream points as well as the effluent discharged point to the water with a rate of 25.64% indicates that the drinking water is most probably contaminated with human and animal feces. This finding, in itself, is not surprising since it is well documented that cattle is the chief reservoir of *E. coli* O157. The stretch of Wupa River studied may be strongly influenced by cattle excrement due to *in situ* herd watering around the River bank. Moreover, the use of enrichment media and high temperature incubation had earlier been shown by Leclerc *et al*. (2011) to increase the sensitivity of *E. coli* O157 isolation from water.

The pathogenic bacteria which were noticed in upstream, discharged effluent and especially the downstream samples are cause for alarm especially in communities which take their water directly from the river. Based on the observed findings, it is evident that

pathogens can be dispersed via sewage treated effluent which may form the basis for environmental pathogen contamination and disease transmission and, this poses a major threat to public health and water confidence levels. The isolation of enteropathogens which include *Escherichia coli*, *Salmonella typhimurium*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Oblitimonas alkaliphila* from the effluent discharged point to the river as well as the downstream site of the wupa River in this study is an indication that although, sewage treatment reduced the pathogens, but does not guarantee the complete elimination of pathogenic bacteria.

Conclusion

There must be continuous monitoring of the efficiency of the wastewater treatment plant so as to enhance biological treatment of wastewater and ensure sustained adherence to permissible standards.

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