Bacteriological Assessment of Pharmaceutical Wastewater and Its Public Health Implications in Nigeria

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A total of 108 wastewater samples were collected for a period of three (3) months and analyzed for bacteriological properties. Wastewaters were collected from the point of discharge (PA), point of contact with the external environment (PB) and downstream of Chanchaga river (PC). The results of this study revealed that the Chanchaga river and its environment were polluted by wastewater discharge from the factory. The downstream of Chanchaga river (PC) had higher bacterial counts than the other sampling sites. The bacteria isolated were *E. coli*, *Salmonella* sp., *Klebsiella* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Clostridium* sp. and *Streptococcus faecalis*. The mean total viable counts ranged from 4.8×10^4 cfu/mL to 3.0×10^8 cfu/mL, 2.0×10^7 cfu/mL to 4.0×10^8 cfu/mL for total coliform counts, 1.3×10^3 cfu/mL to 3.0×10^8 cfu/mL for *Salmonella/Shigella* counts, 340 MPN/100 mL to ≥ 1600 MPN/100 mL for fecal coliform (*E. coli*) and no *Clostridium* were detected in PA, while PB and PC had *Clostridium* counts of 2.0×10^3 cfu/mL and 1.0×10^3 cfu/mL, respectively, only in June. Analysis of variance (ANOVA) of the data showed that there were significant differences between the counts at 5% level of significance ($P < 0.05$), while there was no significant difference between the mean total viable counts, total coliform counts and *Salmonella/Shigella* counts for PA and PB. The PC fecal coliform (*E. coli*) counts were higher than the acceptable maximum limits (0 cfu/mL) prescribed by WHO for potable water. The results of this study revealed that discharged untreated pharmaceutical wastewater into the environment and Chanchaga river pollutes the river with pathogenic bacteria. This poses a health risk and could be hazardous to human health, especially to the communities that use water from the river for domestic purposes. Therefore, there is a need for wastewater treatment facility to be installed in the pharmaceutical factory to reduce the risk of health hazard to the users of Chanchaga river and for constant monitoring of the industrial wastewater discharged into the environment.

Keywords: Pharmaceutical, Wastewater, Bacteria, Environment, River

Introduction

Wastewater is used water draining out of homes and industries that contains a wide variety of chemicals, debris and microorganisms. It contains large amounts of solid waste, dissolved

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organic matter and toxic chemicals that pose a health risk. It is composed of all the materials that flow from household plumbing systems, including washing water, toilet waste, bathing water, domestic wastewater, ground, surface and atmospheric waters that enter the sewage system (Prescott *et al.*, 2005; Talaro, 2005; and Prescott *et al.*, 2008). Pharmaceutical wastewaters are liquid waste generated by the pharmaceutical industries during the process of drugs manufacturing. The steps involved in the compounding of drugs generate air emission, liquid waste and solid waste (Ulamen and Robert, 2006).

Among such wastes discharged as partially treated or untreated in Nigeria are pharmaceutical wastewaters. Drugs are designed to stimulate a physiological response in human, animals, bacteria and other organisms (Kummerer, 2003). Many pharmaceuticals and personal care products (as well as their metabolites and byproducts) can enter the environment and finally the food chain following ingestion or application by the user or administration to domestic animals. Aquatic environment serves as the major ultimate receiving end for these chemicals, of which little is known with respect to their actual or potential adverse effects. During the past decade, concern has grown about the adverse effect that the use and disposal of pharmaceuticals might potentially have on human and ecological health (Kummerer, 2003). In the last 15 to 20 years, there have been several reports of pharmaceuticals in the environment: human and veterinary drugs were detected in river water and even in drinking water (Richardson and Bowron, 1985; and Halling-Sorensen *et al.*, 1998). Although reported levels are very low, effects were observed, with a noteworthy example being hormone disruption in fish due to the presence of estrogens in the environment. Drug substances may reach the environment via use or disposal. Patients will usually excrete a drug or its metabolites, which will then pass on to a sewage treatment plant. There, it may be (partially) degraded, it may absorb to the sludge or it may remain in the effluent. After processing in the sewage treatment plant, the sludge is usually incinerated, but it may also be spread on the land and then leach into the soil and eventually into the groundwater. In the case of disposal, depending on the route (drain, household or industrial waste), pharmaceutical wastewater may enter the groundwater and surface water via a sewage treatment plant or by leaching from a land fill site (Halling-Sorensen *et al.*, 1998).

There has been no wastewater treatment system constructed for managing the wastewater from the factory. The wastewater is discharged in the environment without treatment through a pipe. Consequently, wastewater flows along a drainage channel and mixes with wastewaters from human settlements around the factory; this eventually empties into Chanchaga river. This poses serious environmental and public health risks. Worst still, there are villages downstream that use the water in this river for their domestic purposes. The people are prone to several infectious waterborne diseases such as cholera, hepatitis, poliomyelitis, typhoid fever, diarrhea, amoebic and bacillary dysentery. Besides, pollutants in pharmaceutical wastewater may be mutagenic or toxic and consequently lead to several human diseases such as cancer, arteriosclerosis, cardiovascular disease and premature ageing (Grover and Kaur, 1999). It has been shown that disposal of untreated pharmaceutical wastewaters into the environment has great implications on public health,

because of the ability to select and enhance the development of resistant bacteria (Lateef, 2004; and Lateef *et al.*, 2005).

There is an increase in the number of pharmaceutical industries in Nigeria. While more hazardous wastes are generated and discharged into the environment, there is a dearth of information on the potential effects that such may have on biota (Lateef and Yekeen, 2006). Therefore, there is a need for an assessment of pharmaceutical wastewater with a view to determining the impact of the discharged wastewater on public health. The results of the findings will evaluate the bacteriological qualities of a pharmaceutical process wastewater and will recommend measures to be used in treating the wastewater. In addition, the results will provide useful data that will guide public health policy formulation and the company. It is believed that such information will assist in the timely formulations of new regimes of environmental regulations to prevent the discharge of untreated wastewaters into the environment, thereby mitigating the risks associated with the exposure to such matrices. This research has the general aim of evaluating the bacteriological qualities of a pharmaceutical process wastewater with a view to determining the impact of the discharged wastewater on public health as well as the safety of the wastewater coming out from the pharmaceutical industry with the specific objectives to enumerate the bacteria in pharmaceutical wastewater, and isolate and characterize the bacterial isolates associated with the pharmaceutical wastewater.

Materials and Methods

A total of 108 samples were aseptically collected in duplicate for the analysis using sterile sample bottles from the designed point of discharge (outlet) (PA), 200 m away from the point of discharge and in contact with external environment designated point B (PB) and at Chanchaga River, 500 m downstream of the river designated point C. At PA, the wastewater was allowed to run for a few minutes through a pipe before sterile sample bottles were used to collect it and quickly corked. At PB, the sample bottles were held facing the wastewater current for the collection. Water samples from Chanchaga river were collected 15 cm below the water surface by holding the sample bottles to face the water current. The wastewater samples were collected between 10 a.m. and 12 p.m. each month for a period of three months (June-August 2009) and transported to the laboratory in an ice box. The samples were analyzed for bacteriological properties within 4 h of collection.

Bacteriological Analysis and Total Count

Total Viable Counts

The spread plate method, as described by Lateef *et al.* (2005), was used. The samples were shaken to obtain a homogenous mixture. 1 mL of the homogenous samples was serially diluted in 9 mL of sterile distilled water to obtain 10^{4} to 10^{9} dilutions.

0.1 mL of the 10^{4} to 10^{9} dilution was aseptically transferred directly onto the plates of solidified Nutrient Agar (NA) medium. The samples were then spread over the surface

of the agar with a sterilized bent glass rod to ensure even distribution of the inoculum on the surface of the agar. The plates were inoculated in duplicate and were incubated at 37 °C for 24-48 h. Colonies which developed on the plates were counted using the colony counter (model 6399/Stuart Scientific Co. Ltd., Great Britain) and expressed as colony forming units per milliliter (cfu/mL) of samples. The colonies differing in size, shape and color were selected and subcultured repeatedly to obtain pure isolates. The pure isolates were maintained on agar slants for further characterization and identification.

Total Coliform Counts

0.1 mL of 10^{-1} to 10^{-8} serially diluted wastewater samples were aseptically plated on Eosin Methylene Blue agar for the enumeration of total coliform counts. The plates were incubated at 37 °C for 24-48 h for colonial growth. Colonies which developed on the plates were counted using the colony counter (model 6399/Stuart scientific Co. Ltd., Great Britain) and expressed as colony forming units per milliliter (cfu/mL) of samples.

Isolation and Enumeration of Clostridium Species

0.1 mL of 10^{-3} serialiy diluted wastewater samples in a conical flask previously heated in thermal regulated water bath (model 72504/2, Searle Company, Greenfield, England) at about 75 °C for 10 min (Itah *et al.*, 1996) to kill non-endospore forming microorganisms were aseptically plated on blood agar plates. They were then incubated anaerobically in an anaerobic jar containing a candle which was lit to remove all the oxygen present in the jar. The lid of the anaerobic jar was screwed tight while the candle was still burning. When the candle light had extinguished, the jar containing the plates were incubated at 37 °C for 24-48 h. The colonies which developed on the plates were counted using the colony counter and expressed as colony forming units per millimeter (cfu/mL) of samples. The colonies developed in the plates were subcultured on fresh media for further characterization and identification of the isolates.

Isolation and Enumeration of Salmonella and Shigella Species

0.1 mL of 10^{-3} to 10^{-8} serially diluted wastewater samples were aseptically inoculated onto Brilliant Green Agar (BGA) and *Salmonella/Shigella* Agar (SSA) plates for the isolation and enumeration of *Salmonella* and *Shigella* species. The plates were incubated at 37 °C for 48 h. The colonies were streaked onto Triple Iron Sugar Agar slant (TISA) for differentiation of *Salmonella* from *Shigella*. The triple iron sugar agar slants were incubated at 37 °C for 24 h. Colonies which developed on the plates were counted using the colony counter and expressed as colony forming units per milliliter (cfu/mL) of samples. The ones that produce black coloration and no coloration were noted as *Salmonella* and *Shigella* species respectively.

Fecal Coliform (E. coli) Counts

The Most Probable Number (MPN) technique was used, as described by Fawole *et al.* (2002) and Bakare *et al.* (2003). Three tubes containing lactose broth were used for the detection of fecal coliform organisms and for determining the Most Probable Number (MPN) of coliform

Bacilli using the McCrady Table Standard Methods for the Examination of Water and Wastewater (SMEWW) (APHA, 1999). The technique consists of three steps:

Presumptive Test: 0.1 mL, 1 mL and 10 mL of each wastewater sample were used to inoculate the lactose broth in five replications. The tubes were incubated at 37 °C for 48 h. For the detection of fecal coliforms, production of acid and gas was taken as positive indication and confirmed the presence of the organism (D'Auriac *et al.*, 2000).

Confirmed Test: Tubes showing positive results from the presumptive test were used to inoculate on MacConkey broth and incubated at 37 °C for 48 h. Gas production in the tubes confirmed the presence of *E. coli* and the tubes were used to determine the MPN value of the fecal coliforms using the McCrady Table.

Completed Test: Tubes with gas production in the confirmed test were used to streak on Eosin Methylene Blue (EMB) agar and incubated at 44.5 °C for 24-48 h (Prescott *et al.*, 2008). Colonies which developed on EMB were identified as *Escherichia coli.*

Characterization and Identification of Isolates

Bacterial Isolates

The bacterial isolates were characterized based on colonial and cell morphology, growth on differential/selective media and biochemical tests which include Gram's reaction, indole tests, methyl red, Voges-Proskauer, citrate utilization, motility, endospore, utilization of carbohydrates such as glucose, sucrose, mannitol, lactose and fructose, oxidase, catalase, coagulase and starch hydrolysis test (Fawole and Oso, 1995; Ogbulie *et al.*, 1998; and Oyeleke and Manga, 2008). The bacteria were identified according to the taxonomic scheme and description in *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974; and Holt *et al.*, 1994).

Statistical Analysis

A one-way analysis of variance (ANOVA) and Duncan Multiple Range (DMR) test were used to determine whether there are significant differences among the values obtained for microbial counts. Statistical Package for the Social Sciences (SPSS package) 15.0, 2006 version was used for data analysis. The statistical analyses were carried out using mean and standard deviation/standard error mean.

Results

The results for bacterial counts in pharmaceutical wastewater and downstream of Chanchaga river are shown in Table 1. Table 1 shows the bacterial counts obtained for the three study sites (PA, PB and PC). The results revealed that the mean for total viable counts ranged from 4.8×10^4 cfu/mL to 3.0×10^8 cfu/mL, 2.0×10^7 cfu/mL to 4.0×10^8 cfu/mL for total coliform counts, 1.3×10^3 cfu/mL to 3.0×10^8 cfu/mL for *Salmonella/Shigella* counts, 340 MPN/100 mL to ≥1600 MPN/100 mL for fecal coliform (*E. coli*) counts and *Clostridium* counts indicated that no *Clostridium* were detected in PA. PB and PC had *Clostridium* counts

Table 1: Mean Values for Bacterial Counts

Parameters	Month	PA	PB	PC
Total Viable Count (cfu/mL)	June	$9.5 \times 10^{4b} \pm 3.6 \times 10^{4}$	$1.0 \times 10^{5ab} \pm 1.0 \times 10^{5}$	$5.0 \times 10^{5a} \pm 2.0 \times 10^{5}$
	July	$1.3 \times 10^{4b} \pm 8.1 \times 10^{4}$	$1.8 \times 10^{4b} \pm 2.1 \times 10^{4}$	$2.0 \times 10^{5a} \pm 1.0 \times 10^{4}$
	August	$3.6 \times 10^{4a} \pm 8.4 \times 10^{3}$	$1.8 \times 10^{4b} \pm 0$	$2.0 \times 10^{5a} \pm 7.7 \times 10^{4}$
	Mean	$4.8 \times 10^{4b} \pm 1.3 \times 10^{4}$	$5.0 \times 10^{4b} \pm 4.0 \times 10^{4}$	$3.0 \times 10^{5a} \pm 7.0 \times 10^{4}$
Total Coliform Count (cfu/mL)	June	$2.4 \times 10^{4b} \pm 1.7 \times 10^{4}$	$4.2 \times 10^{4ab} \pm 2.7 \times 10^{4}$	$6.0 \times 10^{5a} \pm 5.0 \times 10^{4}$
	July	$1.0 \times 10^{4b} \pm 1.0 \times 10^{4}$	$5.0 \times 10^{4b} \pm 1.6 \times 10^{4}$	$7.0 \times 10^{5a} \pm 2.0 \times 10^{4}$
	August	$2.6 \times 10^{4b} \pm 1.7 \times 10^{3}$	$3.9 \times 10^{4ab} \pm 7.9 \times 10^{3}$	$3.1 \times 10^{5a} \pm 1.4 \times 10^{5}$
	Mean	$4.0 \times 10^{4b} \pm 4.0 \times 10^{4}$	$2.0 \times 10^{4b} \pm 3.8 \times 10^{4}$	$4.0 \times 10^{5a} \pm 5.0 \times 10^{4}$
Salmonella/ Shigella Count (cfu/mL)	June	$1.5 \times 10^{4a} \pm 5.0 \times 10^{3}$	$4.0 \times 10^{3ab} \pm 2.7 \times 10^{3}$	$5.0 \times 10^{2b} \pm 3.0 \times 10^{2}$
	July	1.0×10^{3a}	$3.0 \times 10^{2b} \pm 3.3 \times 10^{2}$	$4.0 \times 10^{2b} \pm 5.0 \times 10^{2}$
	August	ND	$3.0 \times 10^{2b} \pm 3.3 \times 10^{2}$	$4.0 \times 10^{2a} \pm 2.0 \times 10^{2}$
	Mean	$1.3 \times 10^{3b} \pm 1.5 \times 10^{2}$	$2.0 \times 10^{2b} \pm 3.3 \times 10^{2}$	$3.0 \times 10^{2a} \pm 4.0 \times 10^{2}$
Faecal Coliform Count (MPN/100 mL)	June	$100^{c} \pm 13$	$880^{b} \pm 190$	$1600^{a} \pm 0$
	July	$130^{c} \pm 14$	$670^{b} \pm 170$	$1600^{a} \pm 0$
	August	$780^{b} \pm 210$	$100^{b} \pm 140$	$1600^{a} \pm 0$
	Mean	$340^{c} \pm 87$	$880^{b} \pm 99$	$1600^{a} \pm 0$
Clostridium Count (cfu/mL)	June	ND	2.0×10^{3}	1.0×10^{3}
	July	ND	ND	ND
	August	ND	ND	ND
	Mean	ND	2.0×10^{3}	1.0×10^{3}

Note: Key: a, b and c: values with different letters on the same row were significantly different from each other ($P < 0.05$). The average values were ± Standard Error of Mean (SEM) from readings taken in three days. PA: Pharmaceutical wastewater (point of discharge: outlet); PB: Discharged wastewater in contact with external environment; PC: Chanchaga river (downstream); ND: not detected; cfu/mL: colony forming unit per milliliter; mg/L: milligram per liter; MPN: Most Probable Number (MPN) method (multiple tube fermentation technique).

of 2.0×10^{3} cfu/mL and 1.0×10^{3} cfu/mL respectively in June, while in July and August no growth was detected. The results showed that downstream of Chanchaga river (PC) had higher bacterial counts than the other sampling sites. A one-way analysis of variance (ANOVA) carried out on all the data showed that there were significant differences between the counts obtained at 5% level of significance ($P < 0.05$), while the mean for total viable counts, total coliform counts and *Salmonella/Shigella* counts for PA and PB were not significantly different from each other.

The bacteria isolated from this study were identified as *E. coli*, *Salmonella* sp., *Klebsiella* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*,

Clostridium sp., and *Streptococcus faecalis*. A total of nine bacterial isolates were identified (Table 2). *E. coli*, *Salmonella* sp., *Klebsiella* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* occurred in all the three sites (PA, PB and PC) throughout the sampling period (Table 2) and had 13.04% frequency of occurrence respectively. *Clostridium* sp. occurred in two sampling sites (PB, PC) and had 8.70% frequency of occurrence respectively, while *Proteus vulgaris* also occurred in two sampling sites (PA, PC) and had

Table 2: Bacterial Isolates of Samples

Bacterial Isolates	Sampling Point			
	PA	PB	PC	Total
E. coli	+	+	+	3
Salmonella sp.	+	+	+	3
Klebsiella sp.	+	+	+	3
Bacillus subtilis	+	+	+	3
Pseudomonas aeruginosa	+	+	+	3
Staphylococcus aureus	+	+	+	3
Proteus vulgaris	+	–	+	2
Clostridium sp.	–	+	+	2
Streptococcus faecalis	–	–	+	1
Total	7	7	9	23

Note: PA: Pharmaceutical wastewater (point of discharge: outlet); PB: Discharged wastewater in contact with external environment; PC: Chanchaga river (downstream); +: Detected ; and –: Not detected.

Table 3: Frequency of Occurrence of Bacterial Isolates

Bacterial Isolates	Frequency of Occurrence	% Frequency of Occurrence
E. coli	3	13.04
Salmonella sp.	3	13.04
Klebsiella sp.	3	13.04
Bacillus subtilis	3	13.04
Pseudomonas aeruginosa	3	13.04
Staphylococcus aureus	3	13.04
Proteus vulgaris	2	8.70
Clostridium sp.	2	8.70
Streptococcus faecalis	1	4.35
Total	**23**	**100%**

8.70% frequency of occurrence respectively. *Streptococcus faecalis* occurred in only one sampling site (PC) and had 4.35% frequency of occurrence. The results showed that *Clostridium* sp. was not detected in PA, *Proteus vulgaris* was also not detected in PB, and *Streptococcus faecalis* was detected in PC only (Table 3). *E. coli*, *Salmonella* sp., *Klebsiella* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphyloccus aureus* had the highest frequency of occurrence (13.04%), followed by *Proteus vulgaris* and *Clostridium* sp., (8.70%), while *Streptococcus faecalis* had the least frequency of occurrence (4.35%) (Table 3).

Discussion

The study revealed that pharmaceutical wastewater (PA), discharged wastewater in contact with external environment (PB) and downstream of Chanchaga river (PC) harbored bacteria of various counts.

The counts were high, revealing that there was a dense population of bacteria in the three study sites. This is probably due to rich nutrients contained in the three sites and also due to the fact that the water samples carried various microorganisms because aquatic ecosystem, among other things, is an embodiment of diverse microorganisms. High total viable bacterial count obtained particularly in the river and the external environment is not unlikely with the advent of rainy season during the period of the study (June to August) because as the rain falls soil microbiota and various microorganisms adhering to vegetations, municipal sewage, garbage, domestic and industrial wastes are washed into the water body and the external environment (Itah *et al.*, 1996; and Obire and Amusan, 2003). The index of the bacterial load was high, and none of the three sites had total bacterial counts less than 10^3 cfu/mL, meaning that the study sites were highly contaminated. Bridges *et al.* (2000) reported that the total bacterial counts higher than 10^2 cfu/mL indicate dangerous contamination and also that the increase in bacterial load may constitute a serious health hazard. In a similar study, Lateef (2004) and Lateef *et al.* (2005) obtained a bacterial count of 2.15×10^5 cfu/mL from pharmaceutical wastewater, while several workers (Chukwura and Okpokwasili, 1997; Adewoye and Lateef, 2003; and Bakare *et al.*, 2003) obtained bacterial counts in the order of 10^5 cfu/mL for bacterial population in some polluted rivers in Nigeria that are exposed to human, agricultural and industrial wastes. These results are in agreement with the findings of the present study, indicating high contamination of the three study sites. Previous studies indicated high bacterial counts from pharmaceutical wastes (Ekhaise and Omavwoya, 2008; and Oyeleke *et al.*, 2008). The result of the fecal coliform (*E. coli*) test indicates that the pharmaceutical wastewater, discharged wastewater in contact with the external environment and downstream of Chanchaga river had evidence of fecal contamination with high mean MPN of 340 MPN/100 mL, 880 MPN/100 mL and ≥1600 MPN/mL respectively. The fecal coliform (*E. coli*) counts recorded within the sampling/study sites were high. This bacteriological quality poses an increased risk of infectious disease transmission to the communities that are dependent on Chanchaga river for household chores. The fecal coliform population density observed in the river and external environment may be due to man's activities along and within the study area, which include direct indiscriminate defecation in or around the river by humans and animals and various

human recreational activities such as swimming, bathing, washing and act of urinating that contribute to high coliform count of the river and the external environment (Eniola and Olayemi, 1999). The fecal coliform density is also due to the advent of the rainy season during the period of the study (June to August). This increase in bacterial load may constitute a serious health hazard. However, fecal contamination of the pharmaceutical wastewater could be due to contamination of the production process by human healthy carriers through handling. This is because the wastewater samples were collected prior to contact with the external environment. The presence of high coliform densities in the wastewater samples during the sampling periods is an indication of fecal pollution of the environment due to human activities. Aluyi *et al.* (2006), in a related study, reported high fecal load with high concentration of *E. coli* in Udu river, Warri, Delta State, Nigeria which was attributed to human activities. Lateef *et al.* (2005) reported evidence of fecal contamination of pharmaceutical wastewater and river with high MPN of × 1800 which is comparable with results obtained from this study. High MPN obtained for river water source is not unlikely because it receives human waste materials (Bakare *et al.*, 2003). High fecal coliform (*E. coli*) of 50 MPN/100 mL, 550 MPN/100 mL and microbial load with a concentration of 10^{7} cfu/mL of 1.8×10^{7} cfu/mL and 3.5×10^{7} cfu/mL were also obtained from pharmaceutical wastewaters (Lateef *et al.*, 2007), while other investigators (Bala, 2006; and Stephen and Ijah, 2006) have also reported high feacal coliform counts indicating the poor microbiological quality of some Nigerian rivers receiving wastewater from industries. *Salmonella/Shigella* counts obtained from the three study sites revealed that no *Salmonella/Shigella* counts were obtained in PA in August, while other sampling sites had higher population of these enteric organisms. This may be attributed to human activities especially in the river and environment, and agrees with previous reports (Theron, 2001; and Obi *et al.*, 2002). The bacteria isolated from pharmaceutical wastewater (PA) discharged wastewater in contact with the external environment (PB) and downstream of Chanchaga river (PC) include *E. coli*, *Salmonella/Shigella* sp., *Klebsiella* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Clostridium* sp. and *Streptococcus faecalis*. *E. coli*, *Salmonella* sp., *Klebsiella* sp., *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated from all the three sites (PA, PB and PC), *Proteus vulgaris* was isolated in two sites (PA, PC), *Clostridium* sp. was isolated in two sites (PB, PC), while *Streptococcus faecalis* was isolated in only one site (PC). These bacteria are the causative agents of various diseases and complications. These findings are comparable to those of Lateef (2004), Lateef *et al.* (2005) and Lateef *et al.* (2007) who had isolated these organisms from pharmaceutical wastewater. In a related research, Ekhaise and Omavwoya (2008) had also reported the isolation of these organisms from the external environment receiving discharged hospital wastewater, while Oyeleke *et al.* (2008) had isolated some of these organisms from hospital solid waste on the environment which could be hazardous to human health. Other researchers (Nevondo and Cloete, 1999; Theron, 2001; Obi *et al.*, 2002; Bakare *et al.*, 2003; Adewoye and Lateef, 2003; Stephen and Ijah, 2006; Bala, 2006; and Nwidu *et al.*, 2008) have also reported the presence of these pathogens in some Nigerian rivers receiving effluent/wastewater from industries which is in agreement with the present study. All these organisms are potential

pathogens of man capable of causing a variety of diseases. *Staphylococcus aureus* causes infections of the skin, deep tissues and organs, pneumonia, enteritis and pseudornembranous enterocolitis and food poisoning; *Proteus* may infect urinary tract and wounds; *E. coli* causes diarrhoea, urinary tract and kidney infections and peritonitis septicaemia; *Pseudomonas* causes infections of wounds, burns, eyes and ears; *Salmonella* causes typhoid fever; *Shigella* causes dysentery; *Streptococcus faecalis* causes urinary tract infection; *Klebsiella* causes respiratory tract infection (bronchitis), urinary tract infections, infection of the blood (septicaemia) and soft tissue infection which may lead to shock and death and *Clostridium* causes tetanus (Pearson *et al.*, 2000; Baker *et al.*, 2001; Ryan and Ray, 2004; Mims *et al.*, 2005; Prescott *et al.*, 2008; and Todar, 2008). Even though many of these bacteria are needed to initiate infections, contaminated water and food are the major means by which they are spread (Prescott *et al.*, 2008; and Todar, 2008). Thus, the presence of these organisms may have serious health implications for the consumers of the water directly from the river and may facilitate widespread infections and can ultimately lead to the outbreak of epidemics. The isolation of these pathogens from the pharmaceutical wastewater is worrisome because the wastewater was collected prior to contact with the external environment. In such a case, it is not impossible to assume that these pathogens were introduced into the production process by human health carriers through handling. The continuous contamination of the process may be enhanced through the processing equipment (Hatcher *et al.*, 1992). The pharmaceutical wastewater, which is discharged in the environment without treatment through a pipe, flows along a drainage channel and eventually empties into Chanchaga river, contaminates nearby streams and food crops in the farm, and inadvertently reaches man. The presence of Cotrimoxazole and other antibiotics will enhance the development of resistant bacteria in the environment and could be dangerous, harmful to human health and pose a health risk to human (gravely). Recent studies have shown that antibiotics can accumulate in the environment, and even persist for up to a year (Zuccato *et al.*, 2002). Thurman and Hostetler (2000) found antibiotics in animal feed and groundwater near lagoons. This could enhance the resistance of bacteria to antibiotics or drugs and also spread bacterial resistance among the inhabitants who may get in contact with the wastewater. Lateef (2004) and Lateef *et al.* (2005) have reported that disposal of untreated pharmaceutical wastewaters into the environment has great implications on public health, because of the ability to select and enhance the development of resistant bacteria. The trend of bacteria obtained in this study has serious public health implications, since major epidemics throughout the world are increasingly found associated with resistant pathogens (Levy, 2001; and Canton *et al.*, 2003). Exposure to pharmaceutical wastewater can represent a risk for health and endangers the wellbeing of the population. Kummerer (2001) reported that after passing through wastewater treatment, pharmaceuticals are released directly into the environment. There is also a relationship between accumulation of heavy metals in the environment and incidence of bacterial resistance. In fact, the potential impact of increased antibiotic resistance due to metal contamination seems to be particularly great considering the very large number of heavy metal-contaminated locations that can favor maintenance and transfer of antibiotic-resistant bacteria (McArthur and Tuckfield, 2000). There seems to be increasing evidence that industrial effluents may

contribute to the emergence, development and spread of resistant strains of bacteria (Aleem *et al.*, 2003; Adewoye and Lateef, 2004; Lateef, 2004; Lateef *et al.*, 2005; and Stepanauskas *et al.*, 2005). In a similar study, Guardabassi *et al.* (1998) reported that while the hospital waste effluent affected only the prevalence of oxytetracycline resistance, the discharge of wastewater from a pharmaceutical plant was associated with an increase in the prevalence of both single- and multiple-antibiotic resistance among *Acinetobacter* species in the sewers. Surface water can pick up solid, liquid and gas either as rainwater or as it percolates through the soil layers. These added substances are broadly classified as biological, chemical (both organic and inorganic), physical and radiological impurities. Others include industrial and commercial solvents, metals and acid salts, sediments, pesticides, herbicides, plant nutrients, radioactive materials, decaying animal and vegetable materials, living organisms such as algae, bacteria, fungi and viruses (Erah *et al.*, 2002). The eventual emergence of this groundwater from aquifer as spring water, rivers, estuaries, or pumping of this water from the aquifer as borehole water may have grave consequence on water quality. Chemical intoxication in drinking water may either be acute or chronic in nature. The acute health effect may be in the form of skin irritation, skin rash, nausea, vomiting, dizziness, etc. Death may ensue if the quantity of chemical consumed is large. Most often, routine examination of water has revealed high level of inorganic chemicals, and the acute effects may not easily be traced by clinicians as symptoms are treated symptomatically. Other chronic effects reported following consumption of inorganic chemicals are cancer, mutagenesis, tetratogenesis, nervousness and immune system disorders (Erah *et al.*, 2002).

Conclusion

Pharmaceutical wastewater harbored pathogenic bacteria. High level of contamination of the wastewater, as revealed in this study, further confirms the danger associated with discharging untreated wastewater into the environment as it affects a variety of ecosystem (aquatic and terrestrial) and poses serious health risks to human beings. This study has drawn attention to the poor bacteriological quality of Chanchaga river. The presence of opportunistic pathogens and enteric organisms is an indication that the water is polluted and poses a serious health risk to its direct consumers.

In view of the fact that little is known about the occurrence, fate and risks that are associated with antibiotics and pharmaceuticals entering the environment (Kummerer, 2003), measures to avoid the release of harmful substances should be incorporated in the design, operation, maintenance and management of pharmaceutical plants, as such efforts will yield both economic and environmental benefits. One study had estimated the costs of bacterial resistance to antibiotics alone to be between $150 mn and $30 bn annually, depending upon how many deaths were caused by resistance (Phelps, 1989).

As industrial wastes are being discharged into aquatic environment directly or through runoff, they may bioaccumulate in aquatic organisms. The ultimate effect will be shown at higher trophic levels due to biomagnifications along the food chain (Odiete, 1999). Thus, there is an urgent need for wastewater treatment facility to be installed to reduce the health hazard the wastewater poses to the users of the Chanchaga river.

Recommendation

Based on the results obtained, the following recommendations are made: pharmaceutical industries should be advised to treat their wastewater properly before discharging into the environment and rivers; regular studies should be carried out on water bodies that receive pharmaceutical wastewater in order to reveal and evaluate its microbial qualities; the pharmaceutical industries should be monitored regularly in order to ascertain the quality of wastewater discharged into the environment; yearly monitoring of the microbiological parameters of the river should be carried out; sewage and wastewater from homes and industries located near Chanchaga river should be treated before being discharged into the river; excessive fertilizer application on farmlands close to the bank of Chanchaga river should be discouraged as they are easily washed into the river by surface runoff; communities around Chanchaga river should be enlightened on the implications of consuming contaminated water, especially by heavy metals and pathogens; proper hygiene should be maintained within the pharmaceutical factory and the environment. Target areas for sanitization should include infrastructure and facilities contained therein, equipment, surrounding areas and most particularly the staff; appropriate technology should be developed for the treatment and recycling of the wastewater for irrigation. Further research should be carried out particularly on the pharmaceutical wastewater and the receiving Chanchaga river.

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