African Scientist Vol. 17, No. 3, September 30, 2016
Printed in Nigeria

1595-6881/2016 $10.00 + 0.00

http://www.niseb.org/afs

AFS 2016047/17307

How Long Can Enteric Pathogens Survive in Polluted Environmental Media?

Olusoji O. Adebisi[1*], Ayodeji J. Olaoye[2], Taiwo V. Senewo[2] and Ifeyinwa S. Obuekwe[3]

[1]Department of Microbiology, Faculty of Life Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria
[2]Easyway Medical Equipment Ltd., 8, Mojidi Street, Off Toyin Street, Ikeja, Lagos State, Nigeria
[3]Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Edo State, Nigeria
[*]Corresponding author: soji-olusegun@unilorin.edu.ng; Tel: +234(0)8060510307;

(Received August 19, 2016)
(Accepted in revised form September 30, 2016)

ABSTRACT: Monitoring pathogen survival in polluted environmental media is useful as an early warning tool to forestall outbreaks of infections and safeguard public health. Survival of clinical strains of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholerae* was monitored for 192 hours in pre-sterilised samples of domestic wastewater and sediment, agricultural wastewater and river water. All pathogens rapidly proliferated in the first 72 hours in all environmental media (2-3 log order increases), but either rapidly or steadily declined thereafter, with *P. aeruginosa* and *V. cholerae* demonstrating the longest persistence and *S. typhi* the least. Survival kinetics of pathogens are similar in domestic wastewater and sediment, though persistence was longer in sediment. While the pathogens did not proliferate as much in agricultural wastewater as compared with the domestic wastewater and sediment, they however, persisted for a longer time in the former. Notably, river water supported the survival of pathogens for a significantly ($p< 0.05$) longer time than any of the wastewaters and sediment. These findings have implications in the prediction and early detection of potential pollution points during epidemiological and source tracking studies.

Keywords: pathogen survival; enteric bacteria; public health; river; microbial source tracking

Introduction

The use of water in homes, industries or farms usually results in the generation of wastewaters which may have obnoxious or harmful effects on the environment and public health. Combination of wastewaters from domestic and agricultural sources constitute one of the most harmful in a community(Obiri-Danso and Jones, 1999) because of the presence of large number of opportunistic and pathogenic organisms most of which are enteric (Avery *et al*., 2008), and are capable of causing infections in humans (Ford, 1999). The non-point discharge of most of these wastewaters also poses serious concerns because fate and transport of pathogens are difficult to monitor (Ferguson *et al*., 2003). Invariably, while it may not be common to see direct discharge of wastewaters into streams and rivers, the

channelling of wastewater from kitchen sinks and toilet sewers onto bare grounds that may later drain into streams and underground aquifer is equally harmful.

There are usually regulations in place for the discharge of wastewater from industrial sources, in developing nations like Nigeria where there is poor infrastructural planning, there are wide gaps in policies and regulations to control domestic wastewater discharges. Drainage systems that collect wastewaters from homes or farms to treatment plants hardly exist even in many of the emerging urban settlements in the developing world (Ford, 1999; Ololade *et al*., 2009). Wastewaters often discharged into the open drainage flow with storm run-offs and empty into streams and rivers that unfortunately serve, most times, also as the source of drinking water for the community. Possibilities of epidemic outbreaks mostly related to enteric pathogens are unavoidable in areas where municipal and agricultural wastewaters are discharged directly into rivers or where river water contaminated by community storm runoffs is used as drinking water sources without treatment (Louis *et al*., 1990; Colwell, 1996; Abraham and Wenderoth, 2005; Avery *et al*., 2008). Waterborne diseases most of which are endemic and infectious, are the major causes of mortality and morbidity in developing countries (Schneider *et al*., 1978; Louis *et al*., 1990; Colwell, 1996). Many of the causal agents are normal flora of the human gastrointestinal tract including *Salmonella typhi, Vibrio cholerae,* and *Escherichia coli* and if ingested through contaminated drinking water or foods may cause serious health issues and even death (Huq *et al*., 1984; Colwell, 1996; Angulo *et al*., 1997; Avery *et al*., 2008).

Bacterial survival in an environment is based on its internal potential and self-induced ability to withstand offensive chemicals and prevail in unfavourable conditions while maintaining its power of reproducibility. This survival rate is usually accessed based on how long or how soon they die-off in an environment (Ferguson *et al*., 2003; Abraham and Wenderoth, 2005). Several works have reported on pathogen survival in different water environments; pristine (Huq *et al*., 1984; Buzoleva and Terekhova, 2002; Khan *et al.*, 2007), treated (Ford, 1999) and contaminated (Abraham and Wenderoth, 2005; Avery *et al*., 2008). However, most of these works are in the temperate regions (Buzoleva and Terekhova, 2002; Abraham and Wenderoth, 2005; Avery *et al*., 2008). Some environmental factors that may contribute to survival have also been evaluated (Huq *et al*., 1984; Khan *et al*., 2007; Avery *etal*., 2008). Notably, ambient temperature influences bacterial growth, and heterotrophic mesophiles that are mostly found in water prefer optimal temperatures above 25°C (Huq *et al*., 1984). Incidence and endemicity of most waterborne pathogens in Africa and other tropical countries have been linked to high water temperature (Huq *et al*., 1984; Colwell, 1996). To our knowledge, no work has been reported that studied the survival of pathogens under such environmental conditions in domestic or agricultural wastewaters. Risk assessments of aetiologies of waterborne diseases require adequate understanding of pathogen distribution and survival strategies within water environments and this would involve methodologies that can detect not only the presence, but also the viability and infectivity of the pathogen (Ford, 1999) and resistance to antibiotics (Abraham and Wenderoth, 2005).

In this paper, we reported the survival at 30°C of certain water-borne pathogens in environmental media including domestic wastewater and sediment, agricultural wastewater and river water. Clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholerae* were inoculated into pre-sterilized wastewater, sediment and water samples and microbial growth determined by standard pour plate technique. The implications of the survival of these pathogens in the environmental media and the public health significance are discussed.

Materials and Methods

Sample Collection and Analysis

Domestic wastewater and sediment samples were collected from the external PVC pipe leading out of the kitchen sink of a popular restaurant in Ilorin, Kwara State, Nigeria. The wastewater is drained into the public open sewerage system which runs through the street. River water samples were collected at two

different points (which were later bulked together) close to areas where residents often collect water used for laundry and other domestic purposes. It was noted that the stream serves as drinking water source for some residents especially during the dry season when the public water supply is usually erratic. Agricultural wastewater was obtained from a small fish pond which also discharges through an underground conduct into the same stream where the river water samples were obtained. Samples were collected, as much as possible, to represent the sources of the pollution. Samples were transported immediately to the laboratory in ice-chest and microbiological analysis carried out within 4 hours of collection.

The physicochemical parameters including temperature and pH values of samples were determined on site using portable hand-held devices (PH Tester PH-107) and turbidity was determined using UV/vis spectrophotometer (Thermo-Scientific Genesys 20) at 460nm. Suspended and dissolved solids concentrations of samples were determined gravimetrically by filtering samples through No. 2 Whatman's filters and then oven-drying residues and filtrates to constant weight respectively. The total solid concentrations were calculated by adding the suspended with the dissolved solids. Moisture content of sediment sample was determined by oven-drying to calculate amount of water lost to evaporation. The mean numbers (3 replicates) of heterotrophic, total and faecal coliform, and *Shigella/Salmonella* counts were obtained on nutrient and selective/differential (Endo-agar, MacConkey agar and Salmonella-Shigella agar) media incubated at appropriate temperatures (37 or 45°C) for 24 and 48 hours (APHA *et al*., 1999). Thereafter, the culture plates were enumerated based on distinct colonies and characteristic fluorescence/pigmentation for total and faecal coliforms, as well as *Shigella/Salmonella*. The mean numbers of heterotrophic, total and faecal coliforms and *Shigella/Salmonella* counts were recorded.

Bacterial Culture Preparation

Clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and *Vibrio cholerae* previously obtained from patients visiting the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria and stocked in the Medical Microbiology and Parasitology Laboratory were used in this study. The stock cultures were first resuscitated in nutrient broth (for 2–3 days to ensure that cells are in their active growth phase) and then incubated on nutrient agar plates. The purity of the isolates was ascertained by a series of sub-culturing on appropriate media and running a battery of definitive morphological and biochemical tests (Cruickshank *et al*., 1980; Holt *et al*., 1994). Axenic bacterial cultures were maintained in separate nutrient broth flasks on rotary shaker for 20–24 hours at 30 ± 1°C. Thereafter, broth cultures were centrifuged at 10,000 *g* for 10 minutes; supernatant was discarded and cell filtrate re-suspended in sterile distilled water and centrifugation repeated a number of times to remove remnants of broth nutrients. Distilled water was used instead of phosphate buffer solution (PBS) to avoid introducing any nutrient or minerals into the harvested cell filtrate. The pure harvested cells were used in the bacterial survival experiments. Cell population was standardised using the McFarland's technique as measured on the UV/vis spectrophotometer (600 nm) to give a population of approximately 1.5×10^6 CFU/ml.

Evaluation of Bacterial Survival

Water and sediment samples were heat-sterilised by repeated autoclaving–re-incubation cycles at 121^oC for 15 mins to ensure that all vegetative organisms are killed. The effectiveness of this sterilization technique was initially verified by plating out. After every autoclaving process, aliquots of samples (1 ml of sterilized water or1 g of sediment) were plated (on nutrient agar and potato-dextrose gar and incubated at 37°C for 24–48 d) to verify the effectiveness of sterilization process and samples left for 48–72 hours before autoclaving again until complete sterilization of samples was achieved. The survival of the clinical isolates was evaluated in the environmental media as follows. One hundred milliliter of sterilized wastewater or water sample or 100 g of sediment sample (wet weight equivalent) placed in each of 250-ml Erlenmeyer flasks were spiked with the pathogens (*ca*. 1.5×10^6 CFU/ml) and incubated on an orbital shaker (100 rpm) in a controlled temperature cubicle (30 ± 1°C). Triplicate plates of *Escherichia coli, Klebsiella pneumoniae* and *Vibrio cholerae* were made on Endo agar and MacConkey agar, *Pseudomonas*

aeruginosa on Nutrient agar and *Salmonella typhi* on Salmonella-Shigella agar, respectively, after 1, 2, 24, 72, 144 and 196 hours of inoculation. The culture plates were incubated at 37°C and enumerated after 24–48 hours.

Statistical Analysis

The survival growth curves for the pathogens are presented as log normalized graphs by transforming the raw data such that the initial cell numbers at time zero were converted to $\log_{10}10$. Comparisons for statistical relationships and significances ($\alpha = 0.05$) of the transformed data were tested for with analysis of variance (ANOVA) performed using the statistical packages of SigmaPlot (SPSS ver. 10, SPSS Inc. Chicago, IL, USA).

Results

Four environmental samples including domestic wastewater and sediment, agricultural wastewater and river water were analysed for their physicochemical and microbiological characteristics (Table 1).

Table 1: Physiochemical and Microbial Characteristics of Environmental Media

Parameters Analysed	Domestic Wastewater	Domestic Sediment	Agricultural wastewater	River water
Appearance	Highly cloudy	Dark humus	Lightly cloudy	Lightly turbid
pH	7.0 ± 0.8	6.6 ± 1.2	7.6 ± 0.2	7.3 ± 0.3
Temperature (°C)	24.2 ± 1.8	23.8 ± 0.6	28.0 ± 2.0	30.2 ± 1.6
Turbidity (Å)	1.548	N.D	0.241	0.040
Suspended Solid (mg/L)	1.2×10^4	N.D	1.75×10^3	1.70×10^2
Dissolved Solid (mg/L)	2.1×10^4	N.D	9.0×10^2	5.0×10^2
Total Solids (mg/L)	3.3×10^4	N.D	2.65×10^3	6.70×10^2
Moisture content (%)	N.D	30	N.D	N.D
Heterotrophic Bacterial Counts ($\times 10^5$ CFU/ml)	190.00 ± 71.10	390.00 ± 26.90	9.43 ± 0.50	3.28 ± 0.14
Total Coliform Counts ($\times 10^5$ CFU/100ml)	215.00 ± 14.90	131.00 ± 12.70	4.82 ± 0.14	3.29 ± 0.80
Faecal Coliform Counts ($\times 10^5$ CFU/100ml)	29.20 ± 2.97	44.50 ± 3.61	28.00 ± 0.28	2.48 ± 0.67
Salmonella/Shigella Counts ($\times 10^3$ CFU/25ml)	155.00 ± 35.40	229.0 ± 33.20	3.10 ± 0.15	7.04 ± 0.35

N.D = Not determined

The mean pH range (6.6 ± 1.2–7.6 ± 0.2) and mean temperature range (23.8 ± 0.6–30.2 ± 1.6°C) recorded in the samples were typically within the range that could promote heterotrophic microbial growth. The domestic wastewater was highly turbid at the time of sampling. The dissolved and suspended solids concentrations in the domestic (2.1×10^4 and 1.2×10^4 mg/L, respectively) and agricultural (9.0×10^2 and 1.75×10^3 mg/L, respectively) waste waters were quite higher than in the river water (5.0×10^2 and 1.70×10^2mg/L, respectively). Heterotrophic bacterial numbers in the samples were generally high (3.28×10^5–3.90×10^7 CFU/ml),with domestic sediment having the highest. Expectedly, the total and faecal coliform populations were higher in the wastewaters than the river water. However, higher *Salmonella/Shigella* count was recorded in the river water than in the agricultural wastewater (Table 1).

The relative abundance of each subset of the bacterial diversities was calculated (Table 2).

Table 2: Relative Abundance of Microbial Community Subsets in Environmental Media

Percent of Subsets to HBC	Domestic Wastewater	Domestic Sediment	Agricultural wastewater	River water
% Abundance of TCC to HBC	1.13	0.34	0.51	1.00
% Abundance of FCC to HBC	0.15	0.11	0.30	0.76
% Abundance of SSC to HBC	0.03	0.02	0.01	0.09
% Abundance of FCC to TCC	13.49	33.97	58.33	75.38
% Abundance of SSC to TCC	2.88	6.99	2.58	8.51
% Abundance of SSC to FCC	21.38	20.58	4.43	11.29

HBC = Heterotrophic Bacterial Counts; TCC = Total Coliform Counts;
FCC = Faecal Coliform Counts; SSC = Salmonella/Shigella Counts

The relative compositions (in percentage) of the total coliform bacteria (TCC) and faecal coliform bacteria (FCC) to the total heterotrophic bacteria (HBC) in all the samples were low (0.34–1.13% and 0.11–0.76%, respectively). However, the percentage abundance of FCC to TCC in river water (75.38%) and agricultural wastewater (58.33%) were high. Table 2 reveals also that there were relatively less *Salmonella-Shigella* cells (SSC) than *E. coli* in all samples, though the proportions of SSC to FCC were higher in the domestic wastewater and sediment samples. Also, the results indicate that some pathogens such as *Salmonella* sp. may persist less than *E. coli* in the environmental media investigated (Table 2).

The survival kinetics of the clinical isolates used in this study are presented as log normalized growth graphs (Figure 1).

In the domestic wastewater, all organisms except *S. typhi* had similar survival patterns where an initial rapid increase in cell populations was followed by an apparent undulating stationary phase which lasted between 72 and 144 hours of inoculation. Only *V. cholerae* and *P. aeruginosa* actually persisted after 192 hours. Interestingly, though *V. cholerae* did not proliferate as much as the other organisms in domestic wastewater, it persisted longer than the others (Figure 1A). The survival kinetics of the pathogens are apparently similar in the domestic wastewater and sediment, though the organisms persisted relatively longer in the sediment (Figure 1B). While *P. aeruginosa* and *K. pneumoniae* persisted, with high cell numbers, after 144 hours, the growth of others were limited after 72 hours and cell numbers became negligible by the 144-hour of inoculation. *V. cholerae* exhibited similar survival kinetic in the sediment as was observed in the domestic wastewater (Figure 1B).

The patterns of survival of the pathogens in the agricultural wastewater were quite different from those in domestic wastewater and sediment. While *K. pneumoniae* and *E. coli* were relatively dormant within the first hour of inoculation, others quickly increased and peaked at 72 hours of inoculation. Thereafter, all the organisms did not significantly decline in cell populations, except *S. typhi* which died-off after 192 hours (Figure 1C). Also, while the organisms did not proliferate as much in the agricultural wastewater (Figure 1C) as compared with the domestic wastewater (Figure 1A) and sediment (Figure 1B), they however, persisted for a longer time. The log normalized survival kinetic of the pathogens in river water is similar to that of agricultural wastewater. *E. coli* and *S. typhi* were dormant within the first two hours while others rapidly increased in cell numbers, peaking at 72-hour of inoculation. Though the cell numbers of all inoculated organisms declined slightly thereafter, their persistence in the river water is significant throughout the 192-hour survival study (Figure 1D).

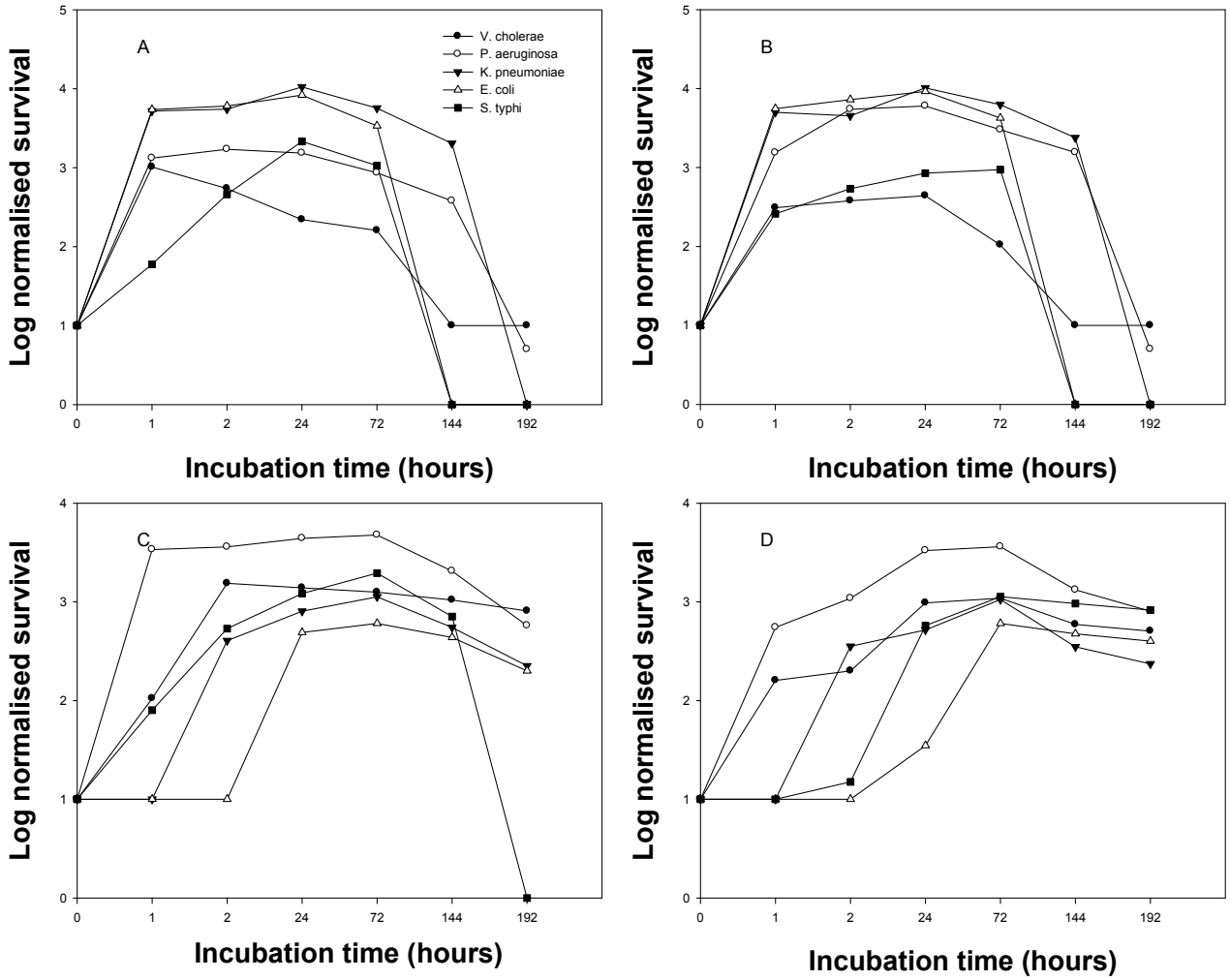

Figure 1: Log normalized survival of pathogens in (a) domestic wastewater, (b) domestic wastewater sediment, (c) agricultural wastewater, and (d) in river water

Discussion

Ranges of pH and temperature values and the high concentrations of suspended and dissolved solids in all the environmental media examined could have encouraged the profusion of heterotrophic bacterial growth. This is usually the case in exposed and polluted environments (Huq *et al*., 1984; Abraham and Wenderoth, 2005; Avery *et al*., 2008; Donovan *et al*., 2008). Suspended and dissolved solids support bacterial growth by providing food, shelter and/or acting as shield for the microbes (Morris *et al*., 1996). Though faecal coliform bacteria were present in moderate numbers in the samples, their presence in the river water which is supposedly used for domestic purposes and to wash fresh vegetables and fruits from farms along the river banks is worrisome. Many of the heterotrophic and enteric bacteria isolated from the environmental samples showed varying multiple resistances to common antibiotics used in the community (data not presented). There is a growing concern about the indiscriminate use and abuse of these antibiotics in the communitywhich could lead to prolonged illnesses or even deaths (Abraham and Wenderoth, 2005).

The comparatively low numbers of the total and faecal coliform bacteria to the total heterotrophic bacteria in all the samples could either mean that the environmental media were remotely exposed to faecal contamination or if exposed, the 'allochthonous' enteric organisms had died-out after previous exposure. The first assumption may be true for the domestic wastewater and sediment because the samples were collected from kitchen sink discharges. However, the higher ratios of faecal to total coliform counts in the agricultural wastewater and river water would support the assumption that the samples had faecal organisms which may have persisted. This could infer that faecal materials were potential sources of contaminants in the river water and agricultural wastewater. Comparatively, the lower cell counts of *Salmonella-Shigella* (SSC) to *E. coli* in all samples and the relatively higher proportions of SSC to FCC (> 20%) in the domestic wastewater and sediment samples further give credence to our assumptions: there is a high possibility that the agricultural wastewater and river water were exposed to faecal materials whereas the domestic wastewater and sediments were only remotely exposed. Also, the results could indicate that some pathogens such as *Salmonella* sp. may persist less than *E. coli* in the environment (Voogt *et al*., 2001; Leclerc *et al*.,2002).

The survival patterns of the isolates showed an initial rapid growth, then, a steady period of growth before gradual death of cells due to depletion of nutrient and oxygen as well as possible build-up of toxic by-products (Pathak and Bhattacherjee, 1994; Leclerc *et al*., 2002; Avery *et al*., 2008) and competition (Buzoleva and Terekhova, 2002). It is noteworthy that these initial vigorous bacterial growths within the first 2 hours peaked by the 24-hour in the domestic wastewater and sediment while it took between 24- and 72-hour in the agricultural wastewater and river water before they troughed. Huq *et al*. (1984) reported similar growth patterns when *V. cholerae* serovar O1 was incubated at 30°C in Chesapeake Bay water over a period of 168 hours. The similarity in the survival patterns of most of the organisms in the domestic wastewater and sediment indicated that the environmental and nutritional conditions could be relatively similar (Donovan *et al*., 2008). The ability of the opportunistic pathogens *K. pneumoniae* and *P. aeruginosa* to persist longer than the other enteric organisms could be both physiological and environmental (Leclerc *et al*., 2002; Panagea *et al*., 2005; Khan *et al*., 2007). The former are known to strive naturally outside the gastro intestinal tract (GIT) (Romling *et al*., 1994; Ford, 1999; Khan *et al*., 2007) while *P. aeruginosa* is known to be non-fastidious (Pellett *et al*., 1983; Pirnay *et al*., 2005). However, the environments proved to be able to support the persistence of the enteric pathogen for at least 72 to 144 hours.*V.cholerae* which did not proliferate much in the wastewater, however, persisted for more than 72 hours. Waterborne enteric pathogens should not proliferate in drinking water though they may remain viable for a very limited period of time (Ford, 1999). The persistence of the organisms was not statistically different in each of the environmental media ($p< 0.05$).

The fact that these pathogens did not die-off in the environmental samples until the 144 hrs especially *E.coli* (survived up to 72 hrs) which is associated with faecal contamination means that there is need for stringent control of the direct discharge of the domestic wastewaters into water bodies (Tyrrel and Quinton 2003). This is especially important since these pathogens are causal agents of cholera, diarrhoea and typhoid (Louis *et al*., 1990; Colwell, 1996; Ford, 1999; Leclerc *et al*., 2002), and account for a large number of infant mortality annually (Colwell, 1996; Leclerc *et al*., 2002). The isolates would have survived better in the agricultural wastewater than in the domestic wastewater because it may contain less complex organic nutrients that can support further microbial growth and allow longer persistence (Soyaslan and Karagüzel, 2008). This suggests that discharge of agricultural wastewaters which have high number of enteric organisms could be more hazardous than domestic wastewaters, though this was not statistically validated by the log-normalized data ($p< 0.05$). Interestingly, the river water supported the persistence of the pathogens more than any of the other three environmental samples used. The persistence in the river water is significant throughout the 192-hour survival study. Longer periods of survival of some pathogens in treated drinking water, rivers and sediments have been reported by some researchers (Ford, 1999; Buzoleva and Terekhova, 2002; Avery *et al*., 2008; Donovan *et al*., 2008). Ambient temperature has been identified as a factor influencing persistence. Recent reports indicated that pathogens survived longer under lower temperature (Buzoleva and Terekhova, 2002; Hallmich and Gehr, 2010) though with less growth (Buzoleva and Terekhova, 2002). However, Huq *et al*. (1984) reported that

higher temperatures encouraged growth and persistence of *V. cholerae* serovar O1 when they compared incubated temperature ranges of 5, 15, 25 and 30°C.

We observed that these point sources of pollution (domestic wastewater/sediment and agricultural wastewater) are the major discharge sources into a stream that runs across the community which many residents use as a drinking water source as well as for various domestic purposes. It has been suggested that about 70% of diarrhoeal cases are contracted through contaminated foods, food utensils, food that have come in contact with contaminated water (Leclerc *et al*., 2002) or from direct ingestion of contaminated drinking water (Ford, 1999; Leclerc *et al*., 2002). In a community where lack of clean safe drinking water is perennial, it is very tempting and economical to use the river water. It was also observed that because of the irrigation farming practice along the banks of the stream, it is not unusual to see farmers washing their farm produce in the stream especially fresh vegetables and fruits which are transported to the market directly from the farm. Food and Food handling have led to several cholera epidemics (Louis *et al*., 1990; Colwell, 1996). Though there is no official report linking waterborne disease cases in the town to use of the river water under study, there is growing concerns on incidences of diarrhoea, dysentery and cholera-like diseases in the community.

The unwholesome practice of using exposed river water to wash farm produce which are sold directly in the market should be discouraged through community health education. Education is very important in safeguarding public health particularly in rural communities where literacy and poverty have greatly impacted the incidence of waterborne communicated diseases (Schneider *et al*., 1978; Colwell, 1996; Leclerc *et al*., 2002; Zobrist *et al*., 2009). Consequently, we recommend that the continued use of the river water for drinking and other domestic purposes and cleaning of farm produce, particularly those that may be consumed without much cooking should be avoided. Moreover, government should prioritize its policy on provision of safe potable water to the public in many parts of the city to support the growing population. More so, responsible authorities should not compromise the need for proper management and disposal of both domestic and agricultural wastes/effluents in order to prevent the epidemics of waterborne diseases.

In conclusion, this study could assist in rapid prediction of possible contamination sources during epidemiological studies as well as in developing surveillance systems. Monitoring survival of pathogens can also be used as early warning tool to identify possible routes of transport in order to forestall outbreaks of water-borne infections in a community.

Authors' Contributions

OOA and ISO designed and planned out the research work. Sample collection and laboratory experiments were carried out by AJO and TVS under the direct supervision of OOA. OOA prepared the initial draft. All authors contributed to the preparation of the final draft of the manuscript.

ACKNOWLEDGEMENTS: The authors are grateful to R.B. Famewo for assisting in the identification of sample sites and collection of samples, and Prof. A.B. Olayemi for his useful suggestions and reviewing the earlier draft of the manuscript. Special thanks to Dr S.K. Babatunde formerly of the Medical Microbiology and Parasitology laboratory, UITH, Ilorin, Nigeria now at Kwara State University, Malete, Nigeria for graciously supplying the clinical isolates.

References

Abraham WR, Wenderoth DF: Fate of Facultative Pathogenic Microorganisms during and after the Flood of the Elbe and Mulde Rivers in August 2002. Acta hydrochimica et hydrobiologica 33(5): 449-454. 2005.

Angulo FJ, Tippen S, Sharp DJ, Payne BJ, Collier C, Hill JE, Barrett TJ, Clark RM, Geldreich EE, Donnell HD, Jr, Swerdlow DL: A community waterborne outbreak of salmonellosis and the effectiveness of a boil water order. Am J Public Health 87(4): 580-584. 1997.

APHA, AWWA, WEF: Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WPCF, Washington, DC 1999.

Avery LM, Williams AP, Killham K, Jones DL: Survival of Escherichia coli O157:H7 in waters from lakes, rivers, puddles and animal-drinking troughs. SciTotal Environ 389: 378-385. 2008.

Buzoleva LS, Terekhova VE: Survivorship of Different Strains of the Bacteria Listeria monocytogenesis and Yersinia pseudotuberculosis in Sea and River Water. Russ JMar Biol 28(4): 259-262. 2002.

Colwell RR: Global Climate and Infectious Disease: The Cholera Paradigm. Science 274: 2025-2031. 1996

Cruickshank R, Daguid JP, Marnion BP, Swam RH: Medical Microbiology. Churchill Livingstone, London. 1980

Donovan EP, Staskal DF, Unice KM, Roberts JD, Haws LC, Finley BL, Harris MA: Risk of Gastrointestinal Disease Associated with Exposure to Pathogens in the Sediments of the Lower Passaic River. Appl Environ Microbiol 74(4): 1004-1018.2008.

Ferguson C, Husman AM, Altavilla N, Deere D, Ashbolt N; Fate and Transport of Surface Water Pathogens in Watersheds. Crit Rev in Env SciTechnol 33(3): 299 - 361.2003.

Ford TE: Microbiological safety of drinking water: United States and global perspectives. Environ Health Persp Suppl 107: 191-206. 1999.

Hallmich C, Gehr R: Effect of pre- and post-UV disinfection conditions on photoreactivation of fecal coliforms in wastewater effluents. Wat Res 44(9): 2885-2893. 2010.

Holt JG, Kieg NR, Sneath PHA, Stanley JE, Williams ST: Bergey's Manual of Systematic Bacteriology. William and Wilkins Company, Baltimore, Maryland. 1994.

Huq A, West PA, Small EB, Huq MI, Colwell RR: Influence of water temperature, salinity, and pH on survival and growth of toxigenic Vibrio cholerae serovar 01 associated with live copepods in laboratory microcosms. Appl Environ Microbiol 48(2): 420-424.1984

Khan N, Ishii Y, Kimata-Kino N, Esaki H, Nishino T, Nishimura M, Kogure K: Isolation of Pseudomonas aeruginosa from Open Ocean and Comparison with Freshwater, Clinical, and Animal Isolates. Microbial Ecol 53(2): 173-186. 2007.

Leclerc H, Schwartzbrod L, Dei-Cas E: Microbial Agents Associated with Waterborne Diseases. Crit Rev Microbiol 28(4): 371 - 409. 2002

Louis MES, Porter JD, Helal A, Drame K, Hargrett-Bean N, Wells JG, Tauxe RV: Epidemic cholera in West Africa: the role of food handling and high-risk foods. Am J Epidemiol 131(4): 719-728. 1990.

Morris RD, Naumova EN, Levin R, Munasinghe RL: Temporal variation in drinking water turbidity and diagnosed gastroenteritis in Milwaukee. Am J Public Health 86(2): 237-239. 1996.

Obiri-Danso K, Jones K:Distribution and seasonality of microbial indicators and thermophilic campylobacters in two freshwater bathing sites on the River Lune in northwest England. J Appl Microbiol 87(6): 822-832. 1999.

Ololade IA, Adewunmi A, Ologundudu A, Adeleye A: Effects of household wastes on surface and underground waters. Int J Phys Sci 4(1): 22-29. 2009.

Panagea S, Winstanley C, Walshaw MJ, Ledson MJ, Hart CA: Environmental contamination with an epidemic strain of Pseudomonas aeruginosa in a Liverpool cystic fibrosis centre, and study of its survival on dry surfaces. J Hosp Infect 59(2): 102-107. 2005.

Pathak SP, Bhattacherjee JW: Effect of pollutants on survival of *Escherichia coli* in microcosms of river water. Bull Environ Contam Toxicol 53: 198-203. 1994.

Pellett S, Bigley DV, Grimes DJ: Distribution of Pseudomonas aeruginosa in a riverine ecosystem. Appl Environ Microbiol 45(1): 328-332. 1983.

Pirnay JP, Matthijs S, Colak H, Chablain P, Bilocq F, van Eldere J, de Vos D, Zizi M, Triest L, Cornelis P: Global *Pseudomonas aeruginosa* biodiversity as reflected in a Belgian river. Environ Microbiol 7: 969-980. 2005.

Romling U, Wingender J, Muller H, Tummler B: A major Pseudomonas aeruginosa clone common to patients and aquatic habitats. Appl Environ Microbiol 60(6): 1734-1738. 1994.

Schneider R, Shiffman M, Faigenblum J: The potential effect of water on gastrointestinal infections prevalent in developing countries. Am J Clin Nutr 31(11): 2089-2099. 1978.

Soyaslan I, Karagüzel R: Investigation of water pollution in the Yalvac basin into Egirdir Lake, Turkey. Environ Geol 55(6): 1263-1268. 2008.

Tyrrel SF, Quinton JN: Overland flow transport of pathogens from agricultural land receiving faecal wastes. J Appl Microbiol 94: 87-93. 2003.

Voogt N, Raes M, Wannet WJB, Henken AM, Giessen AW: Comparison of selective enrichment media for the detection of Salmonella in poultry faeces. LettAppl Microbiol 32(2): 89-92. 2001.

Zobrist J, Sima M, Dogaru D, Senila M, Yang H, Popescu C, Roman C, Bela A, Frei L, Dold B, Balteanu D: Environmental and socioeconomic assessment of impacts by mining activities - a case study in the Certej River catchment, Western Carpathians, Romania. Environ Sci Pollut R 16: 14-26. 2009.