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QUALITY ASSESSMENT OF ABATTOIR EFFLUENTS FROM THE DISCHARGED POINT INTO A RECEIVING WATERSHED IN IKPOBA RIVER COMMUNITY: A POTENTIAL SOURCE OF MICROBIAL CONTAMINANT

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ABSTRACT

Effluent discharges into receiving watershed and increasing adverse environmental problems have received a lot of attention because of the rapid industrialization of modern society. This study aims to ascertain the discharge of untreated abattoir effluents as a potential source of microbial contamination in the environment. Standard culture-dependent techniques with selective media were used for the detection of the bacterial pathogen. Escherichia coli O.157 were detected at $2.57\times10^{4}\pm0.35$ cfu/mL in location 1 and $5.00\times10^{3}\pm0.33$ cfu/mL at location 2 at the discharged point. Total coliform count was $2.90\times10^{3}\pm0.44$ cfu/mL; $5.56\times10^{3}\pm0.61$ cfu/mL upstream and $5.70\times10^{3}\pm0.80$ cfu/mL; $5.90\times10^{3}\pm0.67$ cfu/mL discharged point at sampling location 1 and 2 respectively. Also, Enterococcus count increased in the downstream from $4.73\times10^{4}\pm0.42$ cfu/mL to $8.40\times10^{4}\pm1.28$ cfu/mL at location 1, and a decreased was observed from $1.04\times10^{5}\pm0.17$ cfu/mL to $6.75\times10^{4}\pm1.03$ cfu/mL at location 2. In the study, the population density of Salmonella at the discharge point and downstream were $1.67\times10^{5}\pm0.15$ cfu/mL; $1.60\times10^{5}\pm0.12$ cfu/mL and $2.00\times10^{5}\pm0.17$ cfu/mL; $1.50\times10^{5}\pm0.13$ cfu/mL for location 1 and location 2 respectively. The detection of indicator organism in the abattoir effluent is of environmental and public health risk. Hence there is a need for continuous monitoring in order to improve standard environmental and public health safety in the community.

1. INTRODUCTION

Effluent discharges into receiving waters and cumulative adverse environmental effects have received a lot of attention because of the rapid industrialization of modern society. Globally, efforts have been directed in preventing escalation of the problems of waste and environmental pollution. In many parts of the world, the issue of environmental health is now being taken with utmost importance. However, in Nigeria, like in many

other developing countries, discharge of untreated wastes into the environment is still a major challenge (Nafarnda *et al*., 2012). In Benin City, abattoirs dispose their wastewater into the nearby surface water bodies (Igbinosa *et al.,* 2017). At present there is no facility for the treatment of abattoir wastewater. The discharge of abattoir wastewater into the nearby river is a public health risk which exposes the public to waterborne diseases especially individuals who depend on the water as a source for diverse domestic functions.

Abattoir wastewater harbours several microorganisms which are washed from animal carcasses during processing. These pathogens comprise of viral, parasitic and bacterial pathogens. The bacteria pathogens include *Escherichia coli, Escherichia coli* O157: H7 *Salmonella* species, *Yersinia enterocolitica* (Sobsey *et al*., 2002), *Pseudomonas* species, *Aeromonas* species and *Enterococcus* species (Igbinosa *et al*., 2012; Igbinosa *et al.,* 2017). Although *E. coli* O157: H7 has been shown to be prevalent in sheep and pig, however, the main reservoir is cattle with no asymptotic features (Nafarnda *et al*., 2012). Abattoirs and aquaculture environment are of interest because they could be a potential reservoir of pathogenic and antibiotic-resistant genes which is of public and environmental health risk (Igbinosa *et al*., 2012; Igbinosa *et al.,* 2017). The microbiological assessment of abattoir effluent is vital to ascertain impact and consequences on the environment and its associated health risk. Due to the huge number of microbes in water, faecal indicator organisms have been used to assess the level of faecal pollution in water for several decades (Byamukama *et al*., 2005; Nafarnda *et al*., 2012). This is of concern because these organisms may cause human infections or foodborne infections as a result of transfer into the food chain ecosystem (Coker *et al*., 2001). These indicator organisms include total coliform, *Escherichia coli*, faecal coliform, *Enterococcus*. There is documented evidence of an association between the occurrence of gastrointestinal ailment and faecal indicator organisms after exposure to recreational water thereby providing needs for water quality monitoring (US-EPA 1986; Nafarnda *et al*., 2012). Realizing the significance of abattoir effluents on the environment and public health, this work seeks to investigate the effects of abattoir effluents discharged into the receiving water body as a source of microbial pollution into the environment.

2. MATERIALS AND METHODS

2.1. Sampling Site

The abattoir facilities are situated at Ikpoba slope community within the Ikpoba-Okha Local Government Area of Edo State. The abattoir facilities receive an estimate of 25 cattle and 30 goats which are slaughtered on a daily basis. The facilities produce an approximate 5000 to 10,000 litres of wastewater daily which is discharged into the receiving waterbody.

2.2. Collection of Water Samples

Water samples were collected from two major abattoirs facilities located in Ikpoba slope community that discharges their effluents into Ikpoba River. The two major abattoirs facilities were designated as location 1 and 2 for confidential purpose. Water samples were collected for a period of three months (February, March and April 2017). Samples were collected between 7 am and 10 am during the peak of activities on the day of sampling from three-point stations in each designated location 1 and 2. The abattoir effluent was collected from station X the discharge point at which effluents enter the stream; station Y 50-meters downstream from discharge point and station Z 50-meter upstream from the discharge point. The samplings were done midstream by dipping each sample bottle at approximately 20-30 cm below the water surface, projecting the opening of the container against the flow direction. The samples were collected in 500 millilitre sterilized containers, placed on ice and transported to the laboratory for microbiological analysis within 2 to 4 h.

2.3. Processing of Samples

Water samples were diluted in the ratio 1:9 with sterile distilled water after which serial dilutions of the diluent was carried out. One millilitre (1 mL) of the diluent was plated on the different selective and differential media used in the study. The media used are Bile Esculin Azide agar (Merck, Germany) *Enterococcus*, Sorbitol MacConkey Agar (Lab M Limited, UK) for *E. coli* O157, m-Endo Agar (Biolab, SA (total coliform), Hektoen agar (Lab M Limited, UK) (*Salmonella*), faecal coliform Agar (m-FC) (faecal coliform), (Biolab, SA), Chromocult Agar, (Merck, SA) (*E. coli*), Nutrient Agar (Lab M Limited, UK) (heterotrophic bacteria). All media were incubated for 24 h at 37 ºC except m-FC agar which was incubated at 44.5 ºC (Kadiri, 2017).

2.4. Culture-based Identification of Bacteria

After 24 h of incubation, the growth of the representative organism on each selective and differential media was presumptively identified based on morphological and cultural characteristics, Gram reaction 3% KOH test. Colonies of representative organisms were expressed and counted as colony forming unit per millilitre (cfu/mL).

2.5. Statistical Analysis

All data were analyzed using the statistical package SPSS version 21.0 and Microsoft Excel 2013. All values were analyzed as mean and standard deviations of the mean using descriptive statistics. The one-way analysis of variance (ANOVA) and Duncan multiple range test (DMRT) was used to test differences among the discharge points, upstream and downstream watersheds receiving the abattoir effluents were compared (p-value < 0.05 or p-value < 0.01) for significant difference and to establish statistical relationship.

3. RESULTS AND DISCUSSION

The intensity of faecal indicator bacteria (FIB) was determined from the discharged point station X, 50 m downstream station Y and 50 m upstream station Z. At the point of discharge, *E. coli* O.157 was detected at $2.57\times10^4\pm$ 0.35 cfu/mL in location 1 and $5.00\times10^3\pm$ 0.33 cfu/mL at location 2, however there was a slight decrease in population density of *E. coli* O.157 at downstream of location 1 ($2.14\times10^4\pm0.11$), though there was no statistical difference between station X and station Y of location 1. An elevated population count was observed for *E. coli* O157 downstream at location 2 ($1.36\times10^4\pm0.16$). On the other hand, a decrease was observed for *E. coli*, faecal coliform, and *Salmonella* count downstream station Y compared to the discharged point, station X at both sampling locations 1 and 2 as shown in Figure 1 a, b and c. The decrease count for these organisms downstream may be attributed to dilution factor downstream as the water flows. Tidal waves of the river may bring about agitation thereby resulting in the further dispersal of the waste into the watercourse.

For several decades, faecal indicator bacteria have been routinely used to evaluate water quality and protect individuals from the myriad of enteric pathogens transmitted via waterborne channel as a substitute of faecal indicators (Ishii and Sadowsky, 2008). Faecal indicator organisms are generally commensal that inhabits the gastrointestinal tracts of many warm-blooded animals; their detection in contaminated water is common because they are dispersed in faeces at high concentration (Byappanahalli *et al.*, 2012).

Total coliform increase downstream station Y compared to discharged point, station X from $5.70\times10^3 \pm 0.80$ cfu/mL to $6.40\times10^3 \pm 1.01$ cfu/mL in location 1 and $5.90\times10^3 \pm 0.67$ cfu/mL to $1.07\times10^4 \pm 0.02$ cfu/mL in location 2. Also, *Enterococcus* count was found to increase downstream from $4.73\times10^4 \pm 0.42$ to $8.40\times10^4 \pm 1.28$ cfu/mL at location 1 but decreased from $1.04\times10^5 \pm 0.17$ to $6.75\times10^4 \pm 1.03$ cfu/mL at location 2 as shown in Table 1.

Table 1: Detection of indicator pathogens from the discharge abattoir effluents and the receiving watershed

Indicator pathogens	Sampling location 1 (mean ± SD cfu/mL)			Sampling location 2 (mean ± SD cfu/mL)		
	Discharge point (X1)	50-m downstream (Y1)	50-m upstream (Z1)	Discharge point (X2)	50-m downstream (Y2)	50-m upstream (Z2)
Total coliform	$5.70\times10^{3}\pm0.80$	$6.40\times10^{3}\pm1.01$	$2.90\times10^{3}\pm0.44$	$5.90\times10^{3}\pm0.67$	$1.07\times10^{4}\pm0.02$	$5.56\times10^{3}\pm0.61$
Enterococcus spp	$4.73\times10^{4}\pm0.42$	$8.40\times10^{4}\pm1.28$	$4.24\times10^{4}\pm0.61$	$1.04\times10^{5}\pm0.17$	$6.75\times10^{4}\pm1.03$	$2.84\times10^{4}\pm0.17$
Heterotrophic bacteria	$2.29\times10^{11}\pm0.84$	$2.90\times10^{11}\pm0.82$	$2.34\times10^{11}\pm0.74$	$2.59\times10^{11}\pm0.52$	$2.62\times10^{11}\pm0.57$	$2.37\times10^{11}\pm0.44$

Total coliform count was $2.90\times10^{3} \pm 0.44$ cfu/mL; $5.56\times10^{3} \pm 0.61$ cfu/mL upstream and $5.70\times10^{3} \pm 0.80$ cfu/mL; $5.90\times10^{3} \pm 0.67$ cfu/mL discharged point at location 1 and 2 respectively which portray impact of abattoir effluents on the receiving water body. At upstream of location 1, faecal coliform and *E. coli* count were higher compared to discharged point Figure 1a and 1b. The high microbial density observed upstream may be due to anthropogenic input such as improper disposal of domestic-related waste into the river.

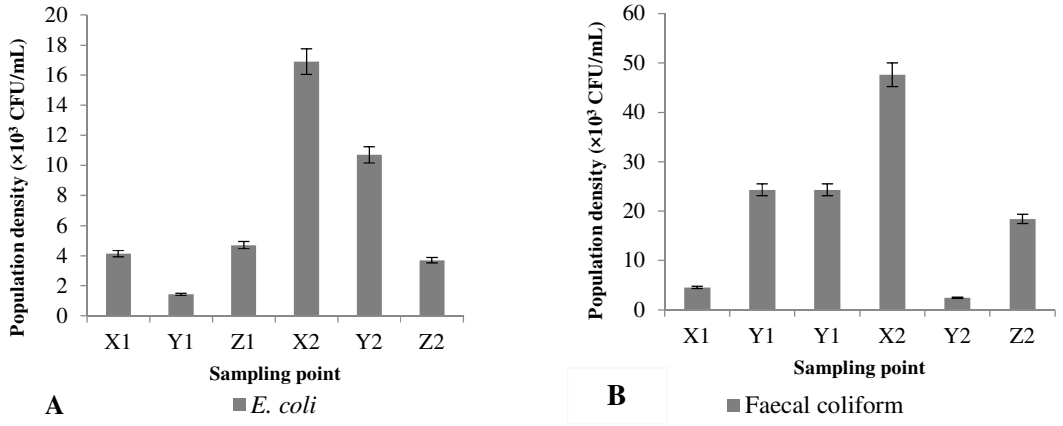

Figure 1: Detection of *E. coli* (A), Fecal coliform (B) from the abattoir effluents and its receiving watercourse. Legend: Sampling station X - the point of discharge, Y- downstream, and Z- upstream at sampling location 1 and 2

Also, rainfall could wash animal faecal deposit around the river banks into the water body. The indicator organism detected in abattoir effluent discharged into the receiving waterbodies exceeded the permissible limit of discharge of effluents into water bodies (FEPA, 1991). Statistical analysis of the data reveals no significant difference between the sampling point of all indicator organism assessed in the study ($p<0.05$). Similar observation of abattoir effluent contamination of waterbody has been documented in previous studies (Benka-Coker and Ojior, 1995; Cadmus *et al*., 1999, Alonge, 2001; Nafarnda *et al*., 2012). This observation also reveals that other non-point sources such as anthropogenic activities impair on the quality of the river.

Enterococci are resident in gut microflora in many animals (Nayak *et al*., 2011; Byappanahalli *et al*., 2012) and are opportunistic pathogens that cause several kinds of infections yearly (Morrison *et al*., 1997). *Enterococci* have emerged as a vital tool of faecal indicator bacteria in diverse water bodies due to their high profusion in human and animal faeces, the easiness of culture, and their relatedness to human health effects (US-EPA 2004). The *Enterococci* are most frequently used as faecal indicator bacteria or general indicators of faecal contamination, but they are also used as surrogates for pathogens and/or health effects in risk

assessment and other modelling applications (Schoen *et al.*, 2011; Sinclair *et al.*, 2012). In this study, it was observed that a higher *Enterococcus* count was observed downstream station Y when compared to upstream station Z. Although researchers have demonstrated that *Enterococci* are generally distributed in different kinds of environments including rivers, marine water, streams, land and sediment (Byappanahalli *et al.*, 2012). However, the higher count observed downstream compared to upstream in the present study reveals that the abattoir effluents have a significant impact on the water quality of the river thereby altering its ecosystem. Numerous studies have demonstrated a correlation between higher concentrations of enterococci and the menaces of humans contracting the gastroenteric infection during recreational water use, predominantly when point source contamination exists (Pruss 1998; US-EPA 2004). Hence the elevated count observed is at risk to environmental and public health especially those individuals who use the water for recreational activities and other domestic purposes.

Escherichia coli is one of the dominant members of the faecal coliform bacteria, they persist in livestock and are easily shed in their faeces. Livestock harbours the bacteria and releases it in their faeces. Their presence in water signals recent faecal contamination and presence of potential pathogens (Ishii and Sadowsky, 2008). A higher count was observed upstream compared to the effluent discharged point. This, therefore, suggests other sources of faecal contamination which could be a faecal deposit of ranch animals which can be easily washed into the river by rainfall. More alarming is the detection of *Escherichia coli* serotype O157 in the abattoir effluent and receiving water body. The main reservoir of *E. coli* serotype O157 is the abdominal tract of ruminants, particularly cattle. They are major carriers of pathogenic bacteria with no evident sickness symptoms (Buchanan and Doyle, 1997). However, *E. coli* O157 can have access to water bodies via wastewater abattoir effluent, cattle-houses and animal farms (Czajkowska *et al.*, 2004). This organism can cause differences in disease symptoms which vary in severity, from haemorrhagic colitis manifested by bloody diarrhoea to haemolytic uremic syndrome/thrombotic thrombocytopenic purpura that can lead to death (Buchanan and Doyle 1997). Hence the detection of *E. coli* O157 is of high risk to environmental and public health as this may result in the outbreak of *E. coli* infections. Such outbreak has been documented (Czajkowska *et al.*, 2004).

Salmonella is recognized as a zoonotic pathogen in humans and animals (Igbinosa, 2015) with a broad spectrum of the host. *Salmonella* can strive well in the environment and animals are known as a possible route of transmission and spread in the environment (Akoachere *et al.*, 2009; Igbinosa, 2015). In the study, the population density of *Salmonella* at the discharge point X and downstream Y were $1.67\times10^5\pm0.15$ cfu/mL; $1.60\times10^5\pm0.12$ cfu/mL and $2.00\times10^5\pm0.17$cfu/mL; $1.50\times10^5\pm0.13$ cfu/mL for location 1 and location 2 respectively. Whereas the population count at upstream were $5.43\times10^4\pm0.75$ cfu/mL and $1.61\times10^5\pm0.11$ cfu/mL at location 1 and location 2 respectively Figure 2.

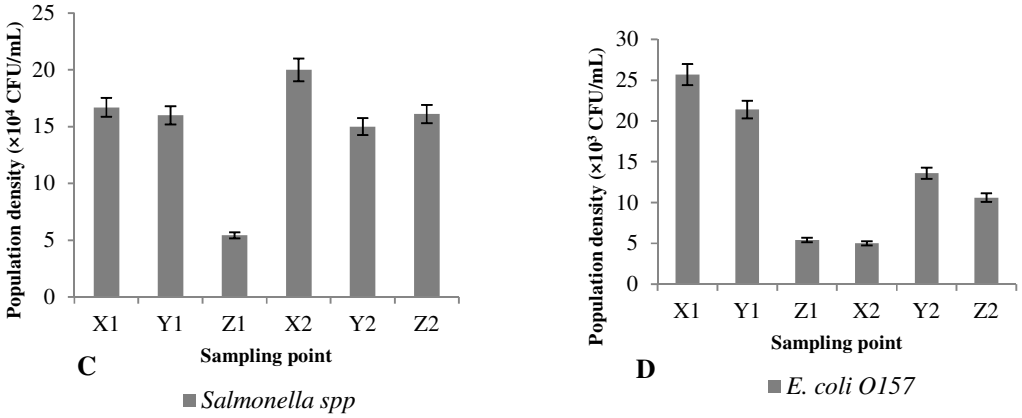

Figure 2: Detection of *Salmonella* (C) and *E. coli* O157 (D) from the abattoir effluents and its receiving watercourse. Legend: Sampling station X - the point of discharge, Y- downstream, and Z- upstream at sampling location 1 and 2

The result obtains in the study corroborate with the finding of Fransen *et al.* (1996) who reported the isolation of *Salmonella* in all slaughterhouses sludge samples assessed in the Netherlands. Also, Barros *et al.* (2007) reported the isolation of *Salmonella* from abattoir environment in Brazil, and Benka-Coker and Ojoir (1995) isolated *Salmonella* from abattoir effluent discharged into a river in southwestern Nigeria. These findings which are in agreement with ours potentiate abattoir effluent as a potential source of *Salmonella* in the environment. An intriguing finding in the study was the detection of *Salmonella* in upstream samples which is in contrast to the findings of Barros *et al.* (2007) where there was no detection of *Salmonella* in the upstream sample during their study. This observation suggests a non-point source of pollution into the river. The detection of *Salmonella* in abattoir effluents and river water samples is a potential risk to public health.

The correlation analysis of the indicator pathogen was carried out as shown in Table 2. *E. coli* O157 positively correlates total coliform (r=0.513) and heterotrophic bacteria (r=0.520) at 0.05 level of significance. Total coliform positively correlates heterotrophic bacteria (r=0.542) at 0.05 level of significance. *Enterococcus* spp. positively correlates faecal coliform (r=0.707) and negatively correlates *Salmonella* spp., (r=-0.549) at 0.01 and 0.05 level of significance respectively. *Salmonella* spp. positively correlates *E. coli* (r=0.571) at 0.05 level of significance.

Table 2: Correlation analysis of indicator pathogens from abattoir and the receiving watershed

Indicator pathogens	*E. coli* O157	Total coliform	*Enterococcus* spp.	Faecal coliform	*Salmonella* spp.	*E. coli*	Heterotrophic bacteria
E. coli O157	1						
Total coliform	0.513*	1					
Enterococcus spp.	-0.004	-0.074	1				
Faecal coliform	-0.141	-0.215	0.707**	1			
Salmonella spp.	0.357	0.366	-0.549*	-0.360	1		
Escherichia coli	-0.055	0.179	-0.435	-0.335	0.571*	1	
Heterotrophic bacteria	0.520*	0.542*	0.315	0.167	0.015	-0.188	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

The positive correlation of *Enterococcus* to faecal coliform observed (Table 2) suggest that the *Enterococcus* detected in the study is of faecal origins, which further support the impact of the abattoir effluent on the receiving water body. The negative correlation observed against *Salmonella* spp, and *E. coli* suggest that *Enterococci* may persist even the absence of these organisms.

4. CONCLUSION

The indicator pathogens detected depicts that the discharged effluents impact significantly on the receiving waterbody. The organisms detected are indicator pathogens of vital importance to public health. It was also observed that another non-point source of contamination contributes to the contamination of the river. Hence there is a need for continuous monitoring in order to develop standard environmental and public health safety. Furthermore, the characterization of the detected organism is needful to ascertain the pathogenic and toxigenic status of the organism.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

REFERENCES

Akoachere, J.T.K., Tanih, N.F., Ndip, L.M. and Ndip, R.N. (2009). Phenotypic characterization of *Salmonella typhimurium* isolates from food-animals and abattoir drains in Buea, Cameroon. *Journal of Health Population and Nutrition,* 27, pp. 612-618.

Alonge, D. O (2001). *Textbook of Meat Hygiene in The Tropics*, Farmcoe Press, Ibadan, Nigeria, 2nd edition.

Barros, L.S.S., Amaral, L.A., Lorenzon, C. S., and Machado, J.L., Neto, J.G. (2007). Potential microbiological contamination of effuents in poultry and swine abattoirs. *Epidemiology and Infection,* 135, pp. 3505-518.

Benka-Coker, M.O. and Ojior, O.O. (1995). Effect of slaughterhouse wastes on the water quality of Ikpoba River, Nigeria. *Bioresource Technology,* 52, pp. 5-12.

Buchanan, R. L. and Doyle, M. P. (1997). Foodborne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli. Food Technology,* 51, pp. 69.

Byamukama, D., Mach, R.L., Kansiime, F.M., Manafi, M. and Farnleitner, A.H, (2005). Discrimination efficacy of faecal pollution detection in different aquatic habitats of a high-altitude tropical country, using presumptive coliforms, *Escherichia coli*, and *Clostridium perfringens* spores. *Applied and Environmental Microbiology,* 71, pp. 65-71.

Byappanahalli, M.N., Nevers, M.B., Korajkic, A., Staley, Z.R., Valerie, J. and Harwoodc, V.J. (2012). *Enterococci* in the environment. *Microbiology and Molecular Biology Reviews,* 76, pp. 685-706.

Cadmus, S.I.B., Olugasa, B.O. and Ogundipe, G.A.T. (1999). The prevalence and zoonotic importance of bovine tuberculosis in Ibadan. In: Proceedings of the 37th Annual Congress of the Nigerian Veterinary Medical Association, pp. 65-70.

Coker, A.O., Olugasa, B. O. and Adeyemi, A.O. (2001). Abattoir wastewater quality in southwestern, Nigeria. In: Scott R. (Edited). People and Systems published by Water Engineering and Development Centre (WEDC), Loughborough University, United Kingdom, pp. 329-331.

Czajkowska, D., Witkowska-Gwiazdowska, A., Sikorska, I., Boszczyk-Maleszak, H. and Horoch, M. (2005). Survival of *Escherichia coli* serotype O157:H7 in water and in bottom-shore sediments. *Polish Journal of Environmental Studies,* 14, pp. 423-430.

Faleke, O.O., Jolayemi, K.O., Igoh, Y.O., Jibril, A.H., and Ayedun, J.O. (2017). *Salmonella* species on meat contact surfaces and processing water in Sokoto main market and abattoir Nigeria. *Macedonian Veterinary Review,* 40, pp. 59-65.

Federal Environmental Protection Agency (FEPA), Guidelines and Standards for Environmental Pollution Control in Nigeria, Ministry of Environment, Lagos, Nigeria, 1991

Fransen, N.G., van den Elzen, A.M., Urlings, B.A., Bijker, P.G. (1996). Pathogenic micro-organisms in slaughterhouse sludge-a survey. *International Journal of Food Microbiology,* 33, pp. 245-256.

Igbinosa, E.O., Odjadjare, E.E., Igbinosa, I.H., Orhue, P.O., Omoigberale, M.N. and Amhanre, N.I. (2012). Antibiotic synergy interaction against multidrug-resistant *Pseudomonas aeruginosa* isolated from an abattoir effluent environment. *Scientific World Journal*, 2012, 308034.

Igbinosa, I.H., Beshiru, A. and Igbinosa, E.O. (2017). Antibiotic resistance profile of *Pseudomonas aeruginosa* isolated from aquaculture and abattoir environments in urban communities. *Asian Pacific Journal of Tropical Disease,* 7, pp. 47-52

Igbinosa, I.H. (2015). Prevalence and detection of antibiotic-resistant determinant in *Salmonella* isolated from food-producing animals. *Tropical Animal Health and Production,* 47, pp. 37-43.

Ishii, S. and Sadowsky, M.J. (2008). *Escherichia coli* in the environment: implications for water quality and human health. *Microbes Environ*, 23, pp. 101-108.

Kadiri, R.A. (2017). Assessment of the impact of discharged abattoir effluent in receiving water body. BSc Dissertation submitted to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin.

Nafarnda, W.D., Ajayi, I.E., Shawulu, J.C., Kawe, M.S., Omeiza, G.K., Sani, N.A., Tenuche, O.Z., Dantong, D.D., and Tags, S.Z. (2012). Bacteriological quality of abattoir effluents discharged into water bodies in Abuja, Nigeria. ISRN *Veterinary Science,* 515689

Nayak, B.S., Badgley, B. and Harwood, V.J. (2011). Comparison of genotypic and phylogenetic relationships of environmental Enterococcus isolates by BOX-PCR typing and 16SrRNA gene sequencing. *Applied Environmental Microbiology,* 77, pp. 5050-5055.

Pruss, A. (1998). Review of epidemiological studies on health effects from exposure to recreational water. *International Journal of Epidemiology,* 27, pp.1-9.

Schoen, M.E., Soller, J.A. and Ashbolt, N.J. (2011). Evaluating the importance of faecal sources in human-impacted waters. *Water Research,* 45, pp. 2670-2680.

Sinclair, R.G., Rose, J.B., Hasham, S.A., Gerba, C.P. and Haas, C.N. (2012). Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. *Applied Environment Microbiology,* 78, pp.1969-1977.

Sobsey, M.D., Khatib, L. A., Hill, V.R., Alocilja, E. and Pillai, S. (2002). Pathogens in animal wastes and the impacts of waste management practices on their survival, transport, and fate. White paper for The National Center for Manure & Agricultural Waste Management, 2002, http://www.mwpshq.org/.

United States Environmental Protection Agency (US-EPA). (1986). Ambient water quality criteria for bacteria. EPA 440/5-84002, Washington, DC, USA, 1986.

United States Environmental Protection Agency (US-EPA). (2004). Implementation guidance for ambient water quality criteria for bacteria. EPA-823-B-04-002. Washington, DC, USA, 2004.