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BACTERIAL ASSESSMENT OF EFFLUENTS FROM SELECTED ABATTOIRS INTO ADJOINING WATER BODIES IN KADUNA METROPOLIS

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ABSTRACT
Abattoir effluents discharged into water bodies have high health implications. The study was carried within Mar to September 2019, to isolate and characterize bacteria from effluents discharged into water bodies from three Local Government Area Kaduna South (Kakuri), Chikun (Sabo-Tasha) and Kaduna North (Kawo) abattoirs within Kaduna metropolis. Three hundred of water samples were collected during the period of study. The samples were analyzed for bacterial content using standard Spread plate technique. The water samples collected content the mixture of blood, urine, piece of bone, faeces, etc. The result obtained from the water samples from the three abattoirs showed a bacterial high means count of 3.5 x 10^3CFU/mL Kakuri abattoirs showed means bacterial count of 2.40x10^3CFU/mL, Sabo abattoirs showed means count of 2.20 x10^3CFU/mL and Kawo abattoir showed means of 1.90 x 10^3 CFU/mL *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., *Shigella* sp. and *Preteus* sp. were isolated from waste water samples obtained from the three abattoirs. Analysis of the water sample obtained from the three abattoirs were observed to have a high numbers of bacterial that are harmful to human like *E. coli*. There is need to study the ecological implication of these bacteria.

Keywords: Abattoirs, Bacterial Content, Characterize, Effluent and Metropolis.

INTRODUCTION

Water is the most relevant natural resource for existence of man which is essential for his survival on earth. The volume of available potable water is found under the ground, in streams, rivers as well as lakes and the proportion of which is only about 3% (Behailu *et al.,* 2017). The available water is often inadequate to meet the needs of ever-growing population and industrial demands (Behailu *et al.,* 2017). This is a common situation in the African continent where majority of the people are living in environments in which the available water resources do not meet global standard (Sawyerr *et al*., 2017). Groundwater, stream and rivers are the commonest potable sources of water around the world (Kanmani *et al.,* 2013). The chemical composition of groundwater is an indicator of suitability for consumption by human beings and animals as well as absorption by plants (Batabyal *et al.,* 2015).

Abattoirs form important components of the livestock industry which provide domestic meat supply and employment opportunities to the teeming population in developing countries including Nigeria (Nafarnda *et al*., 2012). Abattoirs also provide useful by-products such as leather and skin, livestock waste spills for leather and agricultural industries. However, livestock waste spills can introduce enteric pathogens and excess nutrients into surface waters which can also contaminate ground waters (Meadows, 1995). These leachates consist largely of solids, microbial organisms which contaminate adjoining bodies of water (Ifeadi, 1982). These abattoirs are also less developed and lack facilities for the treatment of effluents that they generate and discharge into water bodies.

Contaminated water by abattoirs effluent can pose health risks like waterborne pathogens which can exist in water waste from the abattoirs (Constance *et al*., 2019).These wastes are generated from unscreened animals that are slaughtered in the abattoirs which in turn are hosts to microorganisms including bacteria. Microorganisms present in abattoir effluents may include *Cryptosporidium parvum*, *Campylobacter* spp., *Yersinia enterocolitica*, hepatitis E virus, *Salmonella* spp., rotaviruses, *Escherichia coli* O157: H7, *Listeria monocytogenes* and *Giardia lamblia* (Kosamu). (Ubwa *et al*., 2013). However, various processes involved in cleaning the animals produce a lot of wastes which are discharged into water bodies without treatment. This study aimed to assessing the bacterial composition of waste water from abattoirs within some part of Kaduna metropolis of Kaduna State.

MATERIALS AND METHODS

Study Area

The study was carried out in Kakuri, Sabo and Kawo abattoirs located in Kaduna South, Chikun and Kaduna North Local Government areas of Kaduna State respectively. These cover major parts of Kaduna metropolis with about 1,039,578 inhabitants and the administrative capital of Kaduna State, Nigeria. It is located between latitudes 90°E 3′ and 11°32′ North of the equator and longitudes 6°05′ and 8°38′ East of the Greenwich meridian (Anon, 2007).

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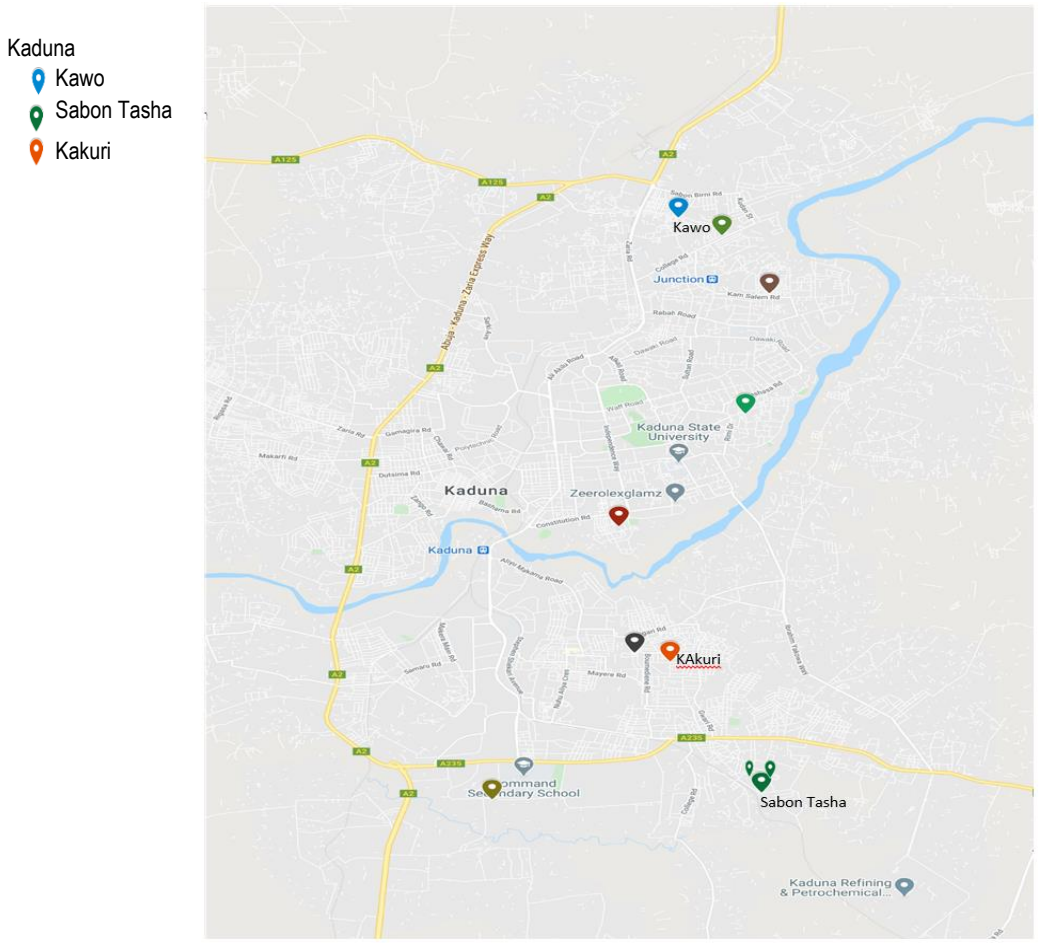

Figure 1: Map showing three site abattoirs

Water Sample Collection (Effluent and Adjoining Water)

The water sample for bacterial analysis were collected in five (5) sterile BOD bottles from three abattoirs at three different points in each of the abattoirs for four (4) weeks within a month from March to September 2019, the sterile BOD bottle was filled with water sample to the top and screw the lid on tightly to prevent leakage this sample were kept in refrigerate until before the analysis. The water samples were analyzed in Zoology laboratory Kaduna state university for present of bacteria. Three hundred (300) waste water samples consisting of one hundred samples from each of the three points were collected between the hours of 6am and 9am using 500mL sterilized BOD bottles Point 1 is the actual point where the effluents from the abattoirs are discharged into the water body. It is a direct effluent filled with blood, faeces, urine, and hair from the slaughtered animals. Point 2 is located upstream from the main point source 200 meters (two hundred meters from the main point source). These were taken as the control samples used to reflect the ambient state of the water body. Point 3 is located 350 metres (three hundred and fifty meter from the point source) downstream from the main point source of the discharged effluent. Water samples were transported in ice blocks to Zoology Laboratory, Department of Biological Sciences, Kaduna state University, Kaduna state. The samples were stored in the refrigerator until required for analyses.

Total Faecal Coliform Count

Serial tenfold dilution of water samples was made in a distilled water that is 1.0M of water sample collected was drop into 9m of distilled water and perform in a further ways, each of the serial dilution was subjected to aerobic plate count using standard spread plate method. Similarly, coliform counts were carried out in Eosin Methylene Blue Agar (EMB) for the presence of *E. coli*, *Klebsiella* sp. and *Enterobacter* sp. while Salmonella Shigella Agar (SSA) for the presence of *Salmonella* sp. and *Shigalla* sp. Macconkey Agar (MCCA) for the presence of lactose bacteria, *Proteus* sp. etc. and if *Salmonilla* sp. are presence within the water samples and Mannitol Salt Agar (MSA) for the presence of *Staphylococcus* sp. and Nutrient Agar (NA) was used to store each of the organism and a successful isolation, all the media were prepared using the manufacture instruction, then each media were dispensed into sterile Petri's dishes swirled gently and allowed to set. The inoculation of the plates was done after a serial tenfold dilution of water samples collected at 10^{-5} each of the media used was prepared by the manufacture instruction, zero-point one millimeter (0.1mL) of diluted sample was inoculated into prepared media using spread plate method. The plates were then inverted and incubated at 37°C and 45°C for 24h to 72h after which bacterial growth were checked and counted. (Cruickshank 1975 and Andrews 2004).

Test for coliform

The following media were prepared according to manufacturer's instruction 'standard plate count agar' Eosin Methylene Blue Agar (EMB), Salmonella Shagella Agar (SSA), Macconkey Agar (MCCA), were sterilized and dispensed into sterile Petri dishes and allowed to set. The inoculation of plates was done after a serial dilution of the samples collected at 10^{-5} using spread plate method. 0.1mL of serial tenfold dilution sample was used for the inoculation. Afterwards, the plates were incubated at 37°C for 24h the following observation was seen EMB agar black bull with green metallic sheen, some of pink colonies, some pink, with mucoid colonies and some colourless growth colonies. For SSA medium the grow colonies with black-green and pointed grow with colourless colonies. MCCA medium was observe pink and colourless grow with foul smell. (Mims *et al*., 2004). Eosin Methylene Blue Agar (EMB), and Macconkey Agar (MCCA), were dispensed into Sterile Petri dishes and allowed to set.

Culturing of effluent samples

The following media were prepared according to manufacturer instruction: Eosin Methylene Blue Agar (EMB), Salmonella and Shigella Agar (SSA) and Macconkey Agar (MCCA), were dispensed into sterile petri dishes and are allowed to set before inoculation. The inoculation of plates was done after a serial tenfold dilution of sample collected at 10^{-5} using spread plate method. 0.1mL of diluted sample was used for the inoculation. Inoculated plates were then incubated at 37°C to 45°C for 24h to72h, afterward each colonies observed were subjected to sub-culturing which depend on the colour, smell and shape observed of the media after 48h, another sub-culture was done after 48h which led to pure culture of this organism in another sterile Petri dishes for each bacterial colony using the same medium (selective, differential and indicator) was used, after obtaining a pure culture of this bacterial, they were store in nutrient agar medium. (Mims *et al*., 2004).

Biochemical Characteristics of Bacteria

Biochemical tests were performed for the identification of bacteria isolates with the help of Bergey's Manual of systematic bacteriology and ABIS 7 online software. The principal tests used for this purpose are gram stain, Indole Test (IND), Oxidase Test (OXI), Catalase Test (CAT), Aerobic and Anaerobic Test (Ae/An).

Gram staining test was performed on each of the isolated bacteria with the following procedure, the fixation of the organism on slide with crystal violet then the slide were pass through smear heat for better fixed, this slide sample was kept for some seconds before two drops of gram iodine on the slide then the slide was passed through the mixture of alcohol and acetone to decolorized the stain the slide were them counter stain with safranin after which each of the slide was kept some minutes, colour stain was check for negative and positive bacteria.(note that all the gram negative showed pink and gram positive showed purple)

Indole Test (IND) was performed by culturing the isolated bacteria organisms in peptone water medium containing tryptophan in a screw capped tube, incubated for 24 h at 37°C.Kovac's 0.5mL was added for each of the bacteria isolated, observed and result recorded accordingly.

Oxidase Test (OXI) test was used to assess the bacteria which produce the enzyme cytochrome Oxidase. Filter paper was moistened with a few drops of 1% tetramethyl-p-phenylene diamine di-hydrochloride. With a wooden applicator, growth from TSA plate was smeared on the paper then the experiment was observed and result recorded accordingly.

Catalase Test (CAT) test was performed by adding a small amount of bacterial isolate into freshly prepared 1% hydrogen peroxide.

Aerobic and Anaerobic Test (Ae/An test) TSA was inoculated and incubated at 37°C in anaerobic jar for 24-48h after which growth was observed (Adesina *et al*., 2018).

Indole test result showed positive results which were indicated by the formation of pink red layer on the broth within seconds of adding Kovac's reagent. OXI result a positive result will development purple color. No color change indicated a negative result.

Catalase test (CAT) and the bubbles of oxygen if appeared the isolate was considered as positive for CAT test.

Aerobic and Anaerobic Test (Ae/An test) negative result was indicated by no black precipitate.

Data Analysis

Data obtained were statistically analyzed using a One-Way Analysis of variance (ANOVA). Least Significant Difference (LSD) test was further performed to compare significant differences between the mean values where differences occurred with P value at $p \leq 0.05$ considered significant. The statistical package used is Statistical Package for the Social Sciences (SPSS) version 25 (Visweswara, 2009).

RESULTS

Enumeration of Bacteria Count

The enumeration count was done using this formula:

$$\text{CFU/mL} = \frac{\text{(No. of colonies x dilution factor)}}{\text{Volume of culture plate.}}$$

The results of the mean values for the TFCC are shown in Table 1. The mean TFCC for effluent point 1, upper point 2 and down point 3 for Kawo abattoir for each point 1, 2, and 3 were 2.1 x 10^3, 2.0 x 10^3 and 1.9 x 10^3 respectively, for Kakuri abattoir at point 1, 2 and 3 were 2.2 x10, 2.1 x $10^3$and 2.2 x 10^3CFU/mL) and Sabo abattoir were 1.90 × 10^3, 1.6 × 10^3and2.1 × 10^3 CFU/mL while water waste respectively. TFCC means value for waste water obtained for points 1, 2 and 3 in water waste for Kawo abattoir at point 1, 2 and 3 were 1.9 x 10^2, 1.3 x 10^2; Kakuri abattoir were 1.30 × 10^3, 1.5 × 10^2 and1.5 × 10^2 (CFU/mL), and 2.0 x 10^2 CFU/mL; for Sabo abattoir at points 1, 2, and 3 were 1.3 x 10^2, 0.9 x 10^2 and 1.9 x 10^2 respectively. The effluent water samples from the three abattoirs were highly contaminated: Kawo abattoir showed mean bacterial count of 190 x10^{-5} CFU/mL, Kakuri abattoir showed mean count of 243 x10^{-5} CFU/mL and Sabo abattoir showed mean count of 220 x10^{-5} CFU/mL. There was no significant difference ($P \leq 0.05$) between the mean bacterial counts and total faecal coliform count of abattoir wastewater discharged into the receiving water body at the beginning and the receiving water bodies at 350 m downstream. (Tables 2-4). On the culture medium the colony with presence of green metallic sheen on EMB agar, pink colony on Macconkey agar and fermentation of medium on MSA was seen on the plates, after sub-culturing which indicated the presence of *Escherichia coli*, for *Klebsiell* sp. On EMB medium the colony with presence of pink, mucoid colonies and for *Enterobacter* sp. Colony with good growth pink without sheen was seen, on Macconkey agar medium the colony with swarm and offensive foul smell, SSA

medium two distill grow was observed, the colourless grow which shown the presence *Shigella* sp. and growth with black thick colonies that shown the presence *Salmonella* sp. Seven isolates were obtained from the three abattoirs under study. They include *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., *Shigella* sp., and *Preteus* sp.

Table 1: Total Faecal Coliform Count (CFU/mL) in water samples obtained from selected abattoirs

Samples points (abattoirs)	Range mean value (CFU/mL)	Mean value (CFU/mL)	Means of mean (CFU/mL)
Main Point 1 Kawo	$1.9X10^2$- $2.8 X 10^3$	$2.1 X 10^3$	
Upper Point 2 Kawo	$1.3 X 10^2$ - $2.6 X 10^3$	$2.0 X 10^3$	$2.0x10^3$
Lower Point 3 kawo	$2.0 X 10^2$- $3.0 X 10^2$	$1.9 X 10^3$	
Main Point 1 Sabo	$1.3 X 10^2$ - $3.0 X 10^3$	$1.9 X 10^3$	
Upper Point 2 Sabo	$0.9 X 10^2$ - $2.2 X 10^2$	$1.6 X 10^3$	$1.8X10^3$
Lower Point 3 Sabo	$1.9 X 10^2$ - $2.3 X 10^3$	$2.1 X 10^3$	
Main Point 1 Kakuri	$2.2x10^2$ - $2.8x10^3$	$2.2x10^3$	
Upper Point 2 Kakuri	$1.9x10^2$-$2.4x10^3$	$2.1x10^3$	$2.1X10^3$
Lower Point 3 Kakuri	$2.1x10^2$-$2.5x10^3$	$2.2x10^3$	

Key: CFU= colony forming unite, point 1 main point of effluent discharge; point 2 upper stream flow; point 3 downstream (upper plus effluent point) In each means value, the values showed no statistically significant difference between the downstream and the point of discharged at ($p<0.05$).

Table 2: Morphological and Biochemical analysis of water samples obtained from selected point of Kawo abattoir

SAMPLE Point	Form	Colour	Gram stain	Cat	Oxi	IND	Ae/ An	*Bacteria*
Pt 1, 2, 3	Circular	Whitish	-	+	-	+	F	*E. coli*
Pt 1,& 3	Circular	Cream	-	+	-	-	F	*Proteus* sp.
Pt 1 & 3	Circular	Yellow	-	+	-	+	F	*Salmonella* sp.
Pt 1 & 3	Irregular	Cream	-	+	-	-	F	*Klebsiella* sp.
Pt 1 & 3	Circular	White	-	+	-	-	F	*Enterobacter aerogenes*
Pt 1,2,3	Circular	Yellowish	+	+	-	-	F	*Staphylococcus* sp.
Pt 1 & 3	Circular	White	-	+	-	+	F	*Shegalla* sp.

Key:
Pt= point, OXI= Oxidase, CAT= Catalase Test, IND = Indole Test, Ae/An= aerobic and anaerobic, F = Facultative

Table 3: Morphological and Biochemical analysis of water samples obtained from selected points of Kakuri abattoir

SAMPLE Point	Form	Colour	Gram stain	Cat	Oxi	IND	Ae/ An	Bacteria
Pt 1 & 3	Circular	Whitish	-	+	-	+	F	*E. coli*
Pt 1,2,3	Circular	Cream	-	+	-	-	F	*Proteus* sp.
Pt 1 & 3	Circular	Yellow	-	+	-	+	F	*Salmonella* sp.
Pt 1 & 3	Irregular	Cream	-	+	-	-	F	*Klebsella* sp.
Pt 1 & 3	Circular	White	-	+	-	-	F	*Enterobacter aerogenes*
Pt 1,2,3	Circular	Yellowish	+	+	-	-	F	*Staphylococcus* sp.
Pt 1 & 3	Circular	White	-	+	-	+	F	*Shegalla* sp.

Key:
Pt = point, OX= Oxidase, CAT= Catalase Test, IND = Indole Test, Test, Ae/An= aerobic and anaerobic, F = Facultative

Table 4: Morphological and Biochemical analysis of water samples obtained from selected point of Sabo abattoir

SAMPLE Point	Form	Colour	Gram stain	Cat	Oxi	IND	Ae/ An	*Bacteria*
Pt 1 & 3	Circular	Whitish	-	+	-	+	F	*E. coli*
Pt 1,2,3	Circular	Cream	-	+	-	-	F	*Proteus* sp.
Pt 1 & 3	Circular	Yellow	-	+	-	+	F	*Salmonella* sp.
Pt 1 & 3	Irregular	Cream	-	+	-	-	F	*Klebsella* sp.
Pt 1 & 3	Circular	White	-	+	-	-	F	*Enterobacter aerogenes*
Pt 1, 2 ,3	Circular	Yellowish	+	+	-	-	F	*Staphylococcus* sp.
Pt 1, 2, 3	Circular	White	-	+	-	+	F	*Shegalla* sp.

Key:
Pt= point, OXI= Oxidase, CAT= Catalase Test, IND = Indole Test, Test, Ae/An= aerobic and anaerobic, F = Facultative

DISCUSSION

Environmental Protection Agency's (2012) state that for maximum contaminant level (MCL) for coliform bacteria in drinking water is zero (no) total coliform per 100mL of water. The number of bacteria colonies found in the incubated effluent water sample showed that the water did not meet the EPA bacteriological standard of zero. At this time, excessive number of other bacteria in a sample interferes with the counting of coliform types. The effluent water samples from the three abattoirs were highly contaminated showed in table 1 rages from 3.0×10^3- 0.9×10^2. This is an indication of contamination of receiving water bodies with abattoir discharged waste water. Similar findings have been reported in other places which include the works of Nafarnda *et al*., (2012), Olaiya *et al*. (2016), Adie *et al*. (2017), Shukri *et al*. (2017) and Njoku *et al*. (2018) who also identified bacterial pathogens in effluent samples from abattoirs. The total coliform in the effluent water samples at point of discharged and at downstream were in exceeded, the limits discharge from industrial effluent into surface waters as set by the Federal Environmental Protection Agency (1999) and Environmental Protection Agency (EPA) (4.0×10^2CFU/mL or 400MPN/100mL) This may be due to the fact that abattoir workers in the study area discharge untreated waste from dressed animals into the water body. This result is in conformity with the findings of Narfanda *et al.* (2012) and Olaiya *et al*. (2016) the presence of these bacteria in intolerable number obviously constitute a serious public health hazard as the presence of these microorganisms is associated with water borne diseases since the waste is discharged into the streams (Olaiya *et al*. 2016).

Seven bacteria species which were characterized and identified from the sites namely *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., *Shigella* sp. and *Preteus* sp. obtained from three abattoirs (Table 2-4), which are indicators of presence of pathogenic and opportunistic microorganisms. The presence of these microorganisms in the three points could be as a result of indiscriminate disposal of untreated abattoir effluents into water bodies by the abattoir workers in these areas. These consist of substance from animals which in turn are hosts to these microorganisms. Similar findings were reported by Ojekunle and Lateef (2017), Kwadzah, and Iorhemen (2015), Njoku *et al*., (2018), Shukri *et al*., (2017); Tekenah *et al*., 2014, Deborah *et al*., (2014), and Sumayya *et al*., (2013).This disagrees with the result of a study conducted in Egypt by El-Gamal and EL-Bahi, 2016 who reported 0% *E coli* and other bacterial from abattoir environmental samples investigated.

No significant difference ($P < 0.0\ 5$) between the means count of total coliform count of abattoir effluent water discharged into the receiving water body from the main point and downstream of the flow where is the point three, although the receiving water bodies at 350m downstream are mixture of effluent and upstream flow which was observed to have almost the same numbers of counts with the point of discharged or main point. This is due to untreated waste water discharge from the abattoir into the flowing water, that showed higher level of bacterial count compared to the upstream flowed which showed less level.

Faecal coliform count was also higher in the abattoir effluent than at other sampling point which was indicative of the contributory effect of the abattoir waste in generalized increase in bacterial contamination of water bodies. Faecal coliforms live in the digestive tract of warm blooded animals and their counts are often used as a surrogate measurement for gastro enteric pathogens. Presence of faecal coliforms in water is evidence that human or animal waste is present in the water. This is a cause of concern as many diseases can be spread through the faecal-oral route. Faecal coliforms in abattoir waste into water have earlier been reported by some researchers like Adebowale *et al.* (2016); Adesina *et al.* (2018) *Escherichia coli*, a major indicator organism for faecal contamination, was also detected in effluent water samples from this work. The discharge of untreated abattoir wastewater could result in an outbreak of *E. coli* infection, as similarly observed by (Olaiya *et al*. 2016) but in disagreement with El-Gamal *et al*. *(*2016).

Conclusion

Enumeration of bacteria in the water samples obtained from three abattoirs in Kaduna metropolis shows that the bacterial load ranges from 0.9×10^2 to 3.0×10^3 (CFU/mL) bacterial counts which is above the recommended level by EPA and FEPA. Wastewater discharged into the water body contains bacteria such as *E coli, Staphylococcus aureus*, *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., *Shigella* sp.*,* and *Preteus* sp. There is need to study the ecological implication of these bacteria.

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