



## Bacteriological Analysis of Water used in Abattoirs within Ilorin metropolis, Kwara State, Nigeria

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*Date Received:* 10-03-2022

*Date Accepted:* 13-05-2022

**DOI:** <https://doi.org/10.48198/NJPAS/22.A09>

### ABSTRACT

Water that is used in the abattoir for meat processing, cleaning procedures, drinking and other activities must meet international standard for potable water. It must not contain chemicals, hard physical components or microorganisms in amounts high enough to cause hazards to health. The bacteriological status and risk posed by the water used in four government owned abattoirs in Ilorin, Kwara State, Nigeria, were evaluated in this study. The bacteriological examination of samples of water from available sources in abattoirs in the study areas were carried out to determine their suitability for use and potability. Twenty-six samples were collected both before (BA) and during (DA) abattoir activities. The pour plate technique was used to ascertain the Total Viable Bacterial Count (TVC) of collected water samples. The Estimated Number of Coliform Count (ECC) was determined using MacConkey broth. The values of TVC and ECC for all the samples ranged from  $2 \times 10^6$  to  $4.8 \times 10^7$  CFU/mL This exceeded the  $1 \times 10^2$  CFU/mL limit recommended by WHO for potable water. A total of 52 isolates belonging to 8 bacteria species were identified; 2 *Acinetobacter* spp. (3.8%), 14 *Bacillus* spp. (26.4%), 3 *Citrobacter* spp. (5.7%), 3 *Enterobacter* spp. (5.7%), 12 *Salmonella* spp. (22.6%), 4 *Shigella* spp. (7.6%), 9 *Staphylococcus* spp. (17%) and 6 *Proteus* spp. (11.2%) were isolated from the water samples. Results showed that water samples from the study areas had high bacterial load, thus posing high public health and food safety risks especially to the public that consume on meat processed with the water from these abattoirs. Governmental/non-governmental organizations and spirited individuals should urgently address the issue of provision of adequate and safe water for abattoir activities in addition to providing facilities for water treatment so as to safeguard the health of the populace. Consumers should properly wash meat and meats products after purchase, boiling, steaming, frying and proper cooking of meat after purchase and before consumption are highly recommended.

**Keywords:** Bacteriological Analysis, Water, Abattoirs

### Introduction

An abattoir is defined as any registered facility that is responsible for the conversion of food animals to meat through slaughtering process (FAO/WHO, 1993). The animals involved include livestock, poultry and special classes of animals like games

and crocodiles. To supply meat for human consumption, slaughtering animals like cattle, sheep, goats, pigs, poultry, camels, donkeys and horses are common practices in our community. The slaughtering process remains critical in

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ensuring that consumers receive hygienically safe products (FAO, 2021).

Good quality/potable water does not contain chemical substances or disease-causing micro-organisms in amount that is deleterious to human health and wellbeing (Adeyemo *et al.*, 2002, Oludairo *et al.*, 2020). Water has diverse physical, microbiological and chemical characteristics resulting in varying quality of different water samples (FAO, 2021). The ability to provide potable water that is adequate is important in the hygienic processing of wholesome meat which is safe and fit for human consumption (Olugasa *et al.*, 2000, Oludairo *et al.*, 2020).

Water is essential in the abattoir for workers' personal use, consumption of animals to be slaughtered, washing of meat, skins, hides that are being processed and routine general cleaning of the abattoir (Adeyemo *et al.*, 2002). International standards should be met by water used in the different abattoir processes and activities so that they do not introduce and constitute health risk to the populace. Poor quality water or polluted water coming in contact with meat has been found to contaminate meat and could serve as vehicles for transmission of major enteric diseases such as Cholera, Typhoid, Hepatitis, Amoebic and bacillary dysentery (WHO, 1996). There are publications that indicate that zoonotic diseases are yet to be eliminated or fully controlled in over 80% of the public abattoirs in Nigeria (Cadmus *et al.*, 1992; Olugasa *et al.* 2000). Inadequate ante and post-mortem inspection of slaughtered animals, substandard water quality, inadequate cleaning and sanitation, poor personal hygiene, improper disposal of abattoir waste and spillage of abattoir waste/contaminants to the environment may be the reason for the persistence of these diseases (Ogbomida *et al.*, 2016).

Water quality describes the physical, chemical, biological and aesthetic properties of water. The quality of water determines its fitness for a variety of use including consumption by man and animal and therefore ensure the protection of the health of

consumers and the public (Luvhimbi *et al.*, 2022). Levels of water qualities are assessed in terms of physical characteristics like color, taste, temperature, turbidity, hardness and smell or odour as well as amount and types of micro-organism presents such as bacteria, protozoa and algae parameters (WHO, 2011, Neboh *et al.*, 2013).

Water is indispensable in abattoir operations, thus, water to be used must be clean, safe and free from microbial contamination because water plays significant role in disease transmission (FAO/WHO, 1993). Bacteriological examination of water is therefore a powerful and foremost indicator tool to determine the presence of microorganisms that might constitute health hazard (Rabah *et al.*, 2008). Microorganisms commonly used as indicators of water quality include: coliforms, Faecal *streptococci*, *Clostridium perfringens* and *Pseudomonas aeruginosa* (Adeyemo *et al.*, 2002, Tyagi *et al.*, 2013).

Bacteria, viruses, parasites and other pathogens present in contaminated water have been responsible for water borne diseases including gastroenteritis, cholera, typhoid fever, giardiasis, hepatitis and cancer (Waage, 1999, Falkenmark, 2020).

In contrast to chemical pollution that results in long-range pollution of groundwater over time, micro-biological pollution of groundwater source has an immediate effect on large number of people. Microbial pollution can be equally detrimental to health and well-being of both plants and animals (Olugasa *et al.* 2000, Adetunde and Glover, 2010, Kaper *et al.*, 2014).

This study investigated the possible bacteria group that may be found in the water supply to the abattoir and the safety/ potability of water used in the abattoir.

## **Materials and Methods**

### ***Sample design***

A cross sectional study design was employed for the study. Samples were collected from all government abattoirs in Ilorin, Kwara State for

microbial detection. The abattoirs are: Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata (8°52'75"N, 4°55'27"E); Baboko/Oja-Tuntun Abattoir (8°48'79"N, 4°53'80"E); Ipata-Opomalu Slaughter slab (8°50'024"N, 4°56'381"E) and Mandate Slaughter slab, Adewole (8°47'799"N, 4°50'421"E). The field study was carried out from January to December 2020.

### **Sample collection**

One hundred mL of water samples were randomly collected from borehole and well water present at the study locations. A total of 26 water samples were collected and analyzed for their bacteriological status using media produced by Oxoid® *Basingstoke UK*. They were collected before the commencement of abattoir activities and towards the end of work. Four and 2 water samples were collected during abattoir activity from borehole and well respectively at Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata. Eight and 2 samples were collected at Baboko abattoir from borehole and well respectively, 5 samples each were collected before and during abattoir activities. At Ipata abattoir, 2 samples were collected from borehole during abattoir activities. Six and 2 samples were collected from borehole and well respectively, at Mandate abattoir. Four samples each before and during abattoir activities. Twenty (20) samples were collected from borehole while 6 were collected from well for this study. Six (23.1%) were collected from Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata; 10 (38.4%) from Baboko abattoir, 2 (7.7%) samples were collected from Ipata slaughter slab while 8 (30.8%) were collected from Mandate abattoir (Table 1).

For collection of tap water sample, the tap was allowed to run for about 2-3min before collection into sterile universal sample bottle. Samples from wells were collected using the abattoir well fetcher to draw out water from the well into sterile sample bottles. Collected samples were well labelled, placed in cool box with ice pack and then

transported to the University of Ilorin Veterinary Public Health and Preventive Medicine Laboratory for microbiological analysis (Adebowale *et al.*, 2010).

### **Protocol of the test**

This was carried out according to Adebowale *et al.* Serial dilutions of 1:10 were used for water samples collected in the study. A 10-fold serial dilution of  $10^{-6}$  and  $10^{-4}$  for normal saline and MacConkey broth respectively were performed as the dilution solution for standard plate count and total coliform count. Using a sterile 10 mL syringe, 9 mL of each test water sample solution were dispensed into each test tubes respectively. One milliliter of sample solution was dispensed into the first test tube containing 9 mL of the diluent and serially diluted up to the last test tube ( $10^{-6}$  and  $10^{-4}$ ) (Adebowale *et al.*, 2010).

### **Inoculation of samples on spread plate method**

Plate count agar (CM0463 Oxoid®) was prepared, allowed to cool and then poured into agar plate. For each agar plate an inoculum of 0.1 mL of the serial dilution of normal saline dilution was dispensed and spread gently over the agar surface. The last two dilution,  $10^{-5}$  and  $10^{-6}$  were plated to have two plates per sample so that the mean of the count could be obtained. Thereafter, the inoculated plates were incubated at 37°C for 24 hours in the incubator.

### **Inoculation of sample into peptone water**

1mL of each sample was dispensed into 5mL of peptone water, it was well capped and thoroughly mixed and then incubated overnight at 37°C.

### **Inoculation of sample into Rappaport broth**

1mL of each sample was dispensed into 5mL of Rappaport broth and incubated at 37°C for 24 hours.

### **Inoculation of cultured sample from peptone water**

MacConkey Agar, Eosin methylene blue Agar and Mannitol salt agar were prepared according to

manufacturer's instructions. Using sterile inoculating wire loop, cultured broth were streaked onto each agar while maintaining a sterile procedure. All samples inoculated on MacConkey agar and Eosin methylene blue Agar were incubated at 37°C and 44°C respectively for 24 hours for TCC and TBC counts while those in Mannitol salt agar were incubated for 18-24 hours at 37°C (Adebowale *et al.*, 2010).

#### *Inoculation of cultured samples from Rappaport broth.*

Using a sterile inoculating wire loop, cultured samples from Rappaport broth were streaked on *Salmonella-Shigella* agar (SSA) plates and incubated at 37°C for 24 hours.

#### *Colony counting and calculations of CFU/mL and log (CFU/ mL)*

After incubation, each cultured plate count agar was counted to determine the number of viable colonies forming units (CFU). This was carried out using the electronic colony counter and all bacteria counts were done according to the standard prescribed by ISO 4833 and ISO 21528-2.

Calculation of the number of bacteria (CFU) per milliliter of sample was done by dividing the number of colonies by the product of the dilution factor and the volume of inoculum.

Hence, the CFU/ mL was calculated using the formula by Bari and Yeasmin, 2022:

$$\text{CFU/mL} = (\text{number of colonies}) / (\text{dilution factor} \times \text{volume of inoculum})$$

Thereafter, the log<sub>10</sub> CFU/mL for each sample was estimated. Data collected were analyzed using frequency count and percentages.

#### *Sugar fermentation*

Sugar fermentation tests were carried out on presumptive isolates as part of the biochemical test for the purpose of differentiation and identification of organisms. Glucose, galactose, fructose, sucrose

maltose and xylose were the sugar used for the tests, phenol-red was used as indicator.

## **Results**

A total of twenty-six (26) water samples were collected from different sources during the period of study. Out of these samples, two (2) (3.8%) were positive for *Acinetobacter spp.*, fourteen (14) (26.4%) were positive for *Bacillus spp.*, three (3) (5.7%) were positive for *Citrobacter spp.*, three (3) (5.7%) were positive for *Enterobacter spp.*, twelve (12) (22.6%) were positive for *Salmonella spp.*, six (4) (7.6%) were positive for *Shigella spp.*, nine (9) (17%) were positive for *Staphylococcus spp.*, and six (6) (11.2%) were positive for *Proteus* species. The Mean Total Bacterial Count of borehole water sample used before abattoir activities were;  $1.6 \times 10^7$  for Baboko and  $1.7 \times 10^7$  for Mandate Abattoir respectively. While the value during abattoir activities were  $7.5 \times 10^6$  for Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata,  $2.3 \times 10^7$  for Baboko and  $5.5 \times 10^6$  for Ipata,  $1.2 \times 10^7$  was recorded for Mandate abattoir (Table 3). The Mean Total Coliform Estimation of water sample collected before abattoir activities were Baboko  $7.38 \times 10^3$ , Mandate  $5.125 \times 10^3$  and Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata  $1.5 \times 10^2$  while the values during abattoir activities were Baboko  $1.0027 \times 10^4$ , Ipata  $5 \times 10^2$  and Mandate  $4.025 \times 10^3$  (Table 4).

Cultural characteristic, staining reaction and biochemical activities of isolates were used in the identification of bacteria isolated from water samples collected.

Table 2 indicated that eight (8) different bacteria were isolated from the 26 samples analyzed. *Bacillus spp.* and one (1) *Staphylococcus spp.* were isolated from two (2) borehole samples collected from Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata while one (1) out of the two well water sample collected contained *Bacillus spp.*

Six (6) out of the seven (7) bacteria isolated were seen in borehole water from Baboko abattoir while the well water sample contained *Bacillus* spp., *Enterobacter* spp., *Salmonella* spp., *Shigella* spp. and *Staphylococcus* spp.

Borehole water samples from Ipata slaughter slab contained *Bacillus* spp, *Salmonella* spp, *Shigella* spp and *Staphylococcus* spp.

Borehole water samples from Mandate abattoir contained *Bacillus* spp, *Citrobacter* spp, *Enterobacter* spp, *Salmonella* spp and *Staphylococcus* spp while well water sample contain *Acinetobacter* spp, *Citrobacter* spp, *Proteus* spp. and *Citrobacter* spp.

Table 3 shows the total bacterial count of  $3.0 \times 10^7$  and mean bacterial count of  $7.5 \times 10^6$  in borehole water sample collected from Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata during abattoir activities while well water sample analyzed contained a total of  $4.4 \times 10^7$  and a mean bacterial count of  $2.2 \times 10^7$ . Water sample was collected before abattoir activities in Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata.

Borehole water sample collected from Baboko before abattoir activities contained a total of  $6.4 \times 10^7$  TBC and a MTBC of  $1.6 \times 10^7$  while during abattoir activities contained a total of  $9.22 \times 10^7$  and  $2.3 \times 10^7$  MTBC. Also, the only well water sample collected before and during activities (due to of restricted access to the sources of water) contained TBC  $2 \times 10^6$ , MTBC  $2 \times 10^6$  and TBC  $4 \times 10^6$ , MTBC  $4 \times 10^6$  respectively.

From Ipata only borehole water sample were obtained during activities and it contained  $1.0 \times 10^1$  TBC and  $5.5 \times 10^6$  MTBC.

A TBC of  $6.8 \times 10^7$  and MTBC of  $1.7 \times 10^7$  were seen in water sample collected from borehole at Mandate abattoir before activities while TBC of  $4.8 \times 10^7$  and MTBC of  $1.2 \times 10^7$  were seen during abattoir activity while of  $8 \times 10^6$  and  $1.8 \times 10^7$  TBC and MTBC were seen before and during abattoir activities respectively.

Table 4 indicated a total coliform estimation of  $2 \times 10^2$  in all four (4) borehole water samples collected from Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata during activities with a mean of  $5 \times 10^1$  coliform estimation while the well water samples collected during activities showed a coliform estimation of  $2 \times 10^2$  mean coliform estimation of  $1 \times 10^2$ .

Coliform estimation of  $1.02 \times 10^4$  with a mean value of  $6.38 \times 10^3$  was seen in borehole water collected before abattoir activities in Baboko while an estimation of  $1 \times 10^4$  and mean value of  $2.75 \times 10^1$  were recorded during abattoir activities. In well water samples coliform estimation of  $1 \times 10^3$  and mean value of  $1 \times 10^4$  were seen before activities while estimation of  $1 \times 10^4$  and mean value of  $1 \times 10^3$  was recorded during activities in Baboko abattoir.

The borehole water sample collected from Ipata during activity had a coliform estimation of  $1 \times 10^1$  and mean value of  $5 \times 10^0$ .

Borehole water samples collected before activities at Mandate abattoir contained an estimated coliform of  $2.01 \times 10^4$  with average of  $5.025 \times 10^3$  count while the samples collected during abattoir activities was  $1.21 \times 10^4$  and mean value was  $3.025 \times 10^3$ . Well water sample collected before abattoir activities contained  $1 \times 10^2$  coliform count while an estimated count of  $1 \times 10^2$  was seen in sample collected during abattoir activities.

**Table 1:** Water samples collected for bacteriological analysis before and during abattoir activities in Ilorin metropolis

S/N	Abattoir	Source of water	Number of samples collected		Total
			Before Abattoir Activities (BA)	During Abattoir Activities (DA)	
1.	Akerebiata	Borehole	*0	4	4
		Well	*0	2	2
2.	Baboko	Borehole	4	4	8
		Well	1	1	2
3.	Ipata	Borehole	*0	2	2
		Well	*0	0	0
4.	Mandate	Borehole	3	3	6
		Well	1	1	2
Total			9	17	26

\*Authors were denied access to the source of water before abattoir activity

**Table 2:** Total bacteria (those isolated Before and during abattoir activity) from water samples collected from various abattoirs within Ilorin Metropolis

S/N	Abattoir	Source of water	Sample collected	<i>Acinetobacter</i> spp	<i>Bacillus</i> spp.	<i>Citrobacter</i> spp.	<i>Enterobacter</i> spp.	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Staphylococcus</i> spp.	<i>Proteus</i> spp
1.	Akerebiata	Borehole	4	-	2	-	-	-	-	1 (0, 1)	-
		Well	2	-	1	-	-	-	-	1 (0, 1)	-
2.	Baboko	Borehole	8	2 (1, 1)	3 (1, 2)	2 (1, 1)	-	6 (3, 3)	1 (1, 0)	3 (1, 2)	-
		Well	2	-	1 (1, 0)	-	2 (1, 1)	2 (1, 1)	1 (0, 1)	1 (0, 1)	-
3.	Ipata	Borehole	2	-	1	-	-	1 (1, 0)	1 (0, 1)	1 (0, 1)	-
		Well	0	-	-	-	-	-	-	-	-
4.	Mandate	Borehole	6	-	4 (2, 2)	1 (0, 1)	1 (1, 0)	2 (1, 1)	-	2 (1, 1)	4 (2, 2)
		Well	2	-	2 (1, 1)	-	-	-	1 (0, 1)	-	2 (1, 1)
Total (%)			26	2 (3.8)	14 (26.9)	3 (5.8)	3 (5.8)	11 (21.2)	4 (7.7)	9 (17.3)	6 (11.5)

**Table 3:** Total and Mean Bacterial count in analyzed water samples collected before and during abattoir activities within Ilorin Metropolis.

S/N	Abattoir	Source of water	No of sample collected		TBC		MTBC	
			BA	DA	BA	DA	BA	DA
1.	Akerebiata	Borehole	0	4	0	$3.0 \times 10^7$	0	$7.5 \times 10^6$
		Well	0	2	0	$4.4 \times 10^7$	0	$2.2 \times 10^7$
2.	Baboko	Bore hole	4	4	$6.4 \times 10^7$	$9.22 \times 10^7$	$1.6 \times 10^7$	$2.3 \times 10^7$
		Well	1	1	$2 \times 10^6$	$4 \times 10^6$	$2 \times 10^6$	$4 \times 10^6$
3.	Ipata	Borehole	0	2	0	$1.0 \times 10^1$	0	$5.5 \times 10^6$
		Well	0	0	0	0	0	0
4.	Mandate	Borehole	3	3	$6.8 \times 10^7$	$4.8 \times 10^7$	$1.7 \times 10^7$	$1.2 \times 10^7$
		Well	1	1	$8 \times 10^6$	$1.8 \times 10^7$	$8 \times 10^6$	$1.8 \times 10^7$
Total			9	17				

BA- Before abattoir activities, DA- During abattoir activities, TBC- Total bacterial count, MTBC- Mean Total Bacterial Count. W.H.O. recommendation: of  $1.0 \times 10^2$  CFU/mL

**Table 4:** Mean and Total Coliform Count in water samples collected in abattoirs before and during activities within Ilorin Metropolis.

1.	Abattoir	Source of water	No of sample collected		TCC		MCC	
			BA	DA	BA	DC	BA	D/A
	Akerebiata	Borehole	0	4	0	$2 \times 10^2$	0	$5 \times 10^1$
		Well	0	2	0	$2 \times 10^2$	0	$1 \times 10^2$
2.	Baboko	Bore hole	4	4	$1.02 \times 10^4$	$1.1 \times 10^2$	$6.38 \times 10^3$	$2.75 \times 10^1$
		Well	1	1	$1 \times 10^3$	$1 \times 10^4$	$1 \times 10^4$	$1 \times 10^3$
3.	Ipata	Borehole	0	2	0	$1 \times 10^1$	0	$5 \times 10^0$
		Well	0	0	0	0	0	0
4.	Mandate	Borehole	3	3	$2.01 \times 10^4$	$1.21 \times 10^4$	$5.025 \times 10^3$	$3.025 \times 10^3$
		Well	1	1	$1 \times 10^2$	$1 \times 10^2$	$1 \times 10^2$	$1 \times 10^3$

BA- Before abattoir activities, DA- During abattoir activities, TCE- Total coliform estimate, MCE- Mean coliform estimate. W.H.O. recommendation: of  $1.0 \times 10^2$  CFU/mL

**Discussion**

The study was conducted to determine the bacteria load of water used in various abattoir within Ilorin metropolis. Water of good drinking quality should comply with certain physical, chemical and microbiological standards, which are designed to ensure that water is potable and safe (Harris, 2010).

The standards for potable water used in abattoirs are usually measured in terms of number of coliform and or number of coli per 100mL of water. Microbiological examinations of water samples are meant to determine the sanitary

quality of the water and its level of contamination with waste

Water samples collected from different sources of supply to the abattoir did not comply with international limits of bacterial load for drinking water as recommended by W.H.O. (1999). All values obtained in this study were higher than the W.H.O. recommendation of  $1.0 \times 10^2$  CFU/mL and 2 coliform count/100mL.

*Bacillus spp*, has the highest percentage prevalence of 50% from borehole water sample collected during abattoir activities at Dr. Abubakar Sola Saraki Ultra-Modern Memorial Abattoir, Akerebiata and Baboko while

*Acinetobacter spp* had 25% prevalence from borehole water sample collected before and during abattoir activities (Table 2).

The presence of *Enterobacter spp.*, *Salmonella spp.*, as well as other organisms suggest unhygienic practices especially due to contamination during abattoir activities which may lead to contamination of borehole pipe, reservoirs as well as water sources indicating the level of microbial diversity in the abattoir environment due to poor hygienic practices employed by abattoir workers.

In water piped supplies, the likelihood of contamination increases as the risk of back siphon-age into the distribution network increase when pipes are at lower pressure than the surrounding soil, which often contains leaked out effluents from leaking sewages (Malta, 2014).

The quality of the water consumed by animal and man is critical in controlling infectious diseases and other health problems (Odeyemi *et al.*, 2012). The conservation of water sources is very important to provide safe/potable water. As much as possible, water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral, protozoan and parasites (Tyagi *et al.*, 2013).

### **Conclusion and Recommendations**

Water supplied for meat processing in the abattoirs used for this study is grossly contaminated and could serve as vehicle for the transmission of infectious diseases to the public. There was high presence of bacteria in borehole water samples collected from various abattoirs within Ilorin metropolis during the period of study. Presence of the pathogenic organisms and indicator organisms of faecal contamination (coliform) makes the water unsafe to consumers' health. Government should address the problem of inaccessibility of the public to adequate and safe drinking water since every country is

working towards achieving the millennium development goal of safe water for all by year 2025. Proper drainage facilities for abattoir effluents should be provided so as to prevent waste spills that can introduce enteric pathogens and excess nutrients into surface water and contamination of the ground waters. Also, proper cleaning of tanks, covering of available wells and water treatment before use should be practiced. Routine microbial quality evaluation should be conducted for all the water supplies used by the public and at the abattoirs. Dissemination of the results of studies like this to abattoir workers should be done to enhance safe and hygienic practices so as to reduce human contamination of water and other products from the abattoir. Water quality control measures should be practiced in other to minimize bacterial load associated with sources water in the abattoir. In actual practice no water has been found to be absolutely bacteria free.

### **Conflict of Interest**

The authors hereby state that there is no existing conflict of interest.

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