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Bacteriological Quality of Water Supplies Utilized in Abattoirs Located in Benin City, Ekpoma and Auchi Municipalities n Edo State, Mid-Western Nigeria

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ABSTRACT

Slaughtering and sanitation activities within abattoirs in Edo State are entirely dependent on a steady supply of presumed clean water. These sources include; groundwater, surface water and precipitation. The heterotrophic, total coliform and *Escherichia coli* counts of sampled raw water supplies used by fifteen slaughter-houses in Benin City, Ekpoma and Auchi municipalities was determined using routine procedures which included serial dilution and multiple tube methods. Duplicate water samples were collected from each establishment once monthly for a period of two seasons (November, 2019, April to August 2020, February to March, 2021). The mean bacteriological counts were subjected to one-way ANOVA and Duncan multiple range tests at 95% probability level. Seasonal variations were analyzed using Mann Whitney test (α = 0.05).The overall mean heterotrophic bacterial and total coliform counts ranged from $9.0 \times 10^4 \pm 8746$ cfu/ml to $1.7 \times 10^5 \pm 13422$ cfu/ml, $1.7 \times 10^5 \pm 19957$ cfu/ml and $1.7 \times 10^5 \pm 5247$ cfu/ml and 83 ± 52 MPN/100 ml to 176 ± 5.2 MPN/100 ml. The variations in these mean values were statistically significant (F=9.275, F=3.664, $p<0.05$). Eight bacterial isolates were tentatively identified and they include; *E. coli*, *Acinetobacter* sp., *Bacillus* sp., *Klebsiella mobilis*, *Micrococcus* sp. *Citrobacter* sp., *Serratia marcescens*, *Enterobacter* sp. and *Staphylococcus aureus*. The detection of coliforms rendered the raw water unfit for both carcass preparation and sanitation purposes by the respective establishments.

Keywords: Bacteriological, Coliform, Culturable, Edo State, Slaughter-house, Water supplies

Introduction

Government licenced abattoirs usually utilize varying amounts of water emanating from various sources for various purposes ranging from carcass dressing to sanitation activities. The sources of these water supplies include; underground aquifers, surface water and precipitation. As such, these facilities are entirely reliant on a steady supply of water located at a close proximity to the slaughtering hall. It has been observed that water is utilized in facilitating the movement of animal derived wastes from the drains to nearby receptacles.[1] For slaughter houses in Edo State, these nearby receptacles vary from surface water bodies, storm water drainages and terrestrial areas. Meat processing facilities in Edo State depend on several water sources such as groundwater, flowing surface water and precipitation for water supplies.

Irrespective of the fact that minimally processed beef cuts emanating from these facilities are cooked prior to consumption by consumers, there is a need to ascertain the microbial quality of raw water utilized in the preparation of these raw meat cuts in these slaughter-houses. It has been opined that bacteriological evaluation of water is a relevant and important approach in determining the presence of microorganisms that could constitute a public health risk.[1,2]

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Examples of prokaryotes routinely utilized as water quality indicators include; coliforms exemplified by *Escherichia coli* and *Klebsiella* spp.[2] It has been stated that an understanding of the causes of fluctuations with respect to the microbial quality of raw water quality is critical, as it will directly affect the need for treatment, treatment efficiency and the resultant health risk linked with the treated water.[3] Generally, the microbial quality of raw water is impacted by both natural and anthropogenic utilization factors. Examples of natural factors include; wildlife, climate, topography, geology and vegetation.[3] Anthropogenic utilization factors entail point sources such as municipal and industrial effluent discharges and non-point sources which include; urban and agricultural runoffs.[3]

Against the backdrop of the importance of the bacteriological quality of raw water supplies, the main objective of this study was to assess the bacteriological profiles of sampled raw water supplies within these establishments.

Materials and Methods

Selection of sample locations

The three municipalities; Benin City, Ekpoma and Auchi serve as a major municipality in the three geopolitical areas of Edo State; Edo South, Edo Central and Edo North. Also, these municipalities are the largest in terms of population density in each of the zones.[4] A total of fifteen functional abattoirs in the three urban areas gave consent for water sample collection. Of this number, twelve were located in Benin, one in Ekpoma and two in Auchi municipality (Table 1, Figure 1, 2 and 3).

Collection of water samples

Sterile 2 litre plastic containers were utilised in the collection of representative water samples from the respective slaughter-houses. Duplicate samples of ground water, well water and surface water as utilised by abattoir workers in preparing and dressing bovine carcass

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were collected from each abattoir once monthly for a period of four months per season for a period of two seasons (November, 2019, April to August 2020, February to March, 2021). The facilities in Benin City utilized ground water stored in plastic tanks while the visited facility used well water stored in a single well. Workers in Abattoir AA used ground water stored in plastic tanks. The coordinates of the stream which served as the only source of water to abattoir J was N07˚ 06.038'E006˚ 16.233'. The samples were collected using labeled five liter sterile plastic containers and were kept in coolers filled with ice packs on route to the laboratory. Prior to bacteriological analysis in the laboratory, the samples were preserved *via* refrigeration.

Bacteriological analysis

The water samples were serially diluted up to 10^{-4} and 1 ml aliquots were plated under aseptic conditions.[5]

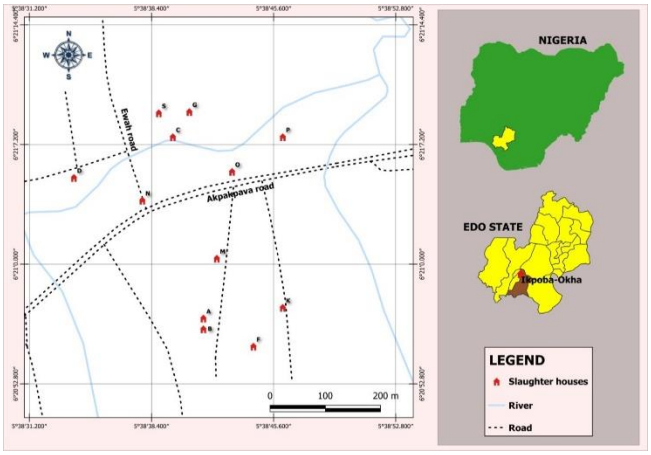

Figure 1: Map of Benin City showing the visited slaughter-houses.

KEY: A: Privately owned slaughter-house A, B: Privately owned slaughter-house B, C: Privately owned slaughter-house C, D: Privately owned slaughter-house D, F: Privately owned slaughter-house F, G: Privately owned slaughter-house G, K: Government owned slaughter-house K, M: Privately owned slaughter-house M, N: Privately owned slaughter-house N, O: Privately owned slaughter-house O, P: Privately owned slaughter-house P, S: Privately owned slaughter-house S

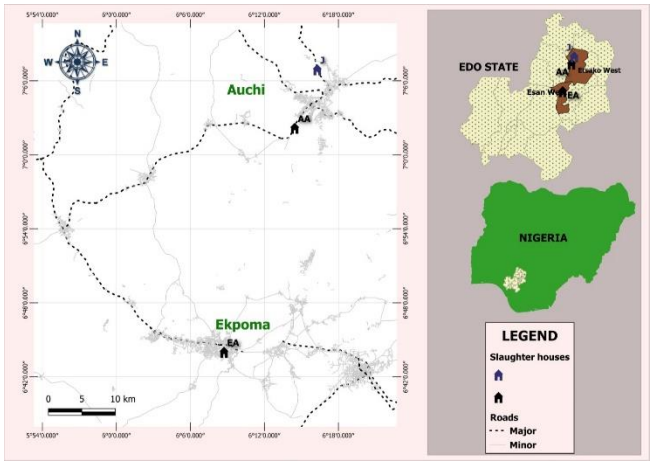

Figure 2: Map of Ekpoma and Auchi showing the slaughter-houses.

KEY: EA: Government owned slaughter-house EA, AA: Government owned slaughter-house AA:, J: Privately owned slaughter-house J

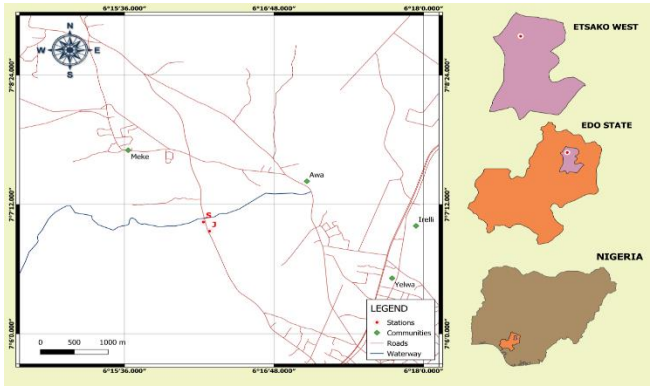

Figure 3: Locational map indicating the geographical position of the stream utilised by privately owned slaughter-house J workers as source of water for carcass washing and sanitary activities

KEY: S: Nearby stream serving as water source for privately owned slaughter-house J, J: privately owned slaughter-house J

Table 1: Location of the abattoirs with their coordinates

Facility	Municipality	Latitude	Longitude
Privately owned slaughter-house A	Benin City	N06˚ 20.946'	E005˚ 38.691'
Privately owned slaughter-house B	Benin City	N06˚ 20.935'	E005˚ 38.691'
Privately owned slaughter-house C	Benin City	N06˚ 21.128'	E005˚ 38.661'
Privately owned slaughter-house D	Benin City	N06˚ 21.087'	E005˚ 38.564'
Privately owned slaughter-house F	Benin City	N06˚ 20.918'	E005˚ 38.722'
Privately owned slaughter-house G	Benin City	N06˚ 21.153'	E005˚ 38.677'
Government owned slaughter-house K	Benin City	N06˚ 20.957'	E005˚ 38.769'
Privately owned slaughter-house M	Benin City	N06˚ 21.006'	E005˚ 38.704'
Privately owned slaughter-house N	Benin City	N06˚ 21.064'	E005˚ 38.631'
Privately owned slaughter-house O	Benin City	N06˚ 21.128'	E005˚ 38.719'
Privately owned slaughter-house P	Benin City	N06˚ 21.128'	E005˚ 38.768'
Privately owned slaughter-house S	Benin City	N06˚ 21.152'	E005˚ 38.647'
Government owned slaughter-house EA	Ekpoma	N06˚ 44.062'	E005˚ 08.725'
Government owned slaughter-house AA	Auchi	N07˚ 02.212'	E006˚ 14.455'
Privately owned slaughter-house J	Auchi	N07˚ 06.953'	E006˚ 16.283'

Pour plating was conducted using duplicate labeled sterile Petri dishes and commercially available Nutrient agar (NA) was utilised in the enumeration of the mean heterotrophic bacterial counts of the diluted samples.[6] The agar plates were incubated at 35ºC for 48 h[6]. The total and faecal or thermo-tolerant (*E. coli*) coliform counts of the samples were evaluated using the multiple tube dilution method.[7] Commercially available culture media utilized for the multiple tube dilution procedure were MacConkey broth and Eosin Methylene blue agar. Upon the expiration of the respective incubation periods, the test tubes and conical flasks were visually assessed for both acid production and gas production, and reference was made to standard McGrady tables to determine the most probable number (MPN) of both the total coliform and faecal coliform count in 100 ml of the respective water samples.[7]
The heterotrophic mean bacterial counts were expressed as cfu/ml. Unique bacterial colonies present on the respective agar plates were purified and sub-cultured into freshly prepared Nutrient agar plates and slants under aseptic conditions using streaking procedures.[8] The cultural characteristics of the sub-cultured isolates were also documented. Further physiological and biochemical tests were conducted on each of the purified isolates to ascertain the tentative identity of the sub-cultured bacterial isolates.[5,9,10] The results of these procedures were collated and compared with documented identification schemes of different bacterial groups[9-,11].

Statistical analysis
All the mean bacteriological values were subjected to one-way ANOVA with the aid of statistical package for social sciences (SPSS version 20). Mean separation was also conducted using a post hoc test; Duncan's multiple range test at 95% level of confidence. The Mann Whitney test was used to ascertain the seasonal differences between the mean counts ($\alpha = 0.05$).

Results and Discussion

Heterotrophic bacterial counts
The mean bacteriological counts recorded for all the examined water samples are presented in Table 2. The overall average heterotrophic bacterial counts ranged from $9.0 \times 10^4 \pm 8746$ cfu/ml for water samples obtained from Slaughter-house S to $1.7 \times 10^5 \pm 13422$ cfu/ml, $1.7 \times 10^5 \pm 19957$ cfu/ml and $1.7 \times 10^5 \pm 5247$ cfu/ml for water samples obtained from Slaughter-house F, Slaughter-house N and Slaughter-house EA . Significant variation in the overall mean heterotrophic bacterial counts values was recorded (F=9.275, $p<0.05$). The enumerated levels of culturable bacterial counts were directly reflective of the non-treatment of the water prior to its usage especially in carcass dressing within the respective facilities.
The routine approach of direct usage of the water abstracted from different sources for carcass dressing by all the abattoir workers is a wrong procedure and has public health implication as the utilized water invariably serve as a source of bacterial contamination with respect to the raw meat prepared in these slaughter-houses. The detection of varying levels of heterotrophic bacterial bio-load for all the examined stored groundwater samples might be attributed to the presence of viable microbial biofilms within the pipes which convey the water under pressure from the underground aquifer to the storage plastic tanks. The widespread occurrence of microbial biofilms in water supply systems and its role as a source of heterotrophic bacteria has previously been described.[12]
The range of heterotrophic bacterial counts observed in this study contrasted with a report which indicated lower range of heterotrophic bacterial counts in respect of tap water samples used by workers in Agege abattoir, Lagos, Nigeria.[13] The ranges of the mean heterotrophic bacterial and coliform counts observed in this study contrasted with data in relation to the bacteriological profile of ground water samples collected from several locations in Benin City, Edo State, Southern Nigeria.[14]

Coliform counts
For the overall average coliform counts, a minimal value; 83 ± 52 MPN/100 ml was recorded for water samples obtained from Slaughter-house B while the maximal value; 176 ± 5.2 MPN/100 ml was recorded for surface water utilized in Slaughter-house J. Significant variation in the overall mean coliform counts was recorded (F=3.664, $p<0.05$). The isolation of culturable coliforms from the water samples was indicative of the unsuitability of the water samples for direct drinking purposes as it did not meet the allowable limit for coliform content in drinking water.[15]

Table 2: Overall bacteriological values of water samples collected from the abattoirs during the sampling period; November 2019 to March 2021

Facility	THBC (cfu/ml)	TCC (MPN/100 ml)	FCC (MPN/100 ml)
Slaughter-house A	$1.3 \times 10^5 \pm 54521^d$	94 ± 60.8^a	80 ± 50.7^a
Slaughter-house B	$1.1 \times 10^5 \pm 13702^a$	83 ± 52.1^a	44 ± 10.5^a
Slaughter-house C	$1.2 \times 10^5 \pm 41823^c$	91 ± 71.7^a	32 ± 19.9^a
Slaughter-house D	$1.0 \times 10^5 \pm 17272^a$	85 ± 58.1^a	33 ± 10.1^a
Slaughter-house F	$1.7 \times 10^5 \pm 13422^f$	93 ± 65.8^a	19 ± 10.5^a
Slaughter-house G	$1.1 \times 10^5 \pm 4229^a$	141 ± 18.8^a	80 ± 10.1^a
Slaughter-house K	$1.6 \times 10^5 \pm 21263^e$	101 ± 63.3^a	32 ± 30.1^a
Slaughter-house M	$1.3 \times 10^5 \pm 12518^d$	105 ± 67.9^a	33 ± 30.1^a
Slaughter-house N	$1.7 \times 10^5 \pm 19957^e$	157 ± 14.4^b	80 ± 10.1^a
Slaughter-house O	$1.0 \times 10^5 \pm 3751^a$	94 ± 60.2^a	25 ± 10.5^a
Slaughter-house P	$1.4 \times 10^5 \pm 16076^b$	89 ± 56.6^a	74 ± 10.5^a
Slaughter-house S	$9.0 \times 10^4 \pm 8746^a$	95 ± 67.6^a	29 ± 10.5^a
Slaughter-house EA	$1.7 \times 10^5 \pm 5247^e$	166 ± 9.2^c	162 ± 12.1^c
Slaughter-house J	$1.4 \times 10^5 \pm 32768^b$	176 ± 5.2^c	91 ± 75^b
Slaughter-house AA	$1.3 \times 10^5 \pm 32993^d$	170 ± 9.6^c	162 ± 11.3^c
Significance	*P<0.05*	*P<0.05*	*P<0.05*
SON (2007) permissible limit	NS	0	0

KEY: * Overall mean ± std. deviation of 8 replicates, SON; Standard Organization of Nigeria, NS; Not stated, THBC; Total heterotrophic bacterial count, TCC; Total coliform count, FCC; Faecal coliform (*E. coli*) count, *P<0.05*-Significant, Different superscripts in the same column indicate significant differences at $p < 0.05$ according to Duncan Multiple Range Test (DMRT)

The overall mean faecal coliform counts varied from 19 ± 10.5 MPN/100 ml for water samples collected from slaughter-house F to 162 ± 12.1 MPN/100 ml and 162 ± 11.3 MPN/100 ml for water samples obtained from Slaughter-house EA and AA respectively. Significant variation in the overall mean faecal coliform counts was recorded (F=6.165, $p<0.05$). *E. coli* detection in the respective water samples was reflective of the un-suitability of the water samples for direct drinking purposes as it did not met the permissible *E. coli* limit for drinking water.[15]

The isolation of coliforms and *E. coli* from the stored groundwater samples is a disturbing trend and would suggest faecal contamination of the groundwater either at the point of origin or during its conveyance. The presence of coliforms in the surface water can be attributed to deposition of surface run-offs into the water body. Also, anthropogenic activities such as bathing and washing of automobiles within the banks of the stream could also contribute to the bacterial and coliform bio-load of the water body.

The isolation of coliforms from the well-water samples could be linked to the suspected presence of microbial biofilms on the surfaces of collecting devices such as the abattoir roof, funnels and pipes which directly convey precipitation into the well or reservoir. The interior walls of the well although cemented were entirely coated with a visible dark coloured slime wall. The presence of this slime coated wall is usually indicative of a thriving microbial biofilm community within the well wall and members of biofilm community are usually present in the stored water content of the well.

It has been stated that the detection of coliforms and *E. coli* in water was suggestive of the possibility that the water might have been contaminated with either human or animal waste materials.[16] However, for lotic or lentic aquatic habitats, it has been opined that known bacterial indicators of faecal pollution; *E. coli* and enterococci may not be reliable for assessing fecal contamination of water in tropical settings.[17] Some of the reasons elaborated include; the possibilities of soil, sediments, water and plants serving as indigenous sources of *E. coli* and enterococci in tropical waters, the ability of fecal indicators to multiply and persist in soil, sediment and water in some tropical and subtropical environments.[17] The range of coliform counts observed in this study was at variance with a study which revealed a range of higher coliform counts for water samples utilized by workers of the Ado-Ekiti Municipal abattoir, Ekiti State.[18]

Seasonal variations in the bacteriological counts

The overall mean total heterotrophic bacterial counts recorded for water samples sourced from slaughter-houses A, B, C, J, P, D, F, K and M in the wet season were significantly higher ($p>0.05$) than corresponding values observed for samples obtained in the dry season (Table 3). However, significantly higher overall mean THBC values ($p>0.05$) were recorded for water samples collected from slaughter-houses N and S during the dry season.

The overall mean total coliform counts observed for water samples sourced from slaughter-houses A, O, B, G, C, J, P, D, F, S, K and M in the wet season were significantly higher ($p > 0.05$) than corresponding mean counts observed for samples obtained in the dry season (Table 3). A similar trend of elevated wet seasonal coliform counts for water samples sourced from several wells in Karu abattoir has also been documented.[19] Identical high coliform counts for water samples collected in the wet season from several locations in Khairpur City, Bangladesh has been recorded[20]

The overall mean faecal coliform counts observed for water samples obtained from slaughter-houses J, A, O, B, G, C, P, D, F, S, M and N in the wet season were significantly higher ($p>0.05$) than corresponding mean counts observed for samples obtained in the dry season.

Possible factors that could have caused the seasonal differences in the bacteriological counts of the water samples include the prevailing environmental temperature associated with the wet season which is generally more favorable for bacterial growth.[20] Other reasons include; higher amounts of surface run-offs in the wet season which can impact both groundwater and surface water and increased infiltration rate of water in the wet season which is known to affect groundwater levels.[21]

Tentatively identified bacterial isolates

Eight (8) bacterial isolates were tentatively identified from the water samples (Figure 4). The identified isolates were; *Escherichia coli*, *Acinetobacter* sp., *Bacillus* sp., *Klebsiella mobilis*, *Micrococcus* sp. *Citrobacter* sp., *Serratia marcescens*, *Enterobacter* sp. and *Staphylococcus aureus*. Amongst the characterized water-borne bacterial isolates, *Enterobacter* sp., *Bacillus* sp., *K. mobilis*, *Micrococcus* sp. and *Citrobacter* sp. were the most frequently isolated bacterial cultures as these isolates were detected in all the water samples (Figure 4). *S. marcescens* was the least frequently isolated bacterial culture as its cumulative percentage frequency of isolation score was 60%.

Table 3: Seasonal variation of bacteriological parameters

Abattoirs		THBC($\times 10^5$cfu/ml)	TCC (MPN/100ml)	FCC (MPN/100 ml)
Slaughter-house A	Wet	1.9	150	155
	Dry	0.8	38	20
	Sig.	0.029	0.029	0.029
Slaughter-house B	Wet	1.1	131	81
	Dry	0.9	34	12
	Sig.	0.029	0.029	0.029
Slaughter-house C	Wet	1.7	158	50
	Dry	0.8	24	14
	Sig.	0.029	0.029	0.029
Slaughter-house D	Wet	1.1	139	64
	Dry	0.9	31	3
	Sig.	0.029	0.029	0.029
Slaughter-house F	Wet	1.8	154	38
	Dry	1.6	31	3
	Sig.	0.029	0.029	0.029
Slaughter-house G	Wet	1.1	157	157

	Dry	1.1	125	125
	Sig.	0.200	0.029	0.029
Slaughter-house K	Wet	1.8	160	60
	Dry	1.4	43	4
	Sig.	0.029	0.029	0.029
Slaughter-house M	Wet	1.4	168	64
	Dry	1.2	42	5
	Sig.	0.029	0.029	0.029
Slaughter-house N	Wet	1.1	150	59
	Dry	1.5	164	2
	Sig.	0.029	0.200	0.029
Slaughter-house O	Wet	1.0	150	53
	Dry	1.0	39	1
	Sig.	0.057	0.029	0.029
Slaughter-house P	Wet	1.6	141	146
	Dry	1.4	36	2
	Sig.	0.029	0.029	0.029
Slaughter-house S	Wet	0.9	158	60
	Dry	1.0	32	2
	Sig.	0.029	0.029	0.029
Slaughter-house EA	Wet	1.7	165	156
	Dry	1.7	168	162
	Sig.	0.057	0.686	0.886
Slaughter-house J	Wet	1.8	175	156
	Dry	1.2	178	22
	Sig.	0.029	0.686	0.029
Slaughter-house AA	Wet	1.6	164	158
	Dry	1.1	176	166
	Sig.	0.057	0.057	0.343

KEY: THBC; Total heterotrophic bacterial count, TCC; Total coliform count, FCC :Faecal coliform count

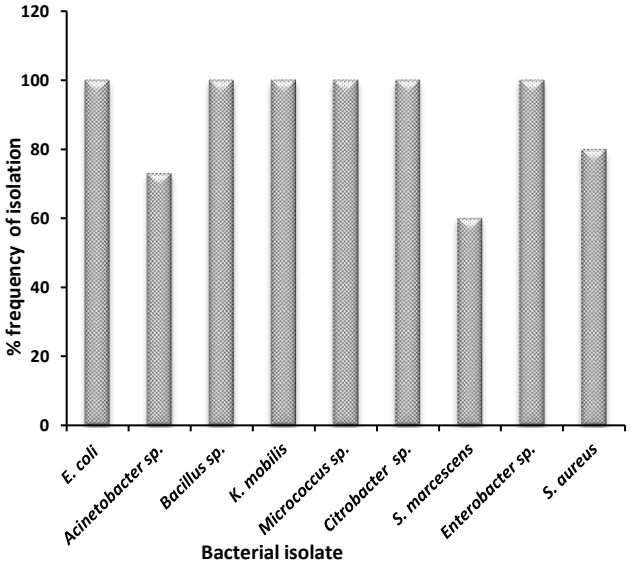

Figure 4: Percentage frequency of isolation of the tentatively identified bacterial cultures

The isolation of these different bacterial genera can be attributed to the capability of the water samples to support the growth of heterotrophic bacteria and coliforms. The detection of coliforms would invariably render the samples non-potable, as the presence of these bacterial indicators would suggest the possibility that the water samples were harboring other likely prokaryotic pathogens. The direct usage of the water supplies which harbored coliforms in carcass dressing and preparation of meat cuts invariably contributed to a trend wherein these facilities were a source of unwholesome meat cuts. This observation should serve as a source of concern to stakeholders in the meat sector within Edo State as these facilities are ideally meant to serve as source of wholesome raw meat cuts even if the final consumers routinely cook purchased raw meat cuts at different temperatures.

However, it should be noted that in the course of conducting facility visitation, it was observed that the abattoir workers did not engage in the direct consumption of the water, as they were completely dependent on commercially available sachet water for drinking purposes.

The differences in the percentages frequencies of isolation of the isolates might be due to the varying concentrations of biofilms on the inner surfaces of the plastic pipes and storage tanks used by the facilities. The detection of *E. coli*, *Acinetobacter* sp., *Bacillus* sp., *Micrococcus* sp. and *S. aureus* from the water samples was in agreement with an earlier report which documented the presence of

these isolates in respect of water samples collected from Agege abattoir, Lagos, Nigeria.[13]

Conclusion and Recommendation

The presence of varying numbers of culturable heterotrophic bacteria and coliforms in water supplies routinely used by the slaughter-houses for carcass dressing and sanitation activities was revealed in this study. The detection of coliforms would render the raw water unfit for both carcass preparation and sanitation purposes by the respective establishments.

It is recommended that prior to usage at these facilities; the water should be subjected to appropriate treatment and disinfection such as heat treatment which would reduce the microbial bio-load associated with the samples. Also, the interior of the water storage tanks and cemented wells should be washed thoroughly with disinfectants at specific intervals so as to reduce the biofilm content at the water and the vessel interfaces.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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