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Microbiological Assessment of Agege Abattoir Situated In Lagos State, Nigeria

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Abstract: *Soil and water samples were collected from a Lagos State Abattoir situated in Agege. The water samples collected for this purpose were tap water and wastewater from different sites of abattoir and the gutter where the workers dispose the waste water. Soil samples were collected from the sites where they dump the solid waste. The pH values of water and soil samples were in the range of 6.10-6.60 and 7.41-7.86 respectively. The Total Bacterial count (TBC) values of water samples and soil samples were in the range of* $4.5x10^4$*-* $2.5x10^9$ *cfu/ml and* $9.0x10^7$*–* $1.2x10^9$*cfu/g respectively. The TCC count for water samples were in the range of 14->1800 (MPN/100ml). The isolated fungi were species of Mucor,Aspergillus, Ventricillium, Rhizopus and Paecilomyces. The bacteria isolated from the collected samples were Bacillus sp., Pseudomonas sp., Escherichia coli, Lactobacillus sp., Listeria sp., Staphylococcus sp., Cornybacterium sp. and Klebsiella sp. Susceptibility test of some of the isolates revealed that most of the isolates are resistant to almost all the antibiotics tested. All the gram negative isolates were sensitive to ofloxacin.*

Key words: *Ofloxacin, Abattoir, TBC, TCC, Susceptibility.*

I. Introduction

Abattoir operation could be very beneficial to man; in that it provides meat for human consumption and other useful by-products, still it can be very hazardous to public health in respect to the waste it generates (Meadows, 1995 [1]; Adeyemi and Adeyemo, 2007 [2]).

Recent studies have shown that zoonoses from abattoir wastes are yet to be fully controlled in more than 80% public abattoirs in Nigeria (Cadmus *et al*., 1999)[3]. Diseases like pneumonia, diarrhea, typhoid fever, asthma, wool sorter diseases, respiratory and chest diseases were reported to be associated with abattoir activities (Bello and Oyedemi, 2009[4].*E .coli* infection source was reported to be undercooked beef which has been contaminated, often in abattoirs, with faeces containing the bacterium. (Encarta, 2005)[5]. These types of diseases can spread from the abattoir to the neighbourhood via vectors or animals. Nwachukwu *et al*., (2011)[6] studied on microbial assessment of surface water and sediment samples from different points (A,B,C) in Otamiri river receiving abattoir wastes. Preliminary identification following the criteria of Holt *et al*., (1994) [7] indicated that the proteolytic bacteria isolates included *Pseudomonas* sp., *Bacillus* sp., *Enterobacter* sp., *Escherichia* sp., *Klebsiella* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Proteus* sp. while lipolytic bacteria were *Pseudomonas* sp., *Moraxella* sp., *Acinetobacter* sp., *Arthrobacter* sp. and *Micrococcus* sp. Some are causative agents of gas gangrene, food poisoning, infantile diarrhea, chronic infections and faecal indicators dehydration, gastrointestinal irritation and infantile of water pollution.

Adebowale *et al.*, 2011[8] conducted a study to assess the different methods of waste disposal at the Lafenwa abattoir and the environmental and public health implication. In addition, the microbial status of the effluent and its receiving surface water were investigated to determine total viable and coliform counts using surface plating and multiple tube test techniques, respectively. The TBC and TCC for waste water during and after slaughtering were 5.2×10^7, 4.9×10^7 and 4.26×10^7, 3.06×10^7 cfu/ml respectively while the receiving surface water had mean TBC and TCC of 4.15×10^7, 3.83×10^7, and 3.89×10^7, 2.87×10^7 cfu/ml respectively. Bacterial organisms isolated from the effluent include *Enterobacter aerogens*, *Hafnia alvei*, Erwinia mallotivora, *Edwardsiella ictaluri, Enterobacter amnigenus* and *Escherichia coli* O157strains while *Proteus mirabilis*, *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Enterobacter intermedius*, *Yersinia aleksiciae*, *Serratia odorifera*, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Eschericia coli* O157 strains were isolated from the surface water. The fungal species isolated were *Trichoderma* spp., *Trichophyton* spp., *Aspergillus* spp., *Scedosporium*spp. and *Coccidioides* spp.

Trond *et al*., (2013)[9] worked on bacteria surviving practical disinfection and compare their survival abilities with representative isolates of the pathogen *Listeria monocytogenes.* The most dominating bacterial genera based on counts on non-selective agar were *Aerococcus*, *Acinetobacter*, *Pseudomonas,Serratia* and *Staphylococcus*. Isolates of *Citrobacter*, *Enterobacter* and *Serratia* dominated on agar plates selective for Enterobacteriaceae.

Moschonas *et al*., (2011)[10] identified 431 psychrophilic or psychrotrophic isolates from commercial Irish beef abattoir environments and "blown packs" of vacuum-packed beef, using PCR and 16S rRNA

sequencing, and estimated their intraspecies genetic diversity using restriction fragment length polymorphism (RFLP) analysis and spacer region PCR (SR-PCR).

Microbial content of wastewater in two abattoirs and the impact on microbial population of receiving soil was studied in Agege and Ojo Local Government Areas in Lagos State, Nigeria by Adesemoye *et al*., 2006[11]. The wastewater samples from both abattoirs were highly contaminated; TheAgege abattoir showed mean bacterial count of $3.32x10^7$cfu/ml and Odo abattoir showed mean count of $2.7x10^7$ cfu/ml. The mean fungal populations were $1.6x10^5$ and $1.2x10^5$cfu/ml for Agege and Odo abattoirs respectively. In the contaminated soil sample, mean bacterial count was $3.36x10^7$ cfu/ml compared to the $1.74x10^6$cfu/ml of the control sample.

At an abattoirsituated in Agegewhich was selected for this study, animals are off loaded and conveyed straight to the slaughter halls. Animals are then slaughtered on the slab, meanwhile different cattle are killed on this same slab without cleaning the slab. With inadequate slaughtering and disposal facilities, the abattoir has also become a source infection and pollution, attracting domestic and wild carnivores, rodents and flies, which are vectors of diseases. The area is rampant with filth and scattered rubbish, which is left uncollected, apart from the blood draining trenches through which the filth is scattered rather than eliminated.

The aim of the study was to isolate and identify organisms from different environmental samples collected from a Lagos State abattoir situated in Agege.

II. Materials And Methods

Study area

The study area selected for this research work is a Lagos State abattoir situated in Agege .According to the butchers working in this abattoir, about 3, 000 cattle are slaughtered on a daily basis; hence, it is one of the biggest slaughter slabs in Nigeria.

The samples were collected between the period of January and February, 2015.

Collection of samples

The different water samples collected for isolation and identification of organisms were:

- Stored water(tap water): A1 and A2 used for cleaning purpose; Waste water from different sites of abattoir: B1,B2, C1,C2 ;Waste water from gutter: D1, D2, E1, E2

Abattoir soil samples collected were:

- Soil samples ST and SD from different soild waste dumping sites
- All the samples were collected in duplicates.

Procedures for collection of samples

The tap water samples were carefully collected by cleaning the outside of the nozzle of the tap and then water was allowed to run for some time, and then wide mouthed sterile bottles were used to collect water samples. All the other water samples (from B,C,D and E) were collected in sterile bottles with the assistance of the butchers and also with approval from the head butcher into the sterile bottles. The samples were labelled properly.

The soil samples collected from two different dumping sites situated at the abattoir. The samples were collected in sterile wide mouthed containers. All the labelled samples after collection were immediately transferred to Microbiology Laboratory of Department of Biological Sciences Covenant University, Ogun state. The samples were put in ice box and were transported within 4 hours to the laboratory.

Determination of pH of the collected samples

The pH values of the samples were determined using the pH meter (Adwa ad1040 pH/Mv.) Ten ml of each of water samples was used for this purpose.

A sample of soil was crushed using a pestle and mortar. 2.0g of the crushed soil was added to a test tube and then filled with distilled water. It was mixed thoroughly and pH was taking using pH meter. This was done 3 times and the average was taken.

Isolation of organisms from collected samples:

One (1) g of soil from each sample was transferred into 9 ml of sterile distilled water, and aseptically, serial dilution as described by Benson (2005)[12] was performed to obtain soil suspension upto 10^{-7}. 1ml of each dilution (10^{-1} -10^{-7}) was inoculated on Nutrient agar medium, Potato dextrose agar medium, MacConkey agar media using a duplicate method: spread plate method and pour plate method. For water samples, the same procedure was followed except 1.0 ml water was used in pace of 1 g of soil. The Nutrient Agar and the MacConkey Agar plates were incubated at 37^oC for 17-24 hrs, whereas the PDA plates were incubated at room

temperature (28±1^{o}C). And the discrete colonies were reinoculated into appropriate media and were kept at 4^{o}C for identification purpose.

Enumeration of bacteria using nutrient agar (NA)

For this purpose, dilutions were made upto 10^{-7} for soil samples for both water and soil samples. One ml of each dilution (10^{-1} -10^{-7}) was inoculated on Nutrient agar medium using pour plate method for water and soil samples.

Enumeration of Total Coliform Count

Most Probable Number (MPN) method was employed to determine the presence of the microorganisms (Kamaldeen and Wahaab, 2011).[13]

Identification of bacterial and fungal isolates.

(a) Identification of bacterial isolates

The colonial morphology of the isolates were examined and then the isolates were identified using gram staining and different biochemical tests (Aneja, 2003)[14].

(b)Identification of fungal isolates:

Pure cultures of fungal isolates were examined under the microscope. The microscope study of each colony was carried out by wet-mount method (Benson,2005)[12]. The somatic and reproductive structures were observed. The relationship of the hyphae, size and shape of the reproductive structures were also noted.

Antimicrobial susceptibility testing

All the isolates were used for this test using streak plate method. Sensitivity disks containing conventional antibiotics like Augmentin (20 µg), Amoxicillin (10 µg), ciprofloxacin (5 µg), cortimoxazole (30 µg), gentamicin (10 µg) andnitrofurantoin (300 µg) manufactured by Abtek Biological Ltd., England were used for sensitivity test. A loopful of growth of each isolate on nutrient agar was suspended in sterile water and then was diluted in steps of 1:10 to give turbidity equivalent to the 0.5 McFarland standard (a density of $1x10^{8}$ cells/ml) before inoculation.Mueller-Hinton agar was inoculated with 0.5 ml suspension of each isolate adjusted to $1x10^{8}$ cells/ml using sterile spreader. Sensitivity discs containing antibiotics were placed on the surface of each Mueller-Hinton agar plate evenly seeded with test organisms and was incubated for 24 h at 37^{o}C (Benson, 2005)[12].

III. Results

pH values of soil and water samples

The pH values of water samples and soil samples were in the range of 6.10-6.60 and 7.41-7.86 respectively (Fig.1 and Fig. 2).

Determination of Total bacterial count (TBC) of water and soil samples

The TBC values of water samples and soil samples were in the range of $4.5x10^{4}$- $2.5x10^{9}$ cfu/mland $9.0x10^{7}$– $1.2x10^{9}$ cfu/g respectively. (Fig.3 and Fig. 4).

Determination of TCC of water samples

The TCC count for water samples A1, A2, B1, B2, C1, C2, D1, D2, E1 and E2 were in the range of 14->1800 (MPN/100ml). The results are shown in Fig.5.

Identification Of Bacteria and fungi from the collected samples

(a) Identification of bacterial isolates

From the gram staining and biochemical characteristics it has been shown that the samples contain species of *Bacillus, Lactobacillus, Pseudomonas,Listeria, Staphylococcus, Cornybacterium, Klebsiella* and *Escherichia coli*.

(b) identification of fungal isolates

The isolated fungi were species of *Mucor, Aspergillus, Ventricillium, Rhizopus* and *Paecilomyces*

Antimicrobial Susceptibility

The results are shown in Table 1. Interpretation of the isolates as sensitive or resistant was based on zones of inhibition according to Clinical and Laboratory Standards Institute guidelines for 2014 (CLSI, 2014)[15].

The antibiotic resistance pattern showed that *Klebsiella*sp. had high resistance to augmentin, cotrimoxazole, amoxicillin, tetracycline, nitrofurantoin (100%) followed by gentamicin (75%) and nalidixic acid (25%). All the isolates were sensitive to ofloxacin.

The *E.coli* isolates had high resistance to nalidixic acid (67%) followed by amoxicillin(50%), cotrimoxazole, augmentin and nitrofurantoin (33%). All *E. coli* isolates were sensitive to ofloxacin.

The *Pseudomonas* isolates had high resistance to augmentin, amoxicillin (100%) followed by gentamicin(20%). All the isolates were sensitive to ofloxacin.

The *Staphylococcus* isolates had high resistance to amoxicillin, nitrofurantoin and augmentin (100%). All the isolates were sensitive to gentamicin, erythromycin, ofloxacin and chloramphenicol.

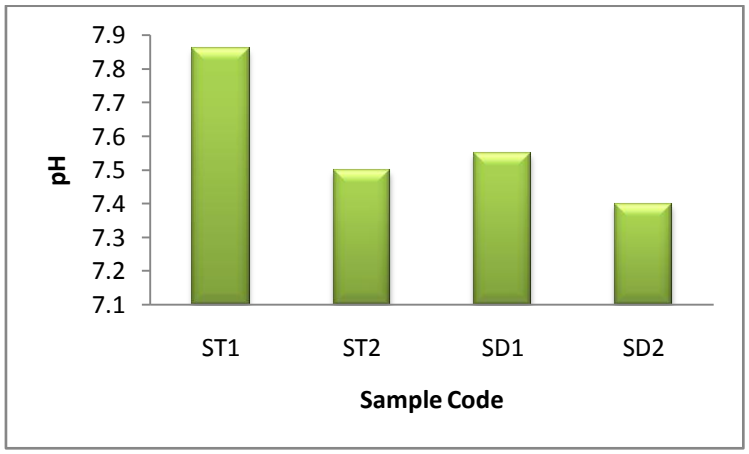

Figure 1: pH values of soil samples

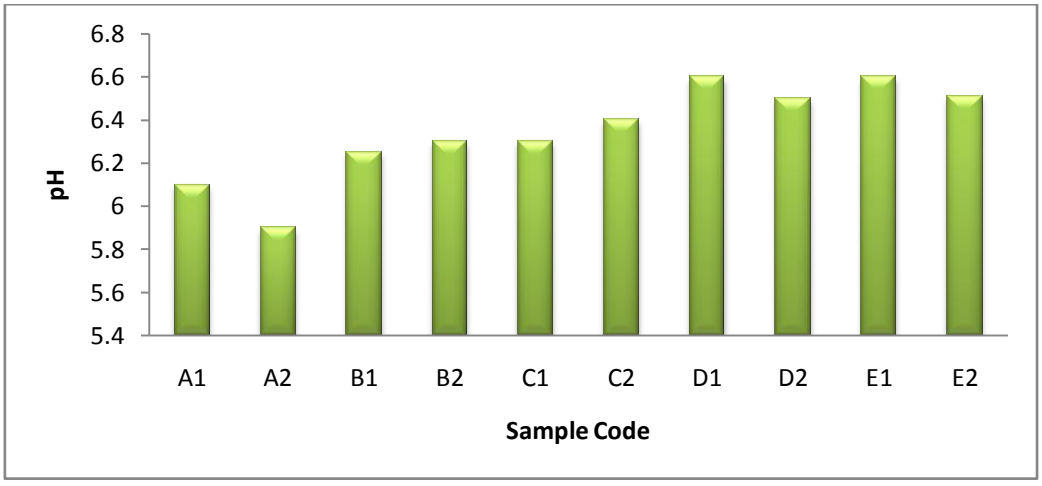

Figure 2: pH values of water samples

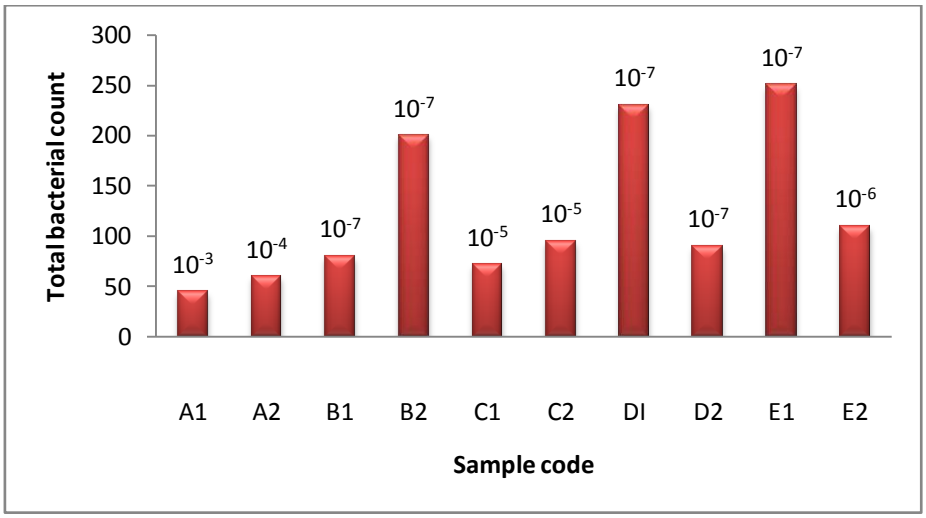

Figure 3: Total bacterial count of water samples

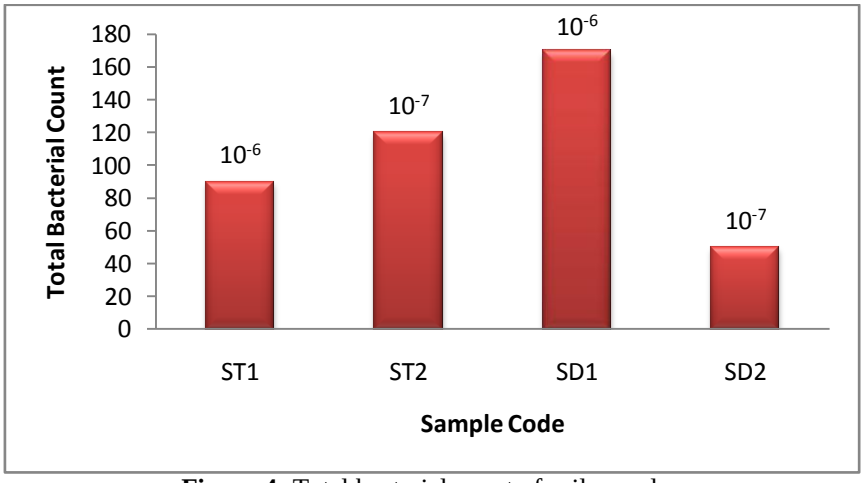

Figure 4: Total bacterial count of soil samples

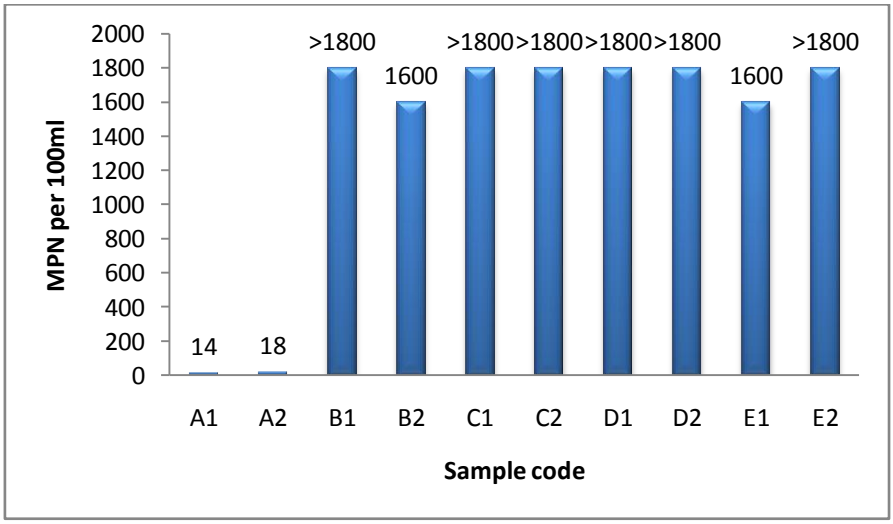

Figure 5: MPN per 100ml of water sample

Table 4.1: Antibiotic sensitivity for *E. coli*, *Pseudomonas* sp., *Klebsiella* sp. and *Staphylococcus* sp. isolates obtained from water samples

IN	Gen	Cot	Aug	Amx	Ofl	Tet	Nit	Ery	Chl	Nal
KSD2	14(i)	r	r	r	18 (i)	r	r	-	-	20(s)
KST1	r	r	r	r	21(s)	r	12(r)	-	-	21(s)
KE2	r	r	r	r	20(s)	r	r	-	-	r
KE1	r	r	r	r	23(s)	r	12(r)	-	-	20(s)
SC1	17(s)	-	r	r	21(s)	12(r)	r	18(s)	20(s)	-
SE2	18(s)	-	12(r)	r	26(s)	14(r)	r	20(s)	21(s)	-
SA1	15(i)	-	r	r	20(i)	14(r)	r	17(s)	22(s)	-
ED1	17(s)	r	r	r	20(i)	12(r)	r	-	-	r
EB1	17(s)	r	r	r	20(i)	11(r)	r	-	-	r
ESD1	20(s)	19(s)	20(s)	14(i)	25(s)	16(s)	16(i)	-	-	r
ED2	20(s)	17(s)	21(s)	13(r)	26(s)	18(s)	18(s)	-	-	r
EE1	15(s)	18(s)	24(s)	14(i)	24(s)	18(s)	17(s)	-	-	24(s)
EE2	18(s)	18(s)	21(s)	14(i)	25(s)	16(s)	15(i)	-	-	20(s)
PSD2	r	-	r	r	20(i)	-	-	-	-	-
PSD1	17(s)	-	r	r	22(s)	-	-	-	-	-
PST1	18(s)	-	r	r	21(s)	-	-	-	-	-
PST2	20(s)	-	r	r	16(r)	-	-	-	-	-
PE2	22(s)	-	r	r	22(s)	-	-	-	-	-

Gen: Gentamycin, Cot: Cotrimoxazole, Aug: Augmentin, Amx: Amoxicillin, Ofl: Ofloxacin, Tet: Tetracycline, Nit: Nitroflurantoin, Ery: Erythromycin, Chl: Chloramphenicol, Nal: Nalidixic acid,s-sensitive, i-intermediate, r-resistant (diameter of zone ≤ 5 mm), "-" not carried out;
PSD2,0PSD1, PST1, PST2 and PE2 - *Pseudomonas* sp., KSD2, KST1, KE2, KE1- Klebsiella sp., SC1, SE2,SA1- *Staphylococcus* sp., ED1, EB1, ESD1, ED2, EE1, EE2- *E. coli* isolates.

IV. Discussion

The study shows the pH values of water and soil samples in agage abattoir were in the range of 6.10-6.60 and 7.41-7.86 respectively. A pH near 7.0 (neutral) plays a part in determining both the qualitative and quantitative abundance of microflora (Federov *et al.*, 1993[16], Edward, 1990[17]).This result is in contrast with the findings of Adesemoye *et al*.,2006 [11] who reported the pH of the abattoir wastewaters was acidic, ranging from 4.3 to 5. It agrees with findings of Nwachukwu *et al.*, 2011[6]who reported that the pH of river water receiving abattoir waste water is slightly alkaline (6.8-7.2). Eze*et al.*, 2013 [18] revealed that the pH value of the soil contaminated with lairage effluent in Umuahia, Abia State was 5.8± 0.5. The TBC values of water samples and soil samples were in the range of $4.5x10^4$ - $2.3x10^9$ cfu/g and $9.0x10^4$ – $2.5x10^9$ cfu/g respectively. The mean total bacterial counts for water and soil samples were high for the studied abattoir. Going by international standard, any water contaminated to this level is neither good for domestic use nor is it supposed to be discharged directly into the environment without treatment. Total bacterial population obtained from the contaminated abattoir soil was high and this could be regarded as destabilization of the soil ecological balance arising from contamination. Quantitative examination of the microorganisms present in water of Aba river in Nigeria where abattoir waste water discharged revealed that as many as $2.05x10^8$ viable bacterial (cfu/ml) were present(Ezeronye and Ubalua, (2005)[19] .The isolated fungi were species of*Mucor*, *Aspergillus*, *Ventricillium*, *Rhizopus* and *Paecilomyces.* The bacteria isolated from the collected environmental samples were *Bacillus* sp., *Pseudomonas* sp., *Escherichia coli*, *Lactobacillus* sp., *Listeria* sp., *Staphylococcus* sp., *Cornybacterium*sp.and *Klebsiella* sp. Ezeronye and Ubalua (2005)[19] isolated *Staphylococcus* sp., *S. faecalis*, *E.coli*, *Salmonella* sp., *Bacillus* and *Clostridium* sp. from water samples of Aba river in Nigeria contaminated with abattoir waste. Meat is the most perishable of all important food since it contain sufficient nutrient needed to support the growth of microorganisms (Magnus, 1981)[20]. The results obtained from this study also indicated that the microbial

population of abattoir facilities was greater than values recommended for sanitary practices of such products and its processing facilities. The high microbial content of the wastewater is also an indication that the water used during the processing of beef in abattoir is not sterile or fit for consumption (Denpster and Cody, 2001)[21]. The high total viable counts recorded in this study showed the microbial diversity in the abattoir environment due to the poor hygienic practice employed by meats sellers and butchers. This determined the variation of bacterial contamination (Clarence *et al*., 2009) [22]. A total of 8 isolates comprising of Gram negative and Gram positive bacteria were isolated in this study. Microorganisms isolated from abattoir facilities in this study have been earlier found in foods, environments and other places, as reported by Enabulele and Uraih (2009)[23]. Nkanga and Uraih (1981)[24] in a study reported high prevalence rate of *E. coli* in raw meat samples from abattoir and traditional open markets, with a prevalence rate of 85.65%. The presence of these organisms in abattoir facilities depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering, processing and packaging of fresh meats. This agrees to previous reports by Okonko *et al*. (2008)[25]. Most of the organisms found in this study are those commonly found in soil and water. The presence of *Escherichia coli* is an indication of faecal contamination of the meat. The presence of *E. coli* and *Staphylococcus* sp.might be due to possible contamination of fresh meats or meat products itself during slaughtering or beef processing or unhygienic handling of the meat right from the slaughtering, butchering plants or due to contamination from the skin, mouth, nose of the handlers which can be introduced directly into foods by process line workers, with lesions caused by *S. aureus* on hands and arms coming into contact with the food, or by coughing and sneezing (Sobukola *et al*., 2009[26] ; Okonko *et al*., 2008[25]). In addition, the isolation of other species may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering, because *Enterobacter* sp. are also inhabitants of dairy products, as reported by Talaro, (2006)[27]. The presence of these organisms is suggestive of impending health hazards.

Fresh meats sold to the public in open markets are grossly contaminated with coliform bacteria as well as other bacteria forms. The finding of this study revealed that this Lagos State Abattoir situated at Agege is contaminated with pathogenic Gram positive and negative bacteria. The possible source of contaminants, are due to the unhygienic manner of handling meat in abattoirs, the environment upon which the meat is slaughtered as well the water used in the processing of the meat. This also implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. Since improper handling and improper hygiene might lead to the contamination of fresh meats and this might eventually affects the health of the consumers (Okonko *et al*., 2008)[25]. The isolates of *Pseudomonas* sp., *Klebsiella* sp., *Staphylococcus* sp. and E. coli obtained from different environmental samples showed multiple antibiotic resistance as shown in Table 1.The presence of multiple resistant organisms in abattoir environment arises from activities in meat production as a result of failure in adhering to good hygienic practices and treatment of waste water before their discharge into receiving water bodies. Antibiotic resistance acquisition due to selective pressure is of public health concerns as resistance genes can be disseminated in nature and transferred to pathogenic counterparts of bacterial species by genetic mobile elements (Wellington *et al*., 2013)[28].

So it may be recommended that the waste water should be treated before discharge because its discharge to water body is a serious public health issue because of the presence of indicator organisms as well as possible pathogens. The abattoir soil was also contaminated so care should be taken to maintain hygienic condition in order to avoid the contamination of meat and meat products ready to sale from abattoir. In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites (Adeyemo, 2002).[29]

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