Distribution of Antibiotic Resistant Bacteria from Abattoir Wastes and its Receiving Waters at Nkwo-ezzamgbo, Ebonyi State, Nigeria

[1]Stanley Chukwudozie Onuoha, [2]Collins Onyebuchi Okafor, [2]Bruno C. Aduo and [2]Faith C. Nwaka

[1]Department of Biotechnology, Ebonyi State University, PMB 053 Abakaliki, Ebonyi State, Nigeria
[2]Department of Biological Science, Evangel University, Akaeze, Ebonyi State, Nigeria

Abstract: Untreated wastes generated by abattoirs and released into the environment, especially water bodies pose a major threat to public health. They have been found to contain large amounts of contaminants, including pathogens of mostly enteric origin such as *Escherichia coli, Klebsiella, Shigella, Salmonella,* etc. and have also been discovered to exhibit resistance to antibiotic treatment. The study is aimed at evaluating the distribution of multidrug resistant bacteria isolated from abattoir wastes and its receiving waters at Nkwo-Ezzamgbo. Wastes were collected from four sites of the abattoir; the slaughter slab, butchering table, rinsing point and midstream of the receiving waters with sterile bottles and swab sticks. Samples were transported to the Microbiology Laboratory for analysis. Isolated organisms were counted, characterized and identified using standard microbiological and biochemical techniques. Antibiotic susceptibility tests were carried out using the disc diffusion method according to National Committee for Clinical laboratory Standards (CSLI). Mean values of bacterial count ranged from 1.86 x 10^4 CFU/mL to 3.42 x 10^4 CFU/mL. Out of the twenty-eight (28) isolates obtained, 8(28.56%) were *P. aeruginosa,* 4(14.28%) *E. coli,* 2(7.14%) *S. aureus,* 2(7.14%) *Klebsiella,* 2(7.14%) *Shigella,* 1(5.57%) *Enterococcus,* 8(28.56%) *Salmonella* and 1(3.57%) *Streptococcus*. All isolated organisms were completely resistant to tetracycline, cephalothin, penicillin G, cefuroxime sodium, erythromycin, nalidixic acid, sulphamethoxazole, cefpirome and oxytetracycline. The most effective antibiotic was azithromycin followed by imipenem. All the organisms had MARI values >0.20, with the highest value exhibited by *Enterococcus* spp. (0.94) and the least by *P. aeruginosa*(0.75). The presence of these multi-drug resistant strains of the isolated organisms in abattoir waste could act as a vehicle in transferring antibiotic resistance to other bacteria. This emphasizes the need for proper treatment and safer disposal of abattoir wastes in Nkwo-Ezzamgbo.

Key words: Abattoir wastes · Ezzamgbo · Antibiotic resistance and Bacteria species

INTRODUCTION

The continuous drive to increase meat production for the protein needs of the ever increasing world population is usually associated with some pollution problems [1]. In Nigeria, the location and operation of several private and government abattoirs, with Ebonyi State not being left out, are generally unregulated. They are usually located near water bodies, where access to water for processing is guaranteed. The animal blood and other wastes are released untreated into the receiving waters and surroundings, while the consumable parts of the slaughtered animal are washed directly into the same water [2].

With water being the second most important necessity of life, contamination of river bodies from abattoir wastes, either directly or indirectly through various processes, could constitute a significant environmental and health hazards, promoting the growth of disease-causing organisms such as bacteria [3]. This further encourages increase in water-borne diseases especially typhoid, diarrhea and dysentery amongst rural dwellers.

Livestock waste spills can introduce enteric pathogens and excess nutrients into surface waters, promoting eutrophication. Equally, these effluents from slaughterhouses could lead to transmission of pathogens to humans and cause zoonotic diseases such as

---

Corresponding Author: Stanley Chukwudozie Onuoha, Department of Biotechnology,
Ebonyi State University, PMB 053 Abakaliki, Ebonyi State, Nigeria.

salmonellosis, brucellosis, coli bacillosis and helminthes infections [4]. According to Bello and Oyedemi [5], medical experts have associated some diseases with abattoir activities which include pneumonia, diarrhea, typhoid fever, asthma, wool sorter diseases, respiratory and chest diseases. *E. coli* infection source was reported to be associated with undercooked beef which has been contaminated at abattoirs with faeces containing the bacterium. These diseases can spread from the abattoir to the neighbourhood via vectors or animals.

Water contaminated with faeces from animals can cause diarrhoea because animal faeces can contain diarrhoea-causing microorganisms [6]. As an example, animal faeces can contain pathogens such as *Escherichia coli* 0157 and *Salmonella* spp., which can infect humans [7]. It has been suggested that waterborne zoonosis can be a bigger problem in developing countries because of the lack of water treatment facilities and use of untreated wastewater [8].

Several studies have revealed that abattoirs in developing countries have an unhygienic environment[9, 10] and detected the presence of pathogens that are known causes of diarrheal diseases and a possible hazard to human health in the abattoir waste and water contaminated by abattoir waste [11, 12]. It has also been suggested that scavengers feeding on abattoir waste can spread pathogens from the waste to new locations [12].

Abattoirs in most developing countries have unhygienic environments that promote the growth of pathogenic microorganisms. Contamination of carcasses with animal wastes (such as dung, blood, etc) and pollution of the receiving waters is a major environmental and public health issue. Furthermore, the recent increase in the development of resistance to antimicrobials by pathogens is a major concern. This study was prompted by the need for safer and healthier abattoir waste management system. It is therefore aimed at determining the distribution of multidrug resistant bacteria from wastes at the abattoir and its receiving waters.

MATERIALS AND METHODS

Description of Study Area: Nkwo- Ezzangbo is one of the largest and most populous markets in Ohaukwu local government area of Ebonyi State. The Nkwo abattoir is located along Enugu-Abakaliki express way. It is a major market for trading donkeys south east of Nigeria. It has an abattoir and several donkeys are slaughtered in this abattoir. On the average, the abattoir produces about 2,071 donkey heads per day. Close to the slaughtering slab is a heap where paunch materials are dumped and have accumulated over the years. The waste materials from the abattoir are washed through drainage, which links the abattoir and the Nkwo stream which is some 450 meters away. Nkwo stream is a tributary to Ogbagu River.
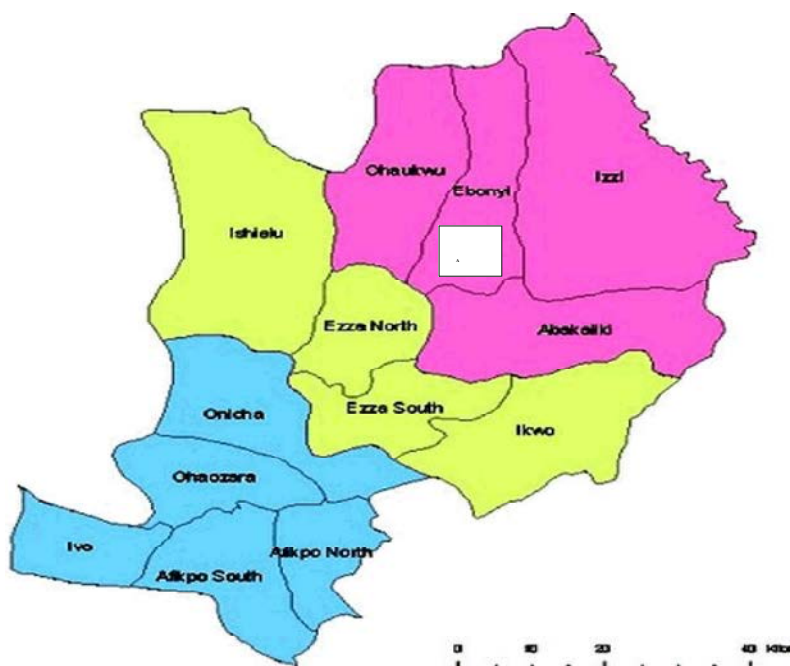

Fig. 1: Map of Ebonyi State showing the sampling locations; A = Location of Ezzangbo Abattoir

Fig. 2: Heap of animal dung and intestinal content at Nkwo-Ezzamgbo abattoir

Fig. 3: Unhygienic slaughter process at the slaughter slab of the abattoir.

Media Preparation: All media used in the study were weighed, dissolved in distilled water and autoclaved or heated according to manufacturers' (Titan Biotech Ltd.) instruction; EMB agar (36 g in 1000 ml), Nutrient Agar (28 g in 1000 ml), MacConkey agar (47 g in 1000 ml), Salmonella-Shigella agar (63g in 1000 ml), Mannitol Salt agar (111 g in 1000 ml), Cetrimide agar (45.3 g in 1000 ml) and Mueller - Hinton agar (38g in 1000ml). 20 ml of each media were dispensed into sterile Petri dishes.

Processing of Wastewater Samples: Swabs from the Butcherstables were first suspended overnight in air-tight test tubes of 5 ml nutrient broth each, before being inoculated with an inoculating loop into nutrient agar plates. The wastewater was agitated to get a homogenous solution and an aliquot (1 ml) of the wastewater from point A was transferred into 9ml of distilled water and diluted serially in test tubes up to 10^{-5} according to the method of Adesemoye *et al*. [13] and labeled appropriately. The same was done for Point B wastewater.

Microbiological Analysis

Isolation of Bacteria: Isolation of bacteria from the abattoir effluent samples was aseptically carried out using standard microbiological techniques as described by Cheesbrough [14]. Dilution factors, 10^{-2} and 10^{-4} from Point A and Point B were pour plated on nutrient agar in four Petri dishes respectively and incubated at 37^0C for 24 hours.

After incubation period, colonies, which developed on the plates, were counted, multiplied by 10 and by the dilution factors and recorded as colony forming units per milliliter (CFU/mL) of the sample. Distinct colonies from the agar stock culture and inocula from the nutrient broth were subcultured (using streak plate method) individually on freshly prepared selective and differential media to obtain pure isolates.

The selective and differential culture media used were *Salmonella-Shigella* agar (for the isolation of *Salmonella* spp. and *Shigella* spp.),Cetrimide selective agar (for the isolation of *Pseudomonas aeruginosa*), Mannitol salt agar (for the isolation of *Staphylococcus aureus*), MacConkey agar (for the isolation of *Enterococcus* and other

Enterobacteriaceae) and Eosin methylene blue agar (for the isolation of *Klebsiella* and *Escherichia coli*). The pure isolates were maintained on agar slants for further characterization and identification.

Identification and Characterization of Isolates: The bacteria isolates were identified by comparing their morphology, microscopy, Gram's reaction and biochemical characteristics with those of known taxa using the schemes of Barrow and Feltham [15]. Morphology, microscopy, Gram's reaction and biochemical tests for *Streptococcus* and *Enterococcus* were similar. They were further differentiated by the ability of *Enterococcus* to ferment lactose and grow well on MacConkey agar containing 6.5% NaCl, producing small dark-red colonies, while *Streptococcus* could not grow on the agar.

Disc Diffusion Susceptibility Test: Bacteria isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by (CLSI, 2005). [16]. In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller-Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface followed by 24 h incubation. After the inoculums were dried, antibiotic impregnated disks were applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated aerobically at 37 °C for 24 h. Finally, the zone of inhibition was measured including the disk diameter. The susceptible and resistant categories were assigned on the basis of the critical points recommended by the CLSI and according to the manufacturer's leaflet attached to them. The standard antibiotic discs (Oxoid, England) and their concentrations used against the isolates were tetracycline (TET) – 10 μg, cephalothin (CEF) – 30 μg, norfloxacin (NOR) – 10 μg, penicillin G (PEN) – 10 μg, azithromycin (AZM) – 15 μg, streptomycin (STR) – 10 μg, cephotaxime (CTX) – 30 μg, gentamycin (GEN) – 30 μg, imipenem (IPM) – 10 μg, cefpirome (CPO) – 30 μg, compound sulphonamides (CS3) - 30 μg, nalidixic acid (NAL) – 30 μg, erythromycin (ERY) – 10 μg, oxytetracycline (OXY) – 30 μg, sulphamethoxazole (SMX) – 25 μg and cefuroxime sodium (CXM) – 30 μg. These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

Multiple Antibiotic Resistance Index (MARI) calculation: MARI values of isolated bacteria against the antibiotics used were computed. MARI is a tool that helps in analyzing health risks and checking antibiotic resistance in a given area. The value of MARI is 0.20 and it differentiates the low risk (<0.20) from the high risk (>0.20). It is calculated by dividing the aggregate resistance of total isolates of an organism to all antibiotics by the product of the total number of antibiotics used and the number of isolates of an organism from the sample site. i.e. x/(y.z), where x represents the aggregate resistance of total isolates of an organism to all antibiotics, y represents the total number of antibiotics used and z represents the number of isolates of an organism from the sample site. This formula was used since the MARI was being calculated from a sample site (environmental sampling) where many isolates were obtained according to the method of Riaz *et al*. [17].

RESULTS

Total Bacterial Count: The result of the total viable counts (TVC) of the bacteria isolated from the abattoir waste and its receiving waters were obtained. The mean values of the colony count for each site were computed and are presented in Table 1. From the results, the number of colonies per ml for the four (4) wastewater sites ranged from 1.86 - 3.42×10⁴ CFU/mL. The highest number of colonies were seen in the waste water obtained from the rinsing point (3.42×10⁴ CFU/mL) followed by the midstream waste sample (2.48×104 CFU/mL) and slaughter slab (2.25×104 CFU/mL), while the least was the butchering table (1.86×10⁴ CFU/mL).(Table 1)

Prevalence and Distribution of Bacterial Species: In this study, a total of eight (8) bacterial organisms were isolated and identified from the samples obtained from Nkwo abattoir wastes and its receiving waters. They include *Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp., *Staphylococcus aureus, Shigella* spp., *Klebsiella* spp., *Enterococcus* spp. and *Streptococcus* spp. The butchering table had the least number of isolates 5(17.85%), while the receiving waters had the highest number of isolates 16(57.12%) (Table 2).

The frequency of each organism isolated varied between waste sites as shown in Table 2. *Pseudomonas aeruginosa* and *Salmonella* spp. had the highest occurrence of 28.56% from the abattoir waste. It was followed by *Staphylococcus aureus, Shigella* spp and *Klebsiella* spp., with prevalence of 7.14%, while the least prevalence of 3.57% was observed in *Enterococcus* spp. and *Streptococcus* spp. (Table 2).

From Table 2, *Shigella* spp., *Klebsiella* spp., *Enterococcus* spp. and *Streptococcus* spp. were only isolated from the waste obtained from the receiving waters, but were not seen at the wastes from the slaughter slab and butchering table. All isolates were present in the receiving waters where the carcasses are being washed, except *Staphylococcus aureus* which was only present in the slaughter slab and butchering table.

Antibiotics Susceptibility/resistance Study: The result of the antibiotics susceptibility studies of the isolates showed that all the bacterial isolates exhibited resistance to more than nine (9) antibiotics as seen in Table 3, although their pattern of resistance varied. All isolated organisms were completely resistant to tetracycline, cephalothin, penicillin G, cefuroxime sodium, erythromycin, nalidixic acid, sulphamethoxazole, cefpirome and oxytetracycline. In addition, the most effective antibiotic was azithromycin, to which all the isolates were susceptible to, except *Enterococcus* spp. (100%) and *Staphylococcus aureus* (50%).

It was also inferred that *E. coli* isolates were 100 % resistant to tetracycline, cephalothin, penicillin G, cephotaxime, cefuroxime sodium, compound sulphonamides, sulphamethoxazole, oxytetracycline, cefpirome, erythromycin and nalidixic acid, it was 75 % resistant to gentamycin and streptomycin respectively, while the least resistance was observed in imipenem (25%). Complete susceptibility was observed in azithromycin.

Pseudomonas aeruginosa was completely susceptible to azithromycin, but was 100% resistant totetracycline, cephalothin, penicillin G, cefuroxime sodium, imipenem,sulphamethoxazoleoxytetracycline, cefpirome, erythromycin, nalidixic acid and compound sulphonamides,moderately resistant to gentamycin (50%) and had the least resistance to streptomycin (12.5%) (Table 3).

Salmonella spp. exhibited high resistance to all the antibiotics used except azithromycin. They were 100% resistant to norfloxacin, compound sulphonamides, sulphamethoxazole, oxytetracycline, cefpirome, erythromycin, nalidixic acid,tetracycline,penicillin G, cephotaxime, cefuroxime sodium,and cephalothin. While, it was 87.5% resistant toimipenem, streptomycin (75%) and the least resistance was observed in gentamycin (62.5%),

Staphylococcus aureus showed moderate resistance (50%) to norfloxacin, gentamycin and azithromycin, but was susceptible to imipenem. Complete (100%) resistance

of *S. aureus* was seen in tetracycline, streptomycin, cephalothin, penicillin G, cephotaxime, cefuroxime sodium, compound sulphonamide, sulphamethoxazole, oxytetracycline, cefpirome, erythromycin andnalidixic acid.

Shigella spp., *Klebsiella* spp., *Enterococcus* spp. and *Streptococcus* spp. were completely resistant (100%) to tetracycline, streptomycin, cephalothin, penicillin G, cephotaxime, cefuroxime sodium sulphamethoxazole, oxytetracycline, cefpirome, erythromycin and nalidixic acid, but were completely susceptible to imipenem, while all except *Enterococcus* spp. (100%) was susceptible to azithromycin. *Klebsiella* spp., *Enterococcus* spp. and *Streptococcus* spp. were completely resistant to gentamycin (100%), while *Shigella* spp. had moderate resistance of 50%. High resistance (100%) to compound sulphonamides was exhibited by *Shigella, Enterococcus* and *Streptococcus,* while *Klebsiella* (50%) showed moderate resistance. On the other hand, moderate resistance to norfloxacin was exhibited by *Shigella* (50%) and *Klebsiella* (50%), while complete resistance was seen in *Enterococcus* (100%) and *Streptococcus* (100%) (Table 3)

A high percentage of all bacterial isolates obtained in this study were highly susceptible to azithromycin with only 7.14% resistance value obtained from the study. These showed that azithromycin is still very effective in treating infections caused by these microorganisms, followed by imipenem with a total resistance value of 57.14%.

Multiple Antibiotic Resistance Indices (MARI): The result from the MARI studies showed that all the organisms had MARI values >0.20, with the highest value exhibited by *Enterococcus* spp. (0.94), followed by *Salmonella* spp. (0.89) and *Streptococcus* spp. (0.88). The least MARI value was seen in *Pseudomonas aeruginosa* (0.75), followed by *Shigella* spp. and *Klebsiella* spp. with 0.81 each (Table 4). *Escherichia coli* and *Staphylococcus aureus* had MARI values of 0.82 and 0.84 respectively.

Table 1: Total viable count (CFU/mL) of bacterial isolates

S/N	Sample point/location	Colony count (CFU/mL)
		($\times 10^4$)
1	Slaughter Slab	2.25
2	Butchering Table	1.86
3	Rinsing Point	3.42
4	Midstream	2.48
	Mean	2.50

Table 2: Distribution and prevalence of isolates from three waste sites within the abattoir.

S/N	Isolates identified	Slab	Table	Water	Prevalence
1	*Escherichia coli*	1(3.57%)	1(3.57%)	2(7.14%)	4(14.28%)
2	*Pseudomonas aeruginosa*	2(7.14%)	1(3.57%)	5(17.85%)	8(28.56%)
3	*Salmonella spp.*	3(10.71%)	2(7.14%)	3(10.71%)	8(28.56%)
4	*Staphylococcus aureus*	1(3.57%)	1(3.57%)	0(0%)	2(7.14%)
5	*Shigella spp.*	0(0%)	0(0%)	2(7.14%)	2(7.14%)
6	*Klebsiella spp.*	0(0%)	0(0%)	2(7.14%)	2(7.14%)
7	*Enterococcus spp.*	0(0%)	0(0%)	1(3.57%)	1(3.57%)
8	*Streptococcus spp.*	0(0%)	0(0%)	1(3.57%)	1(3.57%)
	Total	7(24.99%)	5(17.85%)	16(57.12%)	28(99.96%)

Table 3: Distribution of antibiotic resistance amongst bacteria isolates from abattoir wastes.

S/N	Isolates identified	Total no. of isolates	Number of resistance exhibited by isolates to antibiotics (%)														
			NOR	GEN	TET	STR	AZM	CEF	PEN	CTX	CXM	IPM	CS3	SMX	OXY	CPO	ERY	NAL
1	*Escherichia coli*	4	2(50)	3(75)	4(100)	3(75)	0(0)	4(100)	4(100)	4(100)	4(100)	1(25)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)
2	*Pseudomonas aeruginosa*	8	2(25)	4(50)	8(100)	1(12.5)	0(0)	8(100)	8(100)	2(25)	8(100)	8(100)	7(87.5)	8(100)	8(100)	8(100)	8(100)	8(100)
3	*Salmonella* spp.	8	8(100)	5(62.5)	8(100)	6(75)	0(0)	8(100)	8(100)	8(100)	8(100)	7(87.5)	8(100)	8(100)	8(100)	8(100)	8(100)	8(100)
4	*Staphylococcus aureus*	2	1(50)	1(50)	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
5	*Shigella* spp.	2	1(50)	1(50)	2(100)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
6	*Klebsiella* spp.	2	1(50)	2(100)	2(100)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	0(0)	1(50)	2(100)	2(100)	2(100)	2(100)	2(100)
7	*Enterococcus* spp.	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
8	*Streptococcus* spp.	1	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
	Total	28	17(60.71)	18(64.29)	28(100)	18(64.29)	2(7.14)	28(100)	28(100)	22(78.57)	28(100)	16(57.14)	26(92.86)	28(100)	28(100)	28(100)	28(100)	28(100)

KEY: NOR = Norfloxacin, GEN = Gentamycin, TET = Tetracycline, STR = Streptomycin, AZM = Azithromycin, CEF = Cephalothin, PEN = Penicillin G, CTX = Cephotaxime, CXM = Cefuroxime sodium, IPM = Imipenem, CS3 = Compound sulphonamides, SMX = Sulphamethoxazole, OXY = Oxytetracycline, CPO = Cefpirome, ERY = Erythromycin, NAL = Nalidixic acid, % = percentage.

Table 4: Multiple Antibiotic Resistance Indices (MARI) of bacteria isolates.

S/N	Isolated organisms	Total no. of isolates	MARI
	Escherichia coli	4	0.82
	Pseudomonas aeruginosa	8	0.75
	Salmonella spp.	8	0.89
	Staphylococcus aureus	2	0.84
	*Shigella*spp.	2	0.81
	*Klebsiella*spp.	2	0.81
	Enterococcus spp.	1	0.94
	Streptococcus spp.	1	0.88

DISCUSSION AND CONCLUSION

Discussion: The result of the total bacterial count from the abattoir waste and its receiving waters analyzed showed that the abattoir wastes had high counts. The bacteriological count ranged from 1.86-3.42×10^{4}CFU/mL for the wastes from the slaughter slab, butchering table, rinsing point of the meat at the receiving waters and midstream sampled points. These values indicate very high microbial load and can be attributed to the poor sanitary and hygienic practices of the abattoir workers and the poor state of health of the slaughtered animals. This is unacceptable by WHO (1999)[18], standard guideline which is supposed to be less than ten (<10) CFU/mL. High count of these organisms in the wastewater could be due to the presence of high whole blood content, which serves as a rich protein medium for bacterial growth.

The rinsing point sample had the highest number of colonies (3.42×104 CFU/mL). This could be due to the presence of lots of animal intestinal matter, since it was the point where the carcasses were washed. Most enteric bacteria are commensals in the intestine of animals and humans. As the wastewaters flows midstream, diffusion enhances the decrease in the number of organisms (2.48×104 CFU/mL) as the waste meets cleaner water from the other end of the river, reducing its concentration. These could be further confirmed from Table 2, where the highest number of isolates, 16(57.12%) was obtained from the receiving waters

The slaughter slab having 2.25×104 CFU/mL, had lesser colonies than the receiving waters, but greater than the butchering table (1.86×104 CFU/mL). This could be owing to the fact that the slaughter slab had more blood content and little traces of animal faeces, whereas the butchering table that harboured carcasses already washed at the rinsing point had lesser amount of blood and no visible traces of faeces.

Various species of bacteria were isolated, with most of them belonging to the family *Enterobacteriaecae*. The bacteria isolated from the abattoir wastes were *Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp., *Staphylococcus aureus, Shigella* spp., *Klebsiella* spp., *Enterococcus* spp. and *Streptococcus* spp. The presence of these pathogenic organisms suggests the presence of other opportunistic and pathogenic bacteria. Thus, the conclusion that the abattoir waste contains only these eight organisms cannot be drawn, since the study was limited and results were based only on the samples analyzed.

This study revealed the presence of *Salmonella* in all the samples analyzed from the abattoir sites, with a prevalence of 28.56%, which is not surprising since *Salmonella* has been reported to be an environmentally persistent pathogen capable of surviving and proliferating in diverse environments [19]. The 28.56% prevalence rate of *Salmonella* obtained in this study is however lower than the 64% prevalence rate by Onuoha *et al*. [20] and the 33.3% prevalence rate by Iroha *et al.*[22]; but higher than that of Narfarnda *et al*. [21], who obtained 12.3% from receiving bodies and 13.2% from vegetables irrigated with waste waters at Yola abattoir, Nigeria.

Pseudomonas aeruginosa had one of the highest prevalence rates of 28.56% from the abattoir waste. This result does not fully agree with the findings of Iroha *et al.* [22], who reported 21.7% for *P. aeruginosa* as the least prevalence frequency obtained from Ogbete abattoir in Enugu State. With the presence of *Shigella* spp only in the receiving waters with a prevalence rate of 7.14%, it is reasonable to suggest that the slaughter waste wasn't the source of the *Shigella* spp isolated from the abattoir receiving waters, since *Shigella* spp are bacteria with humans and primates as hosts [23]. More likely, its source could be faecal contamination from humans indicating that abattoir workers or other people residing at the abattoir area defecate into the water and its surroundings.

Furthermore, presence of *Staphylococcus aureus* in the waste samples gotten from only the slaughter slab and butchering table and not the receiving waters could be attributed to contamination from the hides of animals and hands and skin of abattoir workers, since *S. aureus* is a normal flora of the skin of healthy mammals and proper hygiene practices were not adhered to during slaughter process.

Klebsiella spp. as seen in Table 3 showed resistance to norfloxacin, nalidixic acid, gentamycin, streptomycin, tetracycline, oxytetracycline and sulphamethoxazole, which agrees with previous studies by Nathisuwan *et al*. [24].

The multidrug resistance and MARI value of 0.84 exhibited by *Staphylococcus aureus* in this study concurs with the fact that incidence of multiple antibiotic resistant *Staphylococcus* has been previously isolated from abattoir, freshly slaughtered animal and unpasteurized milk in South Africa [25].

From this study, the highest resistance pattern and MARI values was exhibited by *Enterococcus* sp., which showed a 100% resistance to fifteen (15) out of the sixteen (16) antibiotics used and susceptibility to only imipenemand a MARI value of 0.94. This result is not surprising as (Xia *et al.*,2011) [26], reports that *Enterococcus* spp. exhibits remarkable and increasing intrinsic resistance to most antimicrobial agents such as semi-synthetic penicillins, cephalosporins, low levels of aminoglycosides and clindamycin, while exhibiting acquired resistance to chloramphenicol, erythromycin, high levels of clindamycin, tetracycline and high levels of aminoglycosides, penicillins, fluoroquinolones and vancomycin.

Results of the antimicrobial susceptibility/ resistance tests in this study demonstrated that bacterial isolates were resistant to antibiotics commonly used as feed additives (tetracycline, streptomycin and sulfonamides) or therapeutics (penicillin and tetracycline).

CONCLUSION

This research study has investigated and described the bacterial profile and antimicrobial resistance pattern of bacteria isolated from the abattoir wastes in Ezzamgbo. On-site observation of the abattoir environment shows that sanitary conditions under which carcasses are being dressed are far from being ideal. Heaps of bones and animal dungs are a major sight at the abattoir (Fig.2). The state and condition of the slaughter slabs (Fig.3), the butchers and workers, the utensils used and the quality of water used were not up to recommended hygiene standards.

Also, untreated abattoir wastes such as animal urine, blood, intestinal contents, fats, undigested food, aborted fetuses, faeces, hairs, etc. discharged into the environment, especially the nearby water bodies was observed in this study. From the research, it was inferred that these wastes contains large amounts of multidrug resistant bacteria that could impact on public health of humans, especially the abattoir workers and residents around the abattoir.

REFERENCES

1. Hinton, A., R. Buhr and K. Ingram, 2000. Reduction of *Salmonella* in the crop of broiler chickens subjected to feed withdrawal. Poultry Science, 79: 1566-1570.

2. Adelegan, J., 2002. Environmental Policy and Slaughterhouse Waste in Nigeria, 228th WEDC Conference Report, Calcutta, India.

3. Osibajo, O. and G. Adie, 2007. Impact of effluent from Bodija abattoir on the physicochemical parameters on Oshunkanye stream in Ibadan City, Nigeria. African Journal of Biotechnology, 6(15): 1806-1811.

4. Cadmus, S., B. Olugasa and G. Ogundipe, 1999. The prevalence of zoonotic importance of borine tuberculosis in Ibadan, Nigeria. Proceedings of the 37th Annual Congress of the Nigerian veterinary medical Association, Kaduna, pp: 883-886.

5. Bello, Y. and D. Oyedemi, 2009. Impact of abattoir activities and management in residential neighbourhoods: A case study of Ogbomoso, Nigeria. Journal of Social Science, 19: 121-127.

6. WHO 2013a: World Health Organization (2013). Water-related diseases.

7. Berger, P. and R. Oshiro, 2003. Source water protection: Microbiology of source water. In: *Encyclopedia of Environmental Microbiology*, G. Bitton, editor in chief, Wiley-Interscience, N.Y, pp: 2967-2978.

8. Dafour, A., J. Bartram, R Bos and V. Gannon, (ed) 2012. Animal Waste, Water Quality and Human Health, IWA Publishing, London

9. Adeyemo, O., 2002. Unhygienic Operation of a City Abattoir in South Western Nigeria: Environmental Implication. African Journal of Environmental Assessment and Management, 4(1): 23-28.

10. Nwanta, J., J. Onunkwo and E. Ezenduka, 2010. Analysis of Nsukka metropolitan abattoir solid waste and its bacterial contents in South Eastern Nigeria: Public health implication. Archives of Environmental and Occupational Health, 65(1): 21-6.

11. Benka-Coker, M. and O. Ojior, 1995. Effect of slaughterhouse wastes on the water quality of Ikpoba River, Nigeria. Bioresource Technology, 52(1): 5-12.

12. Adeyemi, I. and O. Adeyemo, 2007. Waste management practices at the Bodija abattoir, Nigeria. International Journal of Environmental Studies, 64(1): 71-82.

13. Adesemoye, A., B. Opere and S. Makinde, 2006. Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria. African Journal of Biotechnology, 5(20): 1963-1968.

14. Cheesbrough, M., 2006. District laboratory practice in tropical countries, Microbiological tests. Chapter 7.In: Cheesbrough M, Ed. pt 2. 2nd Ed. Cambridge: Cambridge University Press. pp: 9-267.

15. Barrow, G. and R. Feltham, eds. 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edition, Cambridge University Press, Cambridge, London, UK.

16. Clinical Laboratory Standards Institute (CLSI), 2005. Performance Standards For Antimicrobial Disc Susceptibility Test; 8th ed. Approved Standards, m2A8, Wayne, Pa (USA). 11: 64-68.

17. Riaz, S., M. Faisal and S. Hasnain, 2011. Antibiotic susceptibility pattern and multiple antibiotic resistances (MAR) calculation of extended spectrum â-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* spp. in Pakistan. African Journal of Biotechnology, 10(33): 6325-6331.

18. WHO 1999. Guidelines for drinking water quality. Health Criteria and other Supporting Information, Geneva, Switzerland, 5: 10-15.

19. Winfield, M. and E. Groisman, 2005. Role of non host environments in the lifestyles of *Salmonella* and *Escherichia coli*. Applied and Environmental Microbiology, 69: 3687-3694.

20. Onuoha, S.C., S.C. Eluu and M.O. Okata, 2016. *In-vitro* Antimicrobial Resistance of *Shigella* and *Salmonella* species Recovered from Abattoir effluents in Afikpo, South Eastern Nigeria. International Journal of Current Microbiology and Applied Sciences, 5(4): 488-497.

21. Narfarnda, W., A. Yaji and H. Kubkomawa, 2006. Impact of abattoir waste water on aquatic life: A case study of Yola abattoir. Global Journal of Pure and Applied Sciences, 12: 31-33.

22. Iroha, I., O. Eromonsele, I. Moses, F. Afiukwa, A. Nwakaeze and P. Ejikeugwu, 2016. *In vitro* antibiogram of multidrug resistant bacteria isolated from Ogbete abattoir effluent in.

23. Strockbine, N.A. and A.T. Maurelli, 2005. P. 811. In: Brenner, D. J., Krieg, N. R., & Staley, J. T. (ed.), Bergey's manual of systematic bacteriology, 2nd ed., vol. 2. The Proteobacteria. Springer-Verlag, New York, NY.

24. Nathisuwan, S., D. Burgess and J. Lewis, 2001. Extended-Spectrum â-lactamases: Epidemiology, Detection and Treatment. Pharmacotherapy, 21(8): 920-928.

25. Manie, T., V. Brovel, W. Veith and P. Gouws, 1999. Antimicrobial Resistance of Bacterial Flora Associated with Bovine Products in South Africa. Journal of Food Protection,

26. Xia, Z., W. Dong and W. Xiao-lan, 2011. Characterization of pathogenic or non-pathogenic *Enterococcus faecalis* isolated from lambs from Xinjiang, a remove North-west province of China. African Journal of Microbiology Research, 5(18): 2827-2833.