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Antibiotic susceptibility and microbial analysis of Enterobacteriaceae from wastewater and sediments from abattoirs in Makurdi, Benue State, Nigeria

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ABSTRACT

In Nigeria, the operation of many abattoirs is unregulated. Waste from these abattoirs therefore portend serious hazard to public health. To ascertain this, wastewater and sediment samples from four abattoirs in Makurdi, Benue State were investigated for antibiotic susceptibility and microbial analysis using the dilution pour plate method on Salmonella-shigella Agar (SSA) and MacConkey Agar for isolation of Enterobacteriaceae. While their antibiotic susceptibility pattern was studied using the method of National Committee for Clinical Laboratory Standards (NCCSLS). The result obtained show that mean values of bacterial count ranged from 2.00×10^6 – 1.37×10^8 cfu/ml for wastewater samples and 1.09×10^7 – 8.66×10^7 cfu/ml for sediment samples. The following genera of Enterobacteriaceae; *Escherichia, Klebsiella, Shigella* and *Salmonella* were isolated. Out of the 106 isolates obtained, 31(29.25%) were *Escherichia* spp., 28(26.42%) *Salmonella* spp., 25(23.58 %) *Shigella* spp., and 22(20.75%) *Klebsiella* spp. All isolated *Salmonella* spp. were completely resistant to ceftazidime, and ceftriazone while *Escherichia coli* and *Klebsiella* spp., were completely resistance to ertapenem, ceftazidime, and ceftriazone. Imipenem was the most potent antibiotic as all bacteria isolates were highly susceptible to it. The results obtained show that pathogenic species such as *Salmonella* and *Shiegella* were present in significant numbers in the abattoir wastewater and sediment with varying degree of resistance to antibiotic tested. Therefore, there is need for treating abattoir waste before discharge.

INTRODUCTION

An abattoir has been defined as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, possessing and effective preservation and storage of meat products for human consumption (Alonge, 2001). In Nigeria, the location and operation of several private and government abattoirs, with Benue State not being left out, are generally unregulated. 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; Adeyemi and Adeyemo, 2007). Abattoirs generate large amounts of solid waste and effluents such as rumen contents, blood and waste water (Ayodele and Agboola, 1981). Abattoirs often have difficulties in disposing of the solid wastes and wastewater in an environmentally acceptable fashion and in many instances untreated rumen contents, blood and/or other abattoir effluents and wastewater are released into the environment (Bellani et al., 1978). The resulting pollution not only cause problems related to odour, flies and hygiene, but surface and ground water can be polluted with pathogens (Shuval et al., 1986).

Since sulfa and penicillin were first introduced during

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the 1930s and 1940s, bacteria have revealed a remarkable ability to develop different types of resistance mechanisms which mediate resistance to antimicrobials that are, from the beginning, quite toxic to them. Antibiotic resistance is today one of the most serious threats to public health, and it is a global problem. The evolutionary consequence of a high selective pressure during the years is that there is no longer any antimicrobial drug for which resistance has not been documented. Antibiotic resistance in bacteria is the ability of such bacteria to grow in the presence of antibiotics and resistance could be natural or acquired (ECPDC, 2013). Infections as a result of resistant bacteria are always difficult to treat because conventional treatment fails and leads to longer time of treatment and sometimes death. It also leads to higher medical costs, according to the World Health Organization (WHO, 2013). Resistant pathogenic microorganisms can be transmitted to human from animals and non-pathogenic antibiotic resistant bacteria that are part of normal flora of the intestinal tract and are able to transfer resistance genes to non-pathogenic ones (Blake, 2003). The study aimed at determining the microbial analysis of Enterobacteriaceae and antibiotic susceptibility of isolates species from wastewater and sediments at Makurdi abattoirs, Nigeria.

Public health significance of Enterobacteriaceae family

The Enterobacteriaceae is a family of Gram-negative, non-spore-forming bacteria and is one of the most important groups of bacteria known to man. They are the largest and most heterogeneous collection of Gram negative bacilli of medical importance. Entero-bacteriaceae family is involved in almost all infections acquired in the intensive care unit (ICU), particularly respiratory infections and urinary tract infections. The primary agents associated with Gram-negative antibiotic resistance is *E. coli*, and *Klebsiella* spp., among others. However, the group has shown high levels of multidrug resistance in clinical strains (Farmer III et al., 2007). They are also dispersed in nature and can be found in plants, soil, water, normal microbiota in the intestinal tract of both man and other animals. Microbiological and medical importance stems from the development of infections, as well as pathogenicity and appearance of multi-resistant bacteria to antibiotics used in therapy (Farmer III et al., 2007; Murray et al., 2010).

MATERIALS AND METHODS

Study site

The study sites were a commercial abattoir located in Makurdi, the capital city of Benue State, Nigeria, which is one of the 23 local government areas in the state. It is located in the north central within the Southern Guinea Savannah on latitude 7° 41´ North and longitude 8° 37´ East. It is characterized by tropical climate, dry and wet season. Dry season last for a minimum of six months, beginning from November to April, while the wet season last from May to October. Main annual rain fall is about 1,290 mm (Gobo, 1988). The four abattoirs used were situated in the Wurukum, Wadata, Modern market and North Bank areas of the state capital.

Sample Collection

Wastewater and sediment samples were aseptically collected from four abattoirs into sterile plastic bottles and polythenes respectively from slabs where animals are being slaughtered and also from the drainage channel, wastewater was collected using sterile disposable micropipettes while sediments were collected using sterile hand trowel. Samples were collected at different point from each abattoir and pooled together. The samples were transported immediately to the laboratory in ice packs for microbiological analyses. Samples were collected weekly from each abattoir for a period of four weeks between November and December, 2016.

Isolation and Identification of Enterobacteriaceae

1 g of sediment from each sample was dispensed into 9 ml of sterile distilled water which serve as the stock solution. Serial dilutions of the abattoir wastewater and sediment samples were plated out using the pour plate technique. 1 ml of the appropriate dilutions were plated out on SSA and MacConkey Agar [selective media for Enterobacteriaceae (Adesemoye et al., 2006)] and labeled appropriately. The same procedure was used for the four abattoirs. The plates were then incubated at 37°C for 18 – 24 h. Typical colonies of *Salmonella and Shigella s*pp., black coloured and colourless isolates respectively, were sub-cultured on SSA selective agar media to obtain pure isolates. Same procedure as above was also used for *Klebsiella and E. coli* species using MacConkey selective agar media. Typical colonies of *Klebsiella* and *E. coli* species, red colour isolates were sub-cultured on MacConkey Agar Selective Agar media to obtain pure isolates. All red colonies were further confirmed for *E. coli* isolates using EMB Agar. All isolates showing green metallic sheen on EMB Agar were confirmed for *E. coli* (Holt et al., 1994). Dilution factors, 10^{-2}, 10^{-4}, 10^{-6} and 10^{-8} from the four abattoirs were also plated out on nutrient agar in four Petri dishes respectively and incubated at 37 C for 24 hours. After an incubation, colonies which developed on the plates were counted, total bacteria were estimated and recorded as

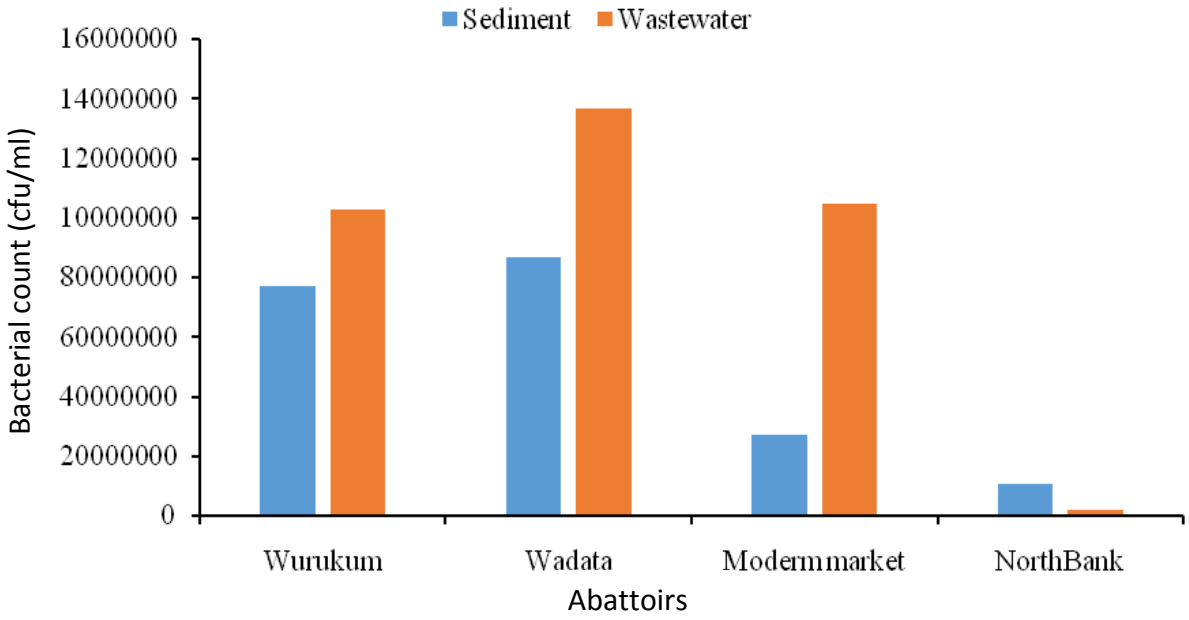

Figure 1. Variation in bacteria counts in wastewater and sediment from four abattoirs.

colony forming units per milliliter (cfu/ml) of the sample. Identification of *Salmonella, Shigella, Klebsiella and E. coli species* were carried out in accordance with standard methods of identification of bacteria of medical importance through microscopy, Gram staining and biochemical tests (Barrow and Feltham, 1993).

Antibiotics susceptibility test of the Isolates

The antibiotics susceptibility test for the Four genera Enterobacteriaceae isolates (Salmonella, Shigella, *Klebsiella* and *E. coli*) was done using the standard disk diffusion technique based on the recommendation of Clinical Laboratory Standards Institute (CLSI, 2014) on Mueller-Hinton agar. The antibiotics used were obtained from Oxoid, U.K. and include: Imipenem 10 µg, Ertapenem 10 µg, ceftazidime 30 µg, ceftriazone 30 µg and ciprofloxacin 5 µg, belonging to the fourth generation cephalosporin and carbapenems. Colonies of 18 – 24 h old culture was picked and suspended in a tube containing sterile normal saline (0.85% NaCl) and the turbidity adjusted to 0.5 (McFarland standards). With the aid of a sterile swab stick, the suspension was uniformly spread over already prepared Mueller Hinton agar plates and with the aid of sterile forceps, the antibiotics were placed carefully on the plates which were inverted and incubated at 37°C for 18 – 24 h. After the incubation period, the zones of inhibition were measured, recorded and compared/interpreted to the CLSI standards (CLSI, 2014). The results were categorized as: R (resistant), I (intermediate) and S (sensitive).

RESULTS

Total bacterial count

The mean values of the colony count for each abattoir are presented in Figure 1. From the results, the number of colonies per ml for the four (4) wastewater sites ranged from 2.00×10^6 – 1.37×10^8 cfu/ml. The highest number of colonies were seen in the wastewater obtained from Wadata abattoir (1.37×10^8 cfu/ml) followed by Modern market abattoir (1.05×10^8cfu/ml) and Wurukum abattoir (1.03×10^8cfu/ml), while the least was from North Bank abattoir (2.00×10^6cfu/ml) (Figure 2). While the number of colonies per ml for the four (4) sediment sites ranged from 1.09×10^7 – 8.66×10^7 cfu/ml. The highest number of colonies were also seen in the sediment obtained from Wadata abattoir (8.66×10^7 cfu/ml) followed by Wurukum abattoir (7.71×10^7 cfu/ml) and Modern market (2.72×10^7 cfu/ml), while the least was also from North Bank abattoir (1.09×10^7 cfu/ml) (Figure 1).

Prevalence and distribution of bacterial species

In this study a total number of 106 isolates belonging to four genera and included 31(29.25%) isolates of *Escherichia*, 28(26.42%) of *Salmonella*, 25(23.58%) of *Shigella* and 22(20.75%) of *Klebsiella* were isolated from

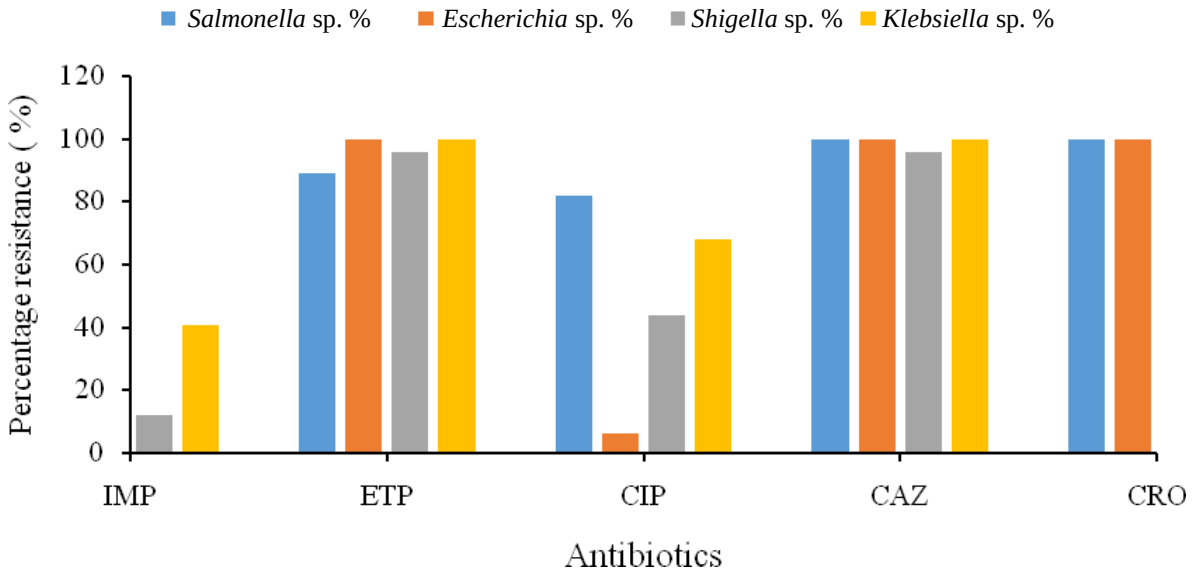

Figure 2. Percentage resistance of isolates to the selected antibiotics.
IMP, Imipenem; **ETP,** Ertapenem; **CIP,** Ciprofloxacin; **CAZ,** Ceftazidime; **CRO,** Ceftriazone.

abattoir wastewater and sediment as shown in Figure 3. The wastewater and sediment had equal number of isolates 53(50.00%). The frequency of each organism isolated varied between wastewater and sediment as shown in Figure 3. *Shigella* spp. had the highest occurrence of 20(37.74%) from abattoir wastewater. It was followed by *E. coli* and *Salmonella* spp. with prevalence of 15(28.30%) and 12(22.64%) while the least prevalence of 6(11.32%) was observed in *Klebsiella* spp. (Figure 3). From the sediment samples in Figure 3, *E. coli, Salmonella* spp. And *Klebsiella* spp. had equal distribution of 16(30.19%) while the least prevalence of 5(9.43%) was observed in *Shigella* spp.

Antibiotics susceptibility/resistance study

The result of the antibiotics susceptibility studies of the isolates showed that all the bacterial isolates exhibited resistance (Table 1), although their pattern of resistance varied. All isolated *Salmonella* spp. were completely resistant to ceftazidime, and ceftriazone while *E. coli* and *Klebsiella* spp., were completely resistance to ertapenem, ceftazidime and ceftriazone. In addition, the most effective antibiotic was imipenem, to which all the isolates were highly susceptible to, *E. coli* (96.77%), *Shigella* spp. (88%), *Salmonella* spp. (57.14%) and *Klebsiella* spp. (59.09%) and ciprofloxacin which is also highly susceptible to *E. coli* isolates (93.55%). It was also inferred that *E. coli* and *Klebsiella* isolates were 100% resistant to ertapenem, ceftazidime and ceftriazone; *Salmonella* spp. were 100% resistant to ceftazidime and ceftriazone. *Shigella* spp. were 96% resistant to ertapenem, ceftazidime and ceftriazone, it was 32% resistant to ciprofloxacin, while the least resistance was observed in imipenem (8%) (Table 1). A high percentage of all bacterial isolates obtained in this study were highly and moderately susceptible to ciprofloxacin. These showed that ciprofloxacin is still very effective in treating infections caused by these microorganisms.

DISCUSSION

The results of the total bacterial count from the four abattoir wastewater and its sediment showed that abattoir wastes had high counts. The microbiological count ranged from 2.00×10^{6} – 1.37×10^{8} and 1.09×10^{7} – 8.66×10^{7} cfu/ml respectively for the waste from Wurukum, Wadata, Modern Market and North Bank. 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) standard guideline which is supposed to be less than ten (<10) cfu/ml. The wastewater samples had the highest number of colonies (Figure 1). 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 forbacterial growth.

The following genera of Enterobacteriaecae were isolated from the abattoir wastes: *E. coli*, *Salmonella* spp., *Shigella* spp. and *Klebsiella* spp. The presence of these pathogenic organisms suggests the presence of

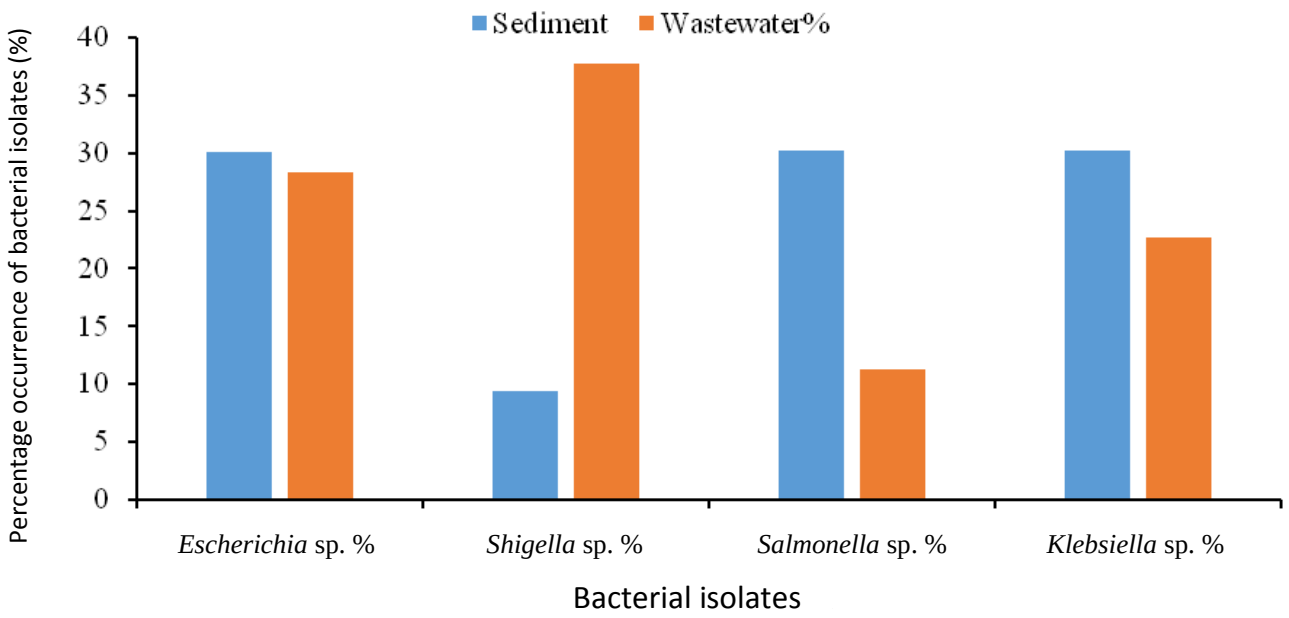

Figure 3. Percentage occurrence of bacterial isolates from wastewater and sediment from four abattoirs.

Table 1. Number and percentage distribution of antimicrobial susceptibility of isolates.

Antimicrobial agents	Disc conc. (µg)	*Salmonella* spp. (n=28)			*Shigella* spp. (n=25)			*Escherichia* spp. (n=31)			*Klebsiella* spp. (n=22)		
		S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
IMP	10	16(57.14)	7(25.00)	5(17.86)	22(88.00)	2(8.00)	1(4.00)	30(96.77)	1(3.57)	0(0.00)	13(59.09)	5(22.73)	4(18.18)
ETP	10	3(10.71)	25(89.29)	0(0.00)	1(4.00)	24(.96.00)	0(0.00)	0(0.00)	31(100.00)	0(0.00)	0(0.00)	22(100)	0(0.00)
CIP	5	5(17.86)	21(75.00)	2(2.14)	14(56.00)	8(32.00)	3(12.00)	29(93.55)	0(0.00)	2(7.14)	7(31.82)	8(36.36)	7(31.82)
CAZ	30	0(0.00)	28(100.0)	0(0.00)	1(4.00)	24(96.00)	0(0.00)	0(0.00)	31(100.00)	0(0.00)	0(0.00)	22(100)	0(0.00)
CRO	30	0(0.00)	28(100.0)	0(0.00)	1(4.00)	24(96.00)	0(0.00)	0(0.00)	31(100.00)	0(0.00)	0(0.00)	22(100)	0(0.00)

IMP, Imipenem; **ETP,** ertapenem; **CIP,** ciprofloxacin; **CAZ,** ceftazidime; **CRO,** ceftriazone; **S,** sensitive; **R,** resistant; **I,** intermediate; **n,** number.

other opportunistic and pathogenic bacteria. Thus, the conclusion that the abattoir waste contains only these four genera 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 the two samples, that is, wastewater and sediment samples analyzed from the abattoir sites, with a prevalence of 22.64 and 30.19%

respectively, which is not surprising since *Salmonella* has been reported to be an environmentally persistent pathogen capable of surviving and proliferating in diverse environments (Winfield and Groisman, 2005). The 22.64 and 30.19% prevalence rate of *Salmonella* obtained in this study is however lower than the 64% prevalence rate reported by Onuoha et al. (2016) and the 33.3% prevalence rate reported by Iroha et al. (2016) from abattoir effluents in Afikpo and Ogbete, Nigeria respectively; but higher than that reported by Nafaranda et al. (2005), who obtained 12.3% from receiving bodies and 13.2% from vegetables irrigated with waste waters at Yola abattoir, Nigeria. With the presence of *Shigella* spp. having the highest occurrence from the wastewater with a prevalence rate of 37.74%, it is reasonable to suggest that the animal waste was the source of the *Shigella* spp. isolated from the abattoir wastewaters, since *Shigella* spp. are bacteria with humans and primates as hosts (Strockbine and Maurelli, 2005). More likely, its source could be faecal contamination from the slaughtered animals.

The bacteria occurrence frequency revealed that *Escherichia* spp., *Klebsiella* spp. and *Salmonella* spp. were dominant in abattoir sediment samples with equal prevalence of 30.19% while *Shigella* spp. was least abundant with prevalence of 9.43%. Pathogenic species of bacteria identified in this study is similar to Coker et al. (2001) who documented pathogenic species of bacteria were identified in abattoir wastewater at South Western Nigeria. Total bacterial populations obtained from abattoir sediment were high and this could be regarded as destabilization of the soil ecological balance arising from contamination. These abattoirs are situated along river Benue where their effluents are constantly discharged. This finding certifies river Benue as unsafe for domestic use (WHO, 1993) due to constant discharge of wastewater from abattoirs. The presence of pathogenic bacteria has been known to cause health hazards (Nafaranda et al., 2005).

The antibiotic susceptibility pattern of this study reveals that most of the isolates were found to be resistant to cephalosporin antimicrobial agents like ciprofloxacin, ceftazidime and ceftriazone and carbapenems like ertapenem and imipenem in varying degrees.

With regard to the antibiogram of *E. coli* isolates, five different antimicrobial discs were used and all the 31 *E. coli* isolates subjected to antimicrobial sensitivity test were found to be resistance 100% to three of the antimicrobial agents except for imipenem and ciprofloxacin which were highly susceptible. The degree of susceptibility is also 100% for ciprofloxacin and 96.77% to imipenem. The absence of resistance against ciprofloxacin shows that it is a drug of choice for the infections caused by *E. coli.*

Multi drug resistance is defined as resistance of an isolate to more than 2 antimicrobials tested (Dominic et al., 2005). Multiple drug resistance was seen in *E. coli* and other bacteria isolates tested. This finding was supported by Bekele et al. (2014), Hiko et al. (2008), Adetunji et al. (2014), Meng et al. (1998) and Schroeder et al. (2002) who reported the existence of multidrug resistant *E. coli*. This corroborates the findings of Ahemed et al. (2006) who also noted that multidrug resistant phenotypes have been spread widely among Gram-negative bacteria. Furthermore, it is stated that studies in other developing countries have shown the trend in enteric pathogens is toward increasing antimicrobial resistance (Hoge et al., 1998). In general, the development of drug resistant *E. coli* isolates and other Gram-negative bacteria can be linked to various aspects including the practice of indiscriminate use of antibiotics in food producing animals (Eliopoulos et al., 2009) and due to the selective pressure to extensive use of antibiotics in the animal industry (Mohammed et al., 2014).

Conclusion

The presence of multiple resistant organisms in abattoir environment may have arisen from failure in adhering to good hygienic practices and treatment of waste water before their discharge into the environment. 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.

RECOMMENDATIONS

The State Environmental Protection Agency should actively monitor activities of the abattoirs and ensure compliance with health and safety standard, and also there is need for improved research in abattoir wastes management in Nigeria.

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