BAJOPAS Volume 15 Number 1, June, 2022

http://dx.doi.org/10.4314/bajopas.v15i1.31

Bayero Journal of Pure and Applied Sciences, 15(1): 220 - 230
Received: April, 2022
Accepted: May, 2022
ISSN 2006 – 6996

POTENTIAL OF CATTLE RUMEN WASTE AS A SOURCE OF ANTIBIOTIC-RESISTANT BACTERIA DISSEMINATION IN THE ENVIRONMENT

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ABSTRACT

The identification of possible sources of antibiotic-resistance dissemination in the environment is one of the ways to tackle the menace of globally challenging antibiotic resistance. This study reported the antibiotic-resistance pattern of bacteria isolated from fresh rumen waste of cattle culled at four privately-owned abattoirs in Osogbo, the Southwestern part of Nigeria. Bacteria were isolated and identified using standard cultural techniques and biochemical characterization tests. The bacterial isolates were tested against twelve antibiotics using the Kirby-Bauer disc diffusion method. The total heterotrophic bacterial count obtained for the four different abattoirs ranged between $2.95 \times 10^{9} \pm 1.14$ CFU g^{-1} and $1.01 \times 10^{11} \pm 1.02$ CFU g^{-1}. Bacterial isolates presumptively identified include Brevundimonas diminuta, Chryseomonas luteola, Citrobacter diversus, Enterobacter intermedius, Escherichia coli, Klebsiella oxytoca, Providencia rettgeri, Pseudomonas sp., Shigella dysenteriae, Stenotrophomonas maltophilia, and Tatumella ptyseos. Thirty-seven (92.5%), eighteen (45%), fourteen (35%), and ten (25%) out of the total 40 bacteria isolated were resistant to augmentin, tetracycline, cotrimoxazole, and gentamicin respectively. The percentage resistance to nalidixic acid (5.9%) and ofloxacin (2.9%) was low among the Gram-negative bacteria, while the percentage resistance to nitrofurantoin was 23.5%. All the Gram-positive bacteria were sensitive to streptomycin while 66.7% were resistant to erythromycin. Multidrug-resistant bacteria isolated were 23 (57.5%). The results of the study showed that rumen waste generated from cattle culled for human consumption at abattoirs in Osogbo metropolis, Nigeria can be a possible source of spreading antibiotic-resistant bacteria in the environment.

Keywords: rumen waste; antibiotic-resistant bacteria; environment; multidrug resistance; public health

INTRODUCTION

In Nigeria, humans purchase meat directly from abattoirs for consumption. Abattoir practices can generate animal wastes such as non-edible cattle parts, hair, blood, hoofs, horns, and rumen wastes. In most developing countries, abattoir wastes are indiscriminately released into the environment without treatment (Olawuni *et al.,* 2017) – (Plate 1). This practice is contrary to what is obtainable in developed nations, where abattoir wastes are channelled through an underground drainage system (Adeyemi and Adeyemo, 2007). The possible negative impacts of discharging abattoir wastes into the environment include obnoxious odours emanating from dumpsites, aesthetic issues, excessive nutrient addition, and increased microbial burden (Ezeoha and Ugwuishiwu, 2011). Moreover, residents around abattoir facilities are exposed to environmental and health risks.

The rumen wastes (pouch contents) make up 3% (10.5 kg) of the total body parts of an average slaughtered White Fulani cattle (Omole and Ogbiye, 2013). The rumen of cattle contains a diverse group of bacteria living symbiotically and aiding food digestion in cattle. On the other hand, among these bacteria are potential human pathogens. Bacteria of public health importance such as *Salmonella* sp., *Escherichia coli*, *Staphylococcus* sp., and *Campylobacter* sp. have been isolated from abattoir wastewater and rumen waste (Franke-Whittle and Insam, 2013; Esemu *et al.,* 2022). Unhygienic cattle processing such as slaughtering and dressing on bare floors (Adjei *et al.,* 2022) as well as washing of carcasses with polluted water from water bodies already contaminated with abattoir

wastes (Olawuni *et al.*, 2017), also increases the chances of spreading bacterial pathogens from abattoir to the environment.

Antibiotic resistance is a global health challenge. The emergence and dissemination of antibiotic-resistant bacteria are major public health concerns that require urgent attention (Aslam *et al.,* 2018). Antibiotic-resistant bacteria can cause serious infections in humans, high mortality, increased length of patients' hospital stay, and economic loss (Mauldin *et al.,* 2010). The ruminants' digestive tracts and microbes in rumen are sources from where antibiotic resistance genes can spread to the environment (Auffret *et al.,* 2017; Sabino *et al.,* 2019; López-Catalina *et al.,* 2021). The indiscriminate use of antibiotics to control pathogens and as food supplements in livestock (Ventola, 2015) encourages the emergence of antibiotic resistant bacteria and high abundance of antibiotic resistance genes in the rumen. Antibiotic usage in livestock production and unhygienic slaughtering, and processing of animals at abattoirs most especially in some parts of Nigeria, underscore the need to study antibiotic resistance in bacteria that are isolated from abattoir environments, including wastewater, landfills, and rumen wastes. This kind of study will provide information on the antibiotic-resistant bacterial pathogens that can spread into the environment from abattoir facilities.

The current study focuses on the antibiotic-resistant patterns of bacteria isolated from rumen waste of cattle slaughtered in four different abattoirs at Osogbo metropolis, Nigeria.

MATERIALS AND METHODS

Sample collection

This study was conducted in four (4) selected privately owned abattoirs located in Osogbo, Southwestern Nigeria. The abattoirs were Bestway (BW), Shasha (SA), Sabo (SB) and Gbonmi (GB). The abattoirs were selected based on their strategic positioning to serve a large number of people (mostly low-income earners) living in Oke-baale and Gbonmi communities where there are no government-owned abattoirs. An average of six heads of cattle is slaughtered per day in each of the abattoirs. Rumen waste samples were collected from 25 randomly selected cattle from each abattoir making a total of 100 samples used for the study. The collection was carried out by aseptically taking one composite sample (mat and liquid mixture) from each slaughtered cow with a sterile spatula into a sterile universal sample bottle. Samples were carefully labelled and transported on ice packs to the laboratory for immediate analysis.

Enumeration, isolation and identification of total heterotrophic bacteria

Heterotrophic bacteria in the rumen waste samples were enumerated using a standard plate count method (Wehr and Franks, 2004). Rumen waste samples were serially diluted using sterile ringer solution as diluent. Then, 100 µL of the 10^{-8} and 10^{-9} dilutions were plated on nutrient agar (Oxoid, England) using the spread plate technique. All plates were incubated in an inverted position for 24 h at 37 ^{o}C. After incubation all the plates with 30 – 300 bacterial colonies were counted and computed using the formula below:

$$N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2)] \times (d)}$$

Where N = Number of colonies per gram of sample, ∑C = Sum of all colonies on all plates counted, n_1 = Number of plates in the first dilution counted, n_2 = Number of plates in the second dilution counted, and d = Dilution from which the first counts were obtained (Niemela, 1983; Maturin and Peeler, 1998).

Identification and characterization of bacterial isolates

Characterization of pure culture was based on conventional phenotypic methods, which include morphology, Gram stain, motility, spore formation, haemolysis, and growth on triple sugar iron (TSI) medium, catalase, oxidase, indole, Voges Proskauer, methyl red, citrate, starch, and sugar fermentation tests. Biochemical tests were interpreted to determine the presumptive nomenclature of the bacteria isolates using Bergey's Manual of Determinative Bacteriology and ABIS online-advanced bacterial identification system (Sorescu and Stoica, 2021). The pure identified bacterial isolates were used for antibiotic susceptibility testing.

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method (Hudzicki, 2009) and according to the standard microbiological guidelines (CLSI, 2012). Bacterial suspensions equivalent to 0.5 MacFarland standard were aseptically spread on solidified Mueller-Hinton agar (Himedia, India) using sterile cotton swab sticks. Inoculated plates were allowed to dry. Afterwards, the antibiotic multi-discs at a spatial orientation of 16 mm (distance between discs) were aseptically placed on the inoculum using sterile forceps. The antibiotics used in this study and their concentrations (Abtek, UK) were amoxicillin (25 µg), cotrimoxazole (25 µg), nitrofurantoin (300 µg), gentamicin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg), ofloxacin (30 µg), augmentin (30 µg), cloxacillin (5 µg), erythromycin (5 µg), streptomycin (10 µg) and

chloramphenicol (10 µg). The results of the diameters of the zones of inhibition were interpreted by comparing them with the Clinical Laboratory Standards Institute standards (CLSI, 2012) and each isolate was recorded as resistant, intermediate or susceptible to the various antibiotics. Multidrug resistance among the isolates was defined as resistance to ≥ 3 classes of antibiotics.

Plasmid profiling of bacterial isolates

Plasmid profiling of twenty-five bacterial isolates including 19 Gram-negative and 6 Gram-positive bacteria was done (result not shown). The selected bacterial isolates include *E. coli* (n=3), *Pseudomonas* spp. (n=7), *Klebsiella oxytoca* (n=2), *Brevundimonas diminuta* (n=1), *Tatumella ptyseos* (n=1), *Citrobacter diversus* (n=1), *Chryseomonas luteola* (n=1), *Enterobacter intermedius* (n=1), *Providencia rettgeri* (n=1), *Shigella dysenteriae* (n=1), *Lactobacillus* sp., (n=1), *L. monocytogenes* (n=1), *Bacillus cereus* (n=1), *Corynebacterium haemolyticum* (n=1) and *Corynebacterium diphtheriae* (n=2). The bacterial isolates were cultured in nutrient broth (Oxoid, England) overnight at 37 ^{o}C. The Plasmid DNA of the bacterial isolates was extracted using the TENS-Mini Prep method. Electrophoresis of extracted plasmid was done in 0.8% agarose gel. The gels were observed under UV-trans-illuminator and the plasmid sizes were compared to the Lambda DNA/Hind III reference marker (Promega, USA).

Plasmid curing of bacterial isolates

Bacterial isolates that possess plasmids were subjected to plasmid curing by culturing them in the presence of a sub-inhibitory concentration of acridine orange (0.10 mg mL^{-1}) in Mueller-Hinton broth (Ojo *et al.,* 2014). Briefly, bacterial isolates were cultured with 0.1 mL of a sub-inhibitory concentration of acridine orange in Mueller-Hinton broth and incubated at 37 ^{o}C for 24 h. Antibiotic susceptibility testing was carried out on the 24 h old culture using the agar disc diffusion method as described above and compared with the initial susceptibility result obtained for the isolates. Plasmid-mediated resistance was determined by the appearance of a zone of inhibition around the antibiotic disc while the absence of a zone of inhibition is an indication of constitutive non-plasmid or chromosome-mediated resistance.

Data Analysis

A descriptive analysis was performed by calculating the means of total heterotrophic bacterial counts (mean ± standard deviation [SD]). Using SPSS 17.0 for Windows, one-way ANOVA, at a 5% significant level, was used to determine whether or not there were significant differences between mean bacterial counts obtained from cattle rumen waste and abattoirs.

RESULTS

Identification and characterization of bacterial isolates

A total of 40 bacterial isolates were identified, including 34 Gram-negative (85%) and 6 Gram-positive (15%) bacteria (Table 1). The isolated Gram-negative bacteria belonging to the family Enterobacteriaceae (42.5%) and Pseudomonadaceae (42.5%) while others are Gram-positive bacteria including *Lactobacillus* sp., *Listeria monocytogenes*, *Corynebacterium* sp. and *Bacillus cereus*. The members of family Enterobacteriaceae isolated in this study include *Providencia rettgeri* (12.5%), *Chryseomonas luteola* (12.5%), *Escherichia coli* (10%), *Klebsiella oxytoca* (7.5%), *Citrobacter diversus* (2.5%), *Enterobacter intermedius* (2.5%), *Shigella dysenteriae* (2.5%) and *Tatumella ptyseos* (2.5%). *Pseudomonas aeruginosa* (10%), *Stenotrophomonas maltophilia* (7.5%) *Brevundimonas diminuta* (5%), *Pseudomonas fluorescens* (5%), and *Pseudomonas borbori* (2.5%) were the members of the family Pseudomonadaceae reported in this study.

Antibiotic resistance profile of bacterial isolates

The antibiotic resistance profile of bacterial isolates obtained from rumen waste samples at four different abattoirs in Osogbo is shown in Figure 1 and Table 2. All the bacteria except members of the genus *Corynebacterium* were resistant to augmentin. Similarly, all the Gram-positive bacteria except *Corynebacterium* exhibited resistance to cotrimoxazole and erythromycin (Figure 1). All the bacterial isolates tested against Amoxicillin (100%) and cloxacillin (100%) exhibited resistance to these antibiotics. *Pseudomonas* spp., *Citrobacter diversus*, *Tatumella ptyseos*, *Enterobacter intermedius* and *Listeria monocytogenes* exhibited 100 % resistance to tetracycline. *C. diversus*, *T. ptyseos* and *L. monocytogenes* exhibited 100 % resistance to gentamicin while *T. ptyseos* and *B. cereus* exhibited 100 % resistance to nitrofurantoin and chloramphenicol, respectively. Generally, 37 (92.5%), 18 (45%), 14 (35%), and 10 (25%) out of the total 40 bacteria isolated were resistant to augmentin, tetracycline, cotrimoxazole, and gentamicin respectively. Percentage resistance to nalidixic acid (5.9%) and ofloxacin (2.9%) was low among the Gram-negative bacteria while percentage resistance to nitrofurantoin was

23.5% (Table 2). All the Gram-negative bacteria except one strain of *P. aeruginosa* were susceptible to ofloxacin. All the Gram-positive bacterial isolates tested against streptomycin were susceptible to the antibiotic.

The most common antibiotype among the Gram-negative bacteria isolates, as observed in Table 3 is the resistance to both amoxicillin and Augmentin (Amx^R, Aug^R) (41.17 %) while among the Gram-positive, it is resistance to Cloxacillin (Cxc^R) (33.33%). In Table 4.0, multi-drug resistance phenotype (i.e. bacterial isolates that exhibited resistance to three or more classes of antibiotics) was observed among 23 (57.5%) of the total bacterial isolates comprising of 12 Genera including *Pseudomonas* (n=7; 30.4%), *Escherichia* (n=3; 13%), *Brevundimonas* (n=2; 8.7%), *Klebsiella* (n=2; 8.7%), *Chryseomonas* (n=2; 8.7%), and 1 each for *Citrobacter*, *Tatumella*, *Enterobacter*, *Providencia*, *Lactobacillus, Listeria* and *Bacillus* (4.3% each).

Plasmid profiling and plasmid curing

Plasmids with a size corresponding to 4,361 bp of Lambda DNA/Hind III reference marker were detected in two bacterial isolates including a strain of *E. coli* (Lane 1) and *P. aeruginosa* (Lane 11) (Figure 2.0). Plasmid bands with sizes less than 2027 bp were detected in four of the Gram-positive bacteria tested. These include *Listeria monocytogenes* (Lane 14), *Bacillus cereus* (Lane 17), *Corynebacterium haemolyticum* (Lane 18), and a strain of *Corynebacterium diphtheriae* (Lane 23) with one band (Figure 3). After plasmid curing, the Gram-positive bacterial isolates, including *L. monocytogenes* (Aug^R, Tet^R) and *B. cereus* (Tet^R) that harbour plasmids were resistant to augmentin and tetracycline (Table 5), while the two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and *Corynebacterium* spp. became sensitive.

Table 1: Percentage Distribution of Bacteria in Rumen Waste Samples obtained from four different abattoirs in Osogbo.

S/N	BACTERIAL ISOLATES	NUMBER (%)
	Gram Negative	
1.	*Providencia rettgeri*	5 (12.5)
2.	*Chryseomonas luteola*	5 (12.5)
3.	*Escherichia coli*	4 (10.0)
4.	*Pseudomonas aeruginosa*	4 (10.0)
5.	*Klebsiella oxytoca*	3 (7.5)
6.	*Stenotrophomonas maltophilia*	3 (7.5)
7.	*Brevundimonas diminuta*	2 (5.0)
8.	*Pseudomonas fluorescens*	2 (5.0)
9.	*Shigella dysenteriae*	2 (5.0)
10.	*Tatumella ptyseos*	1 (2.5)
11.	*Enterobacter intermedius*	1 (2.5)
12.	*Citrobacter diversus*	1 (2.5)
13	*Pseudomonas borbori*	1 (2.5)
	Gram Positive	
14.	*Corynebacterium diphtheriae*	2 (5.0)
15.	*Lactobacillus* sp.	1 (2.5)
16.	*Listeria monocytogenes*	1 (2.5)
17.	*Corynebacterium haemolyticum*	1 (2.5)
18.	*Bacillus cereus*	1 (2.5)
	TOTAL	40

Table 2: Percentage resistance of Bacteria isolated from Rumen Waste Samples to different Antibiotics

Antibiotics (Concentrations)	Gram-negative isolates = 34 (% resistance)	Gram-positive isolates = 6 (% resistance)	Total number of bacterial isolates = 40 (% resistance)
Augmentin (30 µg)	34 (100.00)	3 (50.00)	37 (92.50)
Tetracycline (25 µg)	16 (47.06)	1 (16.67)	17 (42.5)
Cotrimoxazole (25 µg)	11 (32.35)	3 (50.00)	14 (35.00)
Gentamicin (10 µg)	8 (23.53)	1 (16.67)	9 (22.50)
Amoxicillin (25 µg)	34 (100.00)	NT	-
Nitrofurantoin (200 µg)	8 (23.53)	NT	-
Nalidixic acid (30 µg)	2 (5.88)	NT	-
Ofloxacin (5 µg)	1 (2.94)	NT	-
Cloxacillin (5 µg)	NT	6 (100.00)	-
Erythromycin (5 µg)	NT	4 (66.67)	-
Chloramphenicol (10 µg)	NT	1 (16.67)	-
Streptomycin (10 µg)	NT	0 (0.00)	-

NT: Not tested

Table 3: Percentage distribution of Antibiotypes among bacteria isolated from rumen waste obtained from abattoirs in Osogbo, Osun State.

S/N.	Antibiotypes	Occurrence (%)	
		Gram-negative	Gram-positive
1	Amx^{R}, Aug^{R}	14 (41.17)	-
2	Amx^{R}, Cot^{R}, Aug^{R}	3 (8.82)	-
3	Amx^{R}, Aug^{R}, Tet^{R}	1 (2.94)	-
4	Amx^{R}, Gen^{R}, Aug^{R}, Tet^{R}	5 (14.70)	-
5	Amx^{R}, Nit^{R}, Aug^{R}, Tet^{R}	1 (2.94)	-
6	Amx^{R}, Cot^{R}, Gen^{R}, Aug^{R}, Tet^{R**}	2 (5.88)	-
7	Amx^{R}, Gen^{R}, Nal^{R}, Aug^{R}, Tet^{R**}	1 (2.94)	-
8	Amx^{R}, Cot^{R}, Nit^{R}, Aug^{R}, Tet^{R**}	4 (11.76)	-
9	Amx^{R}, Nit^{R}, Gen^{R}, Aug^{R}, Tet^{R**}	1 (2.94)	-
10	Amx^{R}, Cot^{R}, Nit^{R}, Nal^{R}, Aug^{R}, Tet^{R**}	1 (2.94)	-
11	Amx^{R}, Cot^{R}, Nit^{R}, Ofl^{R}, Aug^{R}, Tet^{R**}	1 (2.94)	-
12	Cxc^{R}	-	2 (33.33)
13	Cxc^{R}, Ery^{R}	-	1 (16.67)
14	Cot^{R}, Cxc^{R}, Ery^{R}, Aug^{R}	-	1 (16.67)
15	Cot^{R}, Cxc^{R}, Ery^{R}, Aug^{R}, Chl^{R**}	-	1 (16.67)
16	Cot^{R}, Cxc^{R}, Ery^{R}, Gen^{R}, Aug^{R}, Tet^{R**}	-	1 (16.67)
	TOTAL	34	6

Legend: **Aug**: Augmentin; **Amx**: Amoxicillin; **Chl:** Chloramphenicol **Cxc:** Cloxacillin; **Cot:** Cotrimoxazole; **Ery:** Erythromycin **Gen:** Gentamicin; **Nal:** Nalidixic acid; **Nit**: Nitrofurantoin; **Ofl:** Ofloxacin; **Tet:** Tetracycline; $^{R:}$ Resistance to

Table 4: Antibiotypes of Bacterial Isolates recovered from rumen waste obtained from abattoirs in Osogbo, Osun State.

S/N.	Bacterial Isolate	Resistance Pattern
Gram-Negative Bacteria		
1.	*Escherichia coli*	Amx^R, Cot^R, Gen^R, Aug^R, Tet^R
2.	*Escherichia coli*	Amx^R, Cot^R, Gen^R, Aug^R, Tet^R
3.	*Escherichia coli*	Amx^R, Gen^R, Aug^R, Tet^R
4.	*Escherichia coli*	Amx^R, Aug^R
5.	*Pseudomonas borbori*	Amx^R, Cot^R, Nit^R, Nal^R, Aug^R, Tet^R
6.	*Brevundimonas diminuta*	Amx^R, Cot^R, Aug^R
7.	*Brevundimonas diminuta*	Amx^R, Gen^R, Nal^R, Aug^R, Tet^R
8.	*Klebsiella oxytoca*	Amx^R, Gen^R, Aug^R, Tet^R
9.	*Klebsiella oxytoca*	Amx^R, Nit^R, Aug^R, Tet^R
10.	*Klebsiella oxytoca*	Amx^R, Aug^R
11.	*Citrobacter diversus*	Amx^R, Gen^R, Aug^R, Tet^R
12.	*Tatumella ptyseos*	Amx^R, Nit^R, Gen^R, Aug^R, Tet^R
13.	*Pseudomonas fluorescens*	Amx^R, Gen^R, Aug^R, Tet^R
14.	*Pseudomonas fluorescens*	Amx^R, Gen^R, Aug^R, Tet^R
15.	*Enterobacter intermedius*	Amx^R, Aug^R, Tet^R
16.	*Pseudomonas aeruginosa*	Amx^R, Cot^R, Nit^R, Aug^R, Tet^R
17.	*Pseudomonas aeruginosa*	Amx^R, Cot^R, Nit^R, Aug^R, Tet^R
18.	*Pseudomonas aeruginosa*	Amx^R, Cot^R, Nit^R, Aug^R, Tet^R
19.	*Pseudomonas aeruginosa*	Amx^R, Cot^R, Nit^R, Ofl^R, Aug^R, Tet^R
20.	*Stenotrophomonas maltophilia*	Amx^R, Aug^R
21.	*Stenotrophomonas maltophilia*	Amx^R, Aug^R
22.	*Stenotrophomonas maltophilia*	Amx^R, Aug^R
23.	*Shigella dysenteriae*	Amx^R, Aug^R
24.	*Shigella dysenteriae*	Amx^R, Aug^R
25.	*Providencia rettgeri*	Amx^R, Aug^R
26.	*Providencia rettgeri*	Amx^R, Cot^R, Aug^R
27.	*Providencia rettgeri*	Amx^R, Aug^R
28.	*Providencia rettgeri*	Amx^R, Aug^R
29.	*Providencia rettgeri*	Amx^R, Aug^R
30.	*Chryseomonas luteola*	Amx^R, Aug^R
31.	*Chryseomonas luteola*	Amx^R, Cot^R, Nit^R, Aug^R, Tet^R
32.	*Chryseomonas luteola*	Amx^R, Aug^R
33.	*Chryseomonas luteola*	Amx^R, Cot^R, Aug^R
34.	*Chryseomonas luteola*	Amx^R, Aug^R
Gram-Positive bacteria		
35	*Lactobacillus* sp.	Cot^R, Cxc^R, Ery^R, Aug^R
36	*Listeria monocytogenes*	Cot^R, Cxc^R, Ery^R, Gen^R, Aug^R, Tet^R
37	*Bacillus cereus*	Cot^R, Cxc^R, Ery^R, Aug^R, Chl^R
38	*Corynebacterium haemolyticum*	Cxc^R
39	*Corynebacterium diphtheriae*	Cxc^R
40	*Corynebacterium diphtheriae*	Cxc^R, Ery^R

Legend: **Aug**: Augmentin; **Amx**: Amoxicillin; **Chl:** Chloramphenicol **Cxc:** Cloxacillin; **Cot:** Cotrimoxazole; **Ery:** Erythromycin **Gen:** Gentamicin; **Nal:** Nalidixic acid; **Nit**: Nitrofurantoin; **Ofl:** Ofloxacin; **Tet:** Tetracycline; **R:** Resistance to

Table 5. Antibiotic resistance phenotype of bacterial isolates after plasmid profiling and curing

Bacterial isolate	**Antibiotic resistance before plasmid curing**	**Antibiotic resistance after plasmid curing**
Escherichia coli (Lane 1)	Amx^R, Cot^R, Gen^R, Aug^R, Tet^R	Sensitive
Pseudomonas aeruginosa (Lane 11)	Amx^R, Cot^R, Nit^R, Ofl^R, Aug^R, Tet^R	Sensitive
Listeria monocytogenes (Lane 14)	Cot^R, Cxc^R, Ery^R, Gen^R, Aug^R, Tet^R	Aug^R, Tet^R
Bacillus cereus (Lane 17)	Cot^R, Cxc^R, Ery^R, Aug^R, Chl^R	Aug^R
Corynebacterium haemolyticum (Lane 18)	Cxc^R,	Sensitive
Corynebacterium diphtheriae (Lane 23)	Cxc^R, Ery^R	Sensitive

Legend: **Aug**: Augmentin; **Amx**: Amoxicillin; **Chl:** Chloramphenicol **Cxc:** Cloxacillin; **Cot:** Cotrimoxazole; **Ery:** Erythromycin **Gen:** Gentamicin; **Nit**: Nitrofurantoin; **Ofl:** Ofloxacin; **Tet:** Tetracycline; **R:** Resistance to

Plate 1: Rumen wastes of cattle being deposited directly on a dumpsite by an abattoir attendant.

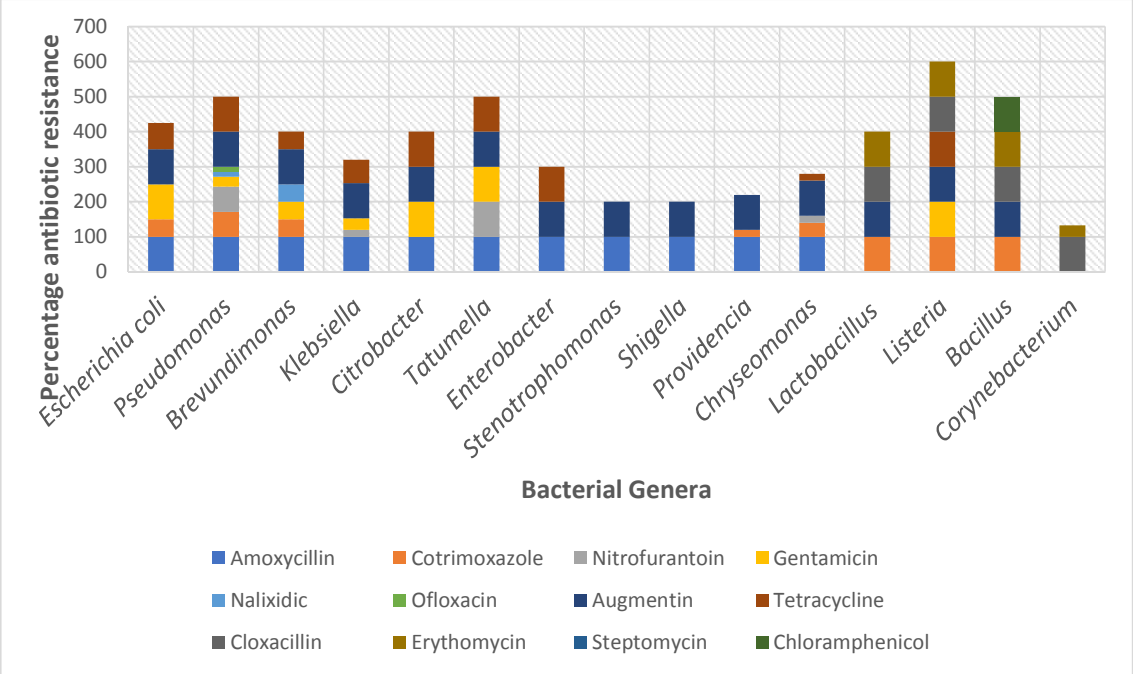

Figure 1: Percentage resistance of forty (40) bacterial isolates made up of 15 bacterial genera isolated from rumen waste to eight antibiotics

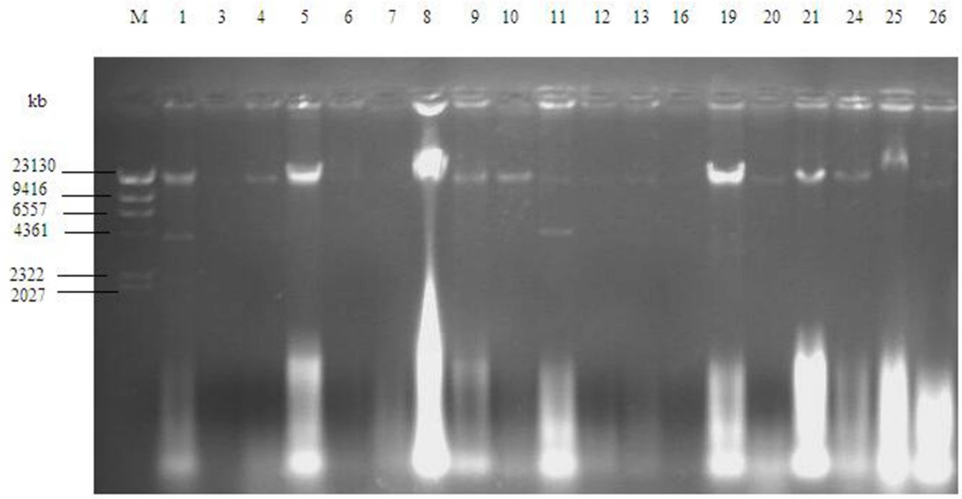

Figure 2: Plasmid Profiles of Gram-Negative Bacteria from Rumen Waste Samples. Lane M is DNA marker (molecular ladder). Lanes 1 – 26 are representative plasmid profiles of the bacterial strains (Lanes 1, 3 and 4=*E.coli*; 5-11=*Pseudomonas* spp; 12=*Brevundimonas diminuta*; 13 & 16=*Klebsiella oxytoca*; 19=*Citrobacter diversus*; 20=*Tatumella ptyseos*; 21=*Chryseomonas luteola*; 24= *Enterobacter intermedius*; 25= *Providencia rettgeri*; 26= *Shigella dysenteriae*)

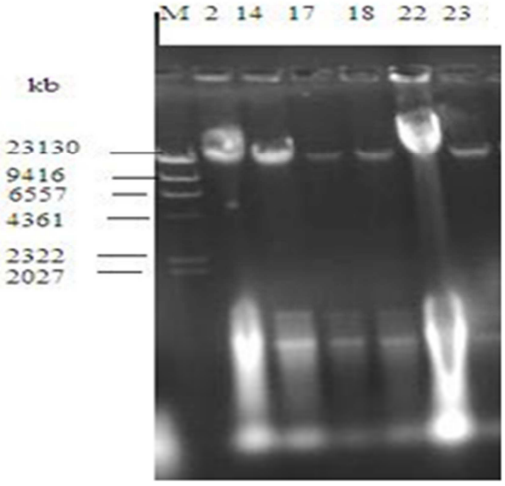

Figure 3: Plasmid Profiles of six Gram-positive Bacteria isolated from Rumen Waste Samples. Lane M is DNA marker (molecular ladder). Lanes 2 (*Lactobacillus* sp.), 14 (*Listeria monocytogenes*), 17 (*Bacillus cereus*), 18 (*Corynebacterium haemolyticum*), 22 (*Corynebacterium diphtheriae*) and 23 (*Corynebacterium diphtheriae*) are plasmid profiles of the bacterial strains.

DISCUSSION

Meat from livestock most especially cattle is one of the cheapest and most readily available sources of proteins to Nigerians (Jabo and Zaharadden, 2018). Abattoir remains one of the places where people purchase meat in Nigeria. Cattle rumen wastes generated in abattoirs are heavily ladened with bacteria that can be potential pathogens to humans. This study focused on isolating and determining the antibiotic-susceptibility pattern of bacteria from cattle rumen wastes obtained from four abattoirs in Osogbo, Osun State, Nigeria.

The members of the group Enterobacteriaceae (42.5 %), including *Providencia rettgeri, Escherichia coli, Klebsiella oxytoca*, *Shigella dysenteriae, Citrobacter diversus*, *Enterobacter intermedius,* and *Tatumella ptyseos* were the bacterial groups isolated from the cattle rumen wastes obtained from the study abattoir followed by the members of the family Pseudomonadaceae (30%) including *Pseudomonas* spp. and *Chryseomonas luteola*. Members of the Genus *Klebsiella*, *Citrobacter*, *Pseudomonas*, *Shigella* and *Providencia* have been isolated from the rumen content of

different breeds of cattle in Nigeria (Akintokun *et al*., 2014). The high prevalence of enteric bacteria obtained in this study is expected because they are normal floral of the small and large intestines of both humans and animals. However, enteric bacteria could either be non-pathogenic or pathogenic. The enteric bacteria isolated from the cattle rumen wastes belong to the common pathogenic Enterobacteriaceae (Rock and Donnenberg, 2014). The pathogenic enteric bacteria are opportunistic, and diseases caused by them are one of the leading causes of death in the developing world (Bublitz *et al*., 2015). For instance, *P. rettgeri* and *E. coli* are the most prevalent members of the Enterobacteriaceae recovered from the cattle rumen waste in this study, and they have been implicated in diarrhoea and urinary tract infections (Kwong *et al*., 2015). Another pathogenic Enterobacteriaceae of public health importance isolated from the cattle rumen waste is *Shigella dysenteriae*. It is highly virulent possessing the ability to produce deadly Shiga-toxin (Mauro and Koudelka, 2011).

Enterobacter intermedius, *Citrobacter diversus*, *Klebsiella oxytoca* and members of the family Pseudomonadaceae are opportunistic pathogens of humans that have been isolated from cattle rumen (Akintokun *et al.,* 2014). Agbaje *et al*. (2011) reported the isolation of *Tatumella ptyseos* in beef. Human infections caused by *T. ptyseos* are not common as only a few clinical cases have been reported (Costa *et al*., 2008). However, *T. ptyseos* has been implicated in diseased conditions such as pneumonitis, asthmatic bronchitis, pulmonary oedema, chronic lung diseases (Hollis *et al*., 1981), pulmonary tuberculosis and sepsis (Costa *et al*., 2008) and gastrointestinal infection (Janda and Abbot, 2006). One of the significant pathogenic Gram-positive bacteria isolated from the cattle rumen is *Listeria monocytogenes*. It can cause abortion in both humans and ruminants (Sahlström, 2003).

Other members of the family Enterobacteriaceae reported in this study have been isolated from wastewater and sediments from abattoirs in some parts of Nigeria (Omoregbe *et al*., 2017). Poor hygienic conditions, management and practices at abattoirs can encourage the spread of the potential bacterial pathogens associated with rumen waste in the environment, more so that cattle rumen wastes generated from abattoirs in Nigeria are washed into nearby waterbodies. Indiscriminately exposed cattle rumen wastes at abattoirs can encourage the transfer of bacterial pathogens by vectors such as houseflies to meat meant for consumption by humans.

The problem of antibiotic resistance is a global concern and it requires urgent attention. Indiscriminate use of antibiotics in veterinary and animal husbandry to improve the health status and growth of food animals increased the emergence of antibiotic-resistant bacteria (Phillips *et al*., 2004). The high level of resistance of bacterial isolates to β-lactam antibiotics including amoxicillin (100%), cloxacillin (100%), and augmentin (92.5%), and even tetracycline (42.5%) could be attributed to the easy accessibility and unregulated use of these antibiotics in veterinary and animal husbandry for the treatment of animal infections and growth promotion. Moreover, tetracyclines and β-lactams are a few of the antibiotics frequently administered to livestock in Southwestern, Nigeria (Adesokan *et al*., 2015). The Gram-positive bacteria isolated in this study also exhibited high resistance to erythromycin (66.67%). Macrolides such as erythromycin are also one of the antibiotics commonly administered for livestock disease treatment in Nigeria (Adesokan *et al*., 2015). In the same study, Adesokan *et al*., (2015) reported an increase in the trend of usage of these antibiotics (tetracyclines, β-lactams, and macrolides) over a period of three years in Osogbo where our study was conducted. Though the Gram-negative bacteria isolated in this study exhibited low resistance to ofloxacin (2.94%), a fluoroquinolone, reports on the increase in fluoroquinolone resistance among bacterial isolates (Lamikanra *et al*., 2011; Adesokan *et al*., 2015) suggests the need to regulate the usage of this class of antibiotics. The consequences of humans getting infected with these antibiotic-resistant bacterial pathogens through the consumption of contaminated meat could be grave including difficulties in infection treatment and economic loss due to an increase in length of hospital stay. The multi-resistant phenotype in bacteria can either be plasmid- or chromosome-mediated. Aside from incessant exposure of bacterial isolates to antibiotics, antibiotic-resistance genes can also spread and persist among bacteria by the exchange of plasmids bearing resistance genes through conjugation (Smillie *et al.,* 2010). Plasmid curing analysis suggested that the antibiotic resistance phenotype of the two Gram-negative bacteria tested is plasmid-mediated while resistance to some antibiotics such as augmentin and tetracycline is chromosome-mediated because resistance to these antibiotics was not lost after plasmid curing. Antibiotic-resistant bacterial pathogens associated with cattle rumen waste can be a possible source of disseminating resistance genes among bacteria

when they harbour plasmids and are released into the environment through the indiscriminate discharge of rumen waste.

CONCLUSION

This study showed that potential antibiotic-resistant bacterial pathogens are residents of cattle rumen waste and there is a high prevalence of plasmid carriage among the Gram-positive bacterial isolates compared to the Gram-negative bacteria. The presence of plasmids in these bacterial isolates can aid the dissemination of antibiotic-resistant genes in the environment if cattle rumen waste is discharged without caution into the environment. It is important to establish laws that will regulate the activities of abattoir owners to prevent the public health problem that can occur through the unguided discharge of rumen waste into the environment. In addition, there is a need to monitor and regulate the use of antibiotics in veterinary and animal husbandry. Lastly, we recommend that cattle rumen waste generated at the abattoir can be used as feedstock for biogas production instead of discharging it indiscriminately into the environment; an activity that can increase the burden of antibiotic-resistant bacterial pathogens in the environment.

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