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Evaluation of resistance determinants in gram-negative bacteria obtained from fish pond and animal-based wastes in South-West, Nigeria

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Abstract

Drug resistance is a global phenomenon in healthcare institutions. However, drug resistance in medically important bacteria from non-clinical settings is rapidly increasing; thus, raising environmental and public health concerns. Hence, the objective of this study was to determine the prevalence of antibiotic resistance determinants in Gram-negative bacteria from selected animal wastes. Animal waste samples (54) were obtained from 18 farms and subjected to bacteriological assessment. Bacteria were isolated and characterized using API 20E kit. Antibiotics susceptibility of the bacteria was determined by disc diffusion method. Resistance genes and plasmid were profiled by PCR and alkaline lysis, respectively. Of 123 bacteria identified, 19.5, 17.1, 14.6, 14.6, 9.8, 8.9, 8.1 and 7.3% were *Enterobacter aerogenes*, *Serratia*, *Providencia stuartii*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Escherichia coli*, *K. oxytoca* and *Pantoea* species, respectively. Susceptibility test indicated that 100, 99, 96, 88, 57, 55 and 50% of the bacteria were resistant to amoxicillin/clavulanate and ceftazidime, gentamicin, cefuroxime, cefixime, ciprofloxacin, nitrofurantoin and ofloxacin, respectively. About 31 and 29% of the bacteria harbored *tetA* and *tetB* genes while 15 and 7% harbored *sul1* and *sul2* genes, respectively. Plasmids with molecular weight of 23.1 kb were found in 16% of the isolates. The study has shown resistant bacteria from animal waste and these bacteria harbor several resistant genes and plasmids. Hence, surveillance system is required to prevent the increasing resistance burden in bacteria within the environment.

Keywords: Antibiotics, API, Integrons, Plasmid, Resistance genes, Wastes

Introduction

Antimicrobial drug resistance is a global problem that affects public health including veterinary medicine (WHO, 2015). This problem results from misuse of antimicrobials used as prophylaxis and growth promoters in animal husbandry (WHO, 2014). It is projected that by 2050, antibiotic-resistance burden will cause approximately 300 million premature deaths cumulatively with an estimate of $100 trillion financial loss to the global economy (Arias & Murray, 2015). The abuse of antimicrobial agents mainly tetracycline and β-lactams (penicillin) in animal husbandry was responsible for the development of resistant organisms (Wright, 2010; Adesokan *et al*., 2015). Development of drug resistance in microorganisms involves transfer or acquisition of resistance genes by either horizontal or vertical route (Ploy *et al*., 2000; Xu *et al*., 2011). Generally, gene transfer between species predominantly in Gram-negative bacteria occurs via mobile genetic elements such as gene cassettes,

plasmids and transposons, insertion sequences and genomic islands (Stokes & Hall, 1989; Recchia & Hall, 1995; Rowe-Magnus & Mazel, 2001; Tauch *et al*., 2002; Iyer *et al*., 2013).

Gram-negative bacteria are known to produce extended-spectrum β-lactamases (ESBLs), especially CTX-M that was responsible for many disease outbreaks (Leflon-Guibout et al., 2008; Woodford, 2008). In addition, plasmids carrying AmpC β-lactamases were found in different species of Gram-negative bacteria (*P. aeruginosa*, *Citrobacter* spp., *E. coli* and others species), and have been associated with multi-drug resistance (Carattoli, 2009). About 17.4% and 41% of Incl1 plasmids are widespread in avian commensal and pathogenic *E. coli*, respectively (Carattoli, 2009). ESBLs are often used by Gram-negative bacteria to metabolize antibiotics (Coque *et al*., 2008; Pitout &, Laupland, 2008) and were implicated in carbapenem resistance that are prevalent in healthcare institutions and in community settings (Kelly *et al*., 2017). ESBLs usually reside on plasmids where genetic exchange between bacteria occurs. There are various ESBLs of which over 150 have been reported worldwide. Nevertheless, class A β-lactamases are the most extensively studied consisting of TEM, SHV and CTX-M β- lactamases (Rupp & Fey, 2003). An estimate of 23% of *K. pneumoniae* and 14% *E. coli* (ESBL producers) were associated with 26,000 infections and 1,700 deaths annually in the United States (Harris, 2015).

Due to the mobility of gene cassettes, integrons play vital roles in the dissemination and spread of resistance genes between different species of bacteria (Tauch *et al*., 2002; Nemergut *et al*., 2008). Integrons are genetic elements that allow the shuffling and mobilization of smaller mobile elements (gene cassettes) (Bennett, 1999; Carattoli, 2001). They facilitate the dissemination of antimicrobial resistant genes in Gram-negative bacteria, especially in members of the family Enterobacteriaceae (Fluit & Schmitz, 1999). There are eight classes of integrons and each is distinguished by the difference in the sequences of their integrase genes (Nield *et al*., 2001). However, Class1, Class 2, Class 3 and Class 4 integrons are implicated in clinical isolates (Hall *et al*., 1999; Rowe-Magnus & Mazel, 2001). The integron classes listed above have been described in Gram-negative bacteria (Martinez-Freijo *et al*., 1998; Yuan *et al*., 2009; White *et al*., 2001). Class 1 is common in clinical settings and is associated with multidrug resistance (Leverstein-van *et al*., 2003). Studies reveal that bacteria that harbor Class 1 integrons are widespread in environment including hospitals (Lévesque *et al*., 1995; Gombac *et al*., 2002), soil (Srinivasan *et al*., 2008), healthy humans (Sepp *et al*., 2009)], food products (Meng *et al*., 2011) and aquatic environments (Rosser & Young, 1999; Moura *et al*., 2007; Wright *et al*., 2008).

Genetic elements are widespread in bacteria resident in various environmental samples, thus facilitating efficient horizontal gene transfer between species especially in environment with high antibiotic pressure. Reports worldwide suggest that antibiotic use in animal husbandry is one of the driving forces behind the development and dissemination of antibiotic resistance in certain pathogenic bacteria (Hamscher *et al*., 2002; Smith *et al*., 2003; Chapin *et al*., 2005. In Nigeria, application of antibiotics in animal feed is common. Thus, there is high tendency for development of antibiotic resistance genes as well as spreading the same across different human, animal and the environmental microbiota. which may constitute a direct threat to public health in this region. Hence, this study aimed to characterize Gram-negative bacteria isolated from different wastes by API 20E kit and evaluate the prevalence of some resistance genes including plasmids in Gram-negative bacteria.

Materials and methods

Sample collection

A total of 54 samples consisting of pond water, poultry dropping and cow dung were collected from three locations each in Ogun, Osun, Ekiti, Ondo, Oyo and Lagos States. The samples were collected in sterile universal bottles and transported to the Microbiology laboratory, Babcock University in an ice bag.

Isolation of bacteria

Ten millilitre aliquot of pond water was mixed with 90 mL of peptone water ((BPW; Oxoid, Basingstoke, England) while 10 g of solid sample (poultry dropping or cow dung) was disolved in 250 ml conical flask containing 90 mL of peptone water. All the prepared samples were homogenized, serially diluted and pour-plated on MacConkey, Salmonella-Shigella, Eosin and Methylene blue agar media (Oxoid, Basingstoke, England). All inoculated plates were incubated aerobically at 37 ºC for 24 h. Thereafter, the developing colonies were sub-cultured on freshly prepared nutrient agar (Oxoid, Basingstoke, England). Purified isolates were stored in 40% glycerol broth at -20 ºC.

Identification of Gram-negative bacteria with API 20E Kit

Isolates were identified using API 20E kit (BioMérieux, France) following the manufacturer's instructions. Briefly, suspension of bacterial colonies was prepared with sterile distilled water and standardized to match 0.5 McFarland standard. The API 20E test cupules were filled with each prepared bacterial suspension. Mineral oil was added into the ADH, LDC, ODC, H_2S and URE compartments to ensure anaerobic environment. Each inoculated stripe in a tray was incubated at 37 °C for 24 h. Thereafter, appropriate reagents were added to the respective TDA, IND and VP compartments and the results were read virtually. The results were converted into a 7-digit profile and matched with the profile index for each species identification.

Antibiotic susceptibility test

Antibiotic sensitivity testing was done according to the disk diffusion method described by the Clinical and Laboratory Standard Institute (CLSI, 2016). Single bacterium colony was inoculated into a test tube containing 2 mL of nutrient broth and incubated overnight at 37 °C. The overnight broth was then standardized to match 0.5 McFarland standard. A sterile swab stick was dipped in the standardized suspension and streaked over the surface of freshly prepared Mueller Hilton agar plates. After drying, commercial antibiotic discs (Abtek Biologicals Limited, United Kingdom were placed on the agar surface maintaining a distance of 30 mm edge to edge and incubated at 37 °C for 24 h. Thereafter, zones of inhibition were measured to the nearest millimeter. The antibiotics used include ofloxacin (OFL, 30 µg), ceftazidime (CAZ; 30 µg), cefuroxime (CRX; 30 µg) ciprofloxacin (CPR; 5 µg), amoxicillin/clavulanate (AUG; 30 µg), gentamicin (GEN; 10 µg), nitrofurantion (NIT; 30 µg) and cetixime (CXM; 5 µg).

Extraction of DNA

Exactly 200 µL of each isolate was transferred into an Ependorff tube. Chromosomal DNA was obtained by using a Qick-DNA^{TM} miniprep plus kit (Zymo research, Biolab, USA) following the manufacturer's instructions. Aliquots (2 µL) of template DNA was used for PCR analysis.

Detection of resistance genes (*sul 1*, sul *2*, *tetA*, *tetB*, *SHV*, *CTX-M*, *TEM* and class 1, 2 and 3 integrons)

Simplex and multiplex polymerase chain reactions (PCR) were used for the detection of resistance genes as previously described elsewhere (Ng *et al*., 2001; Rezaee *et al*., 2011). An aliquot of 2.0 µl of DNA suspension was added to PCR mixture containing 12.5 µL OneTaq Quick-Load 2X Master Mix (New England BioLabs), 0.5 µl of 10 mM primers synthesized by Inqaba Biotech, South Africa (Table 1) and 10.5 µl sterile distilled water. Polymerase Chain Reaction assays was carried out with a negative control (the reagents without DNA template). DNA amplification was carried out using 9700 thermocycler with cycling profile for 30 cycles: initial denaturation at 95^{o}C for 3 min, denaturation 94^{o}C for 30 s, annealing temperature for 30 s, extension 68^{o}C for 30 s and final extension 68^{o}C for 5 min. After PCR amplification, 10 µl of PCR product was resolved by agarose gel electrophoresis.

Plasmid DNA extraction

LB broth supplemented with antibiotics was inoculated with a single colony of test organism. The cultures were grown overnight at 37^{o}C in a shaking incubator (Gallenkomp, England). About 1.5 ml of each overnight culture was transferred into 2 ml vial and centrifuged for 1 min at 14,000 rpm. The resulting pellet was re-suspended with 200 µl of solution I (50mM Tris pH 8.0 with HCl, 10mM EDTA, 100µg/ml RNase A). An aliquot (200 µl) of solution II (200mM NaOH, 1% SDS) was subsequently added and mixed by inversion. Again, 200 µl of solution III (3.0M Potassium Acetate, pH5.5) was added and mixed by inversion. Each vial was centrifuged for 10 min at 14,000 rpm and the supernatant was transferred to fresh vial. Exactly 900 µl of 100% ethanol was added to the supernatant in each vial and mixed by inversion. Thereafter, the vials were centrifuged at 14,000 rpm for 20 min and the supernatant discarded. The DNA pellet was washed with 100 µl of ice cold 75% ETOH and centrifuged again for 30 sec. Thereafter, the supernatant was discarded. The pellet was air dried for 10-30 min and re-suspended in 50 µl of sterile ddH_2O or TE buffer. DNA was resolved in gel electrophoresis using 5 µl of the samples (Birnboim & Doly J. 1979).

Table 1: Primer details used in this study

Primers	Sequence; 5'-3'	Genes	Weight (bp)	Tm (^{o}C)	Reference
hep35	TGCGGGTYAARGATBTKGATTT	Intergrase genes	491	37	White *et al*., 2001
hep36	CARCACATGCGTRTARAT				
hep58	TCATGGCTTGTTATGACTGT	Class I integron cassette region	Variable	46	White *et al*., 2001
hep59	GTAGGGCTTATTATGCACGC				
hep74	CGGGATCCCGGACGGCATGCACGATTTGTA	Class 2 integron cassette regions	491	52	White *et al*., 2001
Hep51	GATGCCATCGCAAGTACGAG				
CTX-1F	CCCATGGTTAAAAAATCACTG	*CTX-M*	891	47	Jeong *et al*.., 2005
CTX-1R	CCGTTTCCGCTATTACAAAC				
tetA F	TTGGCATTCTGCATTCACTC	*tecA*	494	48	Adesiji *et al*., 2014
tetA R	GTATAGCTTGCCGGAAGTCG				
tetB F	CAGTGCTGTTGTGTCATTAA	*tecB*	571	46	Adesiji *et al*., 2014
tetB R	GCTTGGAATACTGAGTGTAA				
sul1 F	TTTCCTGACCCTGCGCTCTAT	*Sul1*	793	52	Adesiji *et al*., 2014
sul1 R	GTGCGGACGTAGTCAGCGCCA				
sul2 F	CCTGTTTCGTCCGACACAGA	*Sul2*	667	51	Adesiji *et al*., 2014
sul2 R	GAAGCGCAGCCGCAATTCAT				
TEM F	ATAAAATTCTTGAAGACGAAA	*TEM*	1080	40	Moosavian & Deiham, 2012
TEM R	GACAGTTACCAATGCTTAATCA				
SHV F	TCGGGCCGCGTAGGCATGAT	*SHV*	660	56	Moosavian & Deiham, 2012
SHV R	AGCAGGGCGACAATCCCGCG				

Results and discussion

Distribution of bacteria in animal wastes

In this study, a total of 123 bacteria were obtained of which 37, 47 and 39 isolates were recovered from pond water, poultry dropping and cow dung, respectively. The percentage occurrences of bacteria from poultry dropping were 19.1, 19.1, 15, 12.8, 10.6, 8.5, 8.5 and 6.4 for *Salmonella enterica*, *Enterobacter aerogenes*, *E. coli*, *Klebsiella pneumoniae*, *Pantoea* spp., *Serratia* spp. , *Klebsiella oxytoca* and *Providencia stuartii* respectively. Likewise, those from cow dung: *P. stuartii* (23.1%), *Serratia* spp. (23.1%), *E. aerogenes* (17.9%), *K. oxytoca* (12.8%), *Pantoea* spp, (10.3%), *E. coli* (5.3%), *K. pneumoniae* (5.1%) and *S. enterica* (2.6%) were found. Finally, pond water contained less species: *Serratia* spp (21.6%), *S. enterica* (21.6%), *E. aerogenes* (21.6%), *P. stuartii* (16.2%), *K. pneumoniae* (10.8%), *E. coli* (5.4%) and *K. oxytoca* (2.7%) (Figure 1).

In this study, *E. coli*, *Serratia* spp, *S. enterica*, *E. aerogenes*, *K. pneumoniae*, *K. oxytoca*, *P. stuartii* and *Pantoea* spp., were recovered from the three types of animal wastes. The recovery of similar bacterial genera from pond water, poultry dropping and cow dung in this study could be attributed to cross contamination in the environment. Specifically, the presence of coliform bacteria in the pond water suggest faecal contamination. Perhaps, faecal contamination was as a result of fertilization of the ponds with animal manure and/or fish excreta (Kay *et al*., 2008). Similar bacterial spectra were previously reported in fish pond water from the Niger Delta region of Nigeria (Njoku *et al*., 2015). The presence of these potential pathogens especially *E. coli*, *Salmonella* and *Klebsiella* suggests that they are widely distributed in this environment. The recovery of potential pathogens including *E. aerogenes*, *Escherichia coli*, *K. oxytoca*, *K. pneumoniae*, *Providencia stuartii* from poultry dropping and cow dung was not surprising because the gastrointestinal tracts of animals contain plethora of microbiota (Thursby & Juge, 2017). Similar bacterial spectra have previously been reported in poultry dropping and cow dung in Nigeria and India respectively, (Sawant *et al*., 2007; Adelowo *et al*., 2009). In this study, *Salmonella* and *Serratia* species were predominant in poultry dropping and cow dung, respectively. This was contrary to a study where *E. coli* strains were the predominant species in poultry dropping and cow dung (Omojowo & Omojasola, 2013). The variation observed could be attributable to differences in sampling areas and isolation techniques.

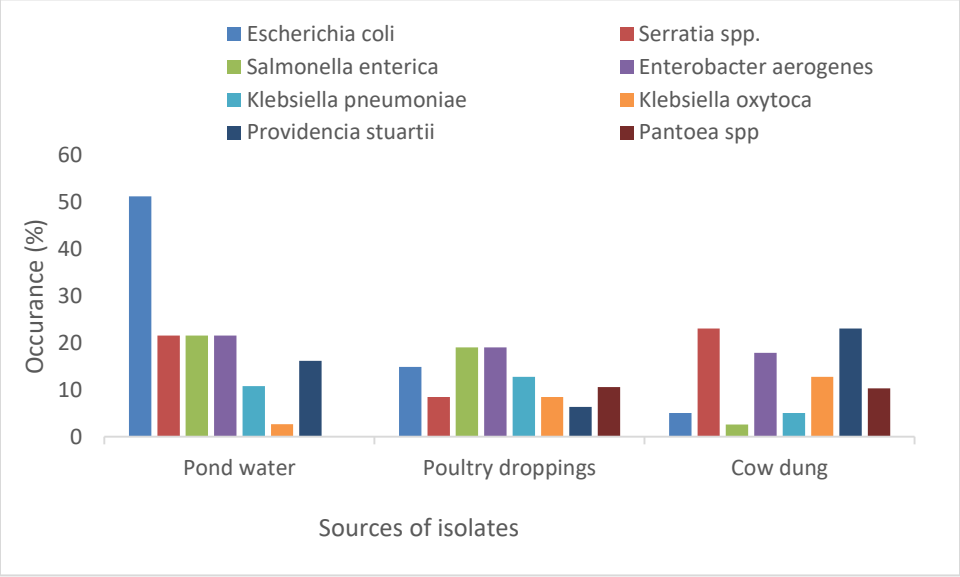

Figure 1: Distribution of Gran-negative bacteria according to their sources

Antibiotic sensitivity pattern

The results showed that all the *E. coli* strains were resistant to CAZ, CRX, GEN, CXM and AUG. compared to other species. However, only 45.0% of *E. coli* were resistant to OFL and NIT while 64% were resistant to CPR. Majority of the isolates were resistant to CAZ, CRX, GEN, CXM and AUG (Table 2). All the isolates were more susceptible to NIT and CPR compared to other antibiotics. Multi-drug resistance was observed in the isolates; 31.1% were resistant against all the antibiotic classes tested. Likewise, 22, 18.7, 22, 43.3 and 4.1% of the isolates were resistant to 7, 6, 5, 4 and 3 antibiotic classes, respectively (Fig 2).

In this study, over 50% of the isolates were resistant to most antimicrobial agents. Specifically, the high percentages of resistance observed in *E. coli* and *K. pneumoniae* in the present study were similar to those reported by Ugwu *et al*. (2018) for 91% *E. coli* and 94% *K. pneumoniae* resistance to CRX. The same report, however, showed that *E. coli* and *K. pneumoniae* were resistant against CAZ (56% and 32%) and gentamicin (46% and 44%) respectively compared to the same species in this study. This variation in resistance observed could be as a result of different strains of the same species encountered as well as different antibiotic pressures in the prevailing environment. More categorically, samples were obtained in abattoirs (meat swabs) rather than droppings as in this study. Higher level of resistance was observed in these isolates against gentamicin compared to results obtained elsewhere (Igbalajobi *et al*., 2015) and this could be due to the abuse and/or misuse of antibiotics by livestock handlers in Nigeria

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Table 2: Resistance pattern of Gram-negative bacteria from animal wastes to different antibiotic classes

Antibiotics	Organisms															
	E. coli n=11		***Serratia.* spp.** n=21		***S. enterica*** n=18		***E. aerogenes*** n=24		***K. pneumonia*** n=12		***K. oxytoca*** n=10		***P. stuartii*** n=18		***Pantoea* spp.** n=9	
	R	**%**	**R**	**%**	**R**	**%**	**R**	**%**	**R**	**%**	**R**	**%**	**R**	**%**	**R**	**%**
CAZ	11	100	21	100	18	100	24	100	12	100	10	100	18	100	9	100
CRX	11	100	20	95	17	94	24	100	10	83	10	100	17	94	9	100
GEN	11	100	21	100	18	100	24	100	11	92	10	100	18	100	9	100
CXM	11	100	18	86	17	94	21	88	9	75	9	90	15	83	8	89
OFL	5	45	16	76	6	33	12	50	4	33	5	50	10	56	4	44
AUG	11	100	21	100	18	100	24	100	12	100	10	100	18	100	9	100
NIT	5	45	16	76	3	17	18	75	5	42	5	50	10	56	6	67
CPR	7	64	13	62	9	50	16	67	5	42	6	60	9	50	5	56

Key: CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CXM- Cefixime, OFL- Ofloxacin, AUG- Amoxicillin/clavulanate, NIT- Nitrofurantoin, CPR- Ciprofloxacin, S- Susceptible, R- Resistant

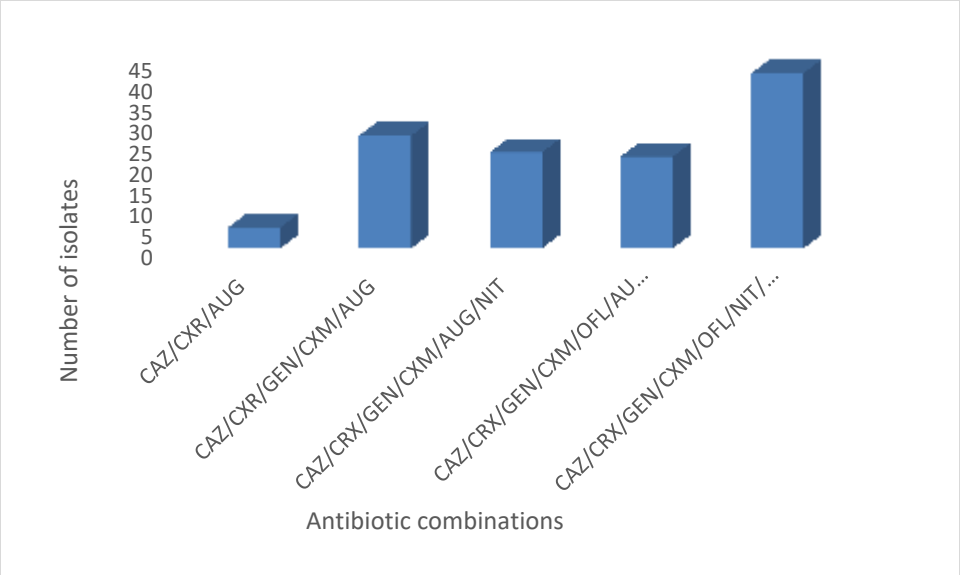

Fig 2: Multidrug resistance profile of Gram-negative bacteria

Key: CAZ- Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CXM- Cefixime, OFL- Ofloxacin, AUG- Amoxicillin/clavulanate, NIT- Nitrofurantoin, CPR- Ciprofloxacin

Resistance determinants (*sul1* and *sul 2, tetA, tetB, SHV, CTX-M, TEM* and plasmid)

PCR analysis results affirmed that 30.9 and 28.5% of the bacteria harbored *tetA* and *tetB*, respectively (Fig 3A and Fig 3B), while 15.4% and 7.3% had *sul1* and *sul2*, respectively. (Fig 3C). However, *SHV*, *CTX-M*, *TEM* and integrons were not detected. Plasmid analysis revealed that only 16.3% of the isolates harbored plasmids with molecular weight of 23.130 kb (Figure 3D).

The detection of sulfanomide (*sul1*and *sul2*) and tetracycline (*tetA* and *tetB*) encoding genes in this current study was consistent with the report of previous work on *E. coli* in poultry farms (Adelowo *et al*., 2014). In another study involving processed bison carcasses (Li *et al*., 2007), presence of sulfonamide encoding genes *sul1* (0.7%), *sul2* (3.6%) and tetracycline encoding genes A (2.2%), B (4.3%) and C (1.4%) were found in *E. coli*. The prevalence of resistance genes and their distributions varied considerably from the one obtained in this study. This was presumptively connected with differences in sampling techniques, locations, antibiotic pressure, batch of the antibiotics used and strains encountered. This study found no ESBL genes. However, a previous study detected bla_{TEM}, bla_{SHV} and *blaCTX-M* in 57.97%, 27.54% and 73.53% strains, respectively, in *E. coli* obtained from broiler farms in Philippines (Gundran *et al*., 2019). In another study, *blaCTX-M-15* was found in 96% and 97% of *E. coli* strains isolated in broiler and human samples, respectively, in Ghana (Falgenhauer *et al*., 2019). Comparable study from China on ESBL-producing *E. coli* obtained from chickens found no SHV genes, rather, *blaCTX-M* (51.7%) and *blaTEM* (79.3%) genes were found (Yuan *et al*., 2009). Hence, several factors aforementioned above may be influencing the gene distribution in different studies.

PCR analysis revealed that none of the isolates harbored integrons. This finding differs with the studies that detected integrons especially those belonging to the class 1 and 2 in bacteria isolated from different environmental samples (Khemtong *et al*., 2008; Adelowo *et al*., 2014; Adesoji *et al*., 2015; Domingues *et al*., 2015; Odetoyin *et al*., 2018). Several gene cassette arrays embedded in class 1 integrons have been found in bacteria isolated from animals and in animal-based food products in Thailand and Portugal (Khemtong *et al*., 2008; Machado *et al*., 2008). A study in Australia identified 86% class 1 integrons (*int1*) and 94% class 2 integrons (*int2*) in bacteria isolated from 50 bovine faecal samples (Barlow *et al*., 2004). The distribution of class 1 integrons has been reported in organisms from different environments associated with human activities. The *int1* dissemination in bacteria from hospital to the environment has been well established

(Wright *et al*., 2008; Gillings *et al*., 2008; Stokes & Gillings, 2011). Also, studies have shown that class 1 integrons can be disseminated among natural bacteria community in humans and animals (Skurnik *et al*., 2006; Nardelli *et al*., 2012). The reason for the absence of integrons in isolates from sampled areas in this study is unknown, but might probably be that the plasmids obtained in this study were not antibiotic resistant plasmids as there was no difference in resistance pattern between bacteria with plasmids and those without. However, it has been established that there are some regions in the world where horizontal genetic transfer mechanisms might be more prevalent, due to historical processes (Nardelli *et al*., 2012).

Plasmids serve as reservoirs of several antibiotic resistance genes; hence, the plasmid profiling in this study. Plasmids were detected in 16.3% of the isolates with molecular size of 23.1 kb. Similarly, the presence of plasmids in Gram-negative bacteria was reported by Ineta *et al*. (2018); therein, 30% of *E. coli* strains harboring plasmids of molecular weight 30 kb were found. In addition, Icgen et al. (2002) in another study reported a range of 3.1 to 32 kb plasmid DNA in 28 *Salmonella* strains isolated from turkey birds. Even larger plasmids with size ranging from 140 to 62 MDa were found in *Salmonella* species isolated from poultry farms and clinical isolates (Begum *et al*., 2016). The latter study maintained that although all the *E. coli* strains with two plasmids (140 Mda and 62 Mda) had different resistance patterns compared to those harboring one plasmid (140 Mda), there was no correlation between drug resistance and isolates with plasmids. The isolates in this current study with no plasmids exhibited multiple drug resistance; high level of resistance is attributable to bacteria with plasmids of molecular size 23-26kb and above (Icgen *et al*., 2002). It has been reported that not all antibiotic resistance genes are located on plasmids; hence, some of the genes encoding resistance may be located on bacterial chromosomes (Ineta *et al*., 2018; Nsofor & Iroegbu, 2013). This could be the case in this present study.

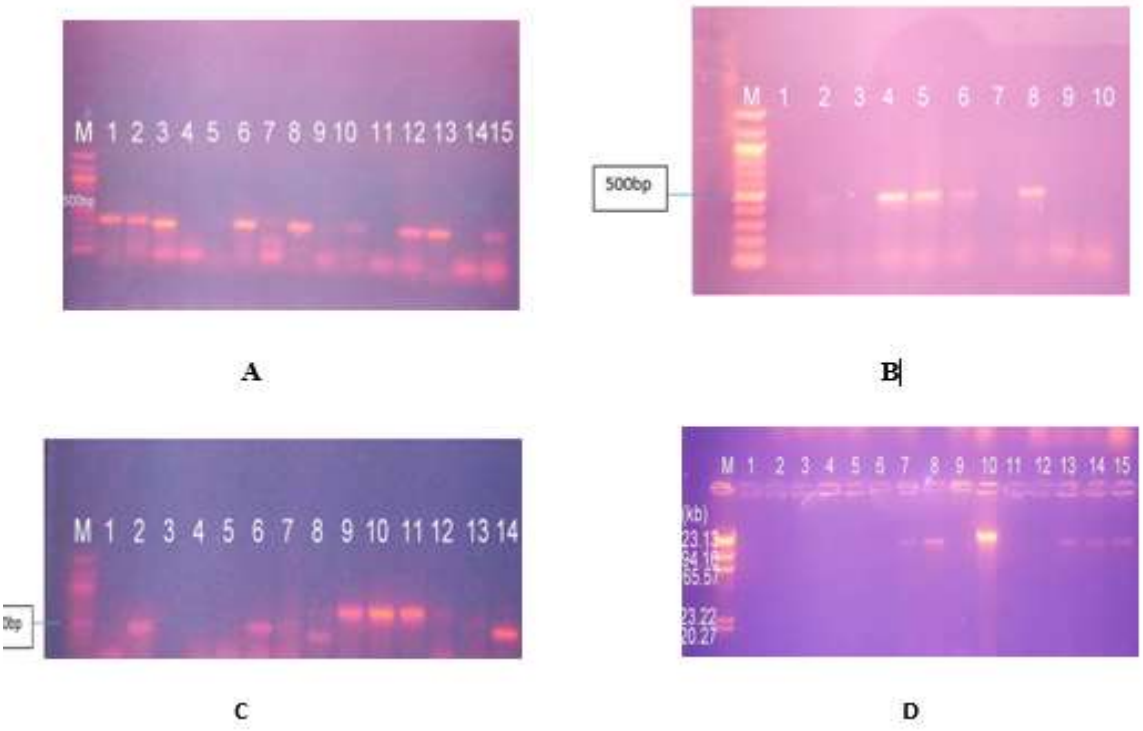

Figure 3: Electrophoregrams of resistance genes and plasmids in Gram-negative bacteria from animal wastes

A: M-Molecular ladder (100bp), *tetA* positive isolates: 1, 2, 3, 6, 8, 10, 12, 13 and 15, Negative control: 5. **B**: *tetB* positive isolates: 4, 5, 6 and 8, Negative control: 1. **C**: *sul2* positive isolates: 2, 6, 8 and 14, *sul1* positive isolates: 9, 10 and 11, Negative control: 3. **D**: M-Hind111 lambda DNA ladder, isolates with plasmid: 7, 8, 10, 13, 14 and 15.

Conclusion

The results revealed multi-drug resistance and high prevalence of resistance determinants in bacteria from the study areas. The resistance determinants are important because they can easily be transferred between bacteria in different environments; thus, increasing the resistance burden in bacteria within the environment. Hence, surveillance system is required to prevent the spread of resistance genes between bacteria while strict regulation should be placed on antibiotic use by regulatory bodies. In addition, awareness campaign amongst livestock farmers on the dangers of indiscriminate use of antibiotics is emphasized.

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