



ANTIBIOTIC RESISTANCE IN BACTERIAL PATHOGENS ISOLATED FROM CATTLE DUNG AND ITS CONTAMINATED SOIL

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ABSTRACT

Cattle dung is used as organic fertilizer and alternative source of fuel or biogas but could also be a source of antibiotic resistance genes in the environment. This study isolated, identified and assessed antibiotic susceptibility pattern of bacteria from cattle dung and its contaminated soil. Bacteria isolation and identification were based on standard techniques while hemolytic activity was used to confirm potential pathogenic bacteria. Antibiotic susceptibility pattern of isolated pathogens were assayed by disk diffusion method. Among isolated bacteria, *Staphylococcus* spp had highest occurrence of 23.8 % while *Micrococcus* spp was the least at 1.3 %. Hemolytic bacteria isolates were *Staphylococcus aureus* (16.5 %), *Bacillus* spp (17.4 %), *Nocardia* spp (4.6 %), *Escherichia coli* (29.4 %), *Pseudomonas* spp (13.8 %), *Serratia marcescens* (2.8 %) and *Salmonella* spp (15.6 %). High resistance (100 %) against Ampiclox (30 µg) was observed in all *Staphylococcus aureus* and *Bacillus* spp isolates while *Pseudomonas aeruginosa* isolates showed 100 % resistance to Ofloxacin (30 µg). Most Gram-positive bacterial isolates were majorly resistant to Beta lactams while Gram negative bacteria were resistant to Fluoroquinolones antibiotics. Multiple antibiotics resistant index (MARI) was measured at greater than 0.2, and was observed in 71.5 % of the hemolytic pathogens. Antibiotics resistance in hemolytic bacterial pathogens from this study is indicative of environmental sources of antibiotic resistance and possible adverse effects on human health.

Keywords: antibiotics, cattle dung, hemolytic bacteria, pathogens, resistance

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INTRODUCTION

Antibiotics have been used as panacea for the treatment of infections in both humans and animals for decades (WHO, 2014). They are often included in feed additives at small doses for growth promotion of animals used for meat, which account for large proportions of the global use of veterinary antibiotics (You and Silbergeld, 2014). The development of large-scale concentrated animal feeding operations (CAFOs) has increased the extensive use of veterinary antibiotics in the treatment of infections, prevention of diseases and promotion of growth (Sarmah *et al.*, 2006; Jechalke *et al.*, 2014; Van Boeckel *et al.*, 2015).

Substantial proportions of the veterinary antibiotics administered are excreted in un-metabolized forms or as active metabolites in the soil (Sarmah *et al.*, 2006). Evidence has shown that antibiotic residues can adversely affect microbial processes in the environment such as nutrient cycling and pollutants degradation (Sarmah *et al.*, 2006; Jechalke *et al.*, 2014). Similarly, antibiotics administered to animals provide selective advantages for antibiotic resistant bacteria (ARBs) to develop in animal intestines, which end up in their dung and eventually in the soil (Muurinen *et al.*, 2017).

Animal manure could be a reservoir of bacteria carrying Antibiotic Resistant Genes (ARGs) and Mobile Genetic Elements (MGEs) such as plasmids (Feng-Hua *et al.*, 2015; Yijun *et al.*, 2018). When manure is used as fertilizer, residual antibiotics and ARGs could disperse into agricultural soils, which may exert selection pressure on antibiotic resistance (Martinez, 2009; Obuekwe and Osariemen, 2020). Antibiotic-resistance genes from the soil resistome can enter the food chain via contaminated crops or groundwater, and have potential consequences for human health if transferred to human pathogens (Marti *et al.*, 2013; Nikolina *et al.*, 2014; Adebisi *et al.*, 2016). There is need to curb this problem by laying emphasis on improving productivity with less dependence on antibiotics especially for developing economies like Nigeria (Chollom *et al.*, 2018). Again, strict control measures should be placed on antibiotics use through government policies and regulations, and where antibiotics are needed, there should be veterinarian prescription before use (Piddock *et al.*, 2016; Chollom *et al.*, 2018). This study assessed antibiotic susceptibility pattern of pathogens from cattle dung and its contaminated soil.

MATERIAL AND METHODS

SAMPLES COLLECTION

Fresh cattle dung, cattle dung manure and dung contaminated soils were collected in five (5) replicates from four (4) cattle settlement/market (Federal Staff road, Dumez junction, Upper Mission and Eyaen) within Benin City. Sterile plastic bags were used for fresh cattle dung and manure collection while cattle dung contaminated soils were collected using soil augers at a depth of 6 inches and then placed in a sterile plastic bag. Fifteen (15) samples of each category were collected for analysis, and the samples were collected during the rainy season in 2018. Methodology flowchart is shown in Figure 1.

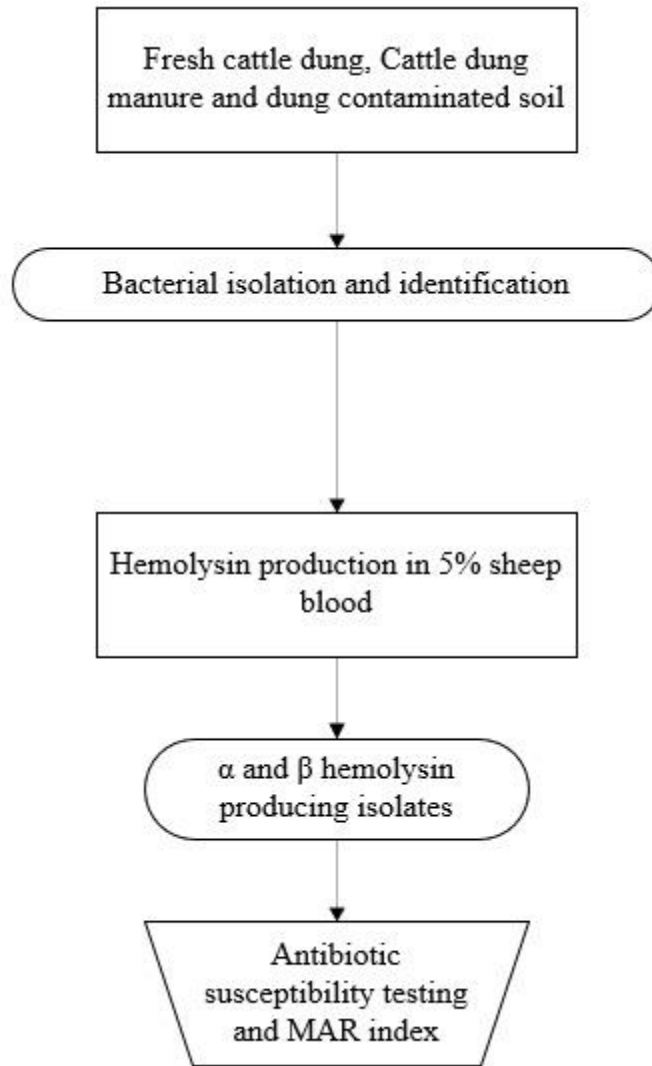


Figure 1: Methodology flowchart

ISOLATION AND IDENTIFICATION OF BACTERIA

The spread plate method was used for bacterial isolation. Ten- fold serial dilution of each sample was prepared aseptically in physiological saline up to 10^{-6} dilutions. Thereafter, 1 ml aliquots of 10^{-5} and 10^{-6} dilutions were plated on nutrient agar (Himedia) and incubated at 37°C for 24 - 48 h under aerobic conditions (APHA, 1999). Further identification was based on Gram's stain and biochemical tests (Cheesebrough, 2006) (Table 1).

HEMOLYSIN PRODUCTION

All bacterial isolates from samples were also cultured on 5 % sheep blood agar (SBA) media as described by Pavlov *et al.* (2004) and Ryan *et al.* (2014). Plates were incubated at 37°C for 24 h and growth was then observed for hemolytic activity of the bacteria. Greenish zone around the culture would indicate α -hemolysis while clear transparent zone indicated β -hemolysis.

ANTIBIOTICS SUSCEPTIBILITY TESTING AND MULTIPLE ANTIBIOTICS RESISTANCE (MAR) INDEX OF BACTERIAL PATHOGENS

Hemolysin producing isolates were sub-cultured on nutrient agar, and pure colonies of pathogenic bacteria were obtained. These were picked to make suspensions in 1ml sterile normal saline that was adjusted to an equivalence of a 0.5 McFarland standard. Subsequently, sterile Mueller-Hinton agar (Oxoid, UK; Bauer *et al.*, 1966) plates were inoculated with them by spreading 0.1ml of each pathogenic bacterial suspension on the entire surface of the plate and then antibiotic discs were inserted. The following antibiotic sensitivity discs and concentrations were used for Gram positive; Ampiclox (30 µg), Amoxicillin (30 µg), Ceftriaxone (25 µg), Cefuroxime (20 µg), Perfloxacin (10 µg), Ciprofloxacin (10 µg), Gentymicin (10 µg), Streptomycin (30 µg), co-trimoxazole (30 µg), Erythromycin (10 µg). While gram negative disc used contained; Augmentin (25 µg), Amoxicillin (30 µg), Perfloxacin (10 µg), Ofloxacin (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Gentamycin (10µg), Streptomycin (30 µg), co-trimoxazole (30 µg) and Chloramphenicol (10 µg). Inoculated plates were allowed to air-dry within 15 minutes and then incubated at 37 °C for 24 h. The zones of growth inhibition were measured and interpreted according to recommended standard (CLSI, 2017).

The multiple antibiotic resistance MAR index was determined for each bacterial pathogen by dividing the number of antibiotics the pathogenic bacteria was resistant to by the total number of antibiotics tested (Adenaike *et al.*, 2016).

RESULTS

A total of sixty (60) samples of fresh cattle dung, manure and dung contaminated soils collected from four settlements within Benin City were microbiologically assessed. Out of these samples, one hundred and fifty-one (151) bacteria were recovered and were identified as *Staphylococcus* spp., *Staphylococcus aureus*, *Micrococcus* spp, *Bacillus* spp., *Nocardia* spp, *Escherichia coli*, *Neisseria* spp., *Pseudomonas* spp, *Serratia marcescens* and *Salmonella* spp (Table 1).

Table 1: Cultural, morphological and biochemical characteristics of bacterial isolates

Characteristics	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
Shape	Irregular	Circular	Irregular	Circular	Circular	Circular	Irregular	Circular	Circular	circular
Colour	Creamy	Creamy	Yellow	Yellow	Whitish	Creamy	Creamy	Creamy	Red	Creamy
Margin	Rhizoid	Entire	Entire	Entire	Entire	Entire	Lobate	Rhizoid	Entire	Rhizoid
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
Wet/ Dry	Wet	Wet	Wet	Wet	Dry	Dry	Wet	Wet	Dry	Dry
Gram Stain	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Negative	Negative
Shape	Cocci	Cocci	Cocci	Rod	Rod	Rod	Cocci	Rod	Rod	Rod
Arrangement	Clustered	Cluster	Pairs	Single	Pairs	Pairs	Single	Single	Single	Pairs
Catalase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative
Coagulase	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Spore	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
Motility	Positive	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Positive	Negative
Indole	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Urease	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
Citrate	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Positive	Positive	Positive
Glucose	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative
Sucrose	Negative	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Negative
Lactose	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative
Maltose	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Positive
Isolates	<i>Staphylococcus spp.</i>	<i>Staphylococcus aureus</i>	<i>Micrococcus spp</i>	<i>Bacillus spp.</i>	<i>Nocardiaspp</i>	<i>E.coli</i>	<i>Neisseria spp.</i>	<i>Pseudomonas spp</i>	<i>Serretiamarcescens</i>	<i>Salmonella spp</i>

However, *Micrococcus* spp, *Nocardia* spp, *Neisseria* spp and *Serretia marcescens* were absent in both fresh cattle dung and cattle dung manure samples but present in dung contaminated soil (Table 2).

Table 2: Frequency of occurrence of bacterial isolates from fresh cattle dung, cattle dung manure and cattle dung contaminated soil samples

Bacterial Isolates	Dung (20)	Manure(20)	Soil (20)	Total (60)	% occurrence
<i>Staphylococcus</i> spp	14 (70 %)	12 (60 %)	10 (50 %)	36 (60 %)	36 (23.8 %)
<i>Staphylococcus aureus</i>	8 (40 %)	7 (35 %)	3 (15 %)	18 (30 %)	18 (11.9 %)
<i>Micrococcus</i> spp	0	0	2 (10 %)	2 (3.3 %)	2 (1.3 %)
<i>Bacillus</i> spp	9 (45 %)	7 (35 %)	3 (15 %)	19 (31.67 %)	19 (12.6 %)
<i>Nocardia</i> spp	0	0	5 (25 %)	5 (8.33 %)	5 (3.3 %)
<i>E.coli</i>	14 (70 %)	9 (45 %)	9 (45 %)	32 (53.33 %)	32 (21.2 %)
<i>Neisseria</i> spp	0	0	4 (20 %)	4 (6.67 %)	4 (2.6 %)
<i>Pseudomonas</i> spp	5 (25 %)	5 (25 %)	5 (25 %)	15 (25 %)	15 (9.9 %)
<i>Serretia marcescens</i>	0	0	3 (15 %)	3 (5 %)	3 (1.9 %)
<i>Salmonella</i> spp	8 (40 %)	5 (25 %)	4 (20 %)	17 (28.33 %)	17 (11.3 %)
Total number of isolates	58	45	48	151	151 (100 %)

In all 151 bacterial samples, *Staphylococcus* spp had the highest percentage occurrence of 23.8 %, followed by *E. coli* (21.2 %) and *Bacillus* spp (12.6 %) while *Micrococcus* spp and *Serretia marcescens* were the least bacterial isolates with percentage occurrence of 1.3 % and 1.9 % respectively (Table 2). These isolates were further tested for virulence factor using hemolysin production and it was observed that a total number of 109 isolates from each sample were pathogenic. *Bacillus* spp, *Pseudomonas* spp, *Staphylococcus aureus*, *Salmonella* spp, *Nocardia* spp, *E. coli* and *Serretia marcescens* were α and β hemolysin positive. However, no hemolysin production was observed with *Staphylococcus* spp, *Micrococcus* spp and *Neisseria* spp (Table 3).

Table 3: Frequency of distribution of hemolytic pathogens from fresh cattle dung, cattle dung manure and cattle dung contaminated soil samples

Bacterial Isolates	Fresh Cattle dung		Cattle dung manure		Cattle dung contaminated soil	
	N	hemolysis +ve	N	hemolysis +ve	N	hemolysis +ve
<i>Staphylococcus</i> spp	14	0	12	0	10	0
<i>Staphylococcus aureus</i>	8	8 (100 %)	7	7 (100 %)	3	3 (100 %)
<i>Micrococcus</i> spp	0	0	0	0	2	0 (0%)
<i>Bacillus</i> spp	9	9 (100 %)	7	7 (100 %)	3	3 (100 %)
<i>Nocardiaspp</i>	0	0	0	0	5	5 (100 %)
<i>E.coli</i>	14	14 (100 %)	9	9 (100 %)	9	9 (100 %)
<i>Neisseria</i> spp	0	0	0	0	4	0 (0%)
<i>Pseudomonas</i> spp	5	5 (100 %)	5	5 (100 %)	5	5 (100 %)
<i>Serratiamarcescens</i>	0	0	0	0	3	3 (100 %)
<i>Salmonella</i> spp	8	8 (100 %)	5	5 (100 %)	4	4 (100 %)
Total number of isolates	58	44 (75.9 %)	45	33 (73.3 %)	48	32 (66.7 %)

Total number of isolates from each sample that are pathogenic = 109

Legend: N/%: Total number of bacteria/ percentages of occurrence; hemolysin +ve: percentage number of bacterial positive for hemolysin production.

Isolated hemolytic bacterial pathogens were subsequently subjected to common antibiotics to assess their resistance patterns as well as their percentage multiple antibiotic resistance indices (Table 4).

Table 4: Percentage Multiple Antibiotics Resistance indices (MARI) > 0.2 Patterns of pathogenic bacterial isolates from fresh cattle dung, cattle dung manure and cattle dung contaminated soil samples

Pathogenic bacterial isolates	Fresh cattle dung		Cattle dung manure		Cattle dung contaminated soil		Total %
	Number of isolates	MARI > 0.2	Number of isolates	MARI > 0.2	Number of isolates	MARI > 0.2	
<i>Staphylococcus aureus</i>	8	7 (87.5 %)	7	3 (100 %)	3	3 (100 %)	13 (72.2 %)
<i>Bacillus</i> spp	9	7 (77.7 %)	7	7 (100 %)	3	2 (66.7 %)	16 (84.2 %)
<i>Nocardia</i> spp	0		0		5	0	0
<i>E. coli</i>	14	8 (57.1 %)	9	7 (77.8 %)	9	6 (66.7 %)	21 (65.6 %)
<i>Pseudomonas</i> spp	5	5 (100 %)	5	4 (80 %)	5	3 (60 %)	12 (80 %)
<i>Serratiamarcescens</i>	0		0		3	0	0
<i>Salmonella</i> spp	8	8 (100 %)	5	4 (80 %)	4	4 (75 %)	16 (94.1 %)
Total	44	35 (79.5 %)	33	25 (75.8 %)	32	18 (56.3 %)	78 (71.5 %)

The resistance profiles of isolated hemolytic pathogens are shown in Figures 2A, 2B, 3A, 3B, 4A and 4B.

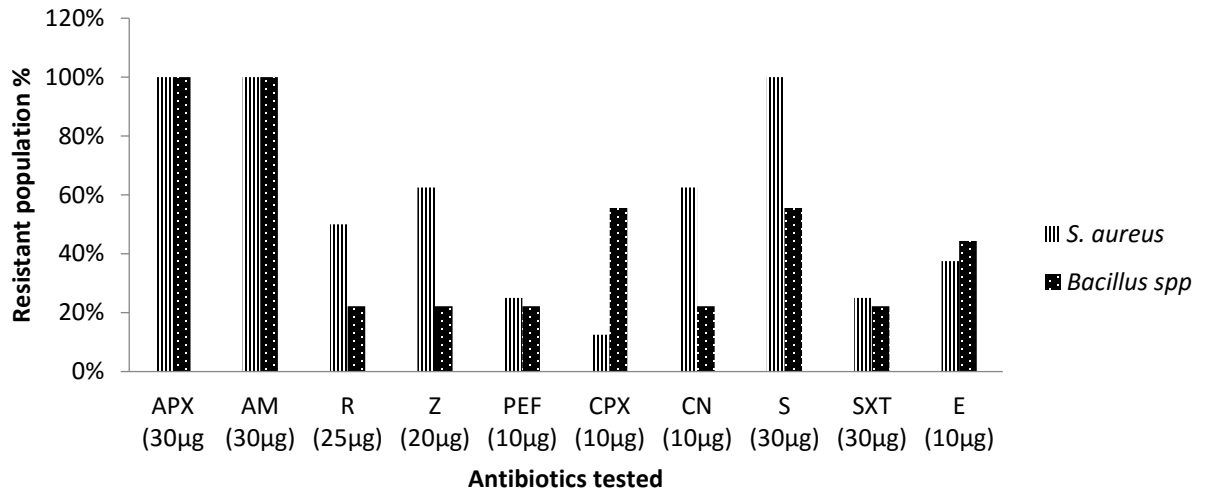


Figure 2A: Percentage number of antibiotics resistant Gram-positive bacteria isolated from fresh cattle dung in Benin City.

Legend: APX: Ampiclox; AM: Amoxicillin; CN: Gentamycin; S: Streptomycin; Z: Cefuroxime; R: Ceftriaxone; E: Erythromycin; SXT: Co-trimoxazole; PEF Perfloxacin; CPX: Ciprofloxacin

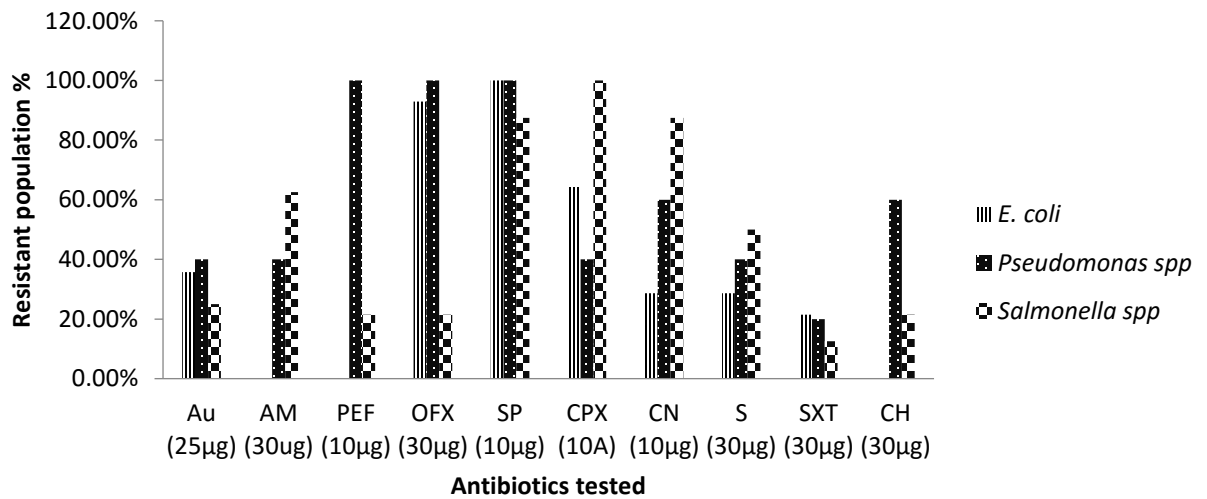


Figure 2B: Percentage number of antibiotics resistant Gram-negative bacteria isolated from fresh cattle dung in Benin City.

Legend: AU: Augmentin; AM: Amoxicillin; CN: Gentamycin; S: Streptomycin; SXT: Co-trimoxazole; CH: Chloramphenicol; OFX: Ofloxacin; PEF Perfloxacin; SP: Sparfloxacin; CPX: Ciprofloxacin

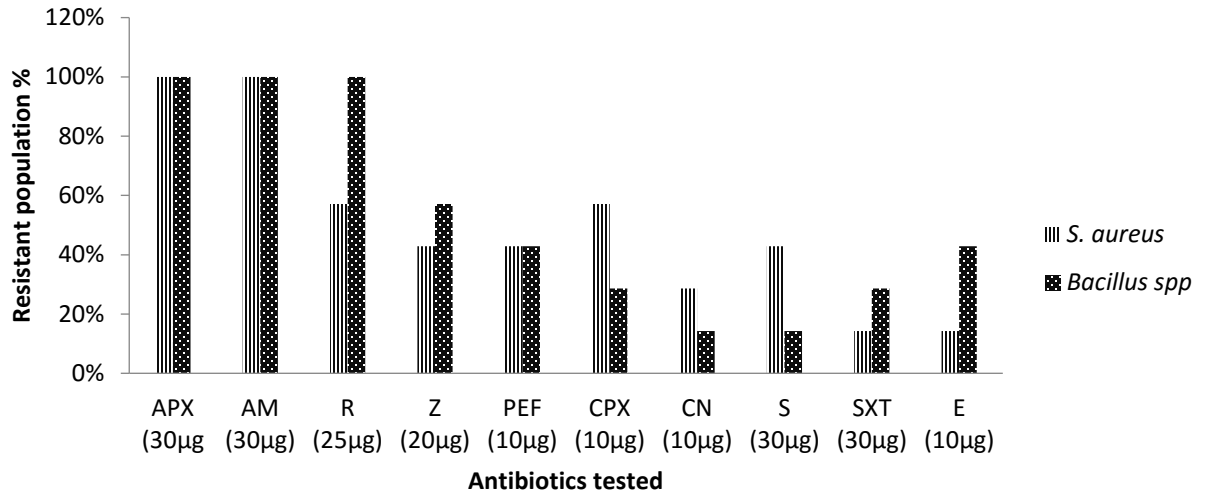


Figure 3A: Percentage number of antibiotics resistant Gram-positive bacteria isolated from cattle dung manure in Benin City.

Legend: APX: Ampiclox; AM: Amoxicillin; CN: Gentamycin; S: Streptomycin; Z: Cefuroxime; R: Ceftriaxone; E: Erythromycin; SXT: Co-trimoxazole; PEF Perfloxacin; CPX: Ciprofloxacin

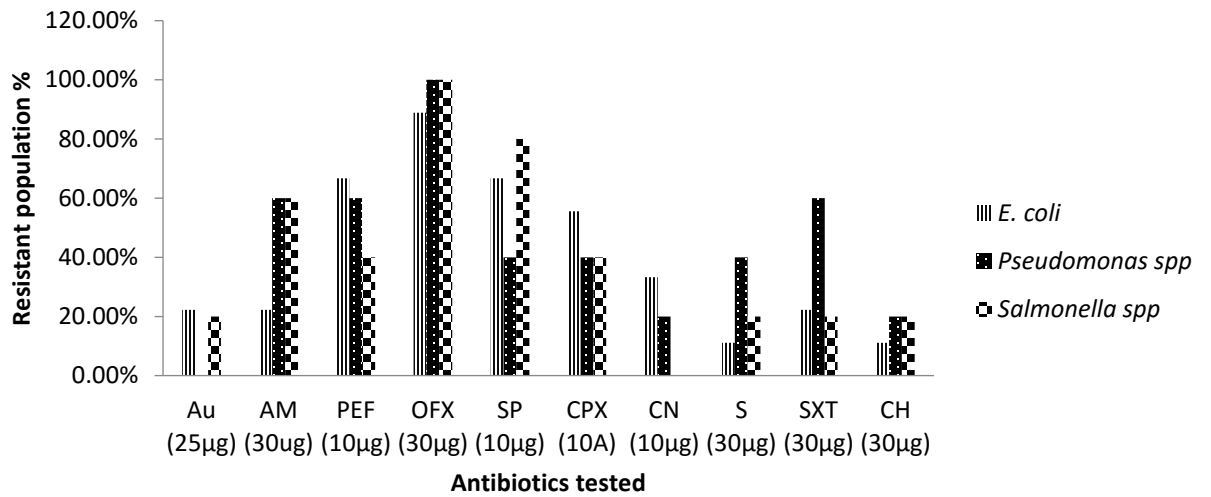


Figure 3B: Percentage number of antibiotics resistant Gram-negative bacteria isolated from cattle dung manure in Benin City.

Legend: AU: Augmentin; AM: Amoxicillin; CN: Gentamycin; S: Streptomycin; SXT: Co-trimoxazole; CH: Chloramphenicol; OFX: Ofloxacin; PEF Perfloxacin; SP: Sparfloxacin; CPX: Ciprofloxacin

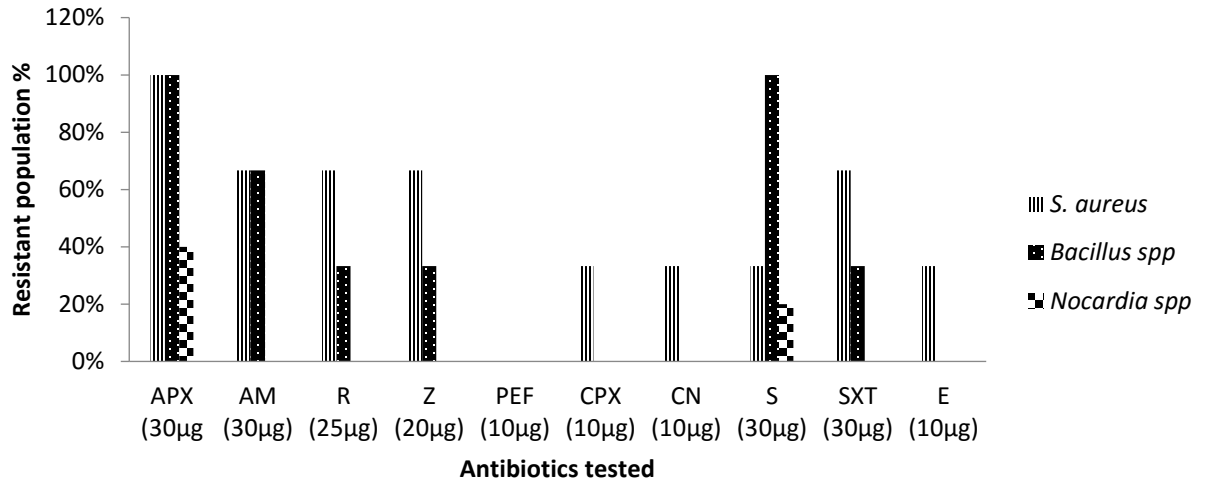


Figure 4A: Percentage number of antibiotics resistant Gram-positive bacteria isolated from cattle dung contaminated soil in Benin City.

Legend: APX: Ampiclox; AM: Amoxicillin; CN: Gentamycin; S: Streptomycin; Z: Cefuroxime; R: Ceftriaxone; E: Erythromycin; SXT: Co-trimoxazole; PEF Perfloracin; CPX: Ciprofloxacin

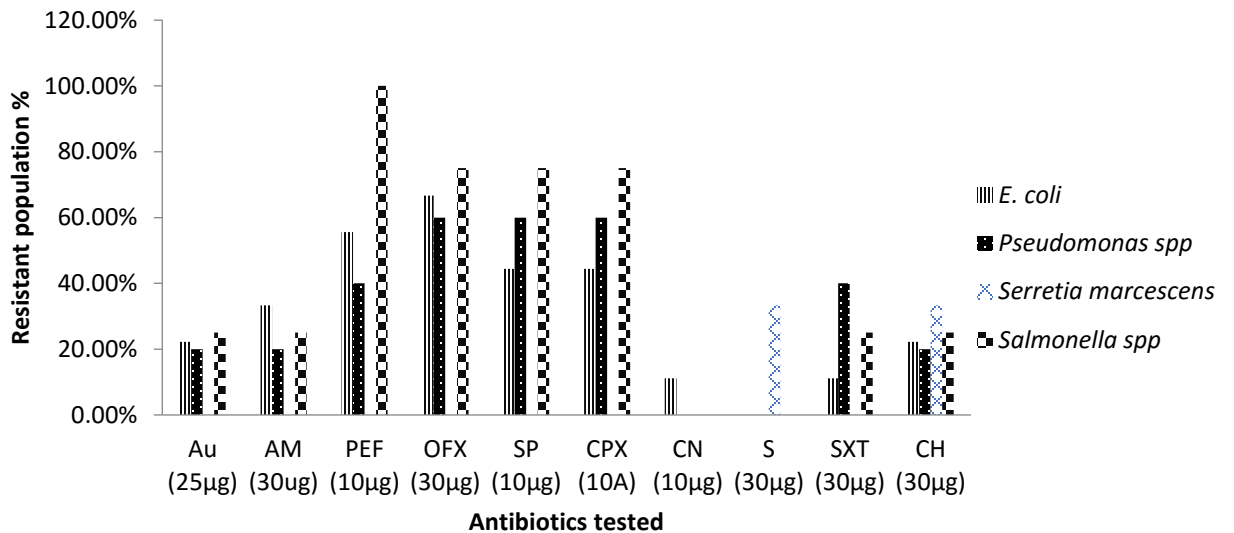


Figure 4B: Percentage number of antibiotics resistant Gram-negative bacteria isolated from cattle dung contaminated soil in Benin City.

Legend: AU: Augmentin; AM: Amoxacillin; CN: Gentamycin; S: Streptomycin; SXT: Co-trimoxazole; CH: Chloramphenicol; OFX: Ofloxacin; PEF Perfloracin; SP: Sparfloxacin; CPX: Ciprofloxacin

Staphylococcus aureus isolated from fresh cattle dung showed 100 % resistance to Ampiclox, Amoxicillin and Streptomycin but was least resistant to Ciprofloxacin (12.5 %). Similar pattern was also observed with *Bacillus spp* which showed 100 % resistance to Ampiclox and Amoxicillin however, were least resistance to Ceftriaxone,

Cefuroxime, Perfloxacin, Gentamycin and co-trimoxazole (22.2 %) (Figure 2A). *Escherichia coli* isolates from fresh cattle dung were resistant to both Sparfloxacin (100%) and Ofloxacin (92.9%) however, it was not resistant to Amoxicillin, Perfloxacin and Chloramphenicol (Figure 2B). On the contrary, *Pseudomonas* spp isolates were completely resistant to Ofloxacin, Perfloxacin and Sparfloxacin while been least resistant to Cotrimoxazole (20 %). Again, *Salmonella* spp isolates were highly resistant to Ciprofloxacin (100 %) followed by Sparfloxacin and Gentamycin (87.5 %) with least resistance to Cotrimoxazole (Figure 2B).

Staphylococcus aureus isolated from fresh cattle dung manure showed high resistance to Ampiclox and Amoxicillin (100 %) and least resistance to Erythromycin and Cotrimoxazole (14.3 %). Similar pattern was also observed with *Bacillus* spp which were resistant to Ampiclox, Amoxicillin and Ceftriaxone but not to Gentamycin and Streptomycin (14.3 %) (Figure 3A). The highest antibiotic resistance in *E. coli* isolates from cattle dung manure was observed with Ofloxacin (88.9 %) however, they were least resistant to Streptomycin and Chloramphenicol (11.1 %). *Pseudomonas* spp and *Salmonella* spp were highly resistant to Ofloxacin (100 %) but *Pseudomonas* spp was least resistant to Augmentin (0 %) while *Salmonella* spp was least resistant to Gentamycin (0 %) (Figure 3B).

Staphylococcus aureus isolated from cattle dung contaminated soil showed highest resistance to Ampiclox (100 %) while the least resistance was observed with Perfloxacin (0 %) (Figure 4A). Conversely, *Bacillus* spp isolates were resistant to both Ampiclox and Streptomycin (100 %) and not to Perfloxacin, Ciprofloxacin, Gentamycin and Erythromycin (0 %) (Figure 4A). *Nocardia* spp were also least resistant to Perfloxacin, Ciprofloxacin, Gentamycin and Erythromycin (Figure 4A). *E. coli* isolates however, showed highest resistance to Ofloxacin (66.7 %) with least resistance to Cotrimoxazole (0 %). Similarly, *Pseudomonas* spp isolates showed highest resistant to Ofloxacin as well as sparfloxacin and Ciprofloxacin (60 %) while *Salmonella* spp were highly resistant to Perfloxacin (Figure 4B). *Serratia marcescens* isolated from cattle dung contaminated soil showed low level of resistance to Augmentin, Amoxicillin, Perfloxacin, Ofloxacin, Sparfloxacin, Ciprofloxacin, Gentamycin and Cotrimoxazole (0 %) (Figure 4B). Amongst the three (3) categories of samples analyzed, fresh cattle dung contained the highest population of antibiotic resistant pathogens, followed by cattle dung manure, then cattle dung contaminated soil.

Multiple antibiotics resistance indices (MARI) of the hemolytic bacterial pathogens from all samples showed that highest percentage multi drug resistant population was observed in *Salmonella* spp (94.1 %). This was followed by *Bacillus* spp (84.2 %), *Pseudomonas* spp (80 %), *Staphylococcus aureus* (72.2 %) and *E. coli* (65.6 %). However, *Nocardia* spp and *Serratia marcescens* had no multi drug resistant population (Table 4).

DISCUSSION

Several bacteria (151) were isolated from cattle dung, manure and dung contaminated soil from cattle settlements in Benin City. *Staphylococcus aureus*, *Bacillus* spp, *Escherichia coli*, *Pseudomonas* spp, and *Salmonella* spp were isolated from all samples while *Micrococcus* spp, *Nocardia* spp, *Neisseria* spp and *Serratia marcescens* were only exclusive to cattle dung contaminated soil. Cattle dung is composed of about 80% water and supports a matrix of undigested plant material that is rich in nutrients, microorganisms and their byproducts (Muhammad and Amusa, 2003). Similar bacterial isolates (*Staphylococcus aureus*, *Bacillus* spp, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella* spp) were isolated from cattle dung in Kampar, Malaysia, and Ekiti state, Nigeria (Teo and Teoh,

2011; Igbalajobi *et al.*, 2015). Again, the lower parts of the gut of cattle contain various microorganisms including *Enterococcus* spp, *B. subtilis*, and *Lactobacillus* spp (Manyi-Loh *et al.*, 2016). Conversely, the presence of *Micrococcus* spp, *Nocardia* spp, *Neisseria* spp and *Serratia marcescens* in dung polluted soil could be from other environmental sources. This could be heightened because of ecological disturbances such as flooding that lead to climate change (Mbah *et al.*, 2016).

In this study *Staphylococcus* spp had the highest percentage occurrence of 23.8 % which may be associated with mastitis (Roberts *et al.*, 2018). This was followed by *E. coli* (21.2 %) and *Bacillus* spp (12.6 %) while *Micrococcus* spp and *Serratia marcescens* were the least bacterial isolates with percentage occurrence of 1.3 % and 1.9 % respectively. This result is similar with the studies of Manyi-Loh *et al.* (2016) who reported high isolation rates of *S. aureus* from cattle manure in relation to other isolated bacteria. Similarly, Spiehs and Goyal (2007) demonstrated that *S. aureus* was the second most numerous species isolated from cattle dung manure. Freshly laid cattle dung and stored domestic cattle dung contain consortia of microorganisms which include *S aureus*, *Bacillus* spp and *E.coli* (Teo and Teoh, 2011; Manyi-Loh *et al.* 2016). *S. aureus*, *E.coli* and other spp are important pathogens in human and veterinary medicine because of their ability to form enterotoxins and cause various diseases (Adebisi *et al.*, 2016; Obuekwe and Osariemen, 2020).

Virulence factor based on hemolysin production by these isolates (*Bacillus* spp, *Pseudomonas* spp, *Staphylococcus aureus*, *Salmonella* spp, *Nocardia* spp, *E. coli* and *Serratia marcescens*) grouped them as pathogenic. Hemolysin is a bacterial protein that breaks down the membrane of red blood cells causing the release of hemoglobin. It acts by integrating into the membrane of the red blood cell and either punching a hole through the membrane or disrupting the structure of the membrane in some other ways (Verdon *et al*, 2009). One hundred and nine (109) out of these bacterial pathogens were positive for α -hemolytic and β -hemolytic production which is an important virulence determinant of a disease outcome, and pathogenicity of commonly recognized pathogens such as Shiga-toxin producing *E. coli*, *Salmonella*, and *Staphylococcus aureus* (Spiehs and Goyal, 2007; Divyakolu *et al.*, 2019; Obuekwe and Osariemen, 2020). *E.coli* is naturally found in the intestinal tract of animals, and shed with faeces however, Pathogenic hemolytic *E. coli* is known to cause many enteric diseases such as traveler's diarrhea and other forms of diarrhea (Carbone *et al* 2002; Adebisi *et al.*, 2016).

Presence of high level of resistance to the tested antibiotics was observed in this study. *S.aureus* and *Bacillus* spp isolated from all three samples were 100 % resistant to Ampiclox. However, only those isolated from fresh cattle dung and cattle dung manure were 100 % resistant to Amoxicillin. Conversely, *E. coli* and *P. aeruginosa* isolates from fresh cattle dung and manure showed high resistance to Ofloxacin while this was observed in *Salmonella* spp from dung manure. This showed that majority of Gram-positive isolated bacteria were resistant to Beta lactams antibiotic while that of Gram negative were more resistant to Fluoroquinolones antibiotics. Studies have shown that *Escherichia coli* and *Pseudomonas aeruginosa* are sources of resistance genes for strains isolated from cattle dung despite the fact that they inhabit different ecosystems (Qingxiang *et al.*, 2013). Resistance gene profiles are believed to play a very important role in mediating and transferring resistance to antibacterial drugs in the bacteria population. They can be localized in discrete transposable elements of DNA called transposons, which are mobile, and can move from one DNA molecule to another (Yijun *et al.*, 2018). This can lead to the rapid spread of antibiotic resistance in a bacteria

population, and explains the emergence of multi-resistant strains (Nain *et al.*, 2015). Bacteria use three main strategies to get protected against β -lactams: alteration in Penicillin Binding Proteins (PBPs) which reduces the affinity of β -lactams, efflux pumps which remove the antibiotic from the bacterial periplasmic space, and production of β -lactamases which hydrolyze the ring of β -lactams (Zapun *et al.*, 2008; Bush, 2013). However, resistance to quinolones has been a problem ever since Nalidixic acid was introduced into clinical medicine more than 40 years ago (Vranakis *et al.*, 2013).

Generally, three mechanisms of resistance to quinolones are currently recognized: mutations that alter the drug targets, mutations that reduce drug accumulation and plasmids that protect cells from the lethal effects of quinolones (Hooper and Jacoby, 2015). Bacteria such as *B. cereus*, *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from cattle dung were reported to be resistant to several antibiotics including Penicillin, Amoxicillin, Ofloxacin, Pefloxacin, Ciprofloxacin, and Tetracycline (Bharti and Maneesha, 2015). Similar resistant pattern was also reported by Owojowo and Omojasola. (2013) in *Escherichia coli*, *Aeromona hydrophila*, *Salmonella typhi*, *Staphylococcus aureus* and *Shigella dysenteriae* from cattle dung with isolates showing high resistance to ampicillin, amoxicillin, gentamicin chloramphenicol and erythromycin. Utilization of antibiotics for other purposes other than therapy can enrich the population of resistant bacteria in the environment capable of infecting humans (Jose Luis Martinez., 2009). Nonetheless, low resistances to tested antibiotics were seen in *Nocardia* spp and *Serratia marcescens* which were exclusively isolated from cattle dung contaminated soil as well as Some *E. coli* isolates that were also susceptible to chloramphenicol. This is in agreement with Barbara *et al.* (2006) who reported antibiotics susceptibility of *Nocardia* spp. Comparably, Abu and Wondikom, (2018) reported the sensitivity of bacterial isolates to similar antibiotics such as Ciprofloxacin and Ofloxacin and opined their value as empiric antibiotic therapy for enteric infections. It has been shown that resistance to ciprofloxacin is usually associated with resistance to other macrolides, lincosamides, and type B streptogramin, and is referred to as MLS resistance (Ndirika *et al.*, 2016).

Multiple drug resistance was seen in pathogenic *Staphylococcus aureus*, *Bacillus* spp, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* spp in this study. This may be attributed to the presence of resistance determinants on plasmids with similar selective markers or as a result of independent, simultaneous development of resistance to different agents (Abu and Wondikom, 2018). These suggest that bacteria have the unique characteristics of being able to transfer resistance genes from one bacterium to another in different population and habitats (Mandal *et al.*, 2011). Multiple antibiotic drug resistance profiles have also been reported in enteric bacteria from both human and animal sources (Ikpeme *et al.*, 2011). Persistent multiple drug resistance of most isolates to appropriate drugs of choice are of great public health concern and calls for periodic monitoring of antibiograms to detect possible changing patterns (Davies, 2014).

CONCLUSION

This study has shown that cattle dung, manure and dung contaminated soil may harbor resistant bacteria pathogens that may pose public health hazard. Cattle dung which is often used as organic manure can be a source of antibiotic resistance pathogenic bacteria in the soil environment. Cattle dung released in the environment should be monitored by farmers as well as national statutory agencies, and awareness programs created to enlighten the public and farmers regarding the safe use and handling of cattle dung. This could be possible through national statutory agencies such as National Environmental Standards and Regulations Enforcement Agency (NESRA). These activities will help prevent dissemination of pathogens in the environment and the transfer of infections to animals and human.

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