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Isolation and Characterization of Bacteria with Multiple Drug Resistance from Pig Dung

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ABSTRACT

Large quantities of antibiotics are used in agriculture, predominantly in animal farming; this has steered the problem of antibiotics resistance in bacteria. This study was aimed at determining the antibiotic resistance pattern of bacterial isolates from pig dung. Large quantities of antibiotics are used in agriculture, predominantly in animal farming; this has steered the problem of antibiotics resistance in bacteria. The most troublesome issue is that resistance can increase with incessant and widespread usage of antibiotics. Pig farms, known to employ the use of antibiotics were considered for sampling, 30 pig dung samples from five pig farms in Ogbomoso were collected using sterile spatula into sampling tubes. Antibiotics used were ceftriaxone (30 µg), cefepime (30 µg), cefixime (5 µg), ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg) and ciprofloxacin (5 µg), all products of Oxoid. The isolates were all resistant to nine of the 10 antibiotics tested, but they all had an intermediate susceptibility pattern to Cefepime. A high percentage (97.35%) of resistance were observed for cefixime, whereas the rate of resistance to cefuroxime was 24.78%. Four of the isolates were identified as *Alcaligenes faecalis* while three were identified as *Achromobacter denitrificans*. The results of this study have revealed an emerging resistance to antimicrobial drugs among bacteria species from pig dung in Ogbomoso; and this may result into serious public health problems as the resistant bacteria make their passages to human populations.

***Keyword*:** *Alcaligenes*, Antimicrobial, Bacteria, Pig dung, Resistance.

Introduction

Intensive, non-therapeutic use of antibiotics for agricultural purposes has resulted in the increase of antibiotic resistance, especially in gut bacteria.[1,2] These resistant bacteria may infect humans, or their resistance genes spread to other bacteria that infect humans.[3] The most worrisome part is that antibiotic resistance can increase with continual and extensive usage of antibiotics.[4] The increase in resistance to antimicrobial can be seen as a global problem in microbial ecology, being the best-known example of a rapid adaptation of bacteria to a new ecosystem.[5]

Large quantities of antibiotics are used in agriculture, especially in animal husbandry; this has occasioned the problem of bacteria resistance to antibiotics.[6] Sulphonamide and beta-lactam antibiotics are amongst the regularly used antibiotics in animal husbandry[7,8] for the control of diarrhoea and many other infectious diseases in pigs.[9,10,11] The usage of antibiotics in animal farming is suspected to affect the bacterial community structure in manure and increase the prevalence of antibiotic resistance of the bacteria in manure.[12,13]

Antimicrobials are not only used therapeutically to treat and as a prophylactic to prevent animal diseases,[14] but are also used extensively at low doses for growth promotion in livestock production[15] throughout the world. This long-term administration of antimicrobials to animals has brought about the evolution of bacteria[8] that are not only resistant to single antimicrobial agents but often to multiple antibiotics.[16] At the present time, however, one-third to half of all antibiotics is either unnecessary or incorrectly prescribed.[17] Antibiotics misuse as well as its overuse did not only facilitates the emergence of drug-resistant bacteria, but also exposes people to needless risk for adverse effects. Widespread use of these drugs has brought about the occurrence of resistance to antibiotic in many important pathogens.[18] The pervasive usage of antibiotics in livestock production is now associated with the development of resistance to antibiotic among bacteria, which can create therapeutic challenges in treating human and animal infections.[19]

The mounting danger from resistant bacteria therefore demands determined efforts for the prevention of the occurrence of new strains with resistant abilities, and the spreading of current ones.[20] Bacteria have already been revealed to freely interchange genetic material in the environment, allowing the transferral of different resistance mechanisms which already are present in environments from one individual bacterium to the another.[21] Horizontally transfer gene, combined with discriminate pressure promotes the extensive propagation of genes for antibiotic resistance; not in only clinical microorganism communities but in also non-clinical environments as well.[22] Therefore, transfer of resistance genes from faecal organisms to indigenous soil and water bacteria can occur.[23] Antimicrobial resistant (AMR) bacteria from animals are, therefore, of serious concern as a potential source of antimicrobial-resistant determinants that may spread to humans through food and water supply.[24]

Transferrable plasmids are significant means of transportation for genes relating to antibiotic resistance[21], particularly plasmids with broad-host range expedite transmission amongst genera, phyla as well

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as domains[25]. The aim of this study was to determine the pattern for antibiotic resistance among bacteria isolated from pig dung.

Materials and Methods

Sample collection

Pig farms, known to engage in antibiotics use were considered for sampling. Thirty pig dung samples from five pig farms in Ogbomoso were collected into sterile plastic containers, packed on ice and transported to the laboratory for microbiological analysis within 1 hour of collection. The pig farms where samples were collected were Agric. farm settlement (Latitude 8° 15′ 53″ N; Longitude 4°12′ 5″ E), LAUTECH farm (Latitude 8° 8′ 26″ N; Longitude 4°16′ 13″ E), Alabi pig farm (Latitude 8° 9′ 37″ N; Longitude 4°13′ 45″ E), Agbomojo pig farm (Latitude 8° 10′ 12″ N; Longitude 4° 14′ 20″ E) and NABES foods pig farm (Latitude 8° 11′ 12″ N; Longitude 4°12′ 39″ E).

Bacteria isolation

The samples (10 g each) were inoculated into 90 mL of peptone water (separately), then incubated at 37°C for 18 – 24 hours.[26] Subsequently, 10 µL of the grown peptone broth was inoculated on Eosin Methylene Blue (EMB) agar and Nutrient agar (Oxoid) plates earlier prepared according to the instruction of the manufacturer; employing the spread plate technique, this was incubated at 37°C for 18 – 24 hours. Pure colonies of a single morphological type were selected and purified after some streaking on Nutrient agar.

Antimicrobial susceptibility testing

In vitro antibiotic sensitivity test of the isolates was conducted on Mueller Hinton agar, employing the disc diffusion method.[27,28] Antibiotics used were ceftriaxone (30 µg), cefepime (30 µg), cefixime (5 µg), ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg) and ciprofloxacin (5 µg), all products of Oxoid. One hundred and thirteen bacteria isolates were tested against these antimicrobial agents belonging to five classes of antibiotics (penicillin, cephalosporins, fluoroquinolone, aminoglycoside and nitrofurans).

Single colony, for each of the isolate was transferred into sterile Nutrient broth and incubated in an incubator shaker at 37°C, until the visible turbidity is equal to that of a 0.5 McFarland standard.[28] The standardized isolates were spread over Mueller Hinton agar plate using sterile cotton swabs; the petri plates were kept at 37°C for 24 hours, after the antibiotic discs have been placed. Susceptibility patterns of the bacteria were determined by measuring the zone of inhibition[29] in millimetre and interpreted according to the Clinical and Laboratory Standard Institute guideline.[28]

***Molecular characterization*:** Bacterial isolates with the highest degree of antibiotic resistance were identified by molecular method.

Deoxyribonucleic Acid (DNA) extraction

The DNA of isolates, each was extracted by using boiling method.[30] First 100 µL of sterilized DNAse and RNAse free water was taken in micro centrifuge tube and approximately loop-full of culture was added. Then denaturation was carried out at 95ºC for 10 min, centrifugation was done to get rid of cell remains and three microliter of the supernatant was used as a template in PCR reaction mixture.[31]

Polymerase Chain reaction (PCR) and 16S rRNA sequencing

A suitable universal primer was used to amplify the target region and sequencing performed using Sanger's method[32]. The primers employed were (518F) CCAGCAGCCGCGGTAATACG and (800R) TACCAGGGTATCTAATCC. The sequence of the product from PCR was compared with known 16S rRNA gene sequences in the GeneBank using the BLAST tool.[33]

Statistical analysis

Data were analysed statistically using SPSS version 22. Mean were separated by Duncan's multiple ranged test ($p \leq 0.05$).

Results and Discussion

The antibiotic susceptibility pattern from pig dung samples obtained from Agric farm settlement (Table 1) revealed that all the bacteria isolates showed resistance to cefixime, eleven (11) isolates were susceptible to ofloxacin, ten (10) were susceptible to ciprofloxacin and nine (9) were susceptible to gentamicin. The highest resistant level amongst the bacterial isolates obtained from the pig dung samples from LAUTECH farm was shown to cefixime while they were most vulnerable to ofloxacin (Table 2). Isolate GB4a obtained from pig dung samples from Alabi pig farm was the most susceptible to the antibiotics tested (resistant only to cefixime); while isolate GB5a had the maximum resistant level to the tested antibiotics as it was resistant to all but one (Table 3). From the samples obtained from Agbomojo pig farm, isolate IS3a showed the maximum resistance level, with susceptibility only to cefipime (Table 4), while isolates IW1a, IW2a, IW2b, IW2a and IB4b were each resistant to just one antibiotic. Out of the isolates obtained from the pig dung samples obtained from NABES food farm, isolate OW4a was the most resistant, with resistance shown to six (6) antibiotics. Isolates OSO4b and OSO8a each was only resistant to one antibiotic (Table 5).

The antibiogram of the isolated bacteria as revealed in table 6 showed that the highest susceptibility was to ofloxacin (82.30%), followed closely by that for gentamicin (80.53%); the highest resistance was shown for cefipime (97.35%) while the highest intermediate pattern was recorded for cefipime (46.02%). Seven bacterial isolates had resistance to nine antibiotic (Table 7), which was the highest in this study. In figure 1, the percentage resistance to cefixime was high among the isolates from samples from the five farms and was the highest in Agric farm settlement samples, LAUTECH farm samples and NABES food farm samples. Four of the seven isolates identified were *Alcaligenes faecalis* while the other three were identified as *Achromobacter dentrificans* (Table 8).

The emergence of β-lactam resistant bacteria in animals and the resistance transfer from isolates to humans pose a potential severe threat to public health.[34] Bacteria obtained from such animals are of particular concern, especially those with resistant ability to extended-spectrum cephalosporins, like as ceftriaxone and ceftiofur, which are critical in veterinary and human medicine in treating bacterial infections.[35]

Isolate AS2a, AS2d, LB5a, LB5c, LB18a and GB5a, all had resistance to nine, out of the 10 antibiotics tested, but they all had an intermediate susceptibility pattern to Cefepime (Table 1, 2 and 3). The results of Yongkiettrakul *et al.*[36] presented extraordinary degree of tetracycline and lincomycin resistance, nonetheless, they reported that a high number of the isolates presented susceptibility to ampicillin, ceftiofur, penicillin, amoxicillin as well as enrofloxacin. A high percentage of resistance were observed for cefixime (97.35%) in Table 6, this complimented the report of Muhammed *et al.*[37] that the abuse of antimicrobials as promoters of growth and in preventing diseases has impressed a discriminating pressure, causing discovery of more resistant bacteria. Adeleke and Omafuvbe[38] noted that all the bacteria isolated from poultry faeces showed high level of antibiotic resistance.

Cefuroxime is one of the most frequently used cephalosporins, approved solely for the treatment of animal diseases like metritis, foot rot and mastitis in cattle, respiratory diseases in ruminants and septicaemia caused by *E. coli* in calves, among others.[39] The resistant rate to cefuroxime (24.78%) was observed to be significantly different from that of cefixime which had the highest resistant rate; it however remains at a high rate compared to the resistance to cefepime which was the lowest in this study. The emergence of the problem of antibiotic resistance is believed to be particularly linked to the amplified use of contemporary cephalosporins in husbandry, which antibiotics are classified by the WHO as critically important in human medicine.[40] Resistance to gentamicin in this study (15.93%) was similar to what was observed by Marculescu *et al.*,[29] who reported that 18.75% of the bacterial strains showed resistance to aminoglycosides.

Some bacterial isolates used in this study also showed resistance to fluoroquinolone (ofloxacin and ciprofloxacin; both had 13.27% resistance). However, Marculescu *et al.*[29] noted that the fluoroquinolones (enrofloxacin) demonstrated efficiency (100%) for

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all tested bacterial strains in their study. It has been established that the use of fluoroquinolones in animals used for food has led to a corresponding antibiotic resistance in bacterial species, leading to infections in man.[41] Resistance of the isolates against augmentin and nitrofurantoin in this study were 14.16% and 18.58% respectively; this differ from the result of Sharma and Bist[42], where all the isolates employed were highly sensitive to Ciprofloxacin (100%) in a uniform manner, but with a 19.20 % resistance occurrences against Nitrofurantoin.

Four of the seven identified isolates (Table 8) were *Alcaligenes faecalis* while three were *Achromobacter denitrificans*. McGann *et al.*[43] had earlier reported an isolate identified as *Alcaligenes faecalis*, showed resistance to all tested β-lactam antibiotics. Most clinical *Achromobacter* and *Alcaligenes* isolates were also reported to show resistance to fluoroquinolones;[44] Neuwirth *et al.*[45] reported the recovery of multidrug-resistant *Achromobacter xylosoxidans* from the sputum with cystic fibrosis.

Table 1: Pattern of antibiotic susceptibility for bacterial isolates from Agric farm settlement pig dung (n = 13)

	CRO	FEP	CFM	CAZ	CRX	GEN	OFL	CPR	AUG	NIT
AW6b	S	S	R	R	R	S	S	S	R	S
AW2a	S	S	R	R	R	S	S	I	S	S
AW12c	S	S	R	R	I	S	S	S	I	S
AS2a	R	I	R	R	R	R	R	R	R	R
AS2b	R	I	R	R	R	S	S	S	R	R
AS2c	I	I	R	R	I	I	S	S	S	I
AS2d	R	I	R	R	R	R	R	R	R	R
AW6a	S	S	R	S	I	S	S	S	S	S
AW2b	S	S	R	S	I	S	S	S	S	S
AB2a	S	S	R	S	R	S	S	S	R	R
AB2b	S	S	R	S	I	S`	S	S	S	I
AS3a	S	S	R	S	S	S	S	S	S	S
AS3b	S	S	R	R	I	I	S	S	I	S

Key: CRO - Ceftriaxone (30µg); FEP -Cefepime (30µg); CFM - Cefixime (5µg); CAZ - Ceftazidime (30µg); CRX - Cefuroxime (30µg); GEN - Gentamicin (10µg); OFL - Ofloxacin (5µg); CPR - Ciprofloxacin (5µg); AUG - Augmentin (30µg); NIT - Nitrofuratoin (300µg); S – Sensitive; I – Intermediate; R – Resistance.

Table 2: Pattern of antimicrobial susceptibility for bacterial isolates from LAUTECH pig dung (n = 42)

	CRO	FEP	CFM	CAZ	CRX	GEN	OFL	CPR	AUG	NIT
Lw10a	I	I	R	S	I	S	S	S	S	S
LB6b	S	I	R	I	I	S	S	S	S	R
LB6a	S	S	R	S	S	S	S	R	S	S
LB14b	S	I	R	I	S	S	S	S	I	S
LB14c	S	I	R	R	I	R	S	S	I	S
LB5b	R	I	R	S	S	S	S	I	S	S
LB5c	R	I	R	R	R	R	R	R	R	R
LW8b	S	I	R	R	I	S	S	S	S	S
LW8c	I	I	R	R	R	S	S	I	S	R
LB7a	S	R	R	S	I	S	S	S	S	S
LW15b	S	S	R	I	S	S	S	S	S	S
LW10b	I	I	R	S	S	S	R	S	I	I
LB6	S	I	R	S	I	S	R	S	I	S
LB14a	I	S	R	S	I	S	R	S	S	R
LB5a	R	I	R	R	R	R	R	R	R	R
LW8a	S	I	R	S	I	S	S	S	S	S
LB7b	S	I	R	S	S	S	S	I	I	S
LS2a	S	S	R	R	I	S	S	S	S	R
LS2b	S	R	R	I	R	S	S	S	I	R

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LS2c	S	I	R	S	I	S	S	S	S	S
LS2d	S	I	R	S	R	S	S	S	S	S
LB18b	R	R	R	S	R	S	S	S	S	S
LB18a	R	I	R	R	R	R	R	R	R	R
LB3a	S	I	R	S	I	R	S	S	S	S
LB3b	S	I	R	I	I	S	R	S	S	S
LB2a	S	I	R	S	S	R	S	S	S	S
LB2b	I	I	R	S	S	I	S	I	S	S
LB2c	S	I	R	S	I	S	S	I	I	S
LW5a	R	I	R	S	S	S	S	S	S	S
LW5b	I	I	R	I	S	S	S	S	S	S
LW15a	S	S	R	I	I	S	S	R	S	R
LB1a	S	S	R	I	S	S	S	S	S	S
LB1b	S	S	R	R	I	S	S	S	S	I
LB9a	S	S	R	I	S	S	S	S	S	S
LB9b	S	S	R	I	S	I	S	R	I	S
LB13a	S	I	R	S	S	S	S	I	R	R
LB13b	S	S	R	I	S	S	S	I	S	S
LB8a	S	I	R	S	I	S	S	S	S	S
LB8b	S	I	R	R	I	S	S	S	S	S
LW4a	S	S	R	S	I	S	R	S	I	S
LW4b	S	S	R	S	R	S	S	S	S	S
LW4c	S	I	R	S	I	S	S	S	I	S

Table 3: Pattern of antimicrobial susceptibility for bacterial isolates from Alabi pig dung (n = 30)

	CRO	**FEP**	**CFM**	**CAZ**	**CRX**	**GEN**	**OFL**	**CPR**	**AUG**	**NIT**
GW12b	R	I	R	S	S	S	S	S	S	S
GB5b	R	I	R	I	I	R	S	S	S	S
GB5c	I	S	R	I	I	R	S	S	S	S
GB3a	S	I	R	S	S	S	S	S	S	S
GB3b	S	I	R	I	I	S	R	S	S	S
GB2a	R	R	R	S	I	S	S	S	S	S
GB2b	R	I	R	I	I	S	S	S	S	R
GB1b	S	S	R	S	I	S	S	S	S	S
GB1a	S	S	I	I	I	R	S	S	S	S
GW12a	R	I	R	R	R	R	R	R	R	S
GB5a	R	I	R	R	R	R	R	R	R	R
GS3a	R	I	R	S	I	R	R	R	R	S
GW3a	S	I	R	S	S	S	S	S	S	S
GB4a	S	S	R	S	S	S	S	S	S	S
GB4b	S	S	R	S	I	S	S	S	S	S
GB4c	S	S	R	S	I	S	S	S	S	S
GB9a	R	I	R	S	I	S	S	I	I	S
GB9b	R	I	R	S	S	R	S	S	S	S

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GW13b	S	R	R	R	S	S	S	S	S	S
GW8a	R	S	R	R	R	S	R	S	I	S
GS3b	R	I	R	R	I	S	I	I	I	R
GW8b	I	I	R	S	I	S	S	S	S	R
GB8a	S	S	R	R	R	S	S	S	S	S
GB8b	S	S	R	S	I	S	S	S	S	S
GW11a	S	S	R	S	I	S	S	S	I	S
GW11b	S	S	R	S	S	S	S	S	S	S
GW11c	S	S	R	S	S	S	S	S	S	S
GW2a	S	S	I	S	I	S	S	S	S	S
GW2c	S	S	R	S	R	S	I	R	S	S
GW2c	S	S	R	S	R	S	S	I	I	R

Table 4: Pattern of antimicrobial susceptibility for bacterial isolates from Agbomojo pig dung (n = 15)

	CRO	**FEP**	**CFM**	**CAZ**	**CRX**	**GEN**	**OFL**	**CPR**	**AUG**	**NIT**
IS1a	I	I	R	R	S	S	I	I	S	S
IW1b	S	S	R	R	I	S	I	R	S	R
IS2a	R	R	R	R	R	S	S	S	I	S
IS3a	R	S	R	R	R	R	R	R	R	R
IS3b	S	S	R	I	I	S	S	S	S	I
IS3c	S	S	R	I	I	R	S	S	S	S
IS1b	S	I	R	S	S	R	S	S	S	S
IW1a	S	S	R	S	I	S	S	S	S	S
IS2b	S	I	R	S	I	S	S	S	S	S
IW2a	S	S	I	I	I	S	S	S	S	S
IW2b	S	S	R	S	I	S	S	S	S	S
IW2a	I	S	R	S	I	S	S	S	S	S
IW2b	S	S	R	S	I	S	S	S	R	I
IB4a	S	S	R	R	R	S	S	S	S	S
IB4b	S	S	R	S	S	S	S	I	S	S

Table 5: Pattern of antimicrobial susceptibility for bacterial isolates from NABES food pig dung (n = 13)

	CRO	**FEP**	**CFM**	**CAZ**	**CRX**	**GEN**	**OFL**	**CPR**	**AUG**	**NIT**
OS2b	R	I	R	R	I	S	S	S	S	R
OS2a	R	I	R	I	R	S	S	S	S	S
OSO4a	S	I	R	R	R	S	S	S	S	S
OSO4b	S	I	R	S	I	S	S	S	S	S
OW4a	R	S	R	R	R	S	S	S	R	R
OW4b	I	I	R	R	S	S	S	S	S	S
OSO8a	I	S	R	S	I	S	S	S	S	S
OSO8b	I	S	R	S	I	S	I	S	I	S
OB2a	R	S	R	I	R	S	S	S	R	S
OB2b	I	S	R	R	R	S	S	S	S	S

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OB2c	R	S	R	I	S	S	S	S	I	S
OB6a	R	S	R	S	S	S	S	S	S	I
OB6b	R	S	R	R	S	S	S	S	S	S

Table 6: Antibiogram of all the bacteria isolated from pig dung

Class of Antibiotics	Antibiotics	Number of Isolates (%)		
		Sensitive	Intermediate	Resistance
Cephalosporins	Ceftriaxone (3rd)	68.00 ± 0.57^{d} (60.18%)	17.00 ± 0.57^{e} (15.04%)	28.00 ± 0.57^{e} (24.78%)
	Cefepime (4th)	55.00 ± 0.57^{b} (48.67%)	52.00 ± 0.58^{g} (46.02%)	6.00 ± 0.57^{a} (5.31%)
	Ceftazidime (3rd)	58.00 ± 0.57^{c} (51.33%)	22.00 ± 1.15^{f} (19.47%)	33.00 ± 0.57^{f} (29.20%)
	Cefuroxime (2nd)	75.00 ± 0.57^{e} (66.37%)	10.00 ± 0.57^{cd} (8.85%)	28.00 ± 0.58^{e} (24.78%)
	Cefixime (3rd)	00.00 ± 0.00^{a} (00.00%)	3.00 ± 0.00^{a} (2.65%)	110.00 ± 0.56^{g} (97.35%)
Aminoglycosides	Gentamicin	91.00 ± 0.57^{h} (80.53%)	4.00 ± 0.66^{ab} (3.54%)	18.00 ± 0.57^{c} (15.93%)
Fluoroquinolones	Ofloxacin	93.00 ± 0.58^{i} (82.30%)	5.00 ± 0.57^{b} (4.43%)	15.00 ± 0.58^{b} (13.27%)
	Ciprofloxacin	87.00 ± 0.58^{g} (76.99%)	11.00 ± 0.58^{d} (9.74%)	15.00 ± 0.57^{b} (13.27%)
Penicillin	Augmentin	76.00 ± 0.57^{e} (67.26%)	21.00 ± 0.57^{f} (18.58%)	16.00 ± 0.57^{b} (14.16%)
Nitrofurans	Nitrofurantoin	83.00 ± 1.15^{f} (73.45%)	9.00 ± 0.58^{c} (7.97%)	21.00 ± 0.57^{d} (18.58%)

Value = Mean ± Standard error. Values that are tailed by same superscript in the column have no significant difference ($p \leq 0.05$).

Table 7: Antibiotic resistance pattern among the bacterial isolates

No of Antibiotics	Resistance pattern	No of isolates
1	CFM	37
	GEN	1
		∑ 38
2	CRO, CFM	6
	FEP, CFM	1
	CFM, CAZ	8
	CFM, CRX	2
	CFM, GEN	5
	CFM, OFL	5
	CFM, CPR	2
	CFM, AUG	1
	CFM, NIT	2
		∑ 32
3	CRO, CFM, GEN	2
	CRO, FEP, CFM	1

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	CRO, CFM, NIT	1
	CRO, CFM, CRX	1
	CRO, CFM, CAZ	1
	FEP, CFM, CAZ	1
	CFM, CAZ, CRX	5
	CFM, CAZ, GEN	1
	CFM, OFL, NIT	1
	CFM, CAZ, NIT	1
	CFM, CRX, NIT	1
	CFM, CPR, NIT	1
	CFM, AUG, NIT	1
	CFM, CRX, CPR	1
		∑ 19
4	CRO, FEP, CFM, CRX	1
	CRO, CFM, CAZ, NIT	2
	CRO, CFM, CRX, AUG	1
	FEP, CFM, CRX, NIT	1
	CFM, CAZ, CRX, AUG	1
	CFM, CRX, AUG, NIT	1
	CFM, CAZ, CRX, NIT	1
	CFM, CAZ, CPR, NIT	1
		∑ 9
5	CRO, CFM, CAZ, CRX, OFL	1
	CRO, FEP, CFM, CAZ, CRX	1
		∑ 2
6	CRO, CFM, CAZ, CRX, AUG, NIT	1
	CRO, CFM, GEN, OFL, CPR, AUG	2
		∑ 3
8	CRO, CFM, CAZ, CRX, GEN, OFL, CPR, AUG	1
		∑ 1
9	CRO, CFM, CAZ, CRX, GEN, OFL, CPR, AUG, NIT	7
		∑ 7

The emergence of β-lactam resistant bacteria in animals and the resistance transfer from isolates to humans pose a potential severe threat to public health.[34] Bacteria obtained from such animals are of particular concern, especially those with resistant ability to extended-spectrum cephalosporins, like as ceftriaxone and ceftiofur, which are critical in veterinary and human medicine in treating bacterial infections.[35]

Isolate AS2a, AS2d, LB5a, LB5c, LB18a and GB5a, all had resistance to nine, out of the 10 antibiotics tested, but they all had an intermediate susceptibility pattern to Cefepime (Table 1, 2 and 3). The results of Yongkiettrakul *et al.*[36] presented extraordinary degree of tetracycline and lincomycin resistance, nonetheless, they reported that a high number of the isolates presented susceptibility to ampicillin, ceftiofur, penicillin, amoxicillin as well as enrofloxacin. A high percentage of resistance were observed for cefixime (97.35%) in Table 6, this complimented the report of Muhammed *et al.*[37] that the abuse of antimicrobials as promoters of growth and in preventing diseases has impressed a discriminating pressure, causing discovery of more resistant bacteria. Adeleke and Omafuvbe[38] noted that all the bacteria isolated from poultry faeces showed high level of antibiotic resistance.

Cefuroxime is one of the most frequently used cephalosporins, approved solely for the treatment of animal diseases like metritis, foot rot and mastitis in cattle, respiratory diseases in ruminants and septicaemia caused by *E. coli* in calves, among others.[39] The resistant rate to cefuroxime (24.78%) was observed to be significantly different from that of cefixime which had the highest resistant rate; it however remains at a high rate compared to the resistance to cefepime which was the lowest in this study. The emergence of the problem of antibiotic resistance is believed to be particularly linked to the amplified use of contemporary cephalosporins in husbandry, which antibiotics are classified by the WHO as critically important in human medicine.[40] Resistance to gentamicin in this study (15.93%) was similar to what was observed by Marculescu *et al.*,[29] who reported that 18.75% of the bacterial strains showed resistance to aminoglycosides. Some bacterial isolates used in this study also showed resistance to fluoroquinolone (ofloxacin and ciprofloxacin; both had 13.27% resistance). However, Marculescu *et al.*[29] noted that the fluoroquinolones (enrofloxacin) demonstrated efficiency (100%) for all tested bacterial strains in their study. It has been established that the use of fluoroquinolones in animals used for food has led to a corresponding antibiotic resistance in bacterial species, leading to

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infections in man.[41] Resistance of the isolates against augmentin and nitrofurantoin in this study were 14.16% and 18.58%, respectively; this differ from the result of Sharma and Bist,[42] where all the isolates employed were highly sensitive to Ciprofloxacin (100%) in a uniform manner, but with a 19.20% resistance occurrences against Nitrofurantoin.

Four of the seven identified isolates (Table 8) were *Alcaligenes faecalis* while three were *Achromobacter denitrificans*. McGann *et al.*[43] had earlier reported an isolate identified as *Alcaligenes faecalis*, showed resistance to all tested β-lactam antibiotics. Most clinical *Achromobacter* and *Alcaligenes* isolates were also reported to show resistance to fluoroquinolones;[44] Neuwirth *et al.*[45] reported the recovery of multidrug-resistant *Achromobacter xylosoxidans* from the sputum with cystic fibrosis.

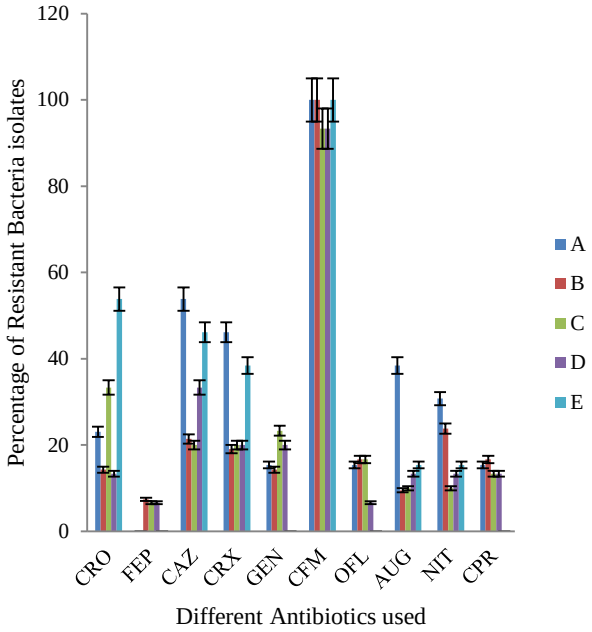

Figure 1: Percentage distribution of resistant bacteria isolates to different antibiotics in the study sites
Key: A= Agric farm settlement; B= LAUTECH piggery farm; C= Alabi piggery; D= Agbomojo piggery and E= NABESfood piggery.

Table 8: Phylogenetic identities of the multi-drug resistant bacteria isolated from the study sites

Isolates Code	Organism Identity	Accession No
ASI2a	*Alcaligenes faecalis*	MH797004
ASI2d	*Alcaligenes faecalis*	MH717107
ISI3a	*Alcaligenes faecalis*	MH818003
LB5c	*Alcaligenes faecalis*	MH801132
LB18a	*Achromobacter denitrificans*	MH718830
LB5a1	*Achromobacter denitrificans*	MH717168
GB5a	*Achromobacter denitrificans*	MH717172

Conclusion

The results of this study has revealed an emerging resistance to antimicrobial drugs among bacteria species from pig dung in Ogbomoso; this may later lead to serious public health issues as the resistant bacteria make their passages to human populations. *Alcaligenes faecalis* and *Achromobacter denitrificans* were the two major groups found with high multiple resistant rates in pig dungs. The possibility of resistant genes transfer between and within bacteria groups, especially in the digestive tract of both animals and humans is high. Consequently, proper control of antimicrobials use in animal farming and strict adherence to the proper methods and time of use needs to be seen to.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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