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Evaluation of antibiotic use patterns among farmers and antibiogram from livestock wastes and fish pond effluents in selected animal farms in Cross River State, Nigeria

Ibiang Arikpo Oka[1], Aniekanabasi Jonathan Okon[2], Lawrence Bassey Etim[1], Inyang Ikpeme Henry[1] and Sylvanus Akpak Upula[1]

[1]Department of microbiology, Cross River State University of Technology, Cross River State, Nigeria
[2]Department of Public Health, University of Calabar, Cross River State, Nigeria

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ABSTRACT: *The extensive and misuse of antibiotics in animal production has become a public health threat. The evaluation of antibiotic use pattern among farmers and antibiogram from livestock wastes and effluent from animal farms was conducted in Cross River State. A descriptive cross-sectional study was undertaken with 379 animal production farmers and multi-stage sampling technique was employed in the selection of farms and respondents. Bacteriological analysis of animal wastes samples and antibiotics susceptibility testing was also conducted. Results revealed that 66.8% of farmers had a good knowledge on the use of antibiotics and its resistance. Majority (91.0%) of the farmers used antibiotics in their animal farms and for purposes of growth promotion, treatment of disease and prevention. Tetracycline, ampicillin, streptomycin, cotrimoxazole, gentamycin and vancomycin were the most frequently used groups of antibiotics. A total of 240 bacteria were isolated and the percentage occurrence of bacterial isolates were: Pseudomonas aeruginosa (14.2%), Escherichia coli (12.5%), Staphylococus aureus (10.8%), while Staphylococcus hominis (2.1%) had the least prevalence of occurrence. All the isolates showed multi-drugs resistance, Staphylococcus aureus showed the highest resistance to several antibiotics commonly used by farmers (80.8% to chloramphenicol, 80.8% to vancomycin and 73.1% of resistance to tetracycline). Statistical analysis of sociodemographic variables with farmers' knowledge and antibiotics use showed that respondents' level of education, years of farming experience, and farm type, were statistically significant (p<0.05). There is a need to improve farmers' knowledge of antibiotics use and the possible consequences of their inappropriate use of antibiotics in farms.*

KEYWORDS: Knowledge, Antibiotics use, bacteria, antibiogram, multi-drug resistance, animal farm wastes

INTRODUCTION

The extensive use and misuse of antibiotics by human and in livestock production and fish farming has become a public health threat. Apart from therapeutic use of antibiotics, animal feed has frequently been supplemented with low concentrations of antibiotics as growth promoters in many countries to improve the feed efficacy, animal health and weight gain. The increased use has been shown to contribute to the increasing prevalence of bacterial antibiotic resistance in the environment (Van Boeckel *et al.*, 2017). Most antibiotics and their antibiotic resistant genes (ARGs) induced in animals are directly discharged from the body through the urine and faeces of animals and subsequently transmitted to environment thereby causing serious environmental pollution, food safety challenges, and ecological toxicity. Recently, the global consumption of antibiotics in livestock has indicated the hotspots of antibiotics use across regions and will have economic and public health impacts (Aslam *et al.*, 2018). The use of antibiotics in animal production farms is known to be a major driver behind increased resistance profiles in bacteria. Untreated animal wastes is a source of antibiotic pollution and antibiotic-resistant bacteria (ARB) in the environment. ARGs are now broadly recognized as emerging environmental pollutants. Some important pathogens commonly found in circulation in different compartments in animal wastes include; *Staphylococcus spp., Enterococcus spp, Campylobacter spp., Salmonella spp. and* Extended Spectrum Beta-Lactamase-producing *Enterobacteriaceae* (Guetiya *et al.*, 2016).

Antibiotic resistance has been reported as a global threat that causes 700,000 deaths annually and is predicted to account for approximately 10 million deaths and US$ 100 trillion economic loss per year by 2050 (Tang, 2017; WHO, 2020). The presence of ARB in livestock waste and fish pond effluents represents a significant concern with respect to the introduction of ARGs to the environment and the development of antibiotic-resistant pathogens (Blau *et al.*, 2019). The factors impelling the emergence, propagation, and spread of bacterial resistance are complex and not fully understood. The numerous gaps in knowledge about antibiotic resistance contributes to the continuing trends of AMR since the statistics about the use of different antibiotics in both health care setting and in animal production farms are not systematically gathered worldwide (FAO 2019; CDC 2019).

Studies have revealed that approximately 13 million households covering 42% of the population in Nigeria own livestock. The population is forecasted to increase significantly by 2050 and the demand for animal farm products is estimated to grow at annual rate by over 3% (FAO, 2019). About 78% of livestock farms in Nigeria uses antimicrobial agents and antibiotics which are restricted in some developed countries due to their impact on environment and in human health, are used in Africa (Kimera *et al.*, 2020). The presence of antimicrobial resistance (AMR) has devastating consequences. Diseases such as Bovine Tuberculosis (TB) are now difficult to treat due to AMR, with high prevalence rate of 10.7% (FAO, 2019).

In Nigeria, antibiotic usage remains inadequately regulated. Currently, data regarding antibiotic use pattern, resistance, and the magnitude of ARGs in livestock is scarce, specifically farmers' knowledge level and perceptions on antibiotic use and its resistance are poorly understood. The apparent potential of ARGs from livestock waste and fish pond effluents to disseminate to receiving environments and eventually transfer to humans underscore the need for a better understanding of how to mitigate in-farm ARGs proliferation. This study therefore evaluate farmers' knowledge of antibiotics use and antibiotic resistance pattern of environmental bacterial isolates from livestock wastes and fish pond effluent in selected animal production farms in Cross River State. Findings from the study will help to generate data on farmers' knowledge on antibiotic use and its resistance, and possible ARB endemic in the region and aid policy makers in decision-making related to its control.

MATERIALS AND METHODS

Study area, design and sampling

The study was conducted in the three senatorial districts of Cross River State, Nigeria. A descriptive cross-sectional study was undertaken with 379 animal production farmers in three farm types to elicit data on farmers' knowledge on antibiotics use and its resistance within the period of 6 months. Multi-stage sampling technique was employed in the selection of farms and respondents. Three senatorial districts were purposively selected and animal farms were stratified into poultry, piggery, and fishery. The farms were selected proportionately based on the number of farm type by simple random sampling. Then the participants were proportionally allocated to each farm based on the number of farms in each farm type.

Sample size determination

The sample size for the cross-sectional household survey was determined using Taro Yamane's formula as cited in Uakarn et al. (2021), to obtain the required 379 sample size and equally distributed to the selected farm types. A sampling frame of all the registered livestock and fish farms was obtained from the Cross River State Ministry of Agriculture, department of livestock development and services.

Method of data collection

The assessment tool was developed and set up in Open Data Kit (ODK) on mobile tablet devices. The questionnaire was designed to assess individuals' knowledge on antibiotics and its resistance, and antibiotics use. The tool included open-ended and closed questions about demographics characteristics, farm characteristics, farmers' knowledge of antibiotics use and its resistance, and antibiotic use. Prior to the study, research assistants in the senatorial districts were trained as enumerators and the questionnaire was piloted with 30 animal production farmers as a first step of validating the tool.

Sample collection for bacteriological analysis

Animal farm wastes (fresh animal droppings and wastewater samples) were aseptically collected from 85 (55.9%) poultry farms, 40(26.3%) fish farms and 27 (17.8%) pig farms in various senatorial districts in Cross river State, Nigeria. The farms were selected proportionately based on the number of farm type in registry of commercial farms in the districts by simple random sampling. Sterile spoons attached to universal sampling bottles were used to collect faecal samples while sterile amber bottles were aseptically used to collect wastewater running off from animal farms and swiftly transferred into an ice container. The samples were collected at 1 month intervals for a period of 3 months from each farm and transported to the laboratory for bacteriological analysis.

Bacterial isolation and identification

The samples obtained were processed in line with standard microbiological and biochemical procedures as reported in similar studies (Shoaib et al., 2020; Ogbor et al., 2019). 10g of animal waste samples were homogenized in 90ml of normal saline and shaken vigorously. The homogenate sample gave 1:10 dilution from which further dilution was made by adding 1ml of homogenate into 9ml of distilled water. For the wastewater samples, ten-fold Serial dilutions of all the wastewater samples were made according to the methods described by Oliveira et al. (2016). 1 ml of the wastewater was aseptically introduced into 9ml of sterile distilled water giving an initial dilution of 1:10ml. Then Aliquots (0.1 ml) of 10^{-3} and 10^{-4} were inoculated in duplicate onto sterile solidified Nutrient agar, MacConkey Agar and Eosin Methylene Blue Agar (EMB) and consistently spread out with a sterile blazed glass spreader. The plates were incubated at 37^{o}C for 18 to 24hours and observed for growth and that of EMB were incubated at 44^{o}C for 24 hours.

The isolates were identified and characterized based on their colonial morphology, gram staining reactions, microscopic appearance and specific biochemical reactions. Biochemical tests such as coagulase test, catalase test, indole production, methyl red test, voges-proskauer test, citrate utilization, urea hydrolysis, catalase test, motility test and Triple sugar iron test are tests used for the identification of bacterial species based on the differences in their biochemical activities. Generally bacterial physiology differs from one type of organism to another. All isolates were further confirmed by VITEK 2 microbial ID/AST system based on standard procedures as described in previous studies (Barman et al. 2018; Bazzi et al., 2017).

Quality control

To validate the accuracy of the bacteria isolates as mean of quality control, uninoculated agar plates were incubated simultaneously. The media plates were observed for microbial growth after incubation, this was to ensure that the isolates obtained after inoculation come from the samples and not due to contamination. Other quality control measures were also ensured throughout the laboratory process.

Antibiotic Susceptibility Testing.

Isolates were subjected to antibiotics susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2021). Thirteen antibiotics disks (Oxoid, UK) used include Trimethoprim/sulfamethoxazole (25μg), Ciprofloxacin (5μg), Amikacin (30μg), Tetracycline (30μg), Cefoxtin (30μg), Ceftazidime (30μg), Gentamicin (10μg), Vancomycin (30μg), Ampicillin (30µg), Chloramphenicol (30µg), Oxacillin (5µg), Streptomycin (10µg) and Levofloxacin (5µg). The antibiotic-impregnated disks were aseptically placed on the inoculated Mueller-Hinton agar plates using sterile forceps and incubated for 18-24h at 37°C. The clear zones of inhibition were measured to the nearest millimetre using a transparent millimetre ruler. Isolates were classified as resistant, Intermediate or susceptible according to CLSI guidelines (CLSI, 2021).

Ethical Approval.

Ethical clearance was obtained from Cross River State Health Ethics Research Committee (CRSH-REC) with REC No. CRSMOH/RP/HREC/2023/401. Written consent was obtained from respondents and reassured of confidentiality of the information provided.

Data analysis

Copies of completed questionnaire were cross-checked to ensure that responses were correct and tick properly. The data were coded and analysed using MS Excel and Statistical Package for Social Sciences (SPSS version 22, 2010). The statistical methods consisting of descriptive statistics of frequency count, percentage, were used and presented in tables and charts. Chi-square test was use for testing associations of selected variables with p value ≤ 0.05 considered statistically significant.

RESULTS

Socio-demographic characteristics of respondents

Investigation of socio-demographic parameters shows that majority of the respondents were males (54.1%), and a good number are at the age of 29-38 years (31.3%). On marital status, majority of respondents were married (53.8%) while a few others were either single, widows or widowers. Based on the educational status of respondents, Majority of the respondents had primary education and a few others had other higher educational attainment while a few others had no formal education. Considering respondents' years of farming experience, a good number had less than 10years farming experience while others had more than 15years farming experience. In terms of respondent's roles in farms, more than half of the respondents (63.9%) were farm workers while others were farm mangers as shown in Table 1.

Table 1**:** Socio-demographic characteristics of respondents in the study area

Variable	Frequency (N)	Percentage (%)
Sex		
Male	205	54.1
Female	174	45.9
Age (Years)		
18 – 28	49	12.9
29 – 38	119	31.4
39 – 48	114	30.1

49 – 58	70	18.5
59 and above	27	7.1
Marital status		
Single	105	27.7
Married	204	53.8
Divorced	37	9.8
Separated	18	4.7
Widowed	15	4.0
Level of education		
No formal education	48	12.7
Primary education	59	15.6
Secondary education	198	52.2
Tertiary	74	19.5
Total	379	100.0
Years of farming experience		
Less than 5years	160	42.2
6 - 10years	174	45.9
11 -15years	36	9.5
15years and above	9	2.4
Role in farm		
Manager	137	36.1
Farm worker	242	63.9
Farm type		
Poultry	212	55.9
Piggery	68	17.9
Fishery	99	26.1

Farmer's knowledge of antibiotics use and its resistance recorded among respondents in the study area

Farmers' knowledge on the use of antibiotics and its resistance recorded among respondents as presented in Figure 1, shows that on the average a good number (66.8%) of the respondents had a good knowledge of antibiotics use and its resistance while a few others (33.2%) had poor knowledge of antibiotics use. Majority of respondents (77.6%) saw antibiotics as antimicrobial agents that kill or inhibit growth of bacteria. More so, (85.5%) of the respondents were aware that improper use of antibiotics in animal farm can cause AMR. Most farmers (83.6%) responded correctly that antibiotics resistance occurs when the body no longer respond to treatment with antibiotics. Additionally, a good number (82.1%) of the respondents were aware that bacteria becomes resistant to antibiotics. However, a proportion of respondents still believe that humans (53.3%) and animals (53.8%) respectively, also shows resistant against antibiotics. About 74% of respondents were aware that resistant bacteria can spread from animals to the environment and (71.5%) had correct knowledge that antibiotics residues in the environment can cause antibiotics resistance (Table 2).

Table 2: Farmers' knowledge on the use of antibiotics and its resistance (N = 379)

Questions	**Correct N (%)**	**Incorrect N (%)**
Antibiotics are antimicrobial agents that kill or inhibit the growth of bacteria	294 (77.6)	85(22.4)
Improper use of antibiotics in animal farm can cause AMR	324(85.5)	55(14.5)
Antibiotics resistance occurs when your body no longer respond to antibiotics	317(83.6)	62(16.4)
Do bacteria become resistant to antibiotics?	311(82.1)	68(17.9)
Do human become resistant to antibiotics?	177(46.7)	202(53.3)
Do animal become resistant to antibiotics?	175(46.2)	204(53.8)
Resistant bacteria can spread from animals to the environment	280(73.9)	99(26.1)
Antibiotics can be used for all types of diseases in animals	132(34.8)	247(65.2)
Antibiotics residues in the environment can cause antibiotics resistance	271(71.5)	108(28.5)
Mean	**253(66.8)**	**126(33.2)**

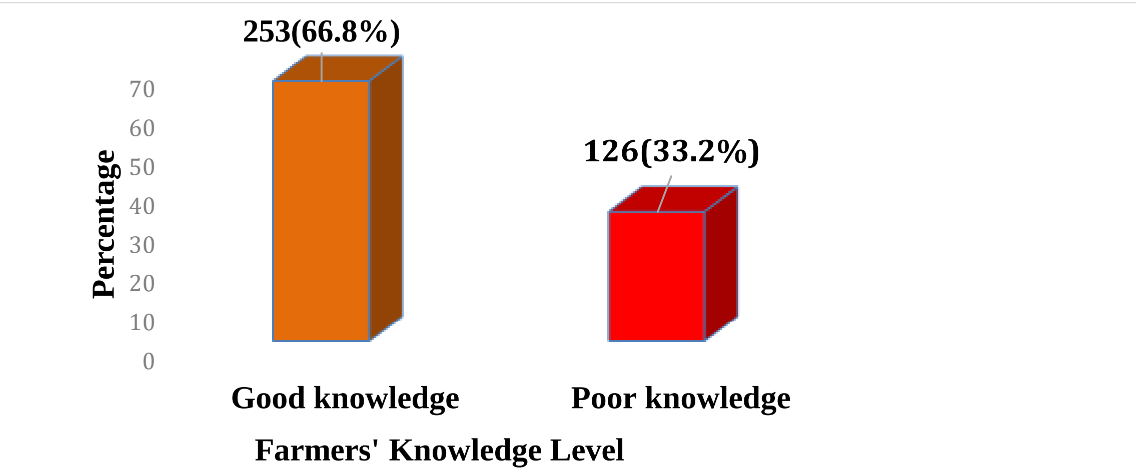

Fig 1: Farmers' knowledge level on the use of antibiotics and its resistance

Table 4: Test of association between socio-demographic characteristics and famers' knowledge level on antibiotics and its resistance

Variable	Knowledge		p-value
	Good	Poor	
Sex			0.071
Male	150(73.2)	55(26.8)	
Female	103(59.2)	71(40.8)	
Age (Years)			0.164
18 – 28	31(63.3)	18(36.7)	
29 – 38	80(67.2)	39(32.8)	
39 – 48	79(69.2)	35(30.8)	
49 – 58	46(65.7)	24(34.3)	
59 and above	17(63.0)	10(37.0)	
Marital status			0.482
Single	74(70.5)	31(29.5)	
Married	144(70.6)	60(29.4)	
Divorced	26(70.3)	11(29.7)	
Separated	5(27.8)	13(72.2)	
Widowed	4(26.7)	11(73.3)	
Level of education			0.031
No formal education	12(25.0)	36(75.0)	
Primary education	40(67.8)	19(32.2)	
Secondary education	135(68.2)	63(31.8)	
Tertiary	66(89.2)	8(10.8)	
Years of farming experience			0.048
Less than 5years	121(75.6)	39(24.4)	
6 - 10years	92(52.9)	82(47.1)	
11 -15years	32(88.9)	4(11.1)	
15years and above	8(88.9)	1(11.1)	
Farm type			0.000
Poultry	177(83.5)	35(16.5)	
Fishery	34(34.3)	65(65.7)	
Piggery	42(61.8)	26(38.2)	

Antibiotics use in animal farms in the study area

Antibiotics use as presented in Table 4, shows that 91.0% of respondents used antibiotics while 9.0% do not use antibiotics in their animal farms. When respondents were asked on the reasons for using antibiotics in farm animals, Majority of the respondents 31.3% mentioned that they used antibiotics for growth promotion, disease treatment and prevention while a low proportion of respondents used antibiotics either for disease treatment, or diseases prevention or growth promotion. Considering the route of antibiotics administration, a reasonable proportion (60.9%) of respondents dissolved the antibiotics in the water for drinking, while others either mixed in animal feed or through injection. Similarly, 58.8% of persons that administered antibiotics to farm animals were farm workers, 24.1% were farm manager, 15.7% were veterinarians while 1.4% were animal health officials. Majority 63.2% of respondents reported that antibiotics are usually used at a given stage of animal growth, 17.4% mentioned usage on sick animal until the recovered while 1.7% of respondents reported the use of antibiotics always. Regarding antibiotics withdrawal period, most of the respondents (69.0%) observed antibiotics withdrawal period while 31.0% did not. Among those that observed withdrawal period, 78.2% said they practice it in animals about to be sold while 21.8% did not complied with the withdrawal period regularly.

Table 3: Antibiotics use pattern in animal farms in the study area (N = 379)

Variable	Frequency (N)	Percentage (%)
Use of antibiotics		
Yes	345	91.0
No	34	9.0
Total	**379**	**100**
Reasons for using antibiotics in farm animals		
To prevent disease	26	7.5
To treat disease	75	21.7
To promote growth	47	13.6
Disease treatment and prevention	23	6.7
Growth promotion and disease prevention	34	9.9
Growth promotion and disease treatment	32	9.3
All of the above	108	31.3
Total	**345**	**100.0**
Frequently used route of administration		
In water	210	60.9
Injection	13	3.8
In feed	122	35.4
Total	**345**	**100.0**
Personnel that administer the antibiotics		
Farm manager	83	24.1
Veterinarian	54	15.7
Animal health officials	5	1.4
Farm worker	203	58.8
Total	**345**	**100.0**
Frequency of antibiotics usage		
All the time	6	1.7
Once a week	7	2.0

At a given stage of their growth	218	63.2
Once in animal life time	54	15.7
Only when animal get sick	60	17.4
Total	**345**	**100.0**
Observation of antibiotics withdrawal period		
Yes	238	69.0
No	107	31.0
Total	**345**	**100.0**
Do you practice it in animals about to be sold?		
Yes	186	78.2
No	52	21.8
Total	**238**	**100.0**

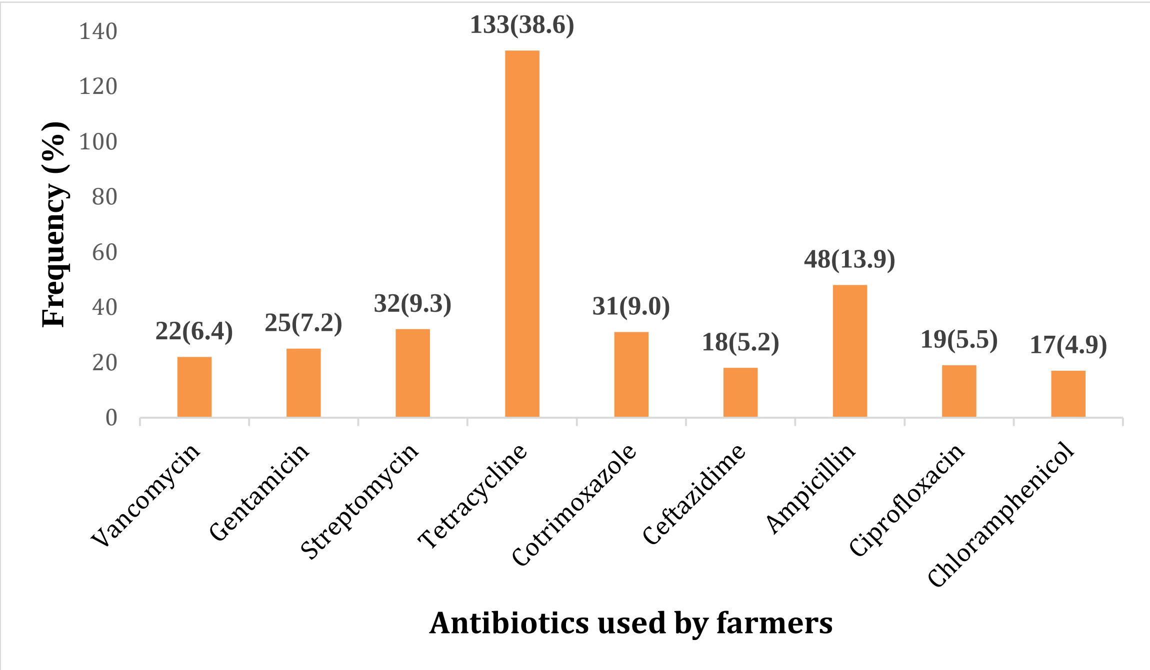

Fig. 2: Commonly used antibiotics by animal production farmers in the study area

Figure 2 shows the antibiotics commonly administer to farm animals. Most of the respondents (38.6%) administered tetracycline, followed by ampicillin (13.9%), streptomycin (9.3%), Cotrimoxazole 31(9.0%), while chloramphenicol 17(4.9%) was the least administered antibiotics.

Table 5: Test of association between socio-demographic characteristics and level of antibiotics use

Variable	Level of antibiotics use			
	Low	**Moderate**	**High**	**p-value**
Sex				0.629
Male	81(43.1)	45(23.9)	62(33.0)	
Female	67(42.7)	44(28.0)	46(29.3)	
Age (Years)				0.417
18 – 28	20(42.6)	12(25.5)	15(31.9)	
29 – 38	43(39.4)	34(31.2)	32(29.4)	
39 – 48	44(43.1)	19(18.6)	39(38.2)	
49 – 58	28(45.2)	16(25.8)	18(29.0)	
59 and above	13(52.0)	8(32.0)	4(16.0)	
Marital status				0.511
Single	46(48.9)	22(23.4)	26(27.7)	
Married	71(37.6)	52(27.5)	66(34.9)	
Divorced	15(48.4)	9(29.0)	7(22.6)	
Separated	8(50.0)	2(12.5)	6(37.5)	
Widowed	8(53.3)	4(26.7)	3(20.0)	
Level of education				0.845
No formal education	17(39.5)	13(30.2)	13(30.2)	
Primary education	22(42.3)	11(21.2)	19(36.5)	
Secondary education	79(43.6)	50(27.6)	52(28.7)	
Tertiary	30(43.5)	15(21.7)	24(34.8)	
Years of farming experience				0.033
Less than 5years	61(42.1)	40(27.6)	44(30.3)	
6 - 10years	64(40.8)	40(25.5)	53(33.8)	
11 -15years	20(57.1)	6(17.1)	9(25.7)	
15years and above	3(37.5)	3(37.5)	2(25.0)	
Farm type				0.000
Poultry	90(42.9)	67(31.9)	53(25.2)	
Fishery	45(66.2)	11(16.2)	12(17.6)	
Piggery	13(19.4)	11(16.4)	43(64.2)	

Bacterial isolates from animal wastes

A total of 240 bacterial isolates belonging to thirteen genera were identified from the wastewater and animal dropping in the three senatorial districts as presented in **Figure 3.** The percentage occurrence of bacterial isolates from wastewater showed that *Pseudomonas* *aeruginosa* (14.2%) had the highest prevalence of occurrence followed by *Escherichia coli* (12.5%), while *Staphylococcus hominis* (2.1%) had the least prevalence of occurrence.

Antibiogram of bacterial isolates

Table 6 shows results obtained in the antibiotic susceptibility test of bacterial isolates from the study area. The results revealed marked differences among bacterial isolates in their susceptibility and resistance patterns to antibiotics. Bacterial resistance to selected antibiotics was observed to be high. All isolates were resistant to one or more of the antibiotics tested. *Staphylococcus aureus* isolated were found to be resistance to several antibiotics such as chloramphenicol (80.8%), vancomycin (80.8%) and tetracycline (73.1%). Among gram-positive bacteria isolated, *Staphylococcus aureus* (88.9%) shows the highest level of resistance to several antibiotics while among the gram negative isolates, *Enterobacter aerogenes* (83.3%), *Klebsiella pneumoniae* (81.3%) and *Escherichia coli* (80.0%) were found to be highly multi-drugs resistant and *P. aeruginosa* (61.8%) was found to be the least resistant isolates as showed in **Fig 4**.

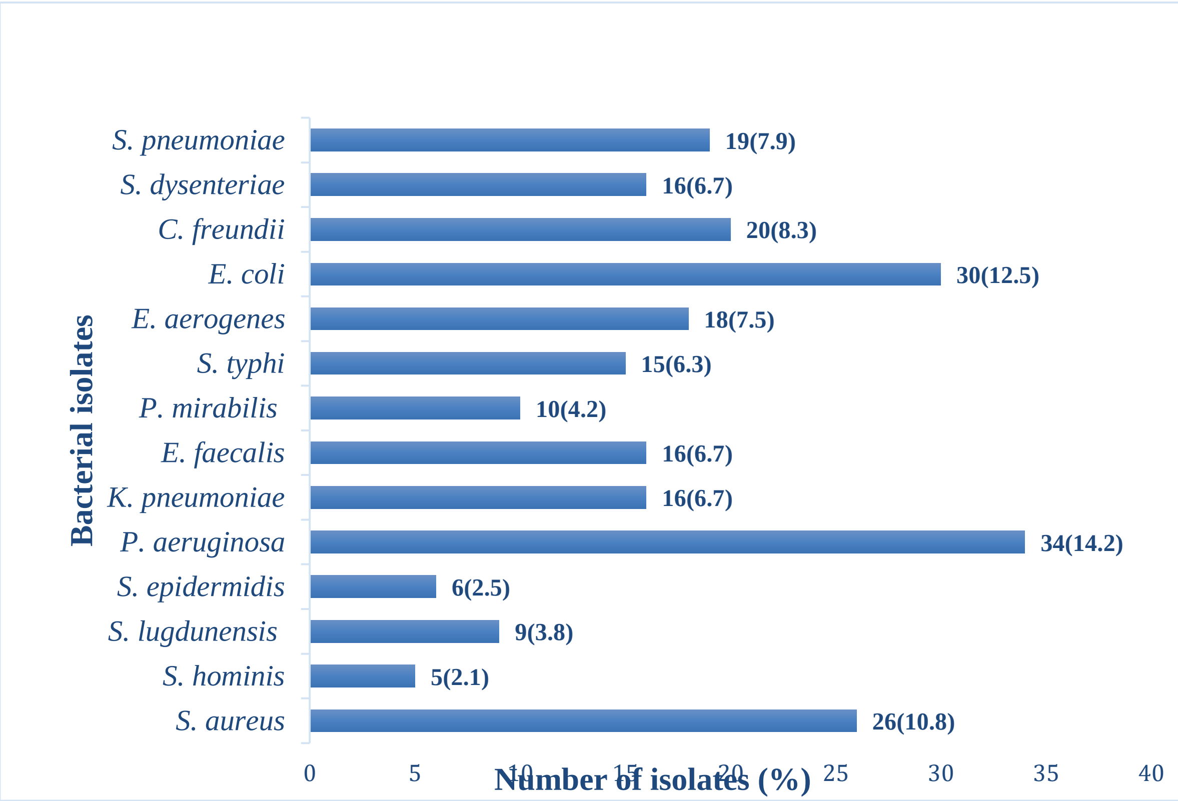

Fig 3: Bacterial isolates recovered from animal farm wastes in the study area

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Table 14: Antibiotics resistance profile of bacterial isolates from farm animal production wastes

	Antibiotics resistance of bacterial isolates N (%)												
Antibiotics class	Aminoglycosides			Cephalosporins		Quinolones & Fluoroquinolones		Penicillins		Phenicols	Tetracyclines	Glycopeptides	Sulfonamides
Bacterial isolates	**GM**	**SRP**	**AK**	**FOX**	**CAZ**	**CIP**	**LEV**	**AMP**	**OXA**	**CHL**	**TE**	**VA**	**SXT**
S. aureus	9(34.6)	12(46.2)	-	-	-	14(53.8)	17(65.4)	-	-	21(80.8)	19(73.1)	21(80.8)	8(30.8)
S. hominis	3(60.0)	2(40.0)	-	-	-	1(20.0)	1(20.0)	-	4(80.0)	3(60.0)	4(80.0)	2(40.0)	1(20.0)
S. lugdunensis	6(66.7)	8(88.9)	-	-	-	4(44.4)	5(55.6)	-	-	7(77.8)	6(66.7)	3(33.3)	2(22.2)
E. faecalis	-	-	-	-	-	8(50.0)	10(62.5)	4(25.0)	-	-	10(62.5)	13(81.3)	-
S. epidermidis	4(66.7)	3(50.0)	-	-	-	2(33.3)	2(33.3)	-	3(50.0)	1(16.7)	2(33.3)	4(66.7)	3(50.0)
S. pneumoniae	10(52.6)	-	-	-	-	-	16(84.2)		17(89.3)	7(36.8)	8(42.1)	13(68.4)	14(737)
P. aeruginosa	14(41.2)	-	16(47.1)	-	18(52.9)	12(35.3)	10(29.4)	-	-	-	-	-	-
K. pneumoniae	11(68.8)	8(50.0)	10(62.5)	12(75.0)	13(81.3)	5(31.2)	8(50.0)	13(81.3)	-	8(50.0)	6(37.5)	-	5(31.3)
P. mirabilis	7(70.0)	6(60.0)	6(60.0)	7(70.0)	6(60.0)	4(40.0)	3(30.0)	7(70.0)	-	7(70.0)	5(50.0)	-	4(40.0)
S. typhi	6(40.0)	8(53.3)	7(46.7)	10(66.7)	11(73.3)	3(20.0)	4(26.7)	11(73.3)	-	7(46.7)	8(53.3)	-	7(46.7)
E. aerogenes	12(66.7)	9(50.0)	13(72.2)	12(66.7)	10(55.6)	7(38.9)	9(50.0)	15(83.3)	-	9(50.0)	10(55.6)	-	12(66.7)
E. coli	14(46.7)	16(53.3)	18(60.0)	18(60.0)	20(66.7)	12(40.0)	14(46.7)	22(73.3)	-	14(46.7)	21(70.0)	-	19(63.3)
C. freundii	16(80.0)	11(55.0)	13(65.0)	11(55.0)	13(65.0)	15(75.0)	14(70.0)	10(50.0)	-	11(55.0)	13(65.0)	-	11(55.0)
S. dysenteriae	12(75.0)	8(50.0)	10(62.5)	12(75.0)	12(75.0)	6(37.5)	8(50.0)	11(68.6)	-	8(50.0)	10(62.5)	-	9(56.3)

Note: **N** –Number of bacterial isolates, **GM** – Gentamicin, **SRP**-Streptomycin, **AK**-Amikacin, **FOX**-Cefoxitin, **CAZ**-Ceftazidime, **CIP**-Ciprofloxacin, **LEV**-Levofloxacin, **AMP**-Ampicillin, **OXA**-Oxacillin, **CHL**-Chloramphenicol, **TE**-Tetracycline, **VA**-Vancomycin, **SXT**-Trimethoprim/sulfamethoxazole

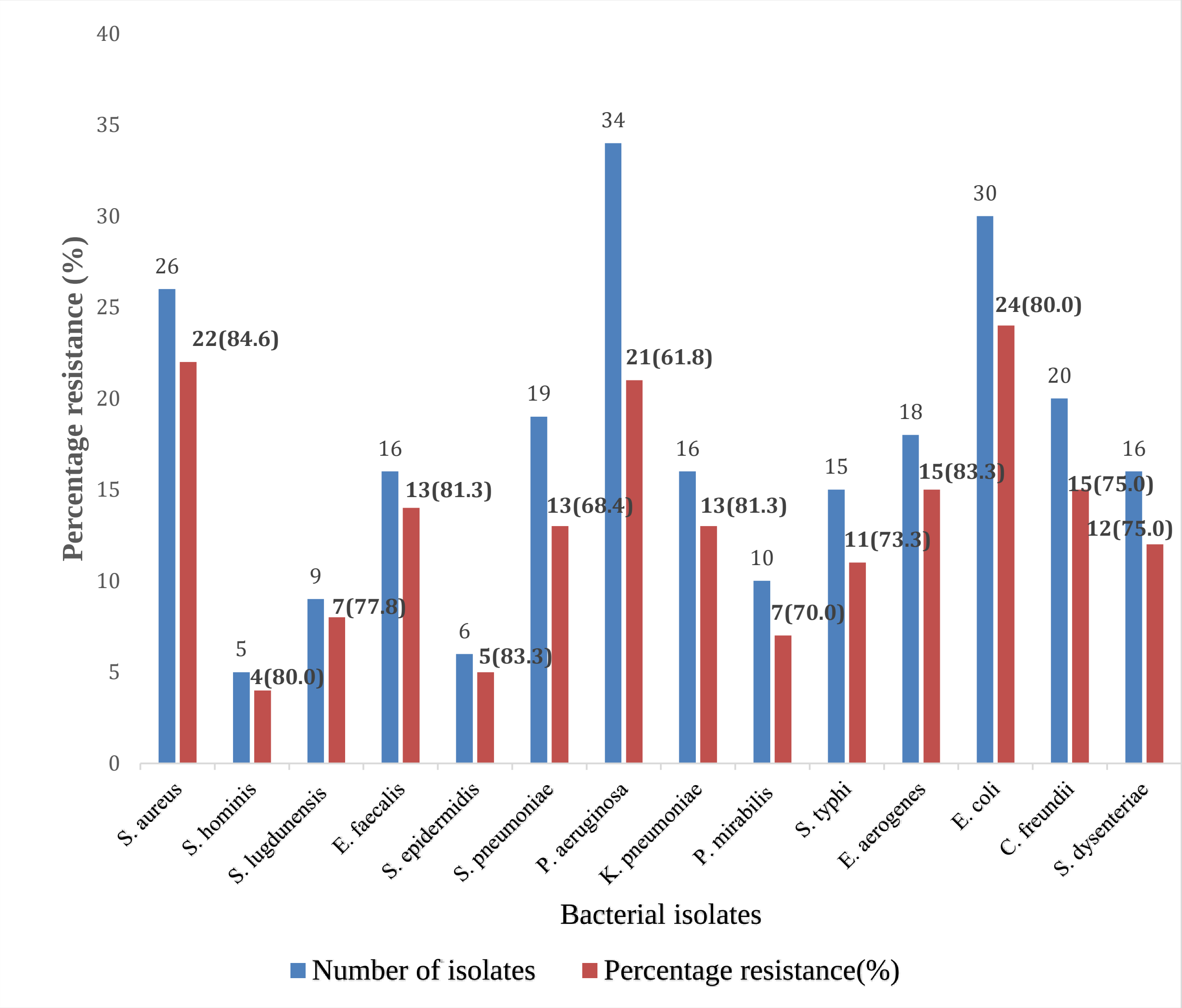

Fig 4: Percentage resistance of bacterial isolates recovered from animal farm wastes in the study area

DISCUSSION

Inappropriate use of antibiotic in animal production farms has momentous consequences for public health and the environment (Caudell et al. 2020). Antibiotic usage varies considerably across animal farm types and locations (Van-Boeckel et al., 2015). In this current study, farmers' knowledge on the use of antibiotics and its resistance recorded among respondents revealed that on the average a good number of farmers had a good knowledge level on the use of antibiotics and its resistance. Majority of the farmers saw antibiotics as antimicrobial agents that kill or inhibit growth of bacteria. More so, respondents were aware that improper use of antibiotics in animal farm can cause AMR. This findings is in line with the report of Geta and Kibret (2021), who revealed that half of the livestock farm owners/workers had good knowledge about ABR and use. Contrary to the findings, Hossain et al. (2022) reported that 41.5%, of farmers possess adequate knowledge of antimicrobial use. The percentage difference may be attributed to their level of education and access to information on antimicrobial agents and its resistance. Similarly, Ozturk et al. (2019) study also revealed that in terms of antibiotic knowledge and appropriate antibiotic use, respondent's level of education is statistically significant to their knowledge on antibiotics use and resistance. Additionally, a good number of the respondents were aware that bacteria becomes resistant to antibiotics. However, a proportion of respondents still believe that humans and animals also shows resistant against antibiotics. A low proportion of respondents were also not aware that resistant bacteria can spread from animals to the environment and had incorrect knowledge that antibiotics residues in the environment can cause antibiotics resistance. This may also be attributed to their level of education and lack of awareness on antibiotics use. The lack of awareness of AMR by farmers is mainly due to a lack of training or education on antimicrobials (Ndukui et al. 2021). Pham-Duc et al. (2019) study also revealed that education levels were positively correlated with increased levels of knowledge around antibiotics, with producers attaining a high school education or found to have better knowledge than those who did not attain a completed high school education. There is a need to provide adequate and suitable information to farmers on antibiotics and the possible consequences of their inappropriate use. The appropriate information can be conveyed to animal farmers through the extension services by veterinarian, training, and educational programs on the use of antimicrobials and the factors that can lead to AMR (McKernan et al. 2021; Moffo et al., 2020). Pearson chi-square test used to assess the relationship between socio-demographic variables and farmer's knowledge of antibiotics use and its resistance, revealed that respondents' level of education (p = 0.031), years of farming experience (p = 0.048), and farm type (p = 0.000) were statistically significant.

Regarding antibiotics use, majority of animal farm owners/workers used antibiotics in their farms. When respondents were asked on the reasons for using antibiotics in farm animals, majority of farmers mentioned that they used antibiotics for treatment and prevention of diseases, while a low proportion uses antibiotics for growth promotion. This findings is in agreement with Pham-Duc et al. (2020), who study revealed that majority of respondents (78%) used antibiotics to treat and prevent disease in pigs. Similarly, Phares et al. (2020) also reported that 86.3% of farmers who administered antibiotics did that to prevent and treat diseases while 13% was for growth promotion. In this study, a good number of respondents used antibiotics for growth promotion, disease treatment and prevention. Many farmers use antibiotics because of the huge demand for animal products such as eggs and chicken meat (Imam et al. 2020). Several studies have reported the inadequate regulation of antibiotics usage in low and middle-income countries, thereby causing significant risks for the environmental development and transfer of AMR due to the release of antibiotics and ARGs to soils and water bodies affected by animal farming operations (Argudın et al., 2017; Van Boeckel et al., 2019). Antibiotics level of use by farmers may be attributed to farmers' years of personal experience, peer-to-peer advice, and information from feed sellers concerning disease prevention, treatment and growth promotion using antibiotics. The use of farmers' personal experience and information gathered from feed sellers have been among the causes of inappropriate use of antibiotics and a contributing factor to the rise of AMR (Hassan et al. 2021; Ndukui et al. 2021).

Considering the route of antibiotics administration, majority of farm owners administered antibiotics by dissolving in animal's drinking water, while a low proportion mixed in feed or through injection. This findings as in consistent with the report of Agoba et al. (2017), which revealed that antibiotics are administered either by mixing with feed or water. Majority of the persons that administered antibiotics to farm animals were farm workers and farm manager, while a low proportion were veterinarians or animal health officials. Phares et al. (2020), also reported that only 8.5% of antibiotic administration was carried out by veterinary officers whilst, 88.5% was done by farm workers and farm managers. A good number of respondents reported the use of antibiotics at a given stage of animal growth, while a low proportion reported the use of antibiotics always. The inappropriate use of antibiotics by farmers may have contributed to the high level of ABR bacteria isolated in the study area. Regarding antibiotics withdrawal period, a good number of farmers do not practice or observed antibiotics withdrawal period. However, the result on the number of farmers that observed withdrawal period is higher than some studies reported for farmers in Bangladesh and a study in Nigeria (Hossain et al. 2022; Ferdous et al., 2019; Alhaji et al., 2018). The differences may be due to farmer's knowledge, years of farming experience and information from veterinarian and animal health officials concerning antibiotics withdrawal period. Most of the respondents were able to mention the brand name of at least one antibiotics that they had frequently administered to their animals. Tetracycline, ampicillin, streptomycin, cotrimoxazole, gentamycin and vancomycin were the most frequently used groups of antibiotics respectively. Several studies also mentioned tetracycline and aminoglycosides as the most commonly used antibiotics (Bedekelabou et al. 2022; Boamah et al. 2016; Gemeda et al. 2020). The test of association using Pearson chi-square test revealed that respondents' years of farming experience (p = 0.033), and farm type (p = 0.000) were statistically significant with farmers level of antibiotics use. Considering the high level of antibiotics resistance in the environment, studies has suggested the devolvement of new antibiotics by pharmaceutical companies (WHO, 2015), while others were on antibiotics alternative. In spite of the development of potential antibiotic alternatives, including vaccines, organic acid, bacteriophages, antimicrobial peptides, plant extracts, prebiotics, enzymes, and probiotics, prudent use of antibiotics remain the best option since antibiotic resistance and tolerance in bacteria are natural evolutionary consequences (Sang and Blecha, 2015; Geta and Kibret, 2021).

Different microbial species of both pathogenic and non-pathogenic organisms were isolated from all the sampled sources examined in this study. A total of 240 bacterial isolates identified from the wastewater and animal dropping is an indication that the animal wastes were grossly contaminated. Prevalence occurrence of bacterial species identified such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Enterobacter aerogenes, Klebsiella pneumoniae, Shigella dysenteriae* and *Enterococcus* *faecalis* are of public health importance. The findings from the present study align with Sule et al. (2016) study that revealed the presence of *Escherichia coli, Staphylococcus aureus* and *Staphylococcus saprophyticus* in wastewater from fish ponds within Ilorin metropolis in Nigeria. The findings also is in line with Umeh et al. (2020) study that revealed the presence of *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, and Salmonella typhi* among others from fish pond water samples in Anambra State, Nigeria. Similarly, (Barua et al. 2021) in an Assam study on bacteriological quality of livestock farm oriented wastewater revealed the presence of *Escherichia coli* in all the wastewater samples. The presence of *K. pneumoniae, E. coli, S. aureus, P. aeruginosa, P. mirabilis, and S. typhi* constitute a public health hazard because animal droppings are used by farmers as a good source of manure for the cultivation of crops and vegetables. The use of the animal droppings especially poultry droppings directly into soils and the watering of vegetables with wastewater from animal farms without any form of treatment poses some public health problems since they may contain pathogenic microorganisms.

Bacterial resistance to selected conventional antibiotics was observed to be high. All isolates were resistant to several antibiotics tested. One of the pathogenic gram positive bacteria (*Staphylococcus aureus*) of medical importance also isolated were found to be resistance to several antibiotics such as chloramphenicol, vancomycin and tetracycline. Also some pathogenic gram negative organisms such as *E. aerogenes, K. pneumoniae, S. typhi,* and *E. coli* were found to show multi-drug resistance. The findings is in line with Sule et al. (2016) study on the bacteriological and physicochemical quality of wastewater from fish ponds within Ilorin metropolis, Nigeria that revealed all the isolated gram negative bacteria were resistant to Ampicillin, and Ceftazidime. Similarly, Fakorede et al. (2020) also found out that bacterial isolates from commercial fish farms in Nigeria were resistant to Ceftazidime, Cefuroxime and Augmentin. In this present study, *P. aeruginosa* was found to be the least resistant isolates. The findings is in agreement with Odoi (2016) study on isolation and characterization of multi-drug resistant *Pseudomonas aeruginosa* in Ashanti Region of Ghana that found out that *Pseudomonas aeruginosa* isolated from poultry litter were all susceptible to levofloxacin. High levels of antimicrobial resistance in food-producing animals have been reported in African countries, with resistance to tetracyclines and penicillin being the most frequently observed (Founou, et al., 2018; Kimera, et al., 2020).

Antibiotic resistance bacteria strains are usually transferred to the general public via food, water bodies, environment and farm workers (Heuer et al. 2011). Infections such as non-typhoidal salmonellosis, and methicillin-resistant *Staphylococcus aureus* (MRSA), which are all capable of spread amongst animals and humans, become more challenging to treat when antibiotic-resistant strains originating from food animals are involved (Bengtsson and Greco, 2014). Moreover from previous studies, *S. aureus* isolates from Africa show a high degree of resistance to penicillin, tetracycline and trimethoprim/sulfamethoxazole, indicating the wide use of these drugs in African countries (Schaumburg et al. 2014).

CONCLUSION

This study revealed that a significant number of farmers have poor knowledge of antibiotics use and its resistance. A proportion of respondents still believe that humans and animals also shows resistant against antibiotics. Many farmers use antibiotics for therapeutic, prophylaxis and growth promotion inappropriately. This has been attributed to their level of education, training acquired and lack of awareness on antibiotics resistance. The inadequate regulation of antibiotics usage in low and middle-income countries causes a significant risks for the environmental development and transfer of AMR due to the release of antibiotics and ARGs to soils and water bodies affected by animal farming operations. There is a need to provide adequate and suitable information to farmers on antibiotics and the possible consequences of their inappropriate use.

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Competing interests

The authors declare that they have no competing interests

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