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Prevalence of Multi-antibiotic Resistant Bacteria in Birds Faeces and Soil Samples from Poultry Farms in Ogbomoso, Oyo State, Nigeria

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Authors' contributions

This work was carried out in collaboration Among all authors. Author AAA designed the study. Authors AAO and IBA wrote the protocols. Author LOO wrote the first draft. Author AAA managed the analysis of the study, while author EAO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was designed to determine the prevalence of multi-antibiotic resistant bacteria in poultry faecal and soil samples from poultry farms in Ogbomoso, Oyo State, Nigeria.
Introduction: The indiscriminate use of antibiotics in the treatment of animals' infection and as growth promoters is increasing the incidence of multi-antibiotic resistant bacteria at an alarming rate. This poses a danger to human health because resistant bacteria can be transferred to human through the food chain.
Methodology: Different isolating media were used to determine the microbial load of faecal and soil samples from two farms and bacterial identification was carried out by standard methods. A susceptibility profile of the bacterial isolates was determined by Kirby-Bauer disc diffusion method and multiple antibiotic resistant (MAR) index was also determined.
Results: Soil samples showed high counts of the microbial load as compared with faecal samples, with count ranging from 0.2 to 10.5 $\times 10^5$cfug-1. Majority of the isolates belonged to Gram-negative

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bacteria (90.9%) and five genera of bacterial isolates were identified in this study (*Klebsiella*, *Citrobacter*, *Escherichia*, *Shigella*, and *Staphylococcus*), while all the isolates showed 12.5 to 100% resistant to all the antibiotics used in this study. The prevalence of multi-antibiotic resistant was 100% and the MAR index ranged from 0.6 to 1.0.

Conclusion: The study revealed that samples from the poultry farms used were reservoirs of multi-antibiotic resistant bacteria and MAR index showed that the resistance observed was from the overuse of antibiotics in the environment. Therefore there should be strict regulations on the use of antibiotics in animals' farms and proper monitoring should be ensured by the government.

Keywords: Faecal sample; multi-antibiotic resistant; Kirby-bauer disk diffusion; gram-negative bacteria.

1. INTRODUCTION

Poultry is one of the fastest growing agricultural sectors worldwide [1] and a major fast-growing source of meat in the world today, representing a quarter of all meat produced. Poultry meat is important in the diet of many people worldwide and it is accepted by all religious beliefs. This worldwide acceptability has made poultry business to be in high demand and this will continue to increase [2]. Studies have shown that poultry meat consumption is steadily increasing worldwide; the last data available indicate that it has reached 14.2 kg per capita per year [3]. The increase in poultry production has also led to the generation of high quantities of poultry wastes usually composed of faeces, feathers, bones, blood and dead birds. These wastes pose serious environmental pollution problems through microbial infection, offensive odour, promotion of flies and rodents breeding [4]. Poultry faeces are the excretory product released as a result of digestion of food taken in by poultry birds [5]. There are several billions of bacteria present in poultry faeces including pathogenic and non-pathogenic species, the normal flora and the opportunistic ones [6]. Faeces from livestock and poultry contain a variety of pathogens; some are highly host-adapted and not pathogenic to humans, while others can produce infections in humans [7].

But in intensively reared food animals, antibiotics are administered for therapeutic purposes and as Antimicrobial growth promoters (AMGPs) to the whole flock rather than individual [8]. The use of antimicrobials in agriculture, especially as growth promoters, chemotherapeutic and prophylactic agents in food animals are of public health implication [9,10]. Acquired resistance against frequently used antibiotics has been observed since the introduction of these antimicrobial agents in human and veterinary medicine [11]. The indiscriminate use of antibiotics is a major factor in emergence, selection, and dissemination of antibiotics resistant microorganisms in both veterinary and human [12]. The rise in antibiotic resistance has been reported in the past two decades [13] and it still remains a global problem until today. In many countries and especially the developing countries, the use of antibiotics in livestock is unregulated and a prescription is not required even for human use [14]. In general, antibiotic use in livestock in Africa [15] is unrestricted. Also in Nigeria, antibiotic use policies are rarely enforced and are widely used in poultry farming without oversight [16,17]. This attitude has led to increase in the development of antibacterial resistant isolates associated with poultry farms in Nigeria and portrays dangers of treatment failures in cases of infection with such isolates.

The main purpose of this research work was to examine the load, types and the multidrug-resistant pattern of bacteria isolated from the faeces of broilers and soil samples within and around two farms from Ogbomoso North local Government, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

Soil samples and faeces (from Layers and Broilers) were collected from two poultry sites (Site 1 and site 2). At each sampling station, fresh droppings were picked into the universal bottle with the use of a sterilized spatula, while soil samples outside the pen were scooped from depths of about 1-10cm into a sterile universal bottle, carefully labeled and transported to the laboratory for microbiological analyses.

2.2 Bacterial Enumeration, Isolation and Identification

10 g of each sample was diluted in 90 ml of sterile normal saline and homogenized. Serial dilution was prepared unto 10^{-5} fold. Then 10 µl was placed on the surface of Nutrient agar, MacConkey agar, Salmonella-Shigella agar, Eosin Methylene agar and Mannitol Salt agar using pour plate technique. Cultured plates were then incubated in the incubator for 24hours at 37°C. Total viable counts were determined for all the isolating media agar used by counting the colonies developed after incubation at 37°C for 24hours [18].

Gram staining reaction, Indole, H_2S, Nitrate reduction, Catalase, Oxidase, methyl red, Voges-Proskauer and sugar fermentation tests were carried out according to standard methods for bacterial identification [19].

2.3 Antibiotic Sensitivity Test

The antibiotic susceptibility profile of the bacterial isolates was determined using the standard Kirby-Bauer disk diffusion method [20]. A total of 13 antibiotics were used and these include; Different generations of Cephalosporin group, Cefuroxime (30 µg), Cefprozil (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefixime (5 µg), Cefepime (30 µg); Aminoglycoside group, Gentamycin (10 µg), Oxacillin (5 µg) and Novobiocin (5 µg) together with Oxacillin(5 µg) (a beta lactamase antibiotics) were used for *Staphylococcus epidermidis* only; Penicillin group, Augmentin (30 µg); Nitrofura group, Nitrofurantoin (300 µg); and Quinolones group; Ofloxacin (5 µg). Bacterial culture equivalent to 0.5 µl McFarland turbidity standard was spread on Muller – Hinton agar plates using a sterile swab and incubated aerobically at 37°C for 24hours. Then, the inhibition zone diameters around the antibiotic disks were measured. The results were expressed as susceptible, intermediate or resistant according to the criteria recommended by the CLSI [21].

2.4 Multiple Antibiotic Resistant (MAR) Indexing

The MAR Index profile was based on isolates and sampling site was performed to evaluate the health risk to the environment. MAR index for test isolates was calculated according to the formula a/b, where 'a' is the No of antibiotics to which the isolate is resistant to and 'b' is the No of antibiotics tested [22]. Therefore, multiple AR was described in this study is the resistance to three or more classes of antibiotic.

3. RESULTS AND DISCUSSION

3.1 Microbial Load

Total Enterobacteriaceae count ranged from 2.6 to 4.0 $\times10^5$cfug-1 with the highest count found in site 2 (Table 1). Total Heterotrophic count varied from 0.7 to 2.8×10^5cfug^{-1}. The highest Staphylococcal count was found in site 1 with a count of 0.9×10^5cfug^{-1}. And the highest Salmonella Shigella count of 1.2×10^5cfug^{-1} was found in the layer faeces of site 2 and the lowest was found in the broiler faeces from site 1 (Table 1).

The study showed that the soil samples from the two sites showed the highest counts for all the isolating media used except in Salmonella Shigella count in which the layers faeces had the highest count of 1.2×10^5cfug^{-1}, while [23] also reported that the highest number of studied microorganisms was isolated in the soil samples collected from the soil within the poultry farms, while [24] also recorded highest count of bacteria (5.4×106 CFU/g) in the soil taken 150m off the poultry facility in their work.

3.2 The Occurrence of Bacterial Isolates

Fig. 1 shows the occurrence of the isolated bacteria in the two sites. *Citrobacter freundii* had the highest occurrence of 26.62% followed by *K. edwardsii* (25.39%), while *S. epidermidis* had the lowest occurrence of 6.67% from site 1. While *Citrobacter freundii* also had the highest occurrence of 42.45% in site 2 followed by *K. oxytoca* (40.40%) and *S. epidermidis* also showed the least occurrence of 2.25%. *E. intermidium* is not found in site 1 and *K. edwardsii* was not found in site 2 too. The higher incidence of Gram-negative reported in this study is expected since these bacteria are normal flora of the intestinal tract of poultry bird [24]. Although some of the common isolates of poultry farms like *Escherichia*, *Shigella* and *Staphylococcus* were isolated from this study but some of the isolates from this work were different to that of [25], while [26] attributed this changes in bacterial populations to differences in geographical location or isolation technique. The low occurrence of *Escherichia* (3.03%) in this

Table 1. Microbial load of microorganisms isolated from the poultry droppings and soil

Samples		Microbial load				
		Bacteria				
		Total enterobacteriaceae count (10^5cfu g^{-1})	Total heterotrophic count (10^5cfu g^{-1})	Total staphylococcal count (10^5cfu g^{-1})	Total feacal coliforms (10^5cfu g^{-1})	Total salmonella shigella count (10^5cfu g^{-1})
Site 1	BROILER (Feaces)	3.2	0.9	0.7	9.2	0.2
	LAYER (Feaces)	2.8	1.1	0.3	8.5	0.9
	SOIL	2.6	2.4	0..9	10.5	0.8
Site 2	BROILER (Feaces)	3.9	0.7	0.2	8.3	0.7
	LAYER (Feaces)	2.7	2.0	0.7	8.6	1.2
	SOIL	4.0	2.8	0.6	9.8	0.9

CFU: Colony forming unit./g

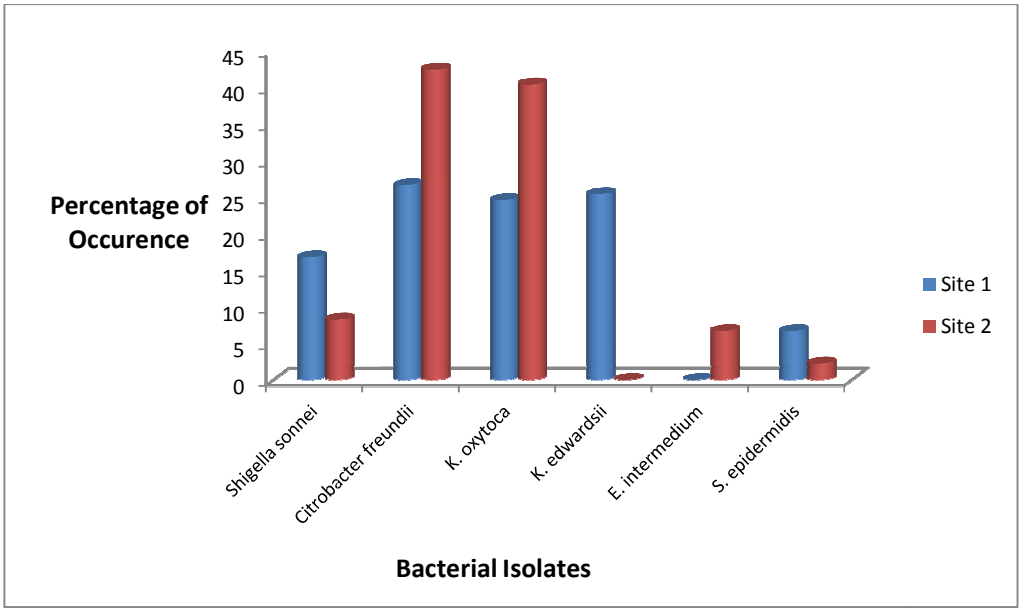

Fig. 1. Percentage of occurrence of bacterial isolates from the sampling sites

study is in agreement with the report of [27] but in contrast with the work of [28] that isolated 75% *Escherichia* species from their work. The presence of *Staphylococcus epidermidis* might be due to the continual entry of poultry house by the workers when feeding the birds or picking their eggs [29]. The results obtained in this work also showed that the faeces from the layers had the lowest occurrence of bacterial isolates, which is also in agreement with work of [30] who reported that bacteria in the layer are slightly lower in population as reported for poultry faeces in Nigeria.

3.3 Antibiotics Susceptibility Tests

All the bacteria isolated in this study showed varying resistant patterns to different classes of antibiotics used. The resistant pattern of bacterial isolates from the two poultry sites used in this study did not reflect much difference (Tables 2a and 2b). Resistant >90% was showed to different generations of Cephalosporin used in this study with the exception of Ceprozil which showed resistant pattern of 12.5 and 35.29% in site 1 and 2 respectively. High resistant (87.5 to 100%) was found against Aminoglycoside (Novobiocin and Gentamycin) and Penicillin. While there was >80% resistant to Nitrofuran (Nitrofurantoin), with the isolates showing 58% resistant to Quinolones class (Ofloxacin), while the *Staphylococcus epidermidis* showed 50 and 100% resistant to Oxacillin in sites 1 and 2 respectively. Generally, Ceprozil showed the least resistant pattern of 12.5 and 35.29% in site 1 and 2 respectively (Tables 2a and b). The antibiogram showed that all the isolates possessed multi-resistant to all the antibiotics used, with the resistant pattern ranging from 6-10, in which three of the isolates showed 100% resistant to the antibiotics used (Table 3). The rise in antibiotic resistance has been reported in the past two decades [13]. This study confirmed previous studies that resistant strains of many bacteria species are present in Nigeria poultry operations [17].

The high occurrence of antibiotic resistant *Staphylococcus* to *Escherichia* observed in this study has also been reported by previous workers [31]. Multiple resistant to antibiotics in *Staphylococcus* has been reported in other studies [16]. *Klebsiella* species isolated in this study showed multiple resistant to antibiotics used, the resistance of *Klebsiella* to different classes of antibiotics has also been reported [32] and various degree of multi-drug resistant among *Klebsiella* have been reported worldwide [32].

MAR index observed in this study ranged from 0.6 to 1.0, and it has been reported that MAR index values higher than 0.2 were considered to have originated from the use of antibiotics within the study area [22].

Table 2a. Antibiotic sensitivity test for bacterial isolates from site1

Organisms	FEP	OXA	CRO	NIT	CPR	CAZ	CRX	GEN	CXM	OFL	AUG	NOV	PEN
Citrobacter freundii	R	-	R	R	S	R	R	R	R	S	R	-	-
Citrobacter freundii	R	-	R	R	S	R	R	I	R	R	S	-	-
Citrobacter freundii	R	-	R	R	S	R	R	R	R	S	R	-	-
Citrobacter freundii	R	-	R	I	S	R	I	R	R	I	R	-	-
Klebsiella oxytoca	R	-	R	R	S	R	R	R	R	I	R	-	-
Klebsiella oxytoca	R	-	R	R	S	R	R	R	R	S	R	-	-
Klebsiella oxytoca	R	-	R	R	I	R	R	R	R	R	R	-	-
Shigella sonnei	R	-	R	R	I	R	R	R	R	R	R	-	-
Shigella sonnei	R	-	R	R	S	R	R	R	R	S	R	-	-
Klebsiella edwarsii	R	-	R	R	S	R	R	R	R	S	R	-	-
Klebsiella edwarsii	R	-	R	R	S	R	R	R	R	S	R	-	-
Klebsiella edwarsii	R	-	R	R	S	R	R	R	R	R	R	-	-
Klebsiella edwarsii	R	-	R	R	S	R	R	R	R	R	R	-	-
Klebsiella edwarsii	R	-	R	R	S	R	R	R	R	R	R	-	-
Staphylococcus epidermidis	-	I	-	S	R	R	R	R	R	S	R	R	R
Staphylococcus epidermidis	-	R	-	I	R	R	R	I	R	S	R	R	R

KEY: FEP: CEFEPIME; CRX: CEFUROXIME; OXA: OXACILLIN
CRO: CEFTRIAXONE; CXM: CEFIXIME
NIT: NITROFURANTOIN; OFL: OFLOXACIN
CPR: CEFPROZIL; AUG: AUGMENTIN
CAZ: CEFTAZIDIME; GEN: GENTAMICIN; NOV: NOVOBIOCIN; PEN: PENICILLIN

Table 2b. Antibiotic sensitivity test for bacterial isolates from site 2

Organisms	FEP	OXA	CRO	NIT	CPR	CAZ	CRX	GEN	CXM	OFL	AUG	NOV	PEN
Escherichia intermedium	R	-	R	R	R	R	R	R	R	R	R	-	-
Klebsiella oxytoca	R	-	R	R	S	R	R	R	R	R	R	-	-
Klebsiella oxytoca	R	-	R	R	R	R	R	R	R	S	R	-	-
Klebsiella oxytoca	R	-	R	R	S	R	R	R	R	I	R	-	-
Klebsiella oxytoca	R	-	R	R	I	R	R	R	R	R	R	-	-
Klebsiella oxytoca	R	-	R	R	S	R	R	R	R	S	R	-	-
Klebsiella oxytoca	R	-	R	R	S	R	R	R	R	S	R	-	-
Shigella sonnei	R	-	R	R	I	R	R	R	R	R	R	-	-
Citrobacter freundi	R	-	R	R	R	R	I	R	R	R	R	-	-
Citrobacter freundi	R	-	R	R	R	R	R	R	R	R	R	-	-
Citrobacter freundi	R	-	R	R	R	R	R	R	R	R	R	-	-
Citrobacter freundi	R	-	R	R	S	R	R	R	R	I	R	-	-
Citrobacter freundi	R	-	R	R	S	R	R	R	R	S	R	-	-
Citrobacter freundi	R	-	R	R	S	R	R	R	R	S	R	-	-
Citrobacter freundi	R	-	R	R	S	R	R	R	R	I	R	-	-
Citrobacter freundi	R	-	R	R	S	R	R	R	R	S	R	-	-
Staphylococcus epidermidis	-	R	-	S	R	R	R	R	R	S	R	R	R

KEY: FEP: CEFEPIME; CRX: CEFUROXIME; OXA: OXACILLIN
CRO: CEFTRIAXONE; CXM: CEFIXIME
NIT: NITROFURANTOIN; OFL: OFLOXACIN
CPR: CEFPROZIL; AUG: AUGMENTIN
CAZ: CEFTAZIDIME; GEN: GENTAMICIN; NOV: NOVOBIOCIN

Table 3. Antibiogram of the resistance pattern of bacterial isolates

Organisms	Resistant pattern	% of resistance	MAR Index
C. freundii[4]	FEP CRO CAZ CXM AUG, GEN	60.00	0.6
C. freundii[2]	FEP CRO NIT CAZ CRX CXM AUG	70.00	0.7
C. freundii[12]	FEP CRO NIT CAZ GEN CXM OFL	70.00	0.7
S. Epidermidis[1]	PEN NOV CPR CAZ CRX CXM AUG	63.63	0.6
K. oxytoca[8]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
S. sonnei[2]	PEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
K. edwarsii[1]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
K. edwarsii[2]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
C. freundii[1]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
C. freundii[3]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
C. freundii[9]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
C. freundii[11]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
K. oxytoca[1]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
K. oxytoca[2]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
K. oxytoca[6]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
K. oxytoca[9]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
C. freundii[8]	FEP CRO NIT CAZ CRX GEN CXM AUG	80.00	0.8
C. freundii[10]	FEP CRO NIT CAZ CRZ GEN CXM AUG	80.00	0.8
K. oxytoca[7]	FEP CRO NIT CAZ CRX GEN CXM AUG OFL	90.00	0.9
K. oxytoca[5]	FEP CRO NIT CPR CAZ CRX GEN CXM AUG	90.00	0.9
S. sonnei[1]	FEP CRO NIT CAZ CRX GEN CXM OFL AUG	90.00	0.9
S. sonnei[3]	FEP CRO NIT CAZ CRX GEN CXM OFL AUG	90.00	0.9
S. Epidermidis[2]	OXA NOV CPR CAZ CRX GEN CXM PEN AUG	81.8	0.7
S. Epidermidis[3]	OXA NOV CPR CAZ CRX GEN CXM PEN AUG	81.8	0.7
C. freundi[5]	FEP CRO NIT CAZ GEN CXM AUG CPR OFL	90.00	0.9
K. edwarsii[3]	FEP CRO NIT CAZ CRX CXM AUG GEN OFL	90.00	0.9
K. edwarsii[4]	FEP CRO NIT CAZ CRX CXM AUG GEN OFL	90.00	0.9
K. edwarsii[5]	FEP CRO NIT CAZ CRX CXM AUG GEN OFL	90.00	0.9
K. oxytoca[3]	FEP CRO NIT CAZ CRX GEN CXM AUG OFL	90.00	0.9
K. oxytoca[4]	FEP CRO NIT CAZ CRX GEN CXM AUG OFL	90.00	0.9
E. intermidium	FEP CAZ CRX CXM AUG CRO NIT CPR GEN OFL	100.00	1.0
C. freundii[6]	FEP CRO NIT CAZ CRX GEN CXM AUG CPR OFL	100.00	1.0
C. freundii[7]	FEP CRO NIT CAZ CRX GEN CXM AUG CPR OFL	10000	1.0

KEY: Citrobacter freundii (1-4): Site1, Citrobacter freundii (5-12):Site 2,Klebsiella oxytoca (1-3) : Site 1, Klebsiella oxytoca (4-9): Site 2, Klebsiella edwarsii (1-5): Site 1, Shigella sonnei (1-2): Shigella sonnei (3) Site2; Escherichia intermidium (1) Site 2; Staphylococcus epidermidis (1-2), S. epidermidis (3) site 2

4. CONCLUSION

This study has revealed that all bacterial isolates used in this study showed multiple resistance to all the antibiotics used. As a result of this, proper checks need to be ensured in the administration of antibiotics to poultry birds in order to reduce the occurrence of multi-antibiotic resistant bacteria in the environment. The MAR index values obtained showed that the resistance of these isolates was due to the high use of antibiotics in the surrounding of poultry farms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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