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Multidrug–resistant *Enterococcus* and coagulase–negative *Staphylococcus* isolates from poultry farms and nearby wells in Ibadan, Nigeria and their resistant genes

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ABSTRACT

Objective: To determine the antibiotic resistance patterns and resistance genes in enterococci and coagulase-negative staphylococci (CoNS) isolated from poultry farms and nearby wells in Ibadan, Nigeria.

Methods: Poultry litter and well water samples were collected from three farms. Isolation of bacterial isolates were done using bile esculin azide agar and presumptive identification was done using conventional methods and confirmed by 16S rRNA using PCR. Antibiotic susceptibility testing was by disk diffusion method, while antibiotics resistance genes (ARGs) was detected by PCR.

Results: Of the 86 Gram-positive cocci isolated, 71 (82.6%) were CoNS comprising *Staphylococcus (S.) sciuri* (69.0%) and *S. xylosus* (31.0%), while the remaining 15 (17.4%) were *Enterococcus* spp. comprising *Enterococcus (E.) faecalis* (60.0%) and *E. faecium* (40.0%). Antibiotics resistance of the isolates showed that 91.5% (CoNS) and 93.0% (enterococci) were resistant to penicillin, 90.1% and 86.7% to tetracycline and 94.4% and 66.7% to erythromycin, respectively. Also, all the 15 (100%) *Enterococcus* spp. and 68 (95.8%) CoNS were multidrug resistant. The ARGs detected in the CoNS include: *tetM* (45.4%), *tetL* (9.0%) and *ermB* (27.3%), while *tet*M (9.1%), *tetL* (4.5%) and *ermB* (4.5%) were found in *Enterococcus* spp.

Conclusions: This study revealed that poultry litters in the study area are pools of antimicrobial resistant *enterococci* and CoNS, as well as, the corresponding ARGs. Therefore, necessary monitoring and ample treatment of poultry waste should be done before their release into the environment.

KEYWORDS: Poultry litter; Well water; Enterococci; Coagulase-negative staphylococci; Antibiotic resistance

1. Introduction

Poultry litter has been identified in several studies as potential source of multiple antibiotic- resistant bacteria, defined as resistance to at least three different classes of antibiotics[1]. The use of poultry litter as fertilizer may lead to the release of resistant bacteria and their related genes into the environment[2]. These contaminants that come from livestock through runoff and leaching can affect water resources in a variety of ways, from the well-known nutrient and pathogen contamination to the less well-known contaminants like antibiotics resistance genes (ARGs)[3]. Hence, contamination of water bodies by microorganisms as a result of animal operations and municipal wastewater treatment facilities is acknowledged as a

Significance

Antibiotic resistant bacteria could find their way into water bodies due to the use of poultry litter as fertilizers leading to public health challenge. Previous studies have focused more on Gram-negative bacteria. The present study revealed that poultry litters are pools of antimicrobial resistant enterococci and coagulase-negative staphylococci as well as antibiotic resistance genes and may be vectors for multidrug resistance bacteria spread.

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significant problem. Antibiotic residues, drug-resistant bacteria, and resistance genes are all recognized as environmental contaminants that are responsible for the persistent public health catastrophe that exists around the globe[4].

Although, the use of antimicrobials in animal farm production could lead to a number of economic benefits, their growth-promoting prophylactic, metaphylactic, and therapeutic properties have generated a variety of antibiotic-resistant bacteria, multidrug resistant (MDR) isolates, and ARGs in the gut of these animals and their wastes[5]. Due to inappropriate placement of wells and other groundwater sources, these resilient pathogens may enter the aquifers through leaching and dust particle contamination, which can cause harmful health issues in humans and other living forms when such water is used for domestic purposes[6]. In Nigeria, compared to Gram-negative bacteria, few studies have focused on Gram-positive cocci bacteria from poultry litter and the nearby wells in spite of growing concerns about their antibiotic resistance. Therefore, the present study was aimed at determining the antibiotic resistance patterns and resistance genes in enterococci and coagulase-negative staphylococci (CoNS) isolated from poultry farms and nearby wells in Ibadan, Nigeria.

2. Materials and methods

2.1. Description of the study area

This study was carried out in three different poultry farms located in Ojoo (a small sized farm with geographical position of 0726555N, 00354342E), Ajibode (a medium-sized farm with geographical position of 0726594N, 00354336E) and Mokola (a medium-sized farm with geographical position of 0724169N, 00353188E) in Akinyele and Ibadan North Local Government Areas, Ibadan, Oyo state. Ibadan is the capital city of Oyo state and the third largest metropolitan area by population in Nigeria, after Lagos and Kano, with a population of over 3 million, and the largest metropolitan geographical area. Ibadan is located between latitude 7o 02’49’’ and 7o 43’ 21” N longitude 3o 31’ 58 and 4o 08’ 20” E. Mean annual temperature is 28 ℃, ranging between 18 ℃ and 37 ℃ and mean annual rainfall is 1 205 mm, with a relative high humidity of about 74.5% all year round.

2.2. Sample collection

Samples were collected between the months of March and April, 2022. Poultry litter samples were aseptically collected at 0-15 cm depth from different points on the poultry farms and transferred into sterile polythene bags, while water samples were aseptically collected from wells at different close distance to the farms. A metal was attached to a one litre sterile container and lowered into the well with a rope. The samples were transported in ice pack to the laboratory in the Department of Microbiology, University of Ibadan for immediate microbiological analysis.

2.3. Isolation of the bacterial isolates

Isolation of the bacterial isolates was carried out using bile esculin azide agar (BEA). Ten grams of poultry litter was suspended in 100 mL sterile distilled water and vortexed vigorously to form a stock. The standard pour plate method was used for the inoculation of the media plates and was inverted and incubated at 35-37 ℃ for 18 to 48 h. Distinct colonies with characteristic black coloration were selected and further sub-cultured to obtain pure culture. The isolates were identified using both the conventional and molecular methods[7,8]. Isolates from bile esculin azide agar not identified as Gram-positive cocci by cellular morphology were excluded from the study.

2.4. Antibiotic susceptibility testing

Antibiotic susceptibility test on the isolates were done using the disc diffusion method on Mueller-Hinton agar as described by Bauer *et al*[9]. Eight different antibiotics discs were used and these include: Penicillin (PG, 10 units), Erythromycin (E, 15 μg), Ciprofloxacin (CIP, 30 μg), Chloramphenicol (C, 30 μg), Nitrofurantoin (F, 300 μg), Meropenem (MEM, 10 μg), Tetracycline (TE, 5 μg), and Ceftriaxone (CRO, 30 μg). These antibiotics were selected based on their use in poultry farming and importance in human therapy against Gram-positive infections. Any isolate that showed resistance to at least three different classes of antibiotics were regarded as MDR.

2.5. Screening of the bacterial isolates for Tetracycline and Erythromycin resistant genes

Some of the MDR isolates that showed resistance to higher numbers of antibiotics were selected for the detection of resistance genes and their DNA were extracted using the protocol of Trindade *et al*[10]. Investigations for the presence of *tetL*, *tetM* and *ermB* genes in MDR isolates (CoNS and *Enterococcus* spp.) were done using Polymerase Chain Reaction (PCR) on the extracted DNA fragments using *tetL*, *tetM* and *ermB* regions specific primers as earlier documented[11,12]. The PCR conditions and controls used for the investigation were as earlier described by Simjee *et al*[13]. The PCR products were loaded into wells and separated on a 1.5%

agarose gel at 120 V with the 100 bp DNA ladder loaded into well 1 and served as molecular weight standard for defining the sizes of the products.

2.6. Molecular identification of selected isolates using 16S rRNA sequencing PCR

Selected *Enterococcus* and CoNS isolates were further identified using 16S rRNA sequencing. The PCR sequencing preparation cocktail consisted of 10 µL of 5x GoTaq colourless reaction, 3 µL of 25 mM MgCl2, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5′- AGAGTT TGA TCMTGGCTCAG-3′ and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8 µL DNA template[8]. The DNA of the isolates was extracted using the protocol of Trindade *et al*[10]. The integrity of the amplified 1.5 Mb gene fragment was checked on a 1% agarose gel ran to confirm amplification. The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Bio-systems using manufacturers' manual, while the sequencing kit used was BigDye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis.

3. Results

Out of the 86 Gram-positive cocci bacteria isolated, 15 (17.4%) were identified as *Enterococcus* species and were obtained from Ojoo and Mokola farms, while, none was isolated from Ajibode farm (Table 1). The remaining 71 (82.6%) isolates were identified as CoNS; they were from the three farms (Ojoo, Ajibode and Mokola), and *S. sciuri* had the highest occurrence rate (Table 2). The resistance profile of the isolates showed that resistance of *Enterococcus* spp. to penicillin, tetracycline and erythromycin were 93.3%, 86.7% and 66.7%, respectively, while for the CoNS isolates, it was 91.5%, 90.1% and 94.4%, respectively (Table 3). Moreover, all (100.0%) of the *Enterococcus* spp. and 95.8% CoNS were MDR, respectively.

Furthermore, the resistance patterns of the *Enterococcus* species and the CoNS isolates showed that there were eight and 16 different antibiotypes, respectively. The highest for the *Enterococcus* spp. was 7 (46.7%) in which four *E. faecalis* and three *E. faecium* showed resistance to a combination of five PG-E-TE-MEM-CRO antibiotics. Moreover, for the CoNS, the highest was 16 (22.5%) in which eleven *S. sciuri* and five *S. xylosus* showed resistance to a combination of four (PG-E-F-TE) antibiotics, respectively; one *S. xylosus* also showed resistant to a combination of seven antibiotics which included PG-C-E-F-TE-MEM-CRO (Table 4 and 5).

Out of 30 MDR isolates selected for resistance genes (*tetL*, *tetM* and *ermB* genes) detection, 22 (63.1%) were positive for either or all the three genes. Specifically, all the three genes were detected in the CoNS isolates, though in varied proportions (Table 6).

Table 1. Occurrence of *Enterococcus* spp. and CoNS across sampled farms.

Farm code	Location	*Enterococcus* spp.	CoNS	Total
		n = 15	*n* = 71	
OP	Ojoo	2 (13.3)	14 (19.7)	16
OW	Ojoo	1 (6.7)	4 (5.6)	5
AP	Ajibode	0 (0)	11 (15.5)	11
AW	Ajibode	0 (0)	6 (8.5)	6
MP	Mokola	4 (26.7)	20 (28.1)	24
MW	Mokola	8 (53.3)	16 (22.5)	24
Total		15 (17.4)	71 (82.6)	86

Note: Data were expressed as *n* (%); OP = Ojoo Poultry; OW = Ojoo Well; AP = Ajibode Poultry; AW = Ajibode Well; MP = Mokola Poultry; MW = Mokola Well; CoNS = coagulase-negative staphylococci.

Table 2. Distribution of *Enterococcus* species and CoNS isolated from the studied farms.

Farms	*E. faecalis*	*E. faecium*	*S. sciuri*	*S. xylosus*	Total
OJOO					
OP	1 (6.7)	1 (6.7)	5 (7.0)	9 (12.7)	16
OW	1 (6.7)	0 (0)	4 (5.6)	0 (0)	5
Sub-total	2 (13.3)	1 (6.7)	9 (12.7)	9 (12.7)	21
AJIBODE					
AP	0 (0)	0 (0)	9 (12.7)	2 (2.8)	11
AW	0 (0)	0 (0)	6 (8.5)	0 (0)	6
Sub-total	0 (0)	0 (0)	15 (21.1)	2 (2.8)	17
MOKOLA					
MP	2 (13.3)	2 (13.3)	14 (19.7)	6 (8.5)	24
MW	5 (33.3)	3 (20.0)	11 (15.5)	5 (7.0)	24
Sub-total	7 (46.7)	5 (33.3)	25 (35.2)	11 (15.5)	
Overall Total	9 (10.5)	6 (6.9)	49 (56.9)	22 (25.6)	86

Note: Data were expressed as *n* (%); OP = Ojoo Poultry; OW = Ojoo Well; AP = Ajibode Poultry; AW = Ajibode Well; MP = Mokola Poultry; MW = Mokola Well; CoNS = coagulase-negative staphylococci.

Table 3. Antibiotic resistance profile of *Enterococcus* species and CoNS from the poultry farms.

Antibiotics	*Enterococcus* spp.			CoNS		
	E. faecalis	*E. faecium*	Total	*S. sciuri*	*S. xylosus*	Total
	n = 9	*n* = 6	*n* =15	*n* = 49	*n* = 22	*n* = 71
PG	8 (88.9)	6(100)	14 (93.3)	46(93.9)	19(86.4)	65(91.5)
C	1 (11.1)	1(17.0)	2 (13.3)	6(12.2)	7(31.8)	13(18.3)
E	5 (55.6)	5 (83.3)	10 (66.7)	47(96.0)	20 (91.0)	67(94.4)
F	1 (11.1)	0 (0.0)	1 (6.7)	26(53.1)	14 (63.7)	40(56.3)
TE	8 (88.9)	5 (83.3)	13 (86.7)	44(89.8)	20 (91.0)	64(90.1)
MEM	7 (77.8)	5 (83.3)	12 (80.0)	9(18.4)	1 (4.5)	10(14.1)
CRO	8 (88.9)	5 (83.3)	13 (86.7)	20(40.9)	7(31.8)	27(38.0)
CIP	0 (0.0)	0(0.0)	0 (0.0)	2(4.1)	0(0.0)	2(2.8)

Note: Data were expressed as *n* (%); PG = Penicillin (10 units); C = Chloramphenicol (30 µg); E = Erythromycin (15 µg); F = Nitrofurantoin (300 µg); TE = Tetracycline (5 µg); MEM = Meropenem (10 µg); CRO = Ceftriaxone (30 µg); CIP = Ciprofloxacin (30 µg); CoNS = coagulase-negative staphylococci.

Table 4. Antibiotype of *Enterococcus* species from the studied farms.

S/N	Antibiotypes	Number of isolates (%)		Total (%)
		E. faecalis	*E. faecium*	
		n = 9	*n* = 6	*n* = 15
1	PG-TE-CRO	1 (11.1)	0 (0.0)	1 (6.7)
2	PG-MEM-CRO	1 (11.1)	0 (0.0)	1 (6.7)
3	TE-MEM-CRO	1 (11.1)	0 (0.0)	1 (6.7)
4	PG-C-E-TE	0 (0.0)	1 (16.7)	1 (6.7)
5	PG-E-MEM-CRO	0 (0.0)	1 (16.7)	1 (6.7)
6	PG-TE-MEM-CRO	1 (11.1)	1 (16.7)	2 (13.3)
7	PG-C-E-F-TE	1 (11.1)	0 (0.0)	1 (6.7)
8	PG-E-TE-MEM-CRO	4 (44.4)	3 (50.0)	7 (46.7)
Total		9 (60.0)	6 (40.0)	15 (100)

Note: Data were expressed as *n*(%); PG = Penicillin (10 units); C = Chloramphenicol (30 µg); E = Erythromycin (15 µg); F = Nitrofurantoin (300 µg); TE = Tetracycline (5 µg); MEM = Meropenem (10 µg); CRO = Ceftriaxone (30 µg).

Table 5. Antibiotype of CoNS from the studied farms.

S/N	Antibiotypes	Number of isolates (%)		Total (%)
		S. sciuri	*S. xylosus*	*n* = 71
1	TE-CRO	0 (0.0)	2(9.1)	2(2.8)
2	PG-E	2 (4.1)	0(0.0)	2(2.8)
3	PG-E-F	5 (10.2)	0(0.0)	5(7.0)
4	PG-E-TE	5 (10.2)	2(9.1)	7(9.9)
5	E-TE-MEM	1(2.0)	0(0.0)	1(1.4)
6	E-TE-CRO	2(4.1)	1(4.5)	3(4.2)
7	PG-C-E-TE	3(6.1)	2(9.1)	5(7.0)
8	PG-E-TE-CRO	2(4.0)	1(4.5)	3(4.2)
9	PG-E-F-TE	11(22.4)	5(22.7)	16(22.5)
10	PG-E-F-MEM	1(2.0)	0(0.0)	1(1.4)
11	PG-TE-CRO-CIP	2(4.1)	0(0.0)	2(2.8)
12	PG-C-E-F-TE	1(2.0)	4(18.2)	5(7.0)
13	PG-E-F-TE-CRO	5(10.2)	2(9.1)	7(9.9)
14	PG-E-TE-MEM-CRO	7(14.2)	0(0.0)	7(9.9)
15	PG-C-E-F-TE-CRO	1(2.0)	0(0.0)	1(1.4)
16	PG-C-E-F-TE-MEM-CRO	0(0.0)	1(4.5)	1(1.4)
Total		48(67.6)	20(28.2)	68(95.7)

Note: PG = Penicillin (10 units); C = Chloramphenicol (30 µg); E = Erythromycin (15 µg); F = Nitrofurantoin (300 µg); TE = Tetracycline (5 µg); MEM = Meropenem (10 µg); CRO = Ceftriaxone (30 µg); C = Ciprofloxacin (30 µg).

Table 6. Occurrence of resistant genes in multidrug resistant *Enterococcus* species and CoNS isolates.

Isolates	Occurrence (%)			Total
	tetL	*tetM*	*ermB*	*n* = 22
	n = 3	*n* = 12	*n* = 7	
E. faecalis	1 (4.5)	2 (9.1)	0 (0.0)	3 (13.6)
E. faecium	0 (0.0)	0 (0.0)	1 (4.5)	1 (4.5)
S. sciuri	1 (4.5)	7 (31.8)	4 (18.2)	12 (54.5)
S. xylosus	1 (4.5)	3 (13.6)	2 (9.1)	6 (27.3)
Total	3 (13.6)	12 (54.5)	7 (31.8)	22 (100)

4. Discussion

The occurrence of multiple antibiotics resistance in *Enterococcus* spp. and CoNS alongside their resistance genes were examined in the present study. Isolation of *Enterococcus* spp. and CoNS from the studied farms is in line with previous reports from which it had been shown that *Enterococcus* spp. and CoNS are among the frequently occurring normal flora in the intestines of birds and other animal species[13]. However, contrary to the findings of Ngbede *et al*[14], wherein *E. faecium* was the most predominant species isolated from poultry manure and cloacal swabs in Zaria, a northern city in Nigeria, *E. faecalis* was found to be the most predominant species in the present study. The distribution of the *Enterococcus* spp. isolated from the studied farms that consisted 10.5% *E. faecalis* and 6.9% *E. faecium* were similar to those reported by Simjee *et al.* [13], where occurrence of *E. faecalis* was reported to be 10.0%. The observation on the occurrence of this bacterial isolate supports previous claim that enterococci makes up a small portion of the Gram-positive bacteria present in poultry samples[15].

Furthermore, Simjee *et al.* had previously noted in a study that CoNS isolates were the only staphylococci species identified from poultry litter samples[13]; this is comparably similar to what was obtained in the present study where CoNS isolates were the only staphylococci identified. Similarly, that *S. sciuri* was the most prevalent CoNS from the latter study also agree with the findings from this present study that showed *S. sciuri* as the most predominant CoNS species obtained. In addition, Boamah *et al.* had also reported a high occurrence of CoNS from poultry litter samples and farm workers in Ghana, with *S. sciuri* having the highest occurrence rate and *S. xylosus* having the least occurrence rate[16]. This observation is in perfect agreement with the findings from the present study. The high incidence of CoNS in the poultry environment suggests that birds are more frequently colonized by CoNS than in mammals, although, assessment of studies involving other animal species is required to validate this data.

The observation from the present study that showed high resistance rate (93.0%) of the enterococci isolates to penicillin, is higher compared to the 61.0% resistance of enterococci to the same antibiotic reported from another study on environmental samples including surface and ground water collected from an area of intensive poultry production[17]. Furthermore, a lower resistance of enterococci isolates (*E. faecalis* and *E. faecium*) to penicillin was also reported from another study on traditional fermented foods[18]. The variation in this observation could be due to the differences in the studied samples and probably as a result of the use of more antibiotics in poultry production than in food production. In addition, the 91.5% of the CoNS in this study that showed

resistance to penicillin is higher compared to the 28.0% reported from a study carried out on samples collected from poultry farms of a neighboring West African country, Ghana[16]. This disparity could be due to differences in farm practices, such as inefficient cleaning of the farm house, incomplete removal of faecal droppings and poor pest control.

The observation from the present study that showed high *E. faecalis* (88.9%) and *E. faecium* (83.3%) resistance to tetracycline corroborates the previous report of 81.0% of enterococci isolated from cloacae swab which were resistant to tetracycline[14]. In addition, the high resistance of *Enterococcus* species obtained in this study to this antibiotics is also comparably similar with the report of Ayeni *et al.* [19] where 81.7% of the enterococci isolates from poultry in Ogun, a neighboring state were reported to be resistant to tetracycline, Similarly, the 89.8% resistance of *S. sciuri* to tetracycline in this study agrees with the 85.3% recently reported on *S. sciuri* isolated from healthy poultry in Portugal[20]. However, the 91.0% resistance observed for *S. xylosus* to tetracycline was higher than the 53.0% resistance of *S. xylosus* isolated from poultry litter in Georgia[13]. The high resistance observed to tetracycline might be due to the previous reports where it had been noted that there is a wide use of this antibiotic in livestock production in Nigeria and other nations[21,22].

The resistance (83.3%) of *E. faecium* to erythromycin observed in this study is comparably similar to the 73.5% resistance reported for *E. faecium* from a study on enterococci isolated from poultry and cloacae swabs in Nigeria[14]. Furthermore, the 55.6% resistance exhibited to erythromycin by *E. faecalis* in this study agrees with the 55.3% previously reported in cattle, pigs and chickens from South Korea[23]. Also, the *S. sciuri* which exhibited a high resistance (96.0%) to erythromycin is similar to the 88.0% resistance of same bacterial isolates from poultry litter to the same antibiotic. However, the 47.0% *S. xylosus* that showed resistance to erythromycin in the latter study is lower compared to what was obtained (91.0%) in this study[13]. This high resistance to erythromycin could probably be as a result of its frequent use in poultry production[24].

The previous report of Simjee *et al.*[13] and Ngbede *et al.*[14] of a low resistance rate of enterococci isolates to the fluoroquinolone antibiotic, such as ciprofloxacin, is in line with the findings of the present study. The reason for this observation might be due to the previous assertion that the fluoroquinolone group of antibiotics is seldom used in animal rearing in Nigeria owing to the high cost[25]. Similarly, the *S. sciuri* and *S. xylosus* that showed relatively low resistance of 4.1% and 0%, respectively, to ciprofloxacin in this study agrees with the 4.0% resistance of CoNS to ciprofloxacin reported from wild birds in Spain[26].

Majority of the *Enterococcus* spp. and the CoNS in the present study were multidrug resistant as shown by their antibiotics resistance patterns. The assertion that the enterococci bacteria could engage in several types of gene transfer processes, such as conjugation, which results in the extensive transmission of resistance determinants via mobile genetic elements like plasmids, may be the cause of multidrug resistance in the *Enterococcus* spp[27]. Also, the high prevalence of MDR CoNS may be an indication of gross misuse of antibiotics in food animal production in the developing countries, including Nigeria, and the ability of CoNS to form biofilms tends to reduce the efficacy of the antimicrobial agents[28,29].

The observation that showed *tetM* gene as the most frequent gene detected in the CoNS isolates in this study agrees with previous reports where this same gene was detected in majority of the investigated CoNS strains in ready-to-eat food of animal origin[30]. The high incidence of *tetM* gene in staphylococci can be due to the presence of the gene on conjugative transposons (Tn916-Tn1545 family) which aids in the spread of this determinant. The low carriage of *tetL* gene observed in the enterococci isolates in the present study compares similarly with the 3.8% reported in enterococci from broilers in Brazil[31]. Also, the screening of CoNS isolates for the presence of this gene showed a low prevalence (6.6%), whereas, Silva *et al.*[20] recently reported that *tetL* gene was the most frequent gene in their study on CoNS from healthy poultry. This disparity might be due to lack of expression of the resistant gene in the isolates in the present study.

Furthermore, resistance of erythromycin is commonly mediated by the *ermB* gene and the location of this gene on mobile genetic elements (MGEs), Tn916 family to be specific, makes its horizontal transfer quite easy[32].The *ermB* gene identified in the CoNS which was in 27.3% of the isolates in the present study, is however lower compared to the 41.0% reported from a similar study on CoNS in Turkey[33]. This disparity might be due to the number of isolates screened for the presence of this gene and also suggests that staphylococci from various geographical locales may have different genetic variations in their erythromycin genes. Similarly, the *ermB* gene was detected in a small percentage (4.5%) of *E. faecium* in this study, and this is in contrast with the report of Cassenego *et al.*[31] where 39.6% of all enterococci isolates obtained from broiler chickens were found to carry the gene. This low prevalence as observed in this study might be an indication that other resistance mechanisms might be occurring in enterococci.

5. Conclusions

The identification of quite a number of multidrug-resistant *Enterococcus* species, as well as, CoNS, from poultry and nearby

wells, suggests that food animals are important reservoirs of antibiotic resistant organisms. This therefore implies a gross misuse of antibiotics in poultry farming. This reality could serve as a threat to public health, as these bacteria have been established as notorious causal agents of many infections plaguing humans. Also, their presence in litter and even nearby wells may lead to the dissemination of antibiotic resistant species in the environment, further compounding the problem of treatment of the opportunistic infections they cause.

Conflict of interest statement

The authors claim there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Falodun OI was involved in the conception, design, manuscript revision and approval for publication, he was also the study overall study supervisor. Nyornebari GB perfumed the experiment, did literature search, analysed the data and wrote the first draft. , Azu V performed the molecular work,. All the authors approved the final draft.

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