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Detection of ESBL-Producing *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus* from Poultry Farms and Feed Shops in Zaria

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Abstract

An increase in small-scale poultry farming, especially in developing countries, increases the risk of inappropriate antibiotic use and the emergence of multidrug-resistant (MDR) pathogens. This study assessed the antibiotic susceptibility patterns of E. coli and S. aureus isolated from different samples (poultry feed, litter, water, and air) from six small poultry farms and three feed-retail shops in Zaria, Kaduna. Selective isolation and characterization, antibiotic susceptibility testing, and screening for ESBL-production (E. coli) and methicillin resistance (S. aureus) were conducted. 8.33% (3/36) and 37.5% (18/48) of E. coli and S. aureus were ESBL- E. coli and MRSA, respectively. ESBL- E. coli were isolated only from litter samples, while for MRSA, 66.7% (12/18), 27% (5/18), and 5.6% (1/18) originated from feed samples from all poultry farms and feed retail shops, litter of four poultry farms, and air of the one poultry farm, respectively. MDR was exhibited by 100%, 41.6%, and 25.0% of E. coli isolates from feed, litter, and water, respectively. For S. aureus, MDR was observed in 91.7%, 58.3%, 33.3%, and 16.7% of isolates from water, feed, air, and litter, respectively. 50-100% of E. coli from feed exhibited high MDR to beta-lactams, quinolones, aminoglycosides, and sulphonamide. Most S. aureus from feeds and air exhibited higher resistance to beta-lactams than to other antibiotics. Poultry litter and feeds were major sources of ESBL-E. coli and MRSA, respectively, and thus pose potential risks of AMR dissemination in the farm environment and among livestock workers. AMR surveillance in livestock settings is required for effective control measures.

Keywords: Antimicrobial resistance, ESBL-producing E. coli, poultry, MRSA, multidrug resistance

INTRODUCTION

The global production of animal-based protein and products is ever-increasing to meet demand, and with this, antimicrobial use in animals is also increasing (Latino et al. 2020). In many countries, antibiotics are used for preventive disease control, growth promotion, and therapeutic purposes in livestock farming (Manyi-Loh *et al.*, 2018; Khmaissa *et al.*, 2024). Inappropriate antibiotic usage is a key contributor to the emergence and spread of multidrug-resistant microorganisms. Consequently, food-producing animals and their environment serve as potential reservoirs of drug-resistant bacteria, including extended-spectrum beta-lactamase (ESBL)-producing bacteria and methicillin-resistant *Staphylococcus aureus* (MRSA) (Beninati *et al.*, 2015). These pathogens threaten animal production, food safety, antimicrobial therapy, and public health (Aliyu *et al.*, 2024). Hence, there is an increased concern about the risks and transmission of drug-resistant pathogens from livestock to humans (Ayinla and Mateus, 2023). Several studies report the detection of MRSA and ESBL-*E. coli* among food-producing animals, including poultry, and their environments (Samutela *et al.*, 2021; Ayinla and Mateus, 2023; Beshiru *et al.*, 2024). The Nigerian poultry industry, a major source of animal protein, is mainly composed of many subsistence-based livestock farms. A high risk of transmission is expected in subsistence-based agricultural communities, where families live in close contact with livestock (Falgenhauer *et al.*, 2019). Notably, the common practice of using poultry litter as organic manure on farms constitutes a risk, especially to handlers and farmers, and contaminates the environment

with pathogens (Moffo *et al.*, 2021; Khong *et al.*, 2022; Ayinla and Mateus, 2023).

There is a paucity of data on ESBL pathogens in livestock farm environments, including poultry farms, in Nigeria and other African countries (Ayinla and Mateus, 2023). Further, there are limited studies on the risks and transmission of antibiotic-resistant bacteria at various stages of the poultry production chain and the waste management process (Falgenhauer *et al.*, 2019). While studies have focused on detecting drug-resistant pathogens in poultry litter and poultry meat, reports on ESBL-*E. coli* and MRSA in poultry feeds, which are supposedly at the beginning of the poultry food chain in the "farm-to-fork" model, are lacking in Nigeria. Consequently, understanding the relative contributions and risks associated with the spread of drug-resistant pathogens through poultry feeds, poultry wastes (poultry litter and wastewater), and air in poultry farms is important. This study assessed the occurrence of ESBL-*E. coli* and MRSA in selected samples from some small-holder poultry farms (water, litter, feed, and air) and poultry feed retail shops (feed and air) in Zaria, Nigeria.

MATERIALS AND METHODS

Sampling sites and sample collection

Six poultry farms and three poultry feed retail shops located in Samaru, Zaria, Kaduna State, Nigeria, were selected for the study. Informed consent was obtained from poultry farm and feed shop owners for sample collection. Samples of poultry litter (10g) and water (20ml) from drinkers were collected from each poultry farm, while poultry feed samples (10g) were collected from the feed retail shops. Samples were collected aseptically from five sampling points and mixed to achieve homogeneity. To isolate airborne *S. aureus*, mannitol salt agar (MSA) plates were exposed in poultry farms and retail feed shops for 20 minutes. All samples and MSA plates were transported immediately to the Department of Microbiology at Ahmadu Bello University, Zaria, Nigeria, for laboratory analysis.

Selective isolation and characterization of *E. coli* and *S. aureus*

For the litter and feed samples, 1 g each was added to 9 mL of sterile physiological saline, homogenized, and then serially diluted to 10^{-3}. An aliquot was streaked onto freshly prepared Eosin methylene blue (EMB) and MSA agar plates, then incubated at 37°C for 24 hours to isolate *E. coli* and *S. aureus*, respectively, in duplicate. Two representative discrete colonies with a green metallic sheen on EMB agar and yellow colonies on MSA were subcultured onto nutrient agar for each sample type and site, and incubated at 37°C for 24 hours. The isolates were Gram-stained and identified by biochemical tests. Presumptive *E. coli* colonies were characterized using indole utilization, methyl red, Voges-Proskauer, motility, and citrate utilization (IMViC) tests, while *S. aureus* isolates were characterized using catalase, coagulase, and DNase tests (Aworh *et al.*, 2019).

Antibiotic susceptibility testing

The antibiotic susceptibility profiles of the confirmed isolates were evaluated using the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. 0.5 McFarland standardized inoculum of each isolate was evenly swabbed on Mueller-Hinton agar, and the following antibiotic discs were gently dispensed on the agar using sterile forceps: Sparfloxacin (SP), Chloramphenicol (CH), Augmentin (AU), Amoxicillin (AM), Perfloxacin (PEF), Ciprofloxacin (CPX), Gentamicin (CN), Streptomycin (S), Ofloxacin (OFX), and Septrin (SXT) for *E. coli*. For *S. aureus*, the antibiotic discs: Gentamicin (10µg), Amoxycillin (30µg), Streptomycin (30µg), Erythromycin (10µg), Pefloxacin (10µg), Ampiclox (30µg), Zinnacef (20µg), Rocephin (25µg), Ciprofloxacin (10µg), and Septrin (30µg) were used. All the plates were incubated at 37°C for 24 hours, and zones of inhibition were interpreted according to CLSI standards (Iwu *et al.*, 2021).

Screening for ESBL *E. coli* and methicillin-resistant *S. aureus*

For *E. coli*, an initial screening test for ESBL production was conducted with ceftazidime (30 µg) and ceftriaxone (30 µg) discs on MHA by the standard disc diffusion method. After incubation at 37°C for 18–24 hours, inhibition zones were measured. Where the zone of inhibition with ceftazidime and ceftriaxone was ≤17 mm and ≤19 mm, respectively, ESBL production was confirmed by the double-disc synergy test with discs containing ceftazidime (30 µg) placed 16–20 mm away from a 20 µg amoxicillin+10 µg clavulanic acid disc using sterile forceps and incubated at 37°C for 18–24 hours. After the incubation period, an increase and distortion of the zone of inhibition around the 20 µg amoxicillin + 10 µg clavulanic acid disc indicate ESBL production (Idris and Afegbua, 2017).

Table 1: Antibiotic resistance pattern and ESBL detection in *E. coli* isolated from samples from selected poultry farms and feed retail shops in Samaru, Zaria.

Site	Sample	Isolate ID	Antibiotic resistance pattern of*E. coli*	MARi	ESBL Screening		
					Initial Screening		Double-discsynergy test
					CRO(30 µg)	CAZ(30 µg)	CRO (30 µg) +AMC (30 µg) +CAZ (30 µg)
Poultry farms	Water	EWA1	AM, AU, CPX, OFX, PEF, SP	0.6	R	R	ND
		EWA2	AM, AU, OFX, PEF, SP	0.5	R	R	ND
		EWB1	AM, AU, CPX, SXT	0.4	S	R	ND
		EWB2	-	0.2	S	R	ND
		EWC1	AM, AU, OFX, PEF, SP	0.5	S	R	ND
		EWC2	SXT, AM, AU	0.3	R	R	ND
		EWD1	-	0	S	R	ND
		EWD2	-	0.1	R	R	ND
		EWE1	AM, AU, OFX, PEF, SP, SXT	0.6	R	R	ND
		EWE2	AM, AU, CPX, S	0.4	R	R	ND
		EWF1	-	0	S	R	ND
		EWF2	-	0	R	R	ND
	Litter	ELA1	CPX	0.1	R	R	ND
		ELA2	SXT	0.1	S	R	ND
		ELB1	-	0	R	R	ND
		ELB2	-	0	S	R	ND
		ELC1	AM, AU, CH, SP, SXT	0.5	R	R	+
		ELC2	AM, AU, CH, SP, SXT	0.5	R	R	ND
		ELD1	-	0	R	R	ND
		ELD2	-	0	S	R	ND
		ELE1	AU, CH, PEF, S, SXT	0.5	R	R	ND
		ELE2	-	0	S	R	ND
		ELF1	AM, AU, SP, S, SXT	0.5	R	R	+
		ELF2	AM, AU, CH, SP, S, SXT	0.6	S	R	+
	Feed	EFF1	AM,AU,CN,OFX, SP, S, SXT	0.7	R	R	ND
		EFF2	AM,CN,S, SXT,SP	0.5	R	R	ND
		EFF3	AM,AU, CH,SP, CN,S	0.6	S	R	ND
		EFF4	AM, CH,SP, SXT,	0.4	R	R	ND
		EFF5	AM, AU, CN,CPX, SP,PEF,OFX,S, SXT	0.9	R	R	ND
		EFF6	AM, CPX, PEF,OFX, SP, SXT,S	0.7	R	R	ND
Feed retail shop		EFR1	AM, SP,SXT	0.3	R	R	ND
		EFR2	AM,AU,CPX,PEF, OFX, SP, CH CN,S	0.9	R	R	ND
		EFR3	AM, SP,CPX,PEF, OFX,S	0.6	R	R	ND
		EFR4	AM, CH,CN,OFX,S	0.5	R	R	ND
		EFR5	AM,AU, CPX,PEF,OFX, SP, SXT,S	0.8	R	R	ND
		EFR6	AM, SP,CPX,OFX,S,	0.5	R	R	ND

Key: F = Poultry feed Sample from Farms, R = Poultry feed sample from retail shops; CRO, Ceftriazone; CAZ, ceftazidine; AMC, amoxicillin/clavulanic acid, R: Resistant; S: Susceptible; ND: No distortion of zone of inhibition. SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxicillin, AU= Augmentin, CN= Gentamicin, PEF= Pefloxacin, OFX= Ofloxacin, S= Streptomycin.

Table 2: Antibiotic resistance pattern of *S. aureus* isolated from water, litter, and feed and air samples from selected poultry farms and feed retail shops in Samaru, Zaria.

Site	Sample	Isolate ID	Antibiotic resistance pattern of *S. aureus*	MARi	MRSA	MDR
Poultry farms	Water	SWA1	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	1	-	+
		SWA2	PEF, CN, APX, Z, AM, R, CPX, S, E	0.9	-	+
		SWB1	PEF, CN, APX, Z, AM, R, S, SXT, E	0.9	-	+
		SWB2	PEF, CN, APX, Z, AM, R, S, SXT, E	0.9	-	+
		SWC1	PEF, CN, APX, Z, AM, R, S, SXT, E	0.9	-	+
		SWC2	PEF, CN, APX, Z, AM, R, S, SXT, E	0.9	-	+
		SWD1	PEF, APX, Z, AM, R, S	0.6	-	+
		SWD2	PEF, CN, APX, Z, AM, R, S, E	0.8	-	+
		SWE1	PEF, APX, Z, AM, R, S, E	0.7	-	+
		SWE2	PEF, CN, APX, Z, AM, R, S, E	0.8	-	+
		SWF1	CN, APX, Z, R	0.4	-	-
		SWF2	PEF, CN, APX, Z, AM, R, SXT, E	0.8	-	+
	Litter	SLA1	APX, Z	0.2	+	-
		SLA2	APX	0.1	+	-
		SLB1	APX, Z, AM, R, E	0.5	-	-
		SLB2	CN, APX, Z, AM, R, E	0.6	-	+
		SLC1	PEF, APX, Z, AM, CPX, SXT	0.6	+	+
		SLC2	AM, CPX	0.2	-	-
		SLD1	PEF, APX, Z, AM	0.4	+	-
		SLD2	-	-	-	-
		SLE1	Z	0.1	-	-
		SLE2	-	-	-	-
		SLF1	APX	0.1	+	-
		SLF2	-	-	-	-
	Feed	SFF1	CPX, R, AM, Z, APX, E, CN	0.7	+	+
		SFF2	SXT, CPX, AM, Z, APX, E	0.6	+	+
		SFF3	AM, Z, APX, SXT, S,	0.5	+	+
		SFF4	AM, Z, SXT, E, CN,	0.5	+	+
		SFF5	AM, Z, APX, CN, E	0.5	+	+
		SFF6	-	0	+	-
		SFR1	AM, Z, APX, R, CN, E	0.6	+	+
		SFR2	AM, Z, APX, E, CN, R	0.6	+	+
		SFR3	AM, Z, APX,	0.3	+	-
		SFR4	AM, Z, APX,	0.3	+	-
		SFR5	AM, Z, CPX, APX	0.4	+	-
		SFR6	AM, Z, APX, CPX	0.4	+	-
	Air	SAF1	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	1	+	+
		SAF2	PEF, CN, APX, Z, AM, R, CPX, SXT, E	0.9	-	+
		SAF3	APX, Z, AM	0.3	-	-
		SAF4	APX, Z, AM	0.3	-	-
		SAF5	APX, Z, AM, R	0.4	-	-
		SAF6	APX, Z, AM, R, S	0.5	-	-
Feed retail shops		SAR1	Z, AM	0.2	-	-
		SAR2	Z, AM	0.2	-	-
		SAR3	AM	0.1	-	-
		SAR4	-	-	-	-
		SAR5	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	1	-	+
		SAR6	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	1	-	+

PEF = Pefloxacin, CN = Gentamicin, APX = Ampiclox, Z = Zinnacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin

For *S. aureus* isolates, MRSA was detected by the cefoxitin disk diffusion test, with a cefoxitin disc (30 µg) placed on an MHA plate containing a standardized inoculum of *S. aureus*. After incubating at 37°C for 18-24 hours, zones of inhibition were measured and zones ≤21 mm were interpreted as resistant, and ≥ 22 mm as sensitive (Alipour *et al.*, 2014).

RESULTS

A total of 36 and 48 isolates of *E. coli* and *S. aureus* were isolated and characterized, respectively (Figure 1). *E. coli* isolates from EMB were characterized as indole positive, methyl red positive, Voges-Proskauer negative, citrate negative Gram-negative bacilli. *S. aureus* isolates from MSA were characterized as catalase-, coagulase-, and DNAase-positive Gram-positive cocci in clusters. Only 8.33% (3/36) of the *E. coli* isolates were ESBL producers, with all originating from litter samples. There was a varying percentage of MDR *E. coli* across the sample types in descending order: feed (100%), litter (66.7%), and water (41.7%) (Figure 2b). 100% of *E. coli* isolates from the feed exhibited multidrug resistance and MAR index ≥0.2, however, a varying resistance to AM (100%), SP (91.6%), S (75%), OF (66.7%), SXT (58.3%), AU, Chloramphenicol and pefloxacin (41.7%), CPX and gentamicin (50%) was observed (Figure 2b). Interestingly, none of the *E. coli* isolates from the feed sample were confirmed to be ESBL-*E. coli*, whereas 25% (3/12) of all *E. coli* isolated from litter samples from two poultry farms were ESBL-*E. coli* based on phenotypic screening (Table 1 and Figure 2b).

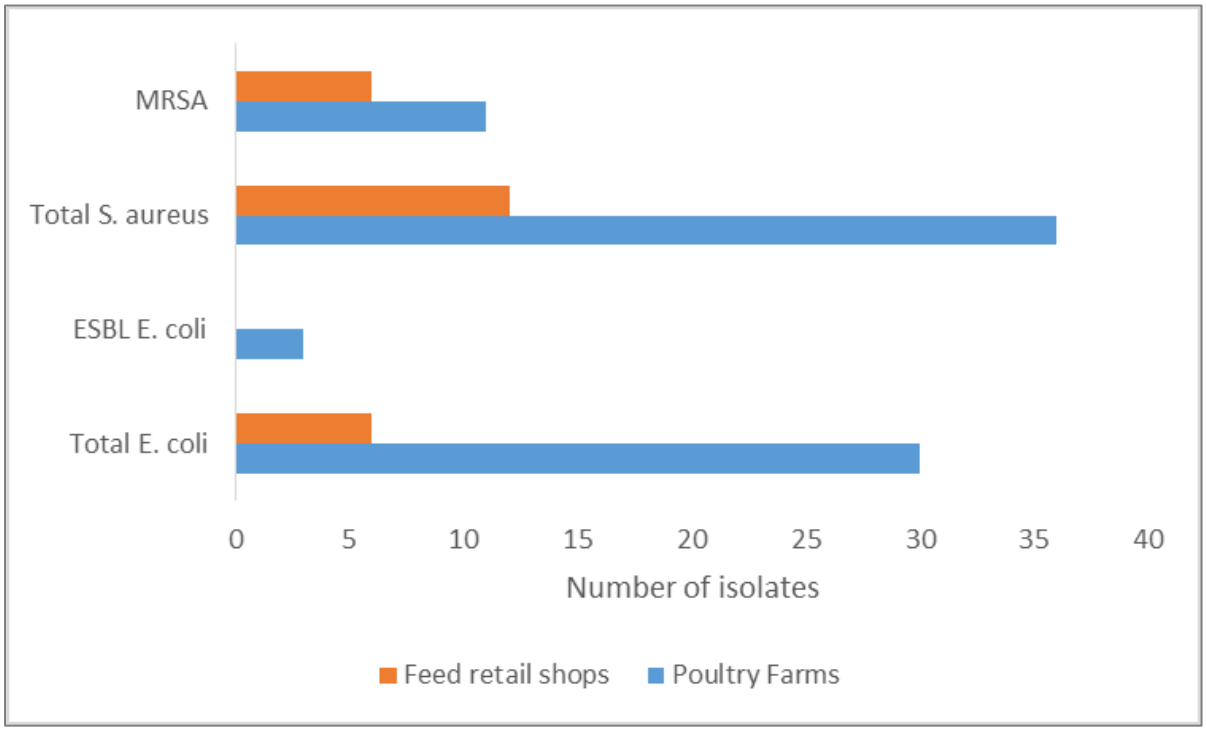


Fig. 1: *E. coli*, ESBL-*E. coli*, *S. aureus*, and MRSA isolated from the six poultry farms and three feed retail shops in Zaria, Kaduna.

Only 37.5% (18/48) of the *S. aureus* isolates were MRSA, with 66.7% (12/18) from feed samples from all the poultry farms and feed retail shops, 27% (5/18) from litter samples of four poultry farms, and 5.6% (1/18) isolated from the air of only one poultry farm A (Table 2). MRSA was detected at higher rates in feed samples (100%; 12/12) than in litter (41.6%; 5/12), air (8.3%; 1/12), or water (0%; 0/12). The multidrug resistance exhibited by *S. aureus* varied with the sample type in the following descending order: water (91.7%), feed (58.3%), air (33.3%), and litter (16.7%) (Figures 3a and 3b). 100% of *S. aureus* isolates from water samples were MDR with a MAR index ≥0.2. Most *S. aureus* from poultry feeds and air exhibited greater resistance to beta-lactams (AM, APX, and Z) than to other antibiotics (Figures 3a and 3b). The number of isolates of *E. coli*, ESBL *E. coli*, *S. aureus*, and MRSA from the poultry farms and feed retail shops is presented in Figure 1.

DISCUSSION

This study isolated *E. coli* and *S. aureus* from poultry litter, poultry feed, water, and air of selected poultry farms and feed retail shops in Samaru, Zaria, and assessed their antibiotic susceptibility patterns. *E. coli* and *S. aureus* isolates were also phenotypically screened for ESBL production and methicillin resistance, respectively. The detection of ESBL-*E. coli* (8.33%; 3/36), and MRSA (37.5%; 18/48) from various samples collected from the poultry farms and feed retails shops is supported by previous findings in poultry farms and slaughterhouses in

Africa (Ayinla and Mateus, 2023), Cameroon (Moffo *et al.*, 2021), South Africa (Fatoba *et al.*, 2022), Brazil (Gazal *et al.*, 2021), and America (Khong *et al.*, 2022). *E. coli*, including ESBL-*E. coli* have been detected in poultry farms in the Kano metropolis (Ibrahim and Habibu 2021), in poultry environments and live-bird markets in Abuja (Aworh *et al.*, 2020).

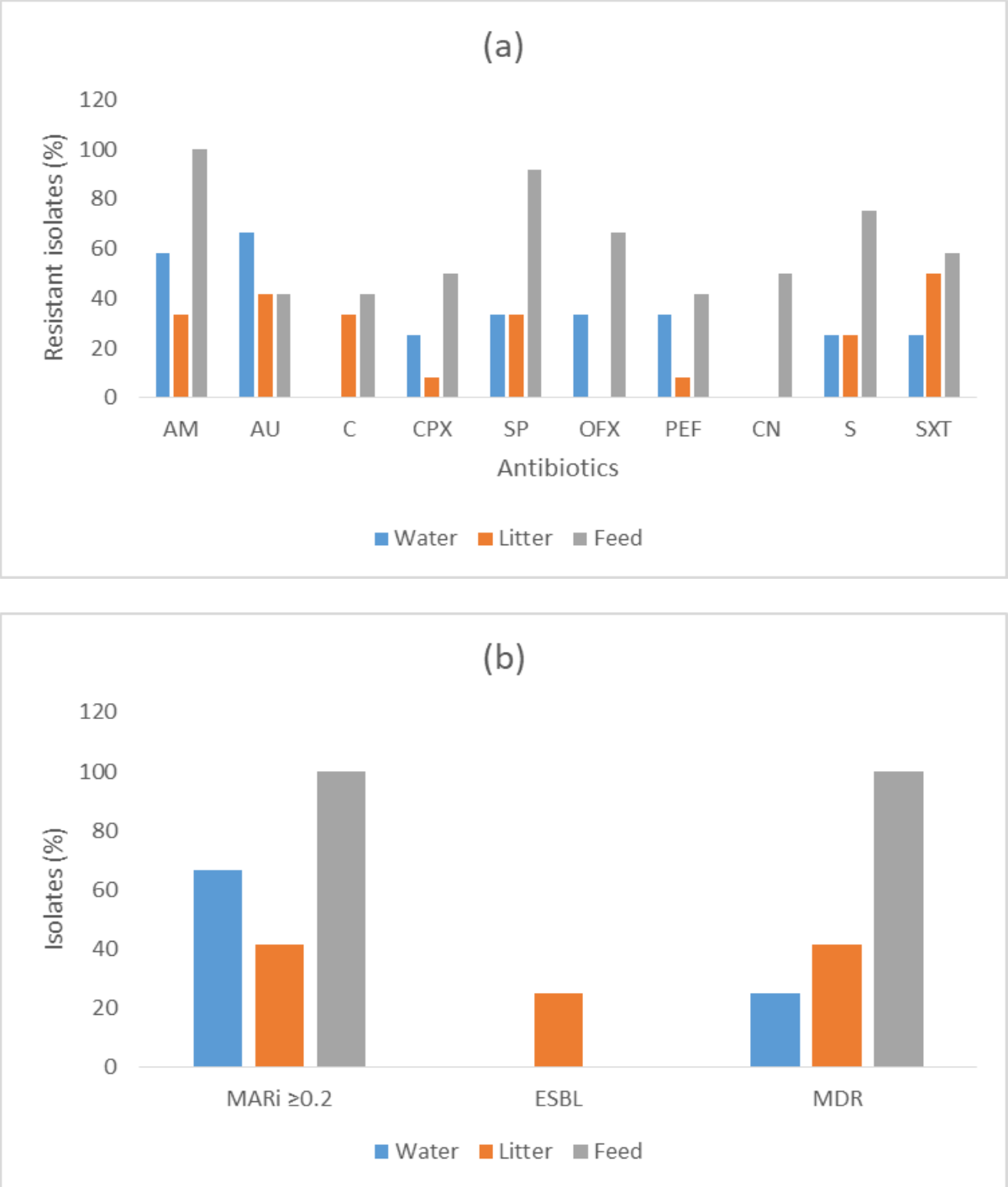
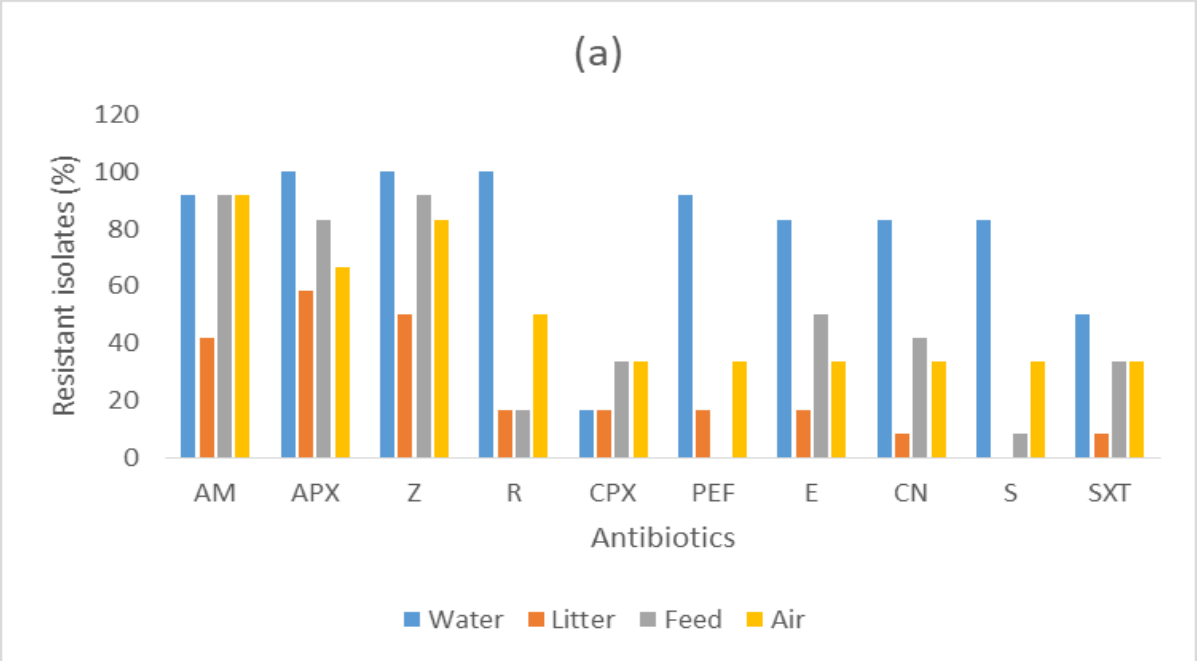


Fig. 2: (a) Antibiotic resistance (%) of *E. coli* isolated relative to sample type and (b) Percentage of *E. coli* isolates with MARi ≥0.2, ESBL and MDR *E. coli* isolated from the different samples (water, litter and feed). SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxicillin, AU= Augmentin, CN= Gentamicin, PEF= Pefloxacin, OFX= Ofloxacin, S= Streptomycin.

Notably, the detection of ESBL-*E. coli* in the litter samples is supported by the findings of Gazal et al. (2021). They recognized poultry litter as a critical point in poultry production, thereby posing a potential health risk to livestock and humans. The detection of MRSA in

feeds (66.7%), poultry litter samples (27%), and air of one poultry farm agrees with previous reports on the presence of livestock-associated-MRSA (LA-MRSA) in livestock settings, including poultry farms (Bounar-Kechih *et al.*, 2018; Samutela *et al.*, 2021; Kasela *et al.*, 2023; Beshiru *et al.*, 2024). MRSA has been reported in various samples from poultry farms in Maiduguri, Borno (Kwoji et al., 2019), and Benin, Edo State (Beshiru *et al.*, 2024).



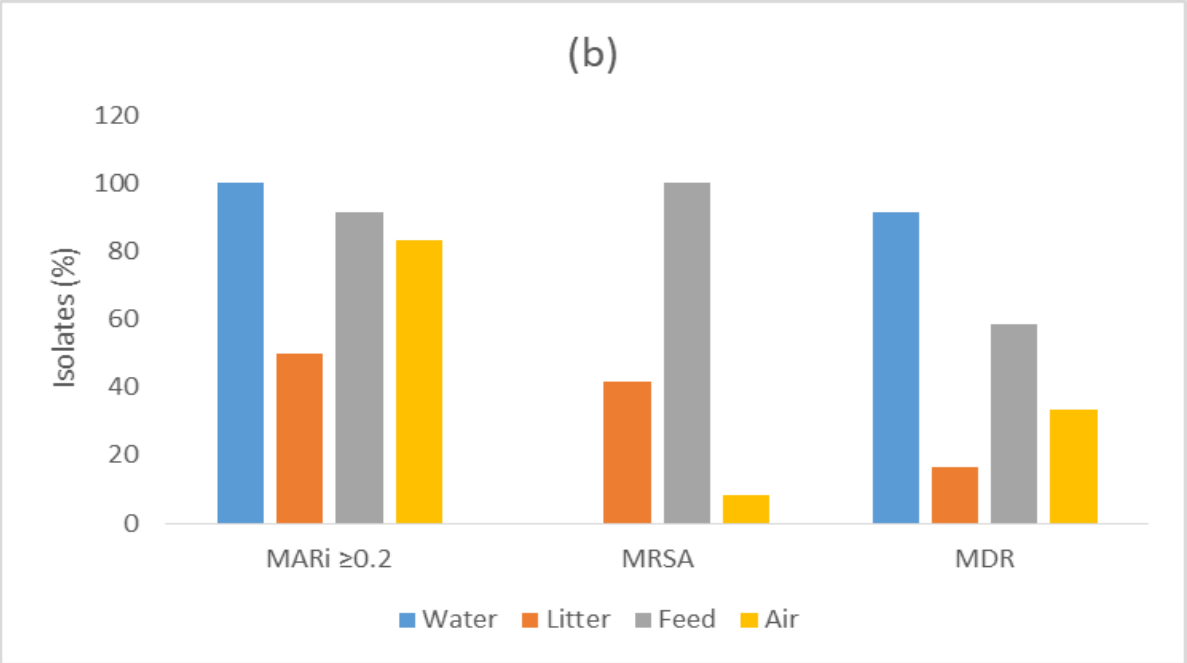


Fig 3. (a) Percentage resistance of *S. aureus* isolated relative to sample (b) Percentage of *S. aureus* isolates with MARi ≥0.2, MRSA, and MDR *S. aureus* isolated from the different sources (water, litter, feed, and air). PEF = Pefloxacin, CN = Gentamicin, APX = Ampiclox, Z = Zinnacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin.

Although *E. coli* isolates exhibited varying multidrug resistance patterns relative to the sample type (feed; 100%, litter; 66.7%, and water; 41.7%), this finding is supported by previous findings on the multidrug resistance of *E. coli* strains from different poultry farms,

including Nigeria (Afunwa *et al.*, 2020; Aworh *et al.*, 2020), Italy (Ghodousi *et al.*, 2015), and Germany (Laube *et al.*, 2013), South Africa (Fatoba *et al.*, 2022). Similarly, the high resistance to beta-lactams and the varying multidrug resistance exhibited by *S. aureus* relative to the sample type (water, feed, air, and litter, which can be attributed to antibiotic use) are supported by previous studies (Silva *et al.*, 2021). Also the finding of MDR MRSA isolates from feed (58.8%) exhibiting a range of resistance to AM and Z (91.7%), APX (83.3%), erythromycin (50%) gentamicin (41.7%) and R (16.7%) Ciprofloxacin and SXT (33.3%), streptomycin (8.3%) and pefloxacin (0%) is supported by the report of multi-drug resistance in 73.3% of MRSA isolates from cloacal and nasal swabs of chickens in Maiduguri, Borno state with 40% of the isolates exhibiting to erythromycin resistance (Kwoji *et al.*, 2019).

E. coli is commonly associated with the intestinal tract of warm-blooded animals and their environments. *E. coli* can be abundant in poultry houses with concentrations of up to 7 log10 CFU g^{-1} in poultry litter (Nguyên *et al.*, 2022). Consequently, the presence of *E. coli* and *S. aureus* in poultry environments, including feed, is expected due to fecal contamination, especially when biosecurity measures are poorly implemented. Poor sanitary and management practices on livestock farms and production systems in Nigeria have been associated with a high prevalence rate of infections.

These infections are increasingly managed through indiscriminate antimicrobial use rather than through improvements in sanitary and biosecurity measures (Kwaghe *et al.*, 2016). The emergence of drug-resistant pathogens in the poultry farm environment is of great concern as they pose risks to livestock workers and consumers (Khong *et al.*, 2022). Multidrug resistance may be attributed to multiple mechanisms, including the presence of large plasmids that contain genes for efflux pumps, antibiotic resistance genes (*bla* genes) for beta-lactamase production, and other enzymes (Khong *et al.*, 2022). Resistance to methicillin and other ß-lactams in *S. aureus* is attributed to the synthesis of penicillin-binding protein (PBP2) from *mec* A gene, resulting in low affinity for methicillin and other ß-lactams (Hsu *et al.*, 2021; Silva *et al.*, 2021).

The detection of ESBL-*E. coli* and MRSA strains could be attributed to antibiotic use for various purposes, including growth promotion in poultry production. The use of antibiotics as growth promoters is not currently banned in Nigeria, but the National Agency for Food and Drug Administration and Control issued a policy statement in 2018 prohibiting their use as growth promoters in animal feeds (Umair *et al.*, 2023). However, some antimicrobials, such as penicillin, erythromycin, and tetracycline, are extensively used for treatment, as prophylactic measures, and as growth promoters, which leads to the development of drug-resistant strains of pathogens (Ayinla and Mateus, 2023; Beshiru *et al.*, 2024).

Consequently, the presence of multidrug-resistant *E. coli* and *S. aureus* in the various samples, particularly in the feeds, raises questions about public awareness and the proper implementation of this policy statement. Although poultry litter processing by composting is an effective biosecurity measure to decrease pathogenic microorganisms before their application as organic manure, litter composting is not a common practice. The presence of MRSA in feed and air also indicates a risk of nasal carriage of LA-MRSA, even with short-term exposure by livestock workers (Gazal *et al.*, 2021).

This study provided information on the occurrence and multidrug resistance patterns of ESBL-producing *E. coli* and MRSA isolated from different samples from selected poultry farms and feed retail shops. Due to financial constraints, a genotypic analysis for the presence of antimicrobial resistance genes in the isolates was conducted.

CONCLUSION

The findings indicate the risk of MDR *E. coli*, *S. aureus*, ESBL-producing *E. coli*, and MRSA dissemination in smallholder poultry farm settings across different samples (poultry litter, water, and feed), and possibly bioaerosol formation containing these pathogens. The relative occurrence of ESBL-producing *E. coli* and MRSA isolated from the different samples varied and may be attributed to individual farm practices (antibiotic use, biosecurity measures, and waste management). Notably, as poultry litter and feeds were major sources of ESBL-*E. coli* and MRSA, respectively, poultry waste management and feed production should be considered important areas for AMR surveillance and quality assurance measures. Hence, the findings reaffirm the need for proper awareness of the importance of responsible antibiotic use, safe poultry waste processing and disposal, ensuring quality assurance in the feed production process, and maintaining adequate biosecurity measures in livestock settings. AMR

surveillance studies that apply omics technologies will be required to understand the role of livestock farms as reservoirs for AMR dissemination in the environment. These will be important for raising awareness of proper antibiotic use, waste management, and the implementation of action plans.

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