

# Poultry Farm and Poultry Products as Sources of Multiple Antimicrobial-Resistant *Salmonella* and *S. aureus*

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## Abstract

**Background:** Poultry production is a key interface for the spread of novel zoonotic and antibiotic-resistant foodborne pathogens. *Salmonella* spp. and *S. aureus* isolates from 2 poultry production were tested for resistance to clinical antibiotics.

**Methods:** One hundred 100 anal swab samples were aseptically collected from 2 small scale poultry farms located within Agulu, during the period of February 2016 to May 2016. The swab sticks were carefully transferred into the buffered peptone water and incubated at 37°C for 24hours and pre-enriched in sterile nutrient broth at 37°C for 24hours. After which, the culture was streaked on selective media *Salmonella*-shigella Agar and Mannitol Salt Agar using a sterile wire loop and further incubated at 37°C for 24 hours. *S. aureus* and *Salmonella* isolates were identified using standard microbiological identification techniques. The isolates were evaluated for antibiotic susceptibility, and for the expression of extended spectrum  $\beta$ -lactamase as well as vancomycin sensitivity.

**Results:** One hundred bacterial isolates (44 *S. aureus* and 56 *Salmonella* spp.) were bacteriologically obtained from the poultry samples. Resistance pattern of the isolates to antibiotics was in the order of ceftazidime>cefuroxime>cloxacillin>augmentin®>ceftriaxone>erythromycin>gentamicin>ofloxacin for *S. aureus* while the *Salmonella* spp. had augmentin®>cefuroxime~ofloxacin>gentamicin>ceftazidime>ceftriaxone. Only 5.3% (3/56) *Salmonella* spp. was ESBL producers while 27.3% *S. aureus* were Vancomycin resistant.

**Conclusion:** Our findings demonstrated that the poultry farm and poultry products could be a source of multiple antimicrobial-resistant *Salmonella* and *S. aureus* and may constitute a public health concern considering the circulation and consumption of livestock and their products, especially chickens and eggs.

**Keywords** Multidrug-resistant; *Salmonella* spp.; *S. aureus*; Poultry products; Public health concern

## Introduction

Foodborne diseases (FBDs) are widespread with great public health and economic concerns. The provision of good sanitation in developed countries has led to a decline in recorded infections as against developing countries, where the greater populace is largely affected by foodborne infections [1-4]. This high burden of FBDs in resource-poor settings is fueled by unhygienic practices, poor knowledge of the existence and influence of microorganisms, unavailability of trained personnel and poor financial support from governments [5,6] In many developing countries Nigeria inclusive, chickens are extensively reared very close to human residential buildings and thus could be involved in environmental contamination [6] Food-producing animals have been reported as the primary reservoir of zoonotic food-borne pathogens, including antimicrobial-resistant bacteria [7-9]. The role of poultry products in the dissemination of antimicrobial-resistant zoonotic bacterial pathogens is well documented [6,10] Poultry is a major global reservoir of *Salmonella*; an important pathogen and egg-associated Salmonellosis has been recognized as a public health problem [11].

Thus, control of this pathogen is of public health importance [12]. Foods of animal origin, especially poultry and poultry products, are often involved in sporadic cases and outbreaks of human salmonellosis [2,13].

Similarly, *S. aureus* has been reported as an important agent of food poisoning [6]. Globally, *S. aureus* associated food poisoning is the third leading cause of food-related illness [8]. *S. aureus* is known to produce six types of enterotoxins (A, B, C1, C2, D, and E) with variable toxicity profile and toxicity is partially related to enterotoxin production. Most food poisoning is caused by heat-stable enterotoxin especially A and D [8,14]. Thus *S. aureus* is a key bacterial organism responsible for FBDs caused by exotoxin production and direct invasion with systemic dissemination [6]. These organisms are resident on the skin, feathers, intestinal and mucosal membrane of the chicken [6,15]. Poultry farm is the main source of Chicken and or egg which are eaten at different homes, restaurants and are sold by several other food vendors. However due to the poor standard of hygiene practice or compliance to standard preparation methods; these microorganisms find their way into consumer causing infections/diseases depending on their virulence and the immune status of the individual. All food safety programs are aimed at preventing contaminated food products from

reaching the consumer and surveillance for FBDs is conducted to delineate the occurrence/burden and it is of important public health concern [16]. It will help in developing innovative tools for predicting and evaluating the risks, the economic, societal and human impacts of FBDs. Thus useful in developing effective strategies to limit outbreaks of this Multi-Drug Resistant (MDR) infections and minimize antimicrobial resistance as Infection-control practitioners and clinicians need the laboratories to rapidly identify and characterize different types of resistant bacteria. This present study was designed to evaluate the prevalence and antibiotic resistance status of *Salmonella* and *S. aureus* isolated from two small scale poultry farms in Agulu, Anambra, Nigeria.

## Materials and Methods

### Study design

The walls of poultry farm Houses were made of wooden stable and blocks which have many wire-meshed windows on the upper section of the walls and. The houses contain feeding troughs, hanged watering pools as well as light sources. Commercially prepared feed concentrate are used and are procured from local animal feed suppliers. The poultry farms sell their products to members of the community, local retailers and restaurants/hotels. The study was approved by the Faculty of Pharmaceutical Science ethical/research committee.

### Sample collection and processing

One hundred (100) anal samples were collected from two small scale poultry farms within Agulu community, Anaocha local government area of Anambra state. Sterile swab sticks were used to collect anal swabs from the poultry farms. The swab sticks were carefully transferred into the sterile nutrient broth and incubated at 37°C for 24 hours. The swab sticks were carefully transferred into the buffered peptone water and incubated at 37°C for 24 hours and were pre-enriched in sterile nutrient broth at 37°C for 24 hours. After which, cultures were streaked on selective media Salmonella-Shigella Agar (SSA) and Mannitol Salt Agar (MSA) using a sterile wire loop and further incubated at 37°C for 24 hours.

### Bacterial isolation and identification

Black colonies on the SSA were presumptive for *Salmonella* while yellow colonies on MSA were considered presumptive for *S. aureus*. They were confirmed with the help of Gram's staining and other biochemical tests (catalase, coagulase, Triple Sugar Iron (TSI) test, Indole test, Citrate utilization), as previously described [17].

### Antimicrobial susceptibility studies

The Antimicrobial Susceptibility of the isolates was evaluated using the Kirby-Bauer disk diffusion assay as per the guideline of clinical and laboratory standard Institute (CLSI) as described by Ugwu et al. [18] Antibiotic disk (ABTEK, Liverpool, UK) containing the following antibiotics was used: Ceftazidime(CAZ) 30 µg, Cefuroxime (CRX) 30 µg, Gentamycin (GEN) 10 µg, Ceftriaxone (CTR) 30 µg, Erythromycin (ERY) 5 µg, Cloxacillin (CXC) 5 µg, Ofloxacin (OFL) 5 µg, Augmentin (AUG) 30 µg was used to test for antibiogram.

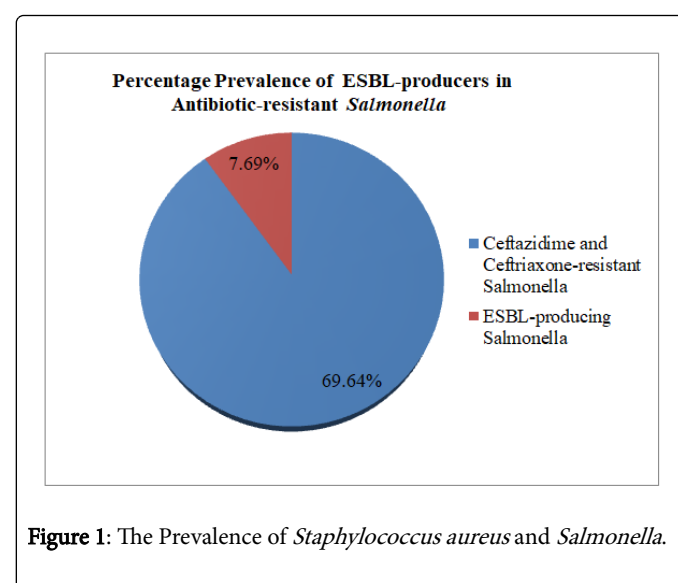
### Double disk synergy test (DDST)

The isolates with inhibition zone diameter of <19 mm for Ceftazidime (CAZ) and <20 mm for Ceftriaxone (CTR) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [19] were further screened for Extended-Spectrum Beta-Lactamases (ESBL) production by DDST on Mueller-Hinton agar (MHA) as described in the above sensitivity testing, using Amoxicillin and Clavulanic Acid (AMC), Ceftriaxone (CTR) and Ceftazidime (CAZ). The amoxicillin and clavulanic acid were placed at the center of the MHA and both cephalosporins were placed 15 mm apart on both sides of the AMC. The Petri dishes were incubated at 37°C for 24 hours. Thereafter the synergies between the disks toward the beta-lactamase inhibitor (AMC) were regarded as positive for ESBL production. The Inhibition Zone Diameter (IZD) of the disc were taken and interpreted and recorded.

### Results

100 samples cultivated in buffered peptone water and pre-enriched in sterile nutrient broth at 37°C for 24 hours, 44% (44/100) were *S. aureus* isolated on Mannitol Salt Agar (MSA) while 56% of the samples (56/100) were *Salmonella* isolated on Salmonella-Shigella Agar (Figure 1).

Table 1 shows the antibiogram of the *S. aureus* isolates.



**Figure 1:** The Prevalence of *Staphylococcus aureus* and *Salmonella*.

S. No.	Antimicrobial agents	Susceptibility	Resistance	Intermediate
1	Ceftazidime (CAZ)	0.0	100	0.0
2	Cefuroxime (CRX)	15.9	84.09	0.0
3	Gentamicin (GEN)	56.82	43.18	0.0
4	Ceftriaxone (CTR)	34.09	65.91	0.0
5	Erythromycin (ERY)	29.55	59.09	11.36
6	Cloxacillin (CXC)	20.45	79.55	0.0
7	Ofloxacin (OFL)	61.36	38.64	0.0

8	Augmentin (AUG)	27.27	72.73	0.0
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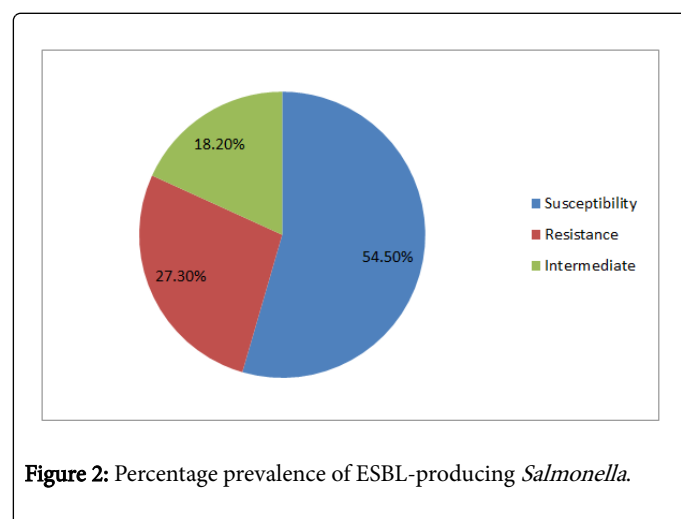
**Table 1:** Percentage Susceptibility for *Staphylococcus aureus* isolates (%).

Most of the isolates were resistant to Ceftazidime (100%), cefuroxime (84.09%), cloxacillin (79.55%) and augmentin® (72.73%). Many (65.91%) were resistant to ceftriaxone, 59.09% (erythromycin) and 43.18% (gentamicin). Ofloxacin (fluoroquinolones) had the least resistance (38.64%). For *Salmonella* (Table 2), it can be seen that augmentin® was least effective with the highest level of resistance (82.14%), followed by ofloxacin and cefuroxime recording the same level of resistance (76.79%).

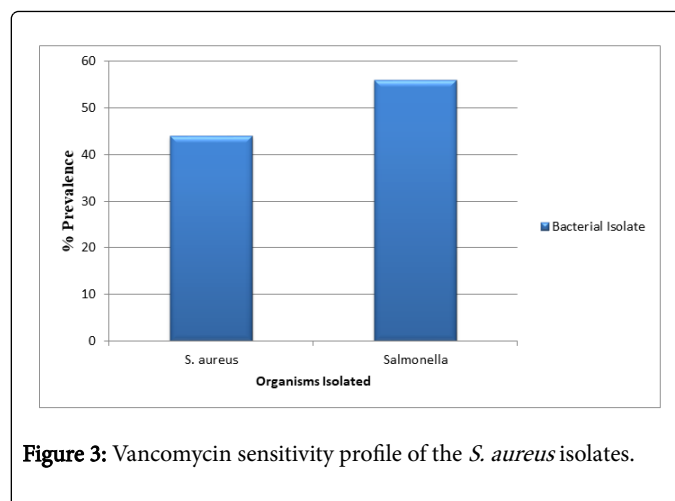
S. No.	Antimicrobial agents	Susceptibility	Resistance	Intermediate
1	Ceftazidime (CAZ)	30.36	57.14	12.5
2	Cefuroxime (CRX)	14.29	76.79	8.93
3	Gentamicin (GEN)	26.79	60.71	12.5
4	Ceftriaxone (CTR)	53.57	33.93	12.5
5	Erythromycin (ERY)	0.0	100	0.0
6	Cloxacillin (CXC)	0.0	1000	0.0
7	Ofloxacin (OFL)	10.71	76.79	12.5
8	Augmentin (AUG)	17.86	82.14	0.0

**Table 2:** Percentage Susceptibility of *Salmonella* isolates (%).

Ceftriaxone had the least resistance (33.93%) while 57.14% and 60.71% of the *Salmonella spp.* were resistant to ceftazidime and gentamicin respectively. The DDST was carried out for 39 (ESBL-screen positives) out of the 56 isolates of *Salmonella* that were resistant to either Ceftazidime (CAZ) or Ceftriaxone (CTR) or both and the result showed that only 5.3% (3/56) of the ESBL-screen positives were ESBL-producers as shown by Figure 2. Similarly, the *S. aureus* isolates were evaluated for Vancomycin sensitivity. Figure 3 shows that 54.5%, 27.3%, and 18.2% were susceptible, resistant and intermediately susceptible to vancomycin.



**Figure 2:** Percentage prevalence of ESBL-producing *Salmonella*.



**Figure 3:** Vancomycin sensitivity profile of the *S. aureus* isolates.

## Discussion and Conclusion

FBDs are defined by WHO as diseases of infectious or toxic nature caused by consumption of contaminated foods and/or water. Epidemiological studies are helpful in developing innovative tools for predicting and evaluating the risks of FBDs as well as its associated economic, societal and human impacts. It will help to develop functional strategies to limit outbreaks of MDR infections with reduced cases of antimicrobial resistance. We evaluated the prevalence and antibiotic-resistance patterns of *Salmonella* and *Staphylococcus aureus* isolates from two small scales poultry farms in Agulu, Anambra, Nigeria. *Salmonella* species in foods of animal origin have been associated with the foodborne pathogen outbreaks [8].

The antimicrobial susceptibility testing revealed an occurrence of multidrug-resistant *S. aureus* in poultry. Similar high resistance to beta-lactams and erythromycin have equally been reported among coagulase-positive *S. aureus* isolated from smallholder flocks in Maiduguri, Northeastern Nigeria [6] and in Zaria North-central Nigeria [20]. In Nigeria, poultry farmers generally use these antimicrobials for the treatment of staphylococcal infections in chickens [20]. The overuse of antibiotics in animal husbandry and for other veterinary purposes encourages resistant microbes to emerge via selective pressure [21-22]. The high resistance of *S. aureus* to beta-lactams may be due to actions of  $\beta$ -lactamase and/or cephalosporins which destroy the  $\beta$ -lactam antibiotics [23-25] or production of PBP2a, an alternative target that is resistant to inhibition by the penicillins [2].

Similarly, in Malaysia Thung et al. [26] reported that all the *Salmonella* isolated from raw chicken meat resisted erythromycin, penicillin, and vancomycin.

The observed high antibiotic resistance is of public health concerns. i). There is a high risk of cross-contamination. Antibiotic-resistant microbes readily contaminate poultry carcasses which often cause contamination of poultry meats and eggs. In addition, these resistant strains may eventually find their way into the environment via wastewater, manure and sewage sludge and momentarily drug-resistant bacteria of animal origin may colonize the human intestine [27-29]. ii). Food producing animals are the key sources of human *Salmonella* infection and fluoroquinolones are considered as the drug of first-line treatment of infections caused by *Salmonella spp.* in human [29,30].

Global incidence of resistance in *Salmonella* spp. isolated from the poultry prods/supply chain is variable and ranges from 21.8% to 96.6% [29,31]. We recorded a low (5.3%) ESBL production among the *Salmonella* isolates from two small scale poultry farms within Agulu. Similar low prevalence of ESBL-producing strains 0.45% (5/1120) were reported by Clemente et al. [32] among *Salmonella* spp. from food items of animal origin in Brazil. Contrarily, a higher prevalence of ESBL production by *Salmonella* spp. isolated from animals has also been reported by Ziech et al. [29]. They established ESBL activity in 45% of the *Salmonella* spp. isolated from the studied broiler processing plants. Despite the low ESBL-producing *Salmonella* isolates reported in this study, microbiologists, laboratory and public health personnel in Agulu and environs should as part of their infection control measures to limit outbreaks of community-onset infection implement a program to detect ESBLs and report cases to the appropriate authority. This is because ESBL resistance genes are primarily carried by plasmids that carry genes encoding resistance to other antibiotic classes and thus treatment options for infections caused by ESBL-producing bacteria are limited [33,34]. The vancomycin resistance test conducted for the coagulase-positive *Staphylococcus aureus* strains showed activity against over three-quarter of the isolates indicating that there is a low prevalence of vancomycin-resistant *Staphylococcus aureus* (VRSA, 27.3%) and vancomycin-intermediate *Staphylococcus aureus* (VISA, 18.2%) within Agulu community. This is line with other documented percentages of vancomycin resistance (9%-46%) among *S. aureus* isolates in animals and food in the African continent as reported by Lozano et al. [35]. In conclusion, this study has shown that foodborne pathogens, *Salmonella* and *S. aureus*, are highly associated with the poultry farms in Agulu and are of potential risks to consumer's health. The high percentage of *Salmonella* and *S. aureus* isolates that were MAR to ceftazidime, cefuroxime, cloxacillin, ofloxacin and augmentin® is suggestive of the misuse of these drugs and has made the poultry and products pose a potential threat to humans. The indiscriminate and off-label use of antibiotics in agriculture is a cause for great concern.

## Conflict of Interest

None

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