

Antibiotics Susceptibility Profile of *Staphylococcus aureus* Isolated from Poultry Birds in Kaduna, Nigeria

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Abstract

Periodic antibiotic susceptibility study is imperative for the treatment and management of disease outbreak in clinics, community and poultry farms. This study evaluated the antibiotic resistant profile of *S. aureus* isolated from poultry farms in Kaduna metropolis. *S. aureus* isolation and biochemical identification were carried out using standard microbiological techniques, while susceptibility test was carried out using agar disc diffusion method. The result showed that out of 670 samples collected from broilers and layers nostril, cloacae, trachea and droppings and the farm workers' ears and nostrils; 164 (55.8%) *S. aureus* were identified. Other *Staph* species identified were *Staph. xylosum* (19.5%), *Staph. hyicus* (6.8%), *Staph. cohnii* (6.8%) and *Staph. intermedius* (3.7%). *S. aureus* was more in layer (51.2%) samples than broilers (48.8%) poultry birds samples in Kaduna metropolis. High resistance was observed against tetracycline (76.8%), ciprofloxacin (60.4%), oxacillin (36.6%), and cotrimoxazole (26.6%). While the isolates showed significant susceptibility to ceftiofur (97.7%), amoxiclav (97.6%) and gentamicin (96.9%). On comparing the percentage resistance of the isolates, this study observed that isolates from farm workers were more resistant to tetracycline, ciprofloxacin, cotrimoxazole and oxacillin than those from broilers while layers were the most resistant. Isolates from layers exhibited high multidrug resistant profile of 81.5% followed by those from farm workers (66.7%), then isolates from broilers (45.9%). High percentage (81.7%) of the *S. aureus* had MAR index ≥ 0.3 and 49.4% (81/164) were multidrug resistant. The most resistance patterns observed among the MDR isolates were: TE, CIP, and OX=12.8% (21); TE, CIP, VA=10.4% (17); TE, CIP, and SXT=7.3% (12); TE, CIP, SXT, and OX=7.3% (12); and TE, CIP, VA, and SXT=6.7% (11). The high resistance to tetracycline and ciprofloxacin showed their frequent use in poultry farm in Kaduna metropolis and immediate action should be taken to correct this anomalies' as this might contribute significantly to community associated multidrug resistance, increased morbidity and economic loss.

Keywords: *S. aureus*; Antibiotics resistance; Poultry birds

Introduction

Staphylococcus aureus is a normal nasal flora microbiota of humans and animals, although they are generally considered commensal bacteria; they have the potential to cause a number of infections which constituted health concerns for women, newborns, elderly, and immunocompromised individuals [1]. In human, *S. aureus* has been implicated in diseases such as dermatitis, pneumonia, septicaemia, osteomyelitis and meningitis in both humans and swine, as well as bovine mastitis in cattle and bumble-foot disease in poultry [2]. In poultry, the disease conditions associated with staphylococcosis vary with the site and route of inoculation in hatchery and poultry farms, and can infect the bones, joints, tendon sheaths, skin, sternal bursa, navel, and yolk sac through breakage of the skin and mucosal membrane of the birds, especially immunocompromised ones are often more prone to staphylococcal infections. Once in the host, *S. aureus* invades the metaphyseal area of the nearest joint, which leads to osteomyelitis and localization within that joint. When *S. aureus* invade the bloodstream, it causes systemic infection in multiple organs, thereby influencing economic losses, which accrued as a result of decreased weight gain, decreased egg production, lameness, mortality, and condemnation at slaughter [3]. Reports have shown that the prevalence of enterotoxigenic *S. aureus* in food handlers that serves as vehicle for zoonotic dissemination of pathogenic *S. aureus* among poultry farm workers, communities and hospitals varies in industries and countries [4]. An estimated prevalence of 2% was reported in a study conducted by Talarico et al. [5] among food handlers in Italy, 12% among flight-catering staff in Finland [6], 19% among restaurant workers in Chile [7] and 62% among fish processing factory workers in India [8]. In Japan, a retail survey performed between 2002 and 2003 found 17.6% of raw chicken meat infested with enterotoxigenic *S. aureus*; an indication of

future and possible staphylococcosis outbreak, which could influence increased mortality and morbidity [9]. To control and manage these diseases/pathogenic *S. aureus*, inappropriate use of antibiotics is employed in both poultry farms and clinical settings. In poultry management, antibiotics are often used in animal food production for growth promotion and routine disease prevention without prescription or control measures. This has necessitated the development of drug resistance superbug such as methicillin resistant *S. aureus* (MRSA), vancomycin intermediate *S. aureus* (VISA), and vancomycin resistant *S. aureus* (VRSA), which are now known as major emerging public health problem [10]. With increase in population density within a particular geographical location, the incidence of both communities associated and hospital associated multidrug resistant *S. aureus* have been observed to increase with time, regardless of hospital size and control measures due to drug abuse and zoonotic transfer of resistance gene mainly located on mobile elements, such as plasmids or prophages and transferable through horizontal gene transfer [11-13]. Therefore, periodic surveillance of antibiotic susceptibility profile of pathogenic strains especially *S. aureus* in poultry farms and hospital is pertinent in

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every geographical location, as this will assist in antibiotic prescription, management and treatment of infections.

Methodology

Sample size determination

A total of 647 samples were randomly collected from 4 Poultry farms representing the 4 geographical zones of Kaduna metropolis using the method describe by Kadam and Bhalerao [14].

$$\text{Thus, } n = Z_a^2 P Z_{1-\beta} / L^2$$

- Where n=Number of samples
- Z_a =Standard normal deviation at 95% confidence limit=1.96
- P=Prevalence=50%
- $Z_{1-\beta}$ =1 - 0.1584=0.8416
- L=Allowable error of 5%=0.05
- $n = Z_a^2 \times P \times Z_{1-\beta} / L^2$
- $n = 1.96^2 \times 0.5 \times 0.8416 / 0.05^2 = 647$

Sample collection and areas

Swab samples from poultry birds (broilers and layers) nostrils, cloacae, droppings, ears and farm workers ear and nostrils were aseptically collected from 4 randomly selected locations within Kaduna metropolis which represent East, South, West and North. The samples were transported to the laboratory for bacteriological analysis in ice packs.

Isolation, identification, purification and biochemical characterization of *S. aureus*

Samples were inoculated into sterile nutrient broth and incubated at 37°C for 24 hours. The overnight cultures were then subcultured on the surface of sterile mannitol salt agar by streaking and incubating at 37°C for 18-24 hours. Cultural characteristics of the resulting colonies were observed and the isolates that produced deep golden yellow coloration were selected and sub-cultural onto increased salt concentrated mannitol salt agar and incubated again overnight. Isolates that grow and still maintained the morphological features of *S. aureus*, were evaluated for catalase, oxidase and other biochemical characteristics using Microgen Staph. ID kit. Isolates that showed the properties of *S. aureus*, were then sub-cultured onto nutrient agar (NA) slants and incubated overnight at 37°C for susceptibility test.

Antibiotic susceptibility test

The antibiotics susceptibility profile of the identified *S. aureus* from poultry and farm workers were determined using the Kirby-Bauer modified disc agar diffusion (DAD) technique, [15,16]. Discrete colonies on NA plate were emulsified in 3 ml of normal saline and the turbidity was adjusted to 0.5 McFarland standard. Using sterile swab sticks, the surface of Muller Hinton agar in 90 mm-diameter plates, were inoculated with the bacteria suspension by streaking the surface of the agar in three directions, rotating the plate approximately to 60° to ensure even distribution. The inoculated plates were then allowed to dry for 10 minutes after which the antibiotic discs were placed on the surface of the agar. The plates were then left at room temperature for the pre-diffusion time before inverted and incubated aerobically at 37°C for 16-18 hrs. The diameter of the zones of growth inhibition were measured to the nearest millimeter and isolates classified as; sensitive, intermediate or resistant based on CLSI interpretative chart zone size [17].

Determination of multiple antibiotic resistance (mar) index

The MAR index will be determined for each isolate by dividing the number of antibiotics to which the isolate is resistant to by the total number of antibiotic tested [18].

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics tested}}$$

Results

Sample collection and processing

A total of 670 poultry samples were randomly collected from 4 farms, which represent the 4 geopolitical zones in Kaduna metropolis. From the birds, 20 samples each were collected from the broilers and layers nostril, cloacae, trachea and droppings while from the farm workers samples were collected from their nostrils and ears (Table 1).

Isolation, identification and biochemical characterization

Out of the 670 samples collected, 530 presumptive organisms with golden yellow colonies grew on mannitol salt agar; of which 454 had the irregular cluster cocci shape, while 362 produced bubbles when tested in H₂O₂ (catalase positive) and 294 formed agglutination when tested with Oxoid latex agglutination kit. Further identification using Microgen Staph. ID showed that 24.5% (164) of the total samples collected were identified as *S. aureus* (Table 2).

Occurrence of other *Staphylococcus* species

Among the organisms identified, *S. aureus*, *S. xylosus*, *S. hyicus*, *S. cohnii* and *S. intermedius* were the predominant *Staphylococcus* species identified. Farm 1 had the highest occurrence of *S. cohnii*, *S. intermedius* and *S. hominis*, while *S. hyicus* was more in Farm 2. Farm 3 had the highest occurrence of *S. aureus*, while *S. xylosus* was predominant in farm 4 (Table 3).

Incidence of *S. aureus* in boiler and layers in Kaduna metropolis

The result below showed that the incidence of *S. aureus* in Kaduna

Farms distribution	Sources of sample collection										Total
	BN	BC	BT	BD	LN	LC	LT	LD	FWE	FWN	
Farm 1 (Kaduna North)	20	20	20	20	20	20	20	20	3	3	166
Farm 2 (Kaduna East)	20	20	20	20	20	20	20	20	2	2	164
Farm 3 (Kaduna South)	20	20	20	20	20	20	20	20	7	7	174
Farm 4 (Kaduna West)	20	20	20	20	20	20	20	20	3	3	166
Total	80	80	80	80	80	80	80	80	15	15	670

BN: Broiler Nostril; BC: Broiler Cloacae; BT: Broiler Trachea; BD: Broiler Droppings; LN: Layers Nostril; LC: Layer's Cloacae; LT: Layers Trachea; LD: Layers Droppings; FEW: Farm Worker's Ear; FWN: Farm Worker's Nostrils.

Table 1: Distribution of sample collection in kaduna metropolis.

Farms (No of samples)	Isolates on mannitol	Microscopy	Catalase Test	Latex agglutination test	Microgen Staph. ID	%
Farm 1 (166)	128	106	81	60	9	5.4
Farm 2 (164)	137	117	96	85	61	37.2
Farm 3 (174)	149	128	98	69	64	36.8
Farm 4 (166)	116	103	87	80	30	18.1
Total (670)	530	454	362	294	164	24.5

Table 2: Identification of *S. aureus*.

metropolis was more in Layer (51.2%) poultry birds than broilers (48.8%) poultry birds (Table 4).

Antibiotics susceptibility testing

High resistance was observed against tetracycline (76.8%), ciprofloxacin (60.4%), oxacillin (36.6%), cotrimoxazole (26.6%) and vancomycin (25%). While the isolates showed significant susceptibility to ceftiofur (2.3%), amoxiclav (2.4%) and gentamicin (3.1%) (Table 5).

Comparative evaluation of the antibiotic susceptibility profile of poultry samples

The isolates from the farm workers were more resistant to tetracycline, ciprofloxacin, cotrimoxazole and oxacillin compared to those from broilers then followed by layers. To ceftiofur, isolates from broilers were more resistant than those from layers and farm workers. To amoxiclav and gentamicin; isolates from farm workers were totally susceptible followed by layers then broilers (Figure 1).

Percentage multidrug resistant

This study showed that isolates from layers exhibited high multidrug resistant profile of 81.5% followed by those from farm

S/N	OIUMS	Farm 1	Farm 2	Farm 3	Farm 4	Total	%
1	<i>S. aureus</i>	9	61	64	30	164	55.8
2	<i>S. Hominis</i>	9	1	0	0	10	3.4
3	<i>S. Cohnii</i>	20	0	0	0	20	6.8
4	<i>S. epidermidis</i>	1	0	0	0	1	0.3
5	<i>S. intermedius</i>	11	0	0	0	11	3.7
6	<i>S. haemolyticus</i>	3	1	1	0	5	1.7
7	<i>S. hyicus</i>	3	14	3	0	20	6.8
8	<i>S. capitis</i>	2	0	0	0	2	0.7
9	<i>S. xylosus</i>	0	6	1	50	57	19.5
10	<i>S. schleiferi</i>	1	0	0	0	1	0.3
11	<i>S. warnei</i>	0	2	0	0	2	0.7
12	<i>M. lylae</i>	1	0	0	0	1	0.3
Total		60	85	69	80	294	100

Table 3: Identified organisms using microgen kit: OIUMS: Organisms Identified Using Microgen S. ID.

Farms	Boiler	Layers	Total
Farm 1	4	5	9
Farm 2	23	38	61
Farm 3	29	35	64
Farm 4	24	6	30
Total (Percentage)	80 (48.8)	84 (51.2)	164 (100)

Table 4: Incidence of *S. aureus* in boiler and layers in Kaduna metropolis.

S/N	Antibiotics	Resistance				Total n=164	% Resistance
		Farm 1 (n=9)	Farm 2 (n=61)	Farm 3 (n=64)	Farm 4 (n=30)		
1	Vancomycin	5	11	21	4	41	25
2	Tetracycline	9	38	50	29	126	76.8
3	Oxacillin	1	18	40	1	60	36.6
4	Cotrimoxazole	5	15	10	13	43	26.6
5	Ciprofloxacin	5	25	49	20	99	60.4
6	Gentamicin	0	2	3	0	5	3.1
7	Amoxiclav	0	4	0	0	4	2.4
8	Ceftiofur	0	2	2	0	4	2.3

Table 5: Antibiotics resistance profile of *S. aureus* isolated from poultry farms in Kaduna metropolis.

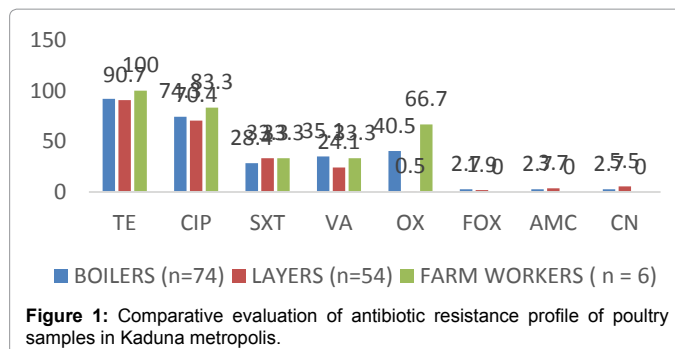


Figure 1: Comparative evaluation of antibiotic resistance profile of poultry samples in Kaduna metropolis.

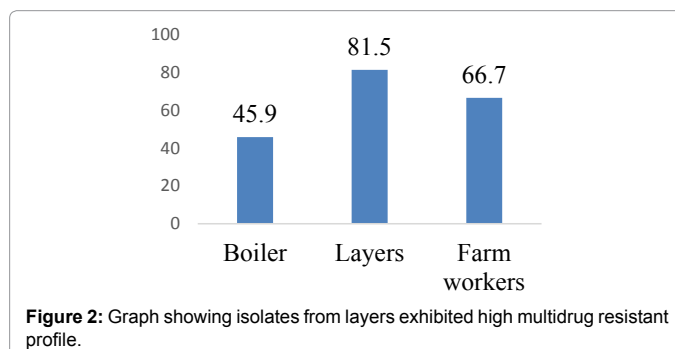


Figure 2: Graph showing isolates from layers exhibited high multidrug resistant profile.

MARI	Frequency	Percentage
0.1	29	17.7
0.2	0	0
0.3	21	12.8
0.4	32	19.5
0.5	44	26.8
0.6	31	18.9
0.7	5	3.1
0.8	0	0
0.9	0	0
1	1	0.6

Table 6: MARI of *S. aureus* from poultry bird.

workers (66.7%), while 45.9% of isolate from broilers were multidrug resistant (Figure 2).

Multiple antibiotic resistance index

Table 6 showed that 81.7% of the *S. aureus* had MAR index ≥ 0.3 while few had MAR index ≤ 0.2 ; an indication that the organisms tested in this study have been pre-exposed to the antibiotics tested (Table 6).

Antibiotic resistance pattern of *S. aureus* from poultry birds

Out of 164 *S. aureus* isolated, 30 were susceptible to all the antibiotics tested. Table 7 showed that 49.4% (81) of the isolates were multidrug resistant (resistant to ≥ 3 class of antibiotics), while 50.6% (83) were not multidrug resistant but resistant to one or two antibiotics. The most resistance patterns observed among the MDR isolates were: TE, CIP, VA, and SXT=6.7% (11), TE, CIP, SXT, and OX=7.3% (12), TE, CIP, VA=10.4% (17), TE, CIP, and OX=12.8% (21), TE, CIP, and SXT=7.3% (12), while 1.2% (2) had TE, SXT, and OX (Table 7).

Discussion

Poultry meat is considered the most commonly reported foodborne pathogens vehicle, followed by the red meat, in which

S/N	Pattern of MDR Resistant	F	NAT	Percentage	Class of R
	CN, FOX, VA, SXT, CIP, AMC, OX, TE	1	8	0.6	MDR
	TE, CIP, and OX	21	3	12.8	MDR
	TE, CIP, and VA	17	3	10.4	MDR
	TE, CIP, and SXT	12	3	7.3	MDR
	TE, CIP, SXT, and OX	12	4	7.3	MDR
	TE, CIP, VA, and SXT	11	4	6.7	MDR
	TE, SXT, and OX	2	3	1.2	MDR
	FOX, AMC, OX	1	3	0.6	MDR
	TE, CIP	18	2	11.0	NMDR
	TE, OX	3	2	1.8	NMDR
	TE, VA	3	2	1.8	NMDR
	TE, CN	2	2	1.2	NMDR
	TE, SXT	2	2	1.2	NMDR
	VA, OX	3	2	1.8	NMDR
	TE	16	1	9.8	NMDR
	OX	3	1	1.8	NMDR
	CIP	1	1	0.6	NMDR
	VA	1	1	0.6	NMDR
	TE, VA, FOX, CIP	1	4	0.6	MDR
	TE, CIP, VA, SXT, OX	1	5	0.6	MDR
	VA, OX, SXT, CIP	1	4	0.6	MDR
	VA, OX, CIP	1	3	0.6	MDR
	Susceptible to all antibiotics	30	0	18.3	NMDR
Total		164		100	

MDR: Multidrug Resistant; NAT: Number of Antibiotic Resistant to; R: Resistance.
Table 7: Antibiotics resistance pattern and classification resistance profile.

S. aureus related food poisoning is the third largest cause of food related illness worldwide [19]. Infections as a result of *Staph aureus* from poultry samples are becoming untreatable due to antibiotics misuse and development of drug resistance strains, such as methicillin resistant *S. aureus* (MRSA) and vancomycin resistant *S. aureus* (VISA) [20]. According to Andreasen [21], disease conditions associated with Staphylococcosis (a bacterial disease that affect a wide range of avian species, including poultry worldwide) vary with the site and route of inoculation and can involve the bones, joints, tendon sheaths, skin, sternal bursa, navel, and yolk sac, which will in-turn influence huge economic losses resulting from decreased weight gain, decreased egg production, lameness, mortality, and condemnation at slaughter of poultry birds. In lieu of this, A total of 670 samples were randomly collected from poultry broilers and layers nostril, cloacae, trachea and droppings in four poultry farms in Kaduna metropolis, Nigeria, of which, a total of 24.5% (164) harbored coagulase positive *S. aureus* (most pathogenic *S. spp*). The percentage of *S. aureus* isolated from this study is lesser than 33% occurrence reported by Bala et al. [10] in Kano, but similar to the work of Nostro et al. [22] who observed 23.8% occurrence of coagulase positive *S. aureus* from poultry sample analysed during a one-year survey in Italy. Among *S. spp.* identified include *S. aureus* (55.8%), *S. xylosum* (19.5%), *S. hyicus* (6.8%), *S. cohnii* (6.8%), *S. intermedius* (3.7%) and *S. hominis* (3.4). This result is also in-line with the finding of Paula et al. [23] who observed an occurrence of 62% *S. aureus* among *S. species*, isolated from poultry samples in Pakistan, followed by *S. intermedius*, *Staph delphini*, and *S. schleiferi* subsp. *coagulans* (10% each) and *S. hyicus* (8%). This study showed that other than *S. aureus* in Staphylococcosis cases, other pathogenic species such as *S. hyicus*, which is the causative agent of osteomyelitis in turkey poultry could also be isolated [21] in our environment. The occurrence of *S. aureus* among layers' samples were more than those among broilers and farm workers. These finding correlates with the

finding of Nworie et al. [24] who observed 51% prevalence among layers and 49% among broilers. Although *S. aureus* is a commensal flora of human skin and animal nasal [10], the high occurrence of pathogenic species of Staphylococcus might have emanated from the hatchery, the general farm environment, and by fomites used in the farm. It has also been reported that the isolation of staphylococci in poultry samples are often connected to poor hygienic practices during slaughtering, transportation, chopping, storage and points of sale by the individuals involved in the production process. This food product can transfer large amounts of staphylococci to stainless steel and polyethylene surfaces with other consumables in the same polythene packs and bags [25]. According to The Poultry Site Journal [26], infection of poultry samples by *S. spp.* is usually via the respiratory route with an incubation period of 2-3 days. Also through wounds, either accidentally or induced by interventions such as beak trimming, and toe trimming may be a portal of entry, which will subsequent spread via the bloodstream to the typical sites of lesions. Damaged skin due to nutritional deficiencies (such as of biotin) may also be a point of entry for microbial infection. These findings further buttress the need for food hygiene during processing, the need for good storage temperature and product discard policy, which should be carefully monitored to ensure that products are microbiologically stable with enhanced safety. Also, it highlighted the need for consumers to be informed and follow the basic instructions regarding storage temperature, cooking to prevent contamination and cross-contamination [27].

According to James et al. [28], the goal of antimicrobial susceptibility testing of significant bacterial isolates is to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The antibiotics susceptibility profile of the *S. aureus* isolates showed that high resistance was observed against tetracycline (76.8%), ciprofloxacin (60.4%), oxacillin (36.6%), and cotrimoxazole (26.6%). Similar patterns of antimicrobial susceptibility have been reported in Zaria, Nigeria by Otolu et al. [29], Italy [30], United States [31] and Ireland [32]. This might be as a result of extensive usage of these antimicrobial agents in animal husbandry over time, which has contributed to the selection of drug-resistant strains [33]. The isolates also showed significant susceptibility to cefoxitin (97.7%), amoxiclav (97.6%), gentamicin (96.9%) and 75% to 5 µg vancomycin. This might be as a result of non-availability/high cost of vancomycin, while the combination therapy of betalactam and betalactamase inhibitor might be the major reason of the low rate of resistance observed against amoxiclav in this study. Also, the parenteral administration of getamicin which involve specialized "hand" for injection, might have curtailed their abused. This finding concurred with the result of Bala et al. [10] who reported that isolates from Kano have varied antibiotic susceptibility pattern and that vancomycin, amoxicillin-clavulanic acid, ciprofloxacin and gentamicin could still be proficient in the management of *S. aureus* infection from poultry farm, but surveillance and publicity should be carried out to curtail the wide spread of antibiotic resistance induced through irrational antibiotic misuse. Resistant to vancomycin (25%) in this study, which has remained the last line of treatment for MRSA infections was also observed in some of the isolates in other studies [34-36]. On comparing the percentage resistance of the isolates, this study observed that isolates from farm workers were more resistant to tetracycline, ciprofloxacin, cotrimoxazole and oxacillin than those from broilers while layers were the list resistant. Isolates from layers exhibited high multidrug resistant profile of 81.5% followed by those from farm workers (66.7%), then isolates from broilers (45.9%). This study concurred with the study of Nworie et al. [24], who also observed a high resistant profile among

S. aureus isolated from the clinic (15.3%) when compared to those from the poultry farm (6.1%) in Ebonyi, Nigeria. This might be as a result of routine use of these antibiotics in clinical therapy which has degenerated to high level of resistance [37]. The classification and evaluation of resistance pattern of the isolates showed that 81.7% of the *S. aureus* had MAR index ≥ 0.3 while 18.3% had MAR index ≤ 0.2 . Also, 45.7% (75) of the isolates were multidrug resistant (resistant to 3 or 4 class of antibiotics), 3.7% (6) were expanded drug resistant (resistant to ≥ 5 antibiotics classes) while 50.6% were not multidrug resistant but resistant to one or two antibiotics. The most resistance patterns observed among the MDR isolates were: TE, CIP, VA, and SXT=6.7% (11), TE, CIP, SXT, and OX=7.3% (12), TE, CIP, VA=10.4% (17), TE, CIP, and OX=12.8% (21), TE, CIP, and SXT=7.3% (12), while 1.2% (2) had TE, SXT, and OX. Suggesting that this resistant isolate originated from a high-risk source of contamination where antibiotics are often used or that a large proportion of the bacterial isolates have been pre-exposed to several antibiotics [38]. Also, the use of antibiotics as prophylaxes, growth promoters or inaccurate dosages given to sick flocks by unqualified personnel may likely have resulted in plasma concentrations that are inconsistent with the desired objectives in the poultry birds, which might have possibly influenced resistance profile of the birds [39].

Conclusion

This study isolated *S. aureus* with high percentage of antibiotic resistant index from poultry samples in Kaduna metropolis, it also showed that in cases of staphylococcal epidemic outbreak in poultry farms around Kaduna, antibiotics such as tetracycline and ciprofloxacin should not be prescribed treatment but rather cefoxitin and amoxicillin-clavulanic acid should be recommended.

Recommendations

Indiscriminate use of tetracycline and ciprofloxacin in poultry farms should be highly restricted, as this could encourage the development of efflux pump, which encourages resistant to structurally unrelated antibiotic with time. Community dissemination of antibiotic resistant genes could be propagated as poultry samples serves as vehicles for dispersal of resistant and pathogenic *S. aureus*. Also, periodic monitoring of resistance profile of *S. aureus* is important as this could help clinicians in diagnosis, treatment of infection and policy formulation.

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