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Research Article

Carriage, Antibiotic Susceptibility, and Beta-Lactamase Production Profiles of Coagulase-Positive *Staphylococcus aureus* Isolated from Chickens in North-Eastern Nigeria

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Abstract

This study investigated carriage, antibiogram and β- lactamase production of coagulase-positive *Staphylococcus aureus* (*S. aureus*) isolated from chicken in north-eastern Nigeria. A total of 238 samples of cloacae and blood swabs collected from 120 poultry farms in some parts of north-eastern Nigeria were screened for *S. aureus* by culture, Gram stain, catalase and coagulase tests. All coagulase-positive isolates were subjected to antimicrobial susceptibility testing by the disk diffusion test against thirteen antibiotics. Resistant isolates were profiled for resistance phenotypes, β-lactamase production, and multiple antibiotic indexes. *Staphylococci* were isolated from 57 farms, with a prevalence of 47.5%, and *S. aureus* carriage or colonization rate of 31.5%. Carriage was observed high in broiler, followed by local, and layer chicken, and in male than female chicken; as well as, high in adult than in young chicken. Antibiotic resistance was commonly observed against tetracycline (82.7%), followed by penicillin (68.0%), and ampicillin (57.3%), and the most common and frequently observed resistance phenotypes were TET-PEN-AP (24.2%), TET-PEN-AP-CIP-LZD (18.2%), and TET-PEN-AP-CDA (15.2%). Evaluation of multiple antibiotic resistance showed that 30.3% of the multi-drug resistant (MDR) isolates had multiple antibiotic resistance index < 0.25, and 69.7% had an index > 0.25. Of the MDR isolates, 30.3% were resistant to 3 antibiotics, 27.3% were resistant to 4, and 24.2% were resistant to 6 antibiotics. Of the isolates, 44% were β- lactamase-producers, with more prevalence in layers (54.2%), than in broilers (42.9%) and local chickens (36.7%). The study revealed an alarming pattern of antibiotic resistance phenotypes and β-lactamase production in *S. aureus* isolates from chicken in northeast Nigeria.

INTRODUCTION

Staphylococcus aureus, a versatile bacteria, is one of the major pathogens associated with clinical infections and food poisoning in humans [1,2] as well as in animal [3]. The skin and mucous membranes, especially of the nasopharyngeal region of birds and mammals constitute predilection and primary reservoirs of the *staphylococcus* [4]. Livestock have been seen as the reservoirs capable of transmitting the bacteria to humans or vice versa [5]. High morbidity and mortality rates have been associated with infections by antibiotic-resistant strains of *Staphylococcus aureus* [6], Although antibiotics and surgical drainages have been employed to treat staphylococcal infections, the appearance of resistance against beta-lactam antibiotics, and methicillin has compromised effective therapy [7,8] Before the introduction of penicillin in 1940, 90% mortality have been reported in human patients due to *S. aureus* infections, but with the advent of penicillin era, remarkable improvement was recorded in both recovery and survival of infected patients [9]. However, not too long after penicillin came into clinical use that the development of resistance against penicillin was reported [10], and thereafter *S. aureus* was found to develop rapid resistance against any drug that it came into contact with. The ability of *S. aureus* to rapidly develop resistance against antibiotics, including beta-lactam agents, is attributed to the possession of a gene called *mec* A, located on the staphylococcal cassette chromosome [11-13] which encodes the production of an altered 75kb penicillin binding protein 2a (PBP2a) responsible for low affinity to all beta-lactam antibiotics and other antimicrobials [14].

Antibiotic resistance is an important public health concern worldwide. Resistance development either in humans or animals by bacteria organisms has been attributed mainly to extreme use or abuse of antibiotics as either growth promoters in chickens or other animals, or misuse in humans [15]. The major mechanism by which *S. aureus* develops resistance to beta-lactam antibiotics is the production of an enzyme called beta-lactamase [16,17], encoded in the blaZ gene located on a transposable part of the large plasmid within the *S. aureus* bacterial cells [9,19]. This gene is said to be easily transferable horizontally from one species to another species of bacteria, due to its location on the plasmid and encodes the production of beta-lactamases (Low, 2003). The beta-lactamases produced by *S. aureus* confer resistance against beta-lactam antimicrobials such as penicillins, cephalosporins and carbapenems. Beta-lactamase *S. aureus* were found to be involved in treatment failures in chicken [16]. Little information on beta-lactamase *S. aureus* have been documented in humans in Nigeria [17-19]. But there is a dearth of published information on beta-lactamase *S. aureus* in animals, particularly in north-eastern Nigeria. This study was therefore carried out to determine the prevalence, antibiotics susceptibility pattern, and production of beta-lactamase in *S. aureus* strains isolated from chickens in some parts of north-eastern Nigeria. Rapid detection of beta-lactamase bacteria in food animals is crucial for control of infections due to *S. aureus* and for therapeutic decision.

MATERIALS AND METHODS

Study area

The study covers three states located in North-eastern Nigeria namely Adamawa, Borno and Gombe states, falling within the conventional arid zone with a land mass of over 136, 000 km^2 (Figure 1), and lies between latitudes 10 and 13°N and longitudes 11 and 15°E. The arid-zone is the largest part of Nigeria, covering the Sudan and Sahel savannah vegetations. The Sahel savannah lies to the north and occupies about 40% of the study area, while the Sudan savannah occupies the southern 60%. The climate here is Sahelian with two distinct seasons: a rainy season which starts from June to September, with a mean annual rainfall of about 600 mm, and the dry cold (harmattan) season from October to February, characterized by low temperatures between 16- 29°c, and the dry hot season from March to May, with temperatures in the range of 46-48°c [20]. The major occupation of the people within this area is arable and livestock farming.

Samples, sample size and sampling

A total of 238 samples comprising of cloacal swab (130) and blood (108) were aseptically collected from 120 chicken farms randomly selected from some parts of the northeastern states in Nigeria between March 2014 and December 2014. Sample size was determined according to Abebe RH [2], for prevalence study in animals. Informed consent was obtained from each poultry farmer prior to sampling. Samples collected were transported to the research laboratory of the department of veterinary medicine university of Maiduguri, in an ice cold sterile container, and preserved at 4°c refrigerator prior to analysis.

Bacterial isolation and identification

Each sample was homogenized in 2ml sterile normal saline and kept at 4°c for 8 hours, and was then added to 2ml nutrient broth No 2 Oxoid® (Basingstoke, Hampshire, UK), and kept overnight for enrichment [21]. The enriched broth was then vortexed and was streaked on blood agar plates supplemented with 5% sheep blood. Plates were incubated at 37°c for 24 hours as described previously [22]. Where present, growth yielding small to medium pink or white colonies were sub cultured onto Manittol salt agar (MSA), Oxoid® (Basingstoke, Hampshire, UK), and incubated overnight. Colonies that yielded characteristic small, round, yellow or golden yellow colour with the MSA were selected as presumptive *staphylococci*. These isolates were subjected to conventional tests including gram stain, catalase and coagulase [23, 24] to differentiate from coagulase-negative *staphylococci*.

Antimicrobial susceptibility testing

Each of the isolates was tested against thirteen antibiotics tigecycline (30µg), imipenem (10µg), linezolide (30µg), norfloxacin 10µg, ciprofloxacin (5µg), penicillin G (10U), ampicillin (10µg), clindamycin (2µg), erythromycin (15µg), gentamicin (10µg), trimethoprim-sulfamethoxazole (25µg), tetracycline (30µg) and chloramphenicol (30µg), obtained from Oxoid® (Basingstoke, Hampshire, UK), using the Kirby Buauer disk diffusion test method according to the clinical laboratory standards institute, CLSI (2014). Briefly; 3 - 4 colonies of the isolate were transferred into 5 ml sterile normal saline in a sterile tube, and vortexed. Tube was prepared such that the turbidity of the bacterial suspension was equivalent to 0.5 McFarland. A sterile swab was dipped into the bacterial suspension and streaked onto Mueller Hinton agar plate, and each antibiotic disk was dispensed using disk dispenser on the surface of the inoculated plate, and Plate was incubated for 24- 48 hours at 37°c. The diameter of inhibition zone was measured in millimeters using a ruler, and result was interpreted according to the CLSI (2014) guidelines. Isolate was considered multiple drugs resistant if it exhibited resistance against more than two antibiotics.

Detection of beta-lactamase production

Beta-lactamase production test was carried out by chromogenic cephalosporin and iodometric methods, as previously described [9].

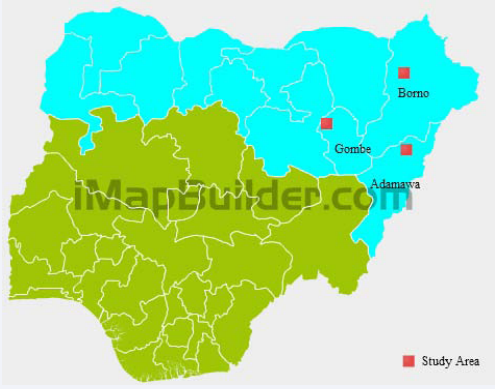

Figure 1 Map of Red boxes: Sampling areas Nigeria showing the study area. Area shaded sky-blue: Arid – zone region.

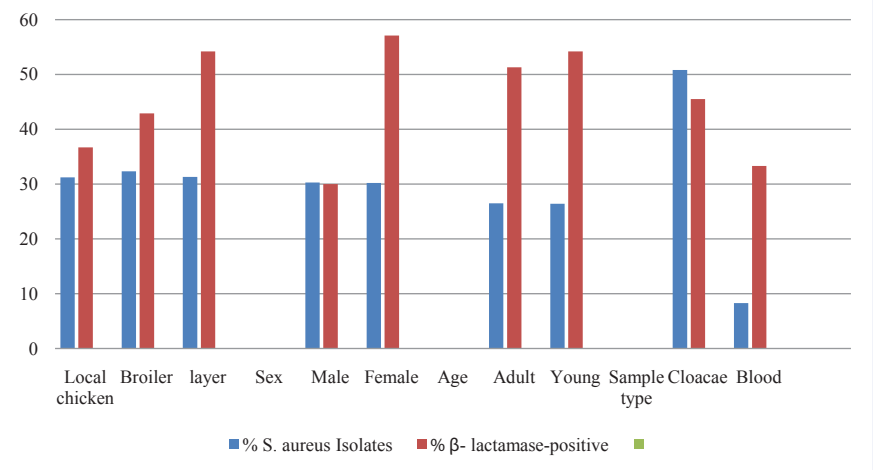

Figure 2 Prevalence of *S. aureus* and beta-lactamase strains, associated with some risk factors in chicken in north-eastern Nigeria. Y-axis: Percentage rate, x-axis: risk factors.

Chromogenic cephalosporin method

Three colonies of the test organism were touched directly with nitrocefin stick, moistened with sterile, distilled water. A change in color from light yellow to deep red color within 20 minutes was identified as positive for beta-lactamase production. No change in color within 20 minutes was considered as negative.

Iodometric method

A concentration of 10,000IU/ml penicillin solution was freshly prepared, and dispensed in 100µl volume into the wells of a 96 wells microtiter tray, and test organisms were suspended in the wells to a concentration of 10^9 cells/ml equivalent to McFarland standard. Two drops of 2 % (w/v) freshly prepared soluble starch were added to each of the wells containing the penicillin solution, and were thoroughly mixed. The tray was then incubated at room temperature for 30 - 60 minutes, after which one drop of iodine solution was dispensed into each of the wells containing the mixture. Solution was then observed for change in colour. Isolates which rapidly decolourized the blue colour of the solution were considered positive for β-lactamase production, and isolates which failed to decolourize the solution were considered negative. Positive (*S. aureus* ATCC 29213) and negative (*S. aureus* ATCC 25923) controls were used in both the chromogenic and iodometric methods.

Data analysis

Data generated were fed into computer, and analyzed by descriptive statistic using Graph Pad In stat version 2009.

RESULTS

The results of carriage or prevalence of *Staphylococcus aureus* in chickens in north-eastern Nigeria are shown in (Table 1). Results showed that a total of 121 (50, 8%) *staphylococci* were isolated: at least one coagulase-positive staphylococcus (CoPS) was isolated from 57 farms, and at least two coagulase-negative *staphylococci* (CoNS) were isolated from 40 farms. The carriage or prevalence rate of *staphylococci* amongst the poultry farms was 80.8%, of which 47.5% were CoPS and 33.3% were CoNS. Of the 130 cloacal samples, 102 (78.5%) yielded *staphylococci*, of which 66 (50.8%) were CoPS, presumptively suspected as *S. aureus*, and 36 (27.7%) yielded coagulase-negative *staphylococci*, considered as CoNS. Of the 108 blood samples, 19 (17.6%) yielded *staphylococci*, of which 9 (8.3%) were CoPS, identified as presumptive *S. aureus*, and 10 (9.3%) were CoNS. In total, 75 (31.5%) of the 238 samples collected yielded *S. aureus* isolates, and 46 (19.3%) yielded CoNS.

The antibiotics susceptibility test result is presented in (Table 2). The General picture depicts majority of the isolates highly susceptible to many of the antibiotics tested. Highest susceptibility was observed to imipenem (98.7%), and lowest to tetracycline (14.7%). The pattern of susceptibility follows in ascending order: tetracycline (14.7%) < penicillin (32.0%) < ampicillin (42.7%) < erythromycin (61.3%) < clindamycin (62.7%) < ciprofloxacin (78.7%) < linezolide (82.7%) = trimethoprim-sulfamethoxazole (82.7%) < tigecycline (84.0%) < chloramphenicol (89.3%) < gentamicin (90.7%) < norfloxacin (92.0%) < imipenem (98.7%). Resistance rates were observed higher against tetracycline (82.7%), followed by penicillin (68.0%), and ampicillin (57.3%). Some percentages of Intermediate resistance were observed against erythromycin (32.0%), clindamycin (18.7%), tigecycline (12.0%), trimethoprim-sulfamethoxazole (10.7%), chloramphenicol (9.3%), and ciprofloxacin (8.0%).

The (Table 3) also shows pattern of antibiotics susceptibility based on the sample site or origin of isolates. It was observed that isolates from cloaca were highly susceptible to almost all the antibiotics: highest rates observed for imipenem (98.5%), norfloxacin, chloramphenicol and gentamicin (90.9%), but high resistance was recorded against tetracycline 81.8%, penicillin (74.2%), and ampicillin (62.1%). All the isolates obtained from blood samples were highly susceptible to almost all the antibiotics tested: imipenem and norfloxacin (100%), tigecycline, linezolide, gentamicin and trimethoprim-sulfamethoxazole (89.9%) respectively, and chloramphenicol, ciprofloxacin, penicillin, and ampicillin (77.8%) respectively. The blood isolates were found

Table 1: Carriage and Prevalence of staphylococci in chickens in some parts of north-eastern Nigeria.

Staphylococci Isolated	Number of farms/ isolates obtained and prevalence (%)obtained and prevalence (%)(n = 120)	Cloaca	Carriage or colonization (% rate) Blood
		(n = 120)	(n = 108)
CoPS	57/75 (47.5)	66 (50.8)	9 (8.3)
CoNS*	40/46 (33.3)	36 (27.7)	10 (9.3)
Total 121 (50.8)	97/121 (80.8)	**102 (78.5)**	**19 (17.6)**

Table 2: Antibiotic Resistance Phenotypes of the Multi-drug Resistant Isolates (n = 33).

Resistance phenotype[a]	Cloaca (n = 30)	Blood (n = 3)	Total (N = 33)
TET-PEN-AP	7 (23.3)	1 (33.3)	8 (24.2)
PEN-AP-CDA	1 (3.3)	0 (0.0)	1(3.0)
PEN-AP-CIP	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-ERY	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-CDA	5 (16.7)	0 (0.0)	5 (15.2)
TET-PEN-AP-LZD	2 (6.7)	0 (0.0)	2 (6.0)
PEN-AP-CIP-LZD	0 (0.0)	1 (33.3)	1 (3.0)
PEN-AP-CIP-CDA-LZD	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-CDA-GM-SXT	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-ERY-CDA-LZD	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-CIP-CDA-LZD	6 (20.0)	0 (0.0)	6 (18.2)
PEN-CHL-GM-CDA-SXT-TGC-LZD	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-CDA-SXT-TGC-LZD	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-CDA-ERY-NOR-LZD	1 (3.3)	0 (0.0)	1 (3.0)
TET-PEN-AP-CIP-CDA-ERY-GM-SXT	0 (0.0)	1 (33.3)	1 (3.0)
TET-PEN-AP-CDA-TGC-ERY-GM-SXT-NOR	1 (3.3)	0 (.0)	1 (3.0)

Table 3: Multiple Antibiotic Resistance Index (MARI).

Number of antibiotics		Resistance Profile (%)		
Isolate is Resistant to	Cloacal isolates	Blood isolates	Total	MARI[a]
	(n = 30)	(n = 3)	(n = 33)	
3	9 (30.0)	1 (33.3)	10(30.3)	0.23
4	8 (26.7)	1 (33.3)	9 (27.3)	0.31
5	1 (3.3)	None	1 (3.0)	0.38
6	8 (26.7)	None	8 (24.2)	0.46
7	3 (10.0)	None	3 (9.1)	0.54
8	None	1 (33.3)	1 (3.0)	0.62
0	1 (3.3)	None	1 (3.0)	0.69

[a] values > 0.25 are adjudged to represent exposure to point source (Krumperman, 1983)

to be resistant to only tetracycline (89.9%). shows the results of antibiotic resistance profiles and multiple antibiotics resistance indices of the multi-drug resistant strains. It was observed that resistance against 3, 4, and 6 antibiotics were most common among the multi-drug resistant (MDR) isolates: 30.3% of the isolates had multiple antibiotic resistance index (MARI) less than 0.25, and 69.6% had MARI greater than 0.25. Results also showed that 10 (30.3%) of the isolates were resistant to 3 antibiotics, and had MARI of 0.23, 9 (27.3%) were resistant to 4 antibiotics, had MARI of 0.31, and 8 (24.2%) isolates were resistant to 6 antibiotics, had MARI of 0.38, while, 3 (9.1%) of the isolates were resistant to 7 antibiotics, and had MARI of 0.54, whereas, 1 (3.0%) of the isolates was found resistant to 5, 8, and 9 antibiotics, had MARI indices of 0.38, 0.62 and 0.69 respectively. The resistance profiles, and MARI based on site or origin of isolates are also shown in the (Figure 2) shows the prevalence of *S. aureus* carriage or colonization, and β-lactamase production based on type, sex, and age of chicken, and type of sample. Prevalence of *S. aureus* was observed slightly high in broilers (32.3%), than in local (31.3%) and layer (31.2%) chicken. Colonization of male chicken (30.3%) was not significantly different from female (30.2%); same was observed in adult (26.5%) and young (26.4%) chicken. On the basis of site of samples, high rate of *S. aureus* colonization was observed in the cloaca (50.8%) than in the blood (8.3%).

Of the 66 isolates obtained from cloacal samples, 30 (45.5%) tested positive for β-lactamase-production, while 3 (33.3%) of the 9 isolates obtained from blood tested positive. Results

also indicate that β-lactamase-positive strains were more prevalent in isolates from layers 13 (54.2%), than isolates from broilers 9 (42.9%), and local chickens 11 (36.7%). Furthermore, β-lactamase-production was observed in majority of the isolates from female 24 (57.1%) than in isolates from male 9 (30.0%) chicken, and in majority of the isolates from young 13 (54.2%) than in isolates from adult 20 (51.3%) chicken.

DISCUSSION

Carriage and prevalence of S. aureus in chickens in northeast Nigeria

Staphylococcus aureus is the most significantly disseminated pathogen worldwide in both human and animals, as well as, in the environment. *S. aureus* isolates in this study were identified based on conventional methods (culture, morphology, Gram stain, catalase and coagulase). Out of the 120 chicken farms studied, *S. aureus* was isolated in 57 farms with a prevalence rate of (47.5%). This result was lower than those reported in many studies across the world. Geidam YA [25] reported very high rate in Selangor area of Malaysia, where 96 *S. aureus* were isolated from 3 poultry farms. Similarly, in Sokoto, northwest Nigeria Kwoji ID [26], reports 72.6% prevalence of *S. aureus* from 136 samples collected from 6 poultry farms. Other similar studies [16,27] carried out previously in Maiduguri, northeast Nigeria reports prevalence rates of 50.8%, and 54% respectively. Most recently, in northern Algeria, 54% prevalence rate was reported by Saliha BK [28]. The result of the present study is higher than those obtained from other studies: Otalu O Jr [29], reports isolation rate of 13 of 400 (3.25%) samples, and Otalu O Jr [30], reports 13 of 1400 (0.93%) samples collected from live poultry and poultry carcasses in Zaria, and Onaolapo JA [31], reports 24% from poultry farms in Kaduna, both northwest Nigeria. In Benin, South-south Nigeria Etinosa OI reports [32], 20% rate from poultry farms Bala HK [33], reports 33% in Kano, north-west Nigeria, while [34], Nostro A reports the rate of 24% from raw chicken meat in Italy, compared to the rate observed in this study.

The discrepancies in the colonization, isolation, or prevalence of *S. auerus* observed with this study and the various studies reviewed, may be influenced by management practices, geographical location, sampling methods, laboratory processes and techniques used [35], as well as, cross-species transmission of *S. aureus*, resulting from production system (mixed farming), breeds, and health status of chickens, at the time of sampling. Studies suggesting variations in *S. aureus* colonization or prevalence in some animal species have also been documented. The rates of 17.1% in large ruminant animals slaughtered for human consumption at Maiduguri abattoir, northeast Nigeria [1] and 12.5% from ready-to-eat food in China [36] 42% in bovine milk samples in Egypt [37], and 73.3% in clinical and 42% in subclinical bovine mastitis in Ethiopia [38], were all lower, compared to the rate observed in chicken in the present study.

Chicken are reared extensively in close proximity to humans (many backyard chickens very common in northern Nigeria), and thus can serve as a potential source or reservoirs of pathogen transfer to humans, playing an important role in environmental contamination with *S. aureus*. Some risk factors such as breed, sex, and age of chicken, and site of sample collection were determined in the present study. It was found that *S. aureus* prevalence was slightly higher in broilers (32.2%), than in local chickens (31.3%) and layers (31.2%); although rates not significantly different ($P > 0.05$). This suggests that *S. aureus* colonization may not necessarily be influenced by breed of birds. Several other studies have either supported or contradicted the finding of the present study. In Zaria, northwest Nigeria Otalu O Jr [29], reported high prevalence in broiler (53.8%) than in layer (38.5%) and local chicken (0%). In northern Algeria, also reports high rates in broiler (42%) than in layer chicken (12%), in agreement with the present study. Previous study [16] in Maiduguri, northeast Nigeria, reports that *S. aureus* were isolated more in Layers (35.0), followed by local (33.0%), than in broilers (32.0%). Other studies in Kaduna, northwest Nigeria Otalu O Jr [30], reports high occurrence in layer (51.2%) than in broiler (48.8%) chicken, in Ebonyi, south-east Nigeria Nworie A [39], reports high prevalence in layer (51.0%) than in broiler (49.0%) chicken. These studies did not support the finding in the present study. In cattle, *S. aureus* prevalence was high in local (Zebu) breed (61.9%), than in pure (Jersey) breed (51.1%) in Ethiopia [37].

The finding in this study also observed insignificant difference in occurrence rate of *S. aureus* in male (30.3%) and female (30.2%), and in adult (26.5%) and young (26.4%) chicken. On the contrary Mamza SA [16], reported high occurrence in young (10.0%), and (19.5%), than in adult (8.0%), and (3.37%) chicken respectively. In cattle, high prevalence was reported in the adults (61.4%) than in the young (15.0%) in Ethiopia [37]. The present study used smaller sample size than the above studies, and this may result in the discrepancies observed. *S. aureus* is ubiquitous and abundant organism in the environment, and local chickens which are usually kept on free range are assumed to be at risk of being frequently colonized than the broilers, and layers, which are reared intensively in confinement under controlled environment. From the finding of the present study, it is difficult to ascertain whether breed, sex, and age, could influence the occurrence of *S. aureus* in chickens, in the advent of indiscriminate overuse of antibiotics for growth promotion, prophylaxis, and chemotherapy, which according to Broens EM [35], has been rampant amongst poultry farmers in Nigeria. Sampling from multiple sites has been observed to reduce the prevalence of *S. aureus* in animals [21]. The present study examined isolates sampled from cloaca and blood, and this might have possibly affected the prevalence rate observed. It was observed that *S. aureus* carriage was more common in the cloaca (50.8%) than in the blood (8.3%) of chicken in this study. The low colonization rate observed from the blood in this study may possibly suggest slow rate of spread or dissemination from the portal of entry to the blood stream.

Antibiotic resistance pattern of S. aureus isolates from chickens in northeast Nigeria

The present study is consistent with several other studies conducted across Nigeria, and the world on *S. aureus* resistance in animals. Multiple antibiotics resistance *S. aureus* were reported in isolates from layer (81.5%), and broiler (45.9%) chicken in Kaduna, northwest Nigeria [31], with 49.4% of the isolates

resistant to more than 3 antibiotics, and 50.6% resistant to less than 2 antibiotics. Isolates obtained from broilers, and layers in northern Algeria were 100% resistant to ≥ 3 antibiotics [28], so also in a study in chickens in Maiduguri [27]. In isolates obtained from cattle in Egypt, 83% were resistant to ≥ 3 antibiotics [37]. In the present study, 44% of the isolates were resistant to more than 3 antibiotics, compared to the above studies. This result reflects fair use of multiple antibiotics combination in poultry in the study area. The pattern of antibiotics resistances observed in this study is in agreement with those of Febler AT, Otalu O Jr [40, 28], who reported multiple resistances across families of antibiotics. In this study, resistances were observed against tetracyclines, β-lactams, macrolides, aminoglycosides, and oxazolidinones. Reports resistances against tetracyclines, β-lactams, and macrolides in support of the finding in the present study. Prevalence of multiple antimicrobial resistances by *S. aureus* isolates across classes of antibiotics may portend opportunistic, and difficult to treat infections in animals, resulting from limited therapeutic option due to antibiotics resistance. This according to Bagcigil FA et al. [41], portends such isolates an important reservoir for bacterial transmission from one host to another, and resistance determinants to non- pathogenic bacteria. However, lack of promoter or other sequences to enable expression of resistance gene [21] by non-pathogenic bacteria could result in susceptibility to antibiotics. Although, proliferation of antibiotics resistance genes on mobile genetic elements in animals according to is a direct result of potential antibiotics overuse, which is widely practiced in poultry production in Nigeria [35].

The antibiotic resistance patterns observed in the present study showed that resistance against tetracyclines (tetracycline; 82.7%) > β-lactam agents (penicillin; 68.0%, ampicillin; 57.3%; linezolide; 17.3%) > lincosamides (clindamycin; 18.7%) > Quinolones (ciprofloxacin; 13.3%) > antifolates (trimethoprim/sulfamethoxazole; 6.7%) = macrolides (erythromycin; 6.7) > aminoglycosides (gentamicin; 5.3%) > amphenicosl (chrloramphenicol; 1.3%). This pattern suggests or reflects the frequency of use, which these antibiotics were subjected to in poultry production in this region. Isolates from other studies tested against similar antibiotics showed comparatively different patterns. Isolates reported from chickens in Zaria by exhibited this pattern: β-lactams (penicillin; 100%) = macrolides (erythromycin; 100%) > tetracycline (46.2%) > amphenicols (chloramphenicol; 46.1%) > antifolates (trimethoprim/sulfamethoxazole; 38.5%) = aminoglycosides (gentamicin; 38.5%) > Quinolones (ciprofloxacin; 15.4%). Isolates from chickens in kaduna (Onaolapo showed the pattern of resistance as tetracycline (76%) > Quinolones (ciprofloxacin; 60.4%) > aminoglycosides (gentamicin; 3.2%). Isolates from chickens in Maiduguri, northeast Nigeria showed β-lactam (ampicillin; 100%) = macrolides (erythromycin; 100%) > lincosamides (clindamycin; 51.9%). Furthermore, isolates from Benin, south-south Nigeria [32], exhibited β-lactam (penicillin; 100%) = antifolates (trimethoprim/sulfamethoxazole; 100%) > lincosamides (clindamycin; 98%) > macrolides (erythromycin; 88%) > aminoglycosides (gentamicin; 30%) Saliha BK reports [28], β-lactam (penicillin; 19.8%) > tetracycline (13.7%) > macrolides (erythromycin; 11.6%) > Quinolones (enrofloxacin; 7.6%) > antifolates (trimethoprim/sulfamethoxazole; 5.7%) > aminoglycosides (gentamicin; 0.7%) in isolates from chicken in northern Algeria, compared to the isolates in the present study.

Studies involving large animals also reported nearly similar patterns of resistance, β-lactam (penicillin; 100%) > tetracycline (77.4%) > macrolides (erythromycin; 18.9%, intermediate resistance), by isolates from bovine mastitis in Ethiopia [38], and β-lactam (ampicillin; 95.2%, penicillin; 83.3%) > antifolates (trimethoprim/sulfamethoxazole; 78.6%) > amphenicols (chloramphenicol; 69.0%) > tetracyclines (tetracycline; 52.4%) > macrolides (erythromycin; 47.6%) > aminoglycoside (gentamicin; 23.8%) > Quinolones (ciprofloxacin; 14.3%) by isolates from mastitic milk in Egypt (Amal [37]. Isolates from humans have also been reported to demonstrate variable pattern of resistance against some common antibiotics tested in this study. In Malaysia, Thung KL described the resistance pattern as β-lactam [42], (penicillin; 100%) > Quinolones (ciprofloxacin; 94%) > aminoglycosides (gentamicin; 76%) > tetracylines (tetracycline; 47%) > lincosamides (clindamycin; 18%), in Romania Ionescu R [43], described tetracycline (99%) > aminoglycosides (gentamicin; 63%) > Quinolones (ciprofloxacin; 58%) > lincosamides (clindamycin; 0%), and in Iran Ruzbahani M [44] reports tetracycline (97%) > lincosamides (clindamycin; 92%) > β-lactams (ampicillin; 70%) > Quinolones (ciprofloxacin; 61%) > aminoglycosides (gentamicin; 50%), while in India Khadri H [45], described, β-lactam (penicillin; 100%) > macrolides (erythromycin; 83%) > aminoglycosides (gentamicin; 73%) > tetracyclines (tetracycline; 61%) > Quinolones (ciprofloxacin; 40%). In human isolates from Nigeria Olowe OA [46], described tetracyclines (tetracycline; 87.5%) = β-lactams (penicillin; 87.5%) > macrolides (erythromycin; 81.1%) > amphenicols (chloramphenicol; 75.0%) > aminoglycosides (gentamicin; 62.5%), in Osogbo, south-west Nigeria. In Keffi, north-central Nigeria [10] Ngwai YB described aminoglycosides (gentamicin; 98%) > macrolides (erythromycin; 86%) > Quinolones (norfloxacin; 78%, ciprofloxacin; 72%)) > amphenicols (chloramphenicol; 73%) > β-lactams (ampicillin; 63%), and from Abuja, north-central Nigeria, the pattern β-lactams (penicillin; 100%) > tetracyclines (tetracycline; 88%) > Quinolones (ciprofloxacin; 62.9%) > macrolides (erythromycin; 60%) > aminoglycosides (gentamicin; 53.6%) > lincosamides (clindamycin; 22.7%) was reported by Abdullahi and Iregbu [47]. It is obvious from these studies, compared with the present study that *S. aureus* isolates from large animals, and humans were more resistant than isolates from chickens. This suggests that resistance pattern of *S. aureus* varies with host species and geographical location.

Phenotypes of antibiotic resistance and multiple antimicrobial resistance index of S. aureus isolates from chickens in northeast Nigeria

The Profiles of phenotype resistance for 33 multiple-drug resistant isolates were determined in this study. It was observed that TET-PEN-AP 8 (24.2%), followed by TET-PEN-AP-CIP-CDA-LZD 6 (18.2%), and TET-PEN-AP-CDA 5 (15.2%) were the most common phenotypes amongst the *S. aureus* isolated from chickens in northeast Nigeria. In this study, an isolate found to be resistant to more than three antibiotics was referred to as multiple antibiotics resistant (MDR). Multiple antibiotics resistance was

evaluated, and it was found that 30.3% of the MDR isolates had MARI of 0.23, while 69.7% had MARI ≥ 0.3. Isolates with MARI > 0.25 were considered to have emerged from a high risk source of contamination [48]; or been pre-exposed to high chunk of multiple antibiotics for preventive or treatment uses [49]. Thus, the isolates with MARI > 0.25 observed in this study portends a serious health threat to humans, as chicken provides a rich source of protein, and forms the greater percentage of meat consumed by the growing population in Nigeria. In addition, multiple drug resistance can limit therapeutic option in the phase of *S. aureus* infections. Multiple antibiotics resistance index was determined by dividing the number of antibiotics an isolate is resistant to by the total number of antibiotics tested [50]. Consistent with the finding in this study, a study in Kaduna, northwest Nigeria [31], Onaolapo JA reports 81.7% of isolates from chicken that had MARI ≥ 0.3, and 17.7% that had MARI ≤ 0.2. In the present study 44.0% of the isolates were multi-drug resistant, compared to 49.4% reported by Onaolapo JA [31]. Although, the present study could not establish sources of contamination, factors such as poor sanitary conditions of poultry houses, hands of farmers, and potential overuse of antibiotics [30-38], as well as non-adherence to antibiotics use policy, might likely be playing a great role.

Prevalence of S. aureus and beta-lactamase strains, associated with some risk factors in chicken in north-eastern Nigeria.

The production of β- lactamases has often resulted in the ability of *S. aureus* to resist a wide range of antibiotics [37]. B-Lactamases are enzymes that have the ability to hydrolyze the β- lactam ring of the β-lactam antibiotic [51], thereby inactivating the antibiotic, and leading to therapeutic failure. The presence of β-lactamase-producing *staphylococci* in chickens portends a major threat to both the consumer and contact persons (farmers, veterinarians), and enhance easy spread of the pathogens in the community, and dissemination within a flock, considering the intensive and backyard poultry practices in Nigeria. Isolates resistant to ampicillin were better producers of β-lactamases, and suggests resistant to oxacillin. In this study, the percentage of penicillin-resistant isolates found to be β-lactamase -producers (84.6%) were lower, compared to 96% reported recently [37]. Incomplete enzymatic reactions, and differences in substrate specification exhibited by different β-lactamase enzymes [52], could result in false-positive or false-negative reactions observed in this study. The percentage of the isolates (81%) in this study that gave true positive reactions for β- lactamase production using the chromogenic cephalosporin method, agree with result of the previous study [52], which reported (82.8%). It was reported that false-positive and false-negative results are unavoidable with the enzyme production methods.

The combination of antimicrobial resistance and β-lactamase production methods carried out in the present study could detect strains positive for the bla Z gene as may be detected by the PCR method described previously [52]. Of important note is that some percentages of the isolates that were penicillin-resistant were found negative for β-lactamase production. This suggests that alternative mechanisms may possibly be playing a role in the antibiotics resistance ability of these isolates other than β-lactamases [21]. Employment of PCR detection of the bla Z gene in these isolates may help give clear decision as to the presence of resistance-encoding gene, better than using just antimicrobial susceptibility and enzyme production tests. The PCR was used to detect 95.7% β- lactamase-producers positive for the bla Z gene from chicken isolates recently [37]. The use of PCR was not employed in the present study. Recent study reports that high percentage of *S. aureus* isolates from layers (16.2%) were positive for β- lactamase production than isolates from broilers (5.9%), and β- lactamase production was high in isolates from young chickens (19.2%) than were isolates from adult chickens (3.4%). In consistence, the present study reports high prevalence of β- lactamase production in isolates from layers (54.2%) than in isolates from broilers (42.9%), and higher in the young (54.2%) than in adult chickens (51.3%), compared to the finding of [26]. The striking finding in the present study is that *S. aureus* colonization appeared not to be significantly varied with risk factors such as breed, sex, and age of the chickens, but β-lactamase production in *S. aureus* suggests variation with breed, sex, and age of chickens. Longitudinal or cross-sectional studies using larger sample sizes are required to investigate this finding. This is important for policy formation and implementation of monitoring and controlling the spread, and early dissemination of resistant strains from the poultry to other animals, humans and the food chain.

CONCLUSION

This study provided a foresight into the prevalence and carriage of multi-drug resistant β- lactamase *S. aureus* in chickens in north-eastern Nigeria. The finding revealed an alarming pattern of phenotype antibiotic resistances and β- lactamase production in *S. aureus* isolates form chickens. It is possible to suggest that some antibiotics norfloxacin, imipenem, tigecycline, gentamicin, clindamycin, and chloramphenicol (although prohibited), which appeared to have potential effect on the *S. auerus* isolates *in vitro* in this study, could be used to control infections caused by *S. aureus* in chickens in this region. Sampling from a number of different sites and larger chicken population will provide more data on multiple antibiotics resistant β -lactamase *S. aureus* carriage, and prevalence which can be generalized over time.

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