

Original Article

Occurrence of methicillin resistant *Staphylococcus aureus* in chickens and farm personnel in Sokoto, North-western Nigeria

Iliya Dauda Kwoji¹, Farouk Muhammad Tambuwal², Mikaeel Bala Abubakar², Yusuf Yakubu³, Asinamai Athliamai Bitrus⁴ and Solomon Jauro¹

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AFFILIATIONS

¹Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Borno State, Nigeria.

²Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria.

³Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria.

⁴Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Malaysia.

CORRESPONDENCE:

Iliya Dauda Kwoji,
Department of Veterinary Microbiology,
Faculty of Veterinary Medicine, University
of Maiduguri, PMB 1069, Borno State,
Nigeria.
E-mail: kojdail28@unimaid.edu.ng

ABSTRACT

Objective: The study was conducted to determine the presence of methicillin resistant *Staphylococcus aureus* (MRSA) in chickens and farm personnel in Sokoto, North-western Nigeria.

Materials and method: A total of 160 samples (136 from chickens and 14 from personnel) were collected and screened for the presence of MRSA using cultural characteristics, biochemical tests and latex slide agglutination test for the presence of Penicillin binding protein 2 α (PBP2 α).

Results: MRSA were detected in 8.82% (n=12/136) of chickens, while 14.29% (n=2/14) in farm personnel. All the isolates were positive for PBP2 α .

Conclusion: This study established for the first time the occurrence of MRSA in both chickens and farm personnel from poultry farms in Sokoto, Nigeria. Thus, the study provided baseline information for further studies on the epidemiology of MRSA.

KEYWORDS

Antibiotics; Chickens; Farms; MRSA

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INTRODUCTION

The livestock census estimate has shown that Nigeria has over 145 million chickens. From a market size perspective, egg production in Nigeria is the largest in Africa, and it has the second largest chicken population after South Africa. In Sokoto State, poultry production is one of the major leading economic activities. The sub-sector tops the priority of the present government as a tool for sustainable development. However, the viability and development of the industry is threatened by infectious diseases partly due to poor sanitary practices and lack of managerial skills. Some of the common poultry diseases encountered in the state include: Newcastle disease, infectious bursal disease, fowl pox, salmonellosis, colibacillosis and various forms of staphylococcal infections ([Ibitoye et al., 2013](#)).

Staphylococci are Gram positive bacteria that range from 0.5-1.5 μm in diameter and exist as irregular grape like clusters ([Harris et al., 2002](#)). These are one of the most prevalent bacteria in both human and animals ([Persoons et al., 2009](#); [Suleiman et al., 2013](#)). *Staphylococcus aureus* is one of the most important pathogens colonizing the skin and mucus membranes of the nares in human and animals incriminated in different disease conditions ranging from minor skin infections, such as furunculosis and carbunculosis to severe and highly debilitating conditions such as pneumonia and endocarditis ([Jensen and Lyon, 2009](#)). The pathogen is endowed with a great variety of virulence markers, which include both structural and secreted products participating in pathogenesis of infection ([Plata et al., 2009](#)). Methicillin resistance emerged among some strains of *S. aureus* immediately after the introduction of the drug for clinical use in the early 1960s ([Sakoulas and Moellering, 2008](#)).

Methicillin resistant *S. aureus* (MRSA) had acquired the *mecA* gene which encodes an alternative penicillin-binding protein 2α with reduced affinity for methicillin ([Nworie et al., 2013](#)). This gene complex also allows cross resistance to non-beta lactam antibiotics such as clindamycin, ciprofloxacin, cotrimoxazole, erythromycin and gentamycin because of the presence of insertion sites for plasmids and transposons ([Nworie et al., 2013](#)). Recently, MRSA has been increasingly reported as a potential zoonotic pathogen ([Bakeet and Darwish, 2014](#)) isolated from a number of animal species such as Dogs, Cats, Horses, Sheep, Pigs and Chickens worldwide ([Becker et al., 2002](#)). The extensive use of antimicrobial agents in food animals' production, where they are often applied at sub-therapeutic doses for growth promotion and routine prophylactics, often result to multidrug-resistant development among bacteria ([Oke and Adewale,](#)

[2013](#); [Bitrus et al., 2016](#)). Since this is a common practice by farmers, it can result to the proliferation of antimicrobials resistant pathogens in poultry ([Oke and Adewale, 2013](#)) thus, threatening the health of farm personnel that are in contact with such animals. The present study aimed at determining the occurrence of MRSA in chickens and farm personnel in Sokoto which was carried out using culture, biochemical tests and detection of PBP 2α .

MATERIALS AND METHODS

Study area: This study was carried out in Sokoto, Nigeria. Sokoto is the capital of Sokoto State located in the extreme Northwestern Nigeria between longitudes 4°8'E and 6°54'E and latitudes 12°N and 13°58'N. It shares boundaries with the Republic of Niger to the North, Kebbi State to the west and Southwest and Zamfara State to the East (**Figure 1**). The climate of the area is semi-arid characterized by cold and hot dry seasons that begins from October and extends up to April. The warm wet season commences from May and end in September with an annual average rainfall ranging between 400 mm–1300 mm. The mean monthly temperature ranges from 13°C in December through February and 40°C–44°C in April and May. The relative humidity on the average is between 10% and 90%. The state is rich in livestock resources with an estimated 3 million sheep, 5 million goats, 4,600 camels, 52,000 donkeys and remarkable number of local and exotic poultry species ([Nworie et al., 2013](#)). The main occupation of the people in the state is arable farming with predominant interest in ruminants farming ([Tambuwal et al., 2011](#)).

Ethical statement: The study protocol was approved by the faculty of Veterinary Medicine postgraduate committee, Usmanu Danfodiyo University Sokoto. Informed consent was obtained from each of the participating poultry farm personnel for inclusion in the study. Ethical clearance was also officially obtained from Sokoto State Ministry of Health for collection of samples from farm personnel.

Study design and sampling procedure/techniques: This was a cross-sectional study and was carried out in Sokoto. It covered Sokoto North, Sokoto South, Dange-Shuni, Kware and Wamako local government areas. Multi-stage random sampling was employed for selection of the farms and systematic random sampling was employed for sample collection in chickens. Six poultry farms were randomly selected and sampled. A total of 150 samples, 136 from chickens and 14 from humans were randomly and purposively collected. Accordingly,

the number of samples collected from chickens comprised 45 layers, 23 pullets, 44 broilers and 24 broiler chicks from an estimated poultry population of 7,035. Similarly, 14 samples were collected from farm workers in varying proportions.

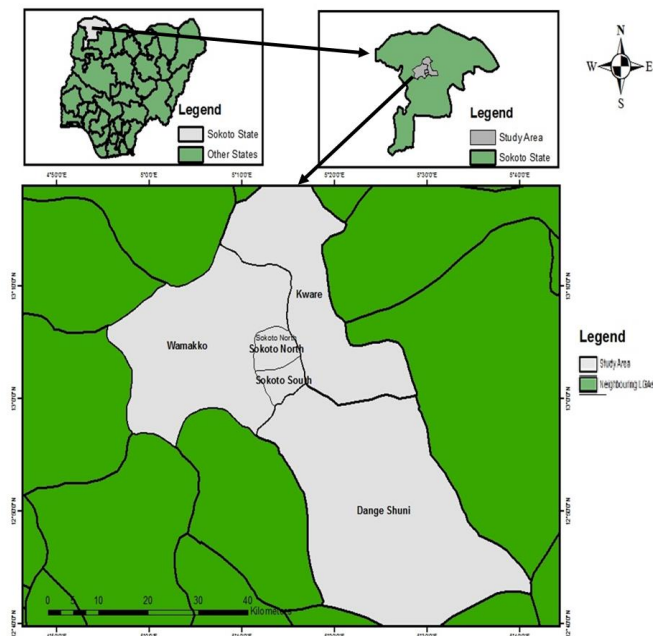


Figure 1: Distribution of sampling points in Sokoto, Nigeria. (Source: Geographical Information Science (GIS) laboratory, Usmanu Danfodiyo University Sokoto)

Sample collection: Random and purposive samplings were respectively used for sample collection from the cloacae of birds and nares of personnel with the aid of sterile swab sticks dipped in peptone using sterile hand gloves. The swab sticks were inserted into the cloaca and nasal cavity and carefully rotated within the sites to ensure sufficient contact. The samples collected were then tightly closed, labeled accordingly and transported to the Veterinary Microbiology laboratory, Usmanu Danfodiyo University, Sokoto for subsequent analysis.

Isolation and identification of *Staphylococcus* spp.: This was carried out by using streak plate method, the prepared mannitol salt agar (MSA) plates were inoculated with the cloacal and nasal swab samples from birds and humans respectively, and incubated at 37°C for 24 h. The suspected *S. aureus* colonies (golden yellow-colonies) on MSA were subjected to Gram's staining and biochemical tests (coagulase, catalase) and hemolysis test for confirmation of *S. aureus*.

Oxacillin resistance screening agar base (ORSAB): Pure culture of *S. aureus* from MSA were sub-cultured on

ORSAB medium (Oxoid), and incubated at 37°C for 18 h. The production of deep blue coloration indicates mannitol fermentation by isolates that are resistant to Oxacillin (Simor et al., 2001).

Latex slide agglutination test (LSAT): This test was carried out for the detection of PBP2 α . It was based on agglutination of latex particles sensitized with monoclonal antibodies against PBP2 α in accordance with the manufacturer's protocol (Oxoid), as described by Felten et al. (2002). Clumping of latex particles by *S. aureus* that showed deep-blue colonies on ORSAB confirms the presence of PBP2 α .

Data presentation and analysis: The data obtained from this study was presented in the form of tables as percentages for descriptive purposes. *Chi*-square analysis was used to check for the level of association between the level of occurrence of MRSA in chickens with age and bird types while logistic regression analysis was carried out to determine the level of association between the levels of occurrence of MRSA in chickens based on farms. The software package used for the analysis was SPSS (Version 22; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

MRSA has been increasingly reported as emerging problem in veterinary medicine, particularly in small animals and poultry (Broens et al., 2011). The presence of MRSA in different poultry farms, slaughter houses, carcasses, or food of poultry origin has been reported (Persoons et al., 2009; Bakeet and Darwish, 2014). This is leading to an increase of reports and interest in MRSA colonization and infection in poultry (Bakeet and Darwish, 2014).

In this study, the occurrence of MRSA in chickens and farm personnel were investigated, the total occurrence of 8.82% was obtained. This occurrence rate ranges from 0.0% to 8.70% with mean of 4.76%. The respective occurrences from farms A, B, C, D, E and F were 8.7, 4.55, 2.61, 8.33, 4.35 and 0.00% (Table 1). The highest occurrence among these farms was found from farm A (8.70%) followed by farm D (8.33%), farm B (4.55%) and farm E (4.35%) while the least was from farm C (2.61%). However, MRSA was not detected from samples in farm F (0.0%) (Table 1). The occurrence of MRSA from birds was highest in chickens less than four weeks of age with significant statistical association ($P < 0.005$, $\chi^2 = 5.999$). However, no statistical difference was observed in the occurrence with respect to chicken types ($P > 0.005$, $\chi^2 = 0.458$) even though the organism was isolated more in

Table 1: Occurrence of *Staphylococcus* spp., *S. aureus* and Methicillin resistant *Staphylococcus* isolated from poultry farms in Sokoto and its environs

| Source (Farms) | <i>Staphylococcus</i> spp. | | <i>S. aureus</i> | MRSA |
|-------------------|---------------------------------|---------------------|---------------------|---------------------|
| | Number of Samples processed (n) | Number Positive (%) | Number Positive (%) | Number Positive (%) |
| A | 23 | 17 (73.91) | 15 (65.23) | 2 (8.70) |
| B | 22 | 18 (81.82) | 16 (72.73) | 1 (4.55) |
| C | 23 | 20 (86.96) | 17 (73.91) | 6 (2.61) |
| D | 24 | 23 (95.83) | 20 (83.33) | 2 (8.33) |
| E | 23 | 21 (91.30) | 20 (86.95) | 1 (4.35) |
| F | 21 | 19 (90.48) | 10 (47.62) | 0 (0.00) |
| Total | 136 | 118 (86.76) | 98 (72.06) | 12 (8.82) |

Table 2: Occurrence of Methicillin resistant *Staphylococcus* in chickens based on farms, age and chicken types in Sokoto and environs

| Factor | Level | Sample (n) | Occurrence (%) | P-value | OR | 95% CI | χ^2 |
|--------------|----------|------------|----------------|---------|-------|--------------|--------------------------|
| Farm | A | 23 | 8.70 | Ref | NA | NA | |
| | B | 22 | 4.55 | 0.583 | 0.500 | 0.042-5.944 | |
| | C | 23 | 2.61 | 0.136 | 3.706 | 0.661-20.765 | |
| | D | 24 | 8.33 | 0.965 | 0.955 | 0.123-7.408 | |
| | E | 23 | 4.35 | 0.558 | 0.477 | 0.04-5.664 | |
| Age | <4 weeks | 47 | 9(19.15) | Ref | NA | NA | |
| | >4 weeks | 89 | 3(3.37) | 0.033 | 0.229 | 0.065-0.808 | 5.999^a |
| Chicken type | Broilers | 68 | 4(5.88) | Ref | NA | NA | |
| | Layers | 68 | 8(16.18) | 0.721 | 0.628 | 0.161-2.441 | 0.458 |

Value(s) with superscript(s) "a" is (are) statistically significant

NA= Not applicable, OR= odd ratio, CI= confidence interval

Table 3: Occurrence of *Staphylococcus* spp., *S. aureus* and Methicillin resistant *Staphylococcus* in personnel based on farms in Sokoto and its environs

| Farms | Sample (n) | <i>S. aureus</i> ^a | MRSA ^b |
|--------------|------------|-------------------------------|---------------------|
| | | Number (%) Positive | Number (%) Positive |
| A | 2 | 1(50.00) | 1(50.00) |
| B | 3 | 1(50.00) | 0(0.00) |
| C | 2 | 0(0.00) | 0(0.00) |
| D | 1 | 0(0.00) | 0(0.00) |
| E | 2 | 1(100.00) | 0(0.00) |
| F | 4 | 2(50.00) | 1(25.00) |
| Total | 14 | 5(35.71) | 2(14.29) |

^a and ^b Expressed as percentages of "n"

layers (5.89%) than broilers (16.18%) (Table 2).

The growths of *S. aureus* isolates on oxacillin-supplemented ORSAB suggest that they are methicillin-resistant strains and also, production of PBP2 α by these isolates confirms that they are methicillin-resistant strains and thus may harbor *mecA* gene which encodes for the expression of PBP2 α (Persoons et al., 2009; Ugwu et al., 2015).

The result of this study is in contrast with that of Neela et al. (2013) who were not able to isolate MRSA from both

poultry farms in Switzerland and Malaysia, respectively. The difference in these occurrence rates could be attributed to the management practices by farmers in the study areas. In the present study, the birds are managed on deep-litter system which by design is difficult for instituting an effective control of disease and pathogens dissemination. The occurrence of MRSA in poultry in this study could also be due to indiscriminate administration of antibiotics to food animals by farmers without prescription by a veterinarian which is a common practice in Nigeria as documented by Broens et al. (2011). The occurrence of MRSA in chickens was significantly

associated with age as chickens younger than four weeks had higher prevalence (19.15%) as compared to those older than four weeks with prevalence of 3.37% ($P < 0.05$, $\chi^2 = 5.999$). This could likely be attributed to misuse or overuse of antibiotics as growth promoters and prophylactics which often results to emergence of drug resistant strains among bacteria. Also, the immune system in young birds is not fully developed as in adult, and maternally derived antibody of chicks is protective only for eighteen days thereby making younger birds more susceptible to diseases than adult birds, as described by [Begum et al. \(2006\)](#).

However, our findings (8.83%) was lower than the 19.4%, as reported by [Lin et al. \(2009\)](#). Similarly, [Akbar and Anal \(2013\)](#) and [De Boer et al. \(2009\)](#) documented this rate as 18.18 and 16.0%, respectively. This variation could be linked to the fact that these authors worked on poultry meat where chances of contamination during meat processing was high. According to [Broens et al. \(2011\)](#), the prevalence of MRSA could vary depending on many factors such as geographical region, sampling methods, and laboratory testing procedures.

The overall occurrence of MRSA in farm personnel estimated in this study was 14.29% (**Table 3**) which nearly agrees with the report of 15% by [Nworie et al. \(2013\)](#), but lower than that of [Oke and Adewale \(2013\)](#) who reported 83.3% occurrence among poultry farm workers in south-western Nigeria during an outbreak of diarrhea and attributed the high incidence to the on-going disease condition. The isolation of MRSA among this occupational group constitutes a serious threat to stakeholders and public health. According to [Rodríguez-Noriega et al. \(2010\)](#) colonization of man and other animals with MRSA constitutes a reservoir and potential source of related infections. Though, MRSA have been detected from farm personnel in this study, the source of infection have not been established, as supported by [Broens et al. \(2011\)](#).

CONCLUSION

This study shows for the first time the occurrence of MRSA in both chickens and farm personnel in poultry farms in Sokoto, Nigeria. Thus, the study provides a baseline data, which can be used for further studies considering epidemiology of MRSA.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

IDK and FMT conceived the study and study design. MBA and YY analyzed data. AAB and SJ wrote and edited the manuscript. All authors were involved in revising the manuscript and approved the final manuscript.

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