

NASAL COLONIZATION OF MULTI-DRUG-RESISTANT *STAPHYLOCOCCUS AUREUS* AND ASSESSMENT OF THE RISK FACTORS AMONG PIGS IN JOS, PLATEAU STATE, NIGERIA

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ABSTRACT

The emergence of multidrug-resistant *Staphylococcus aureus* (*S. aureus*) in food animals poses a growing threat to both animals and public health due to its zoonotic potential. There is limited available data on the assessment of the nasal colonization of multidrug-resistant *Staphylococcus aureus* and the risk factors among pigs in Jos, Plateau state, Nigeria. This study was carried out to determine the nasal carriage of *S. aureus* in pigs and evaluate the antibiotic susceptibility profile of the isolates. A total of 250 nasal swabs were collected from pigs in Jos-South Local Government Area, and analyzed using standard microbiological procedures. Positive isolates were subjected to antibiotic susceptibility testing and prevalence compared across age, sex and breed. Out of the 250 samples 38 (15.2%) were positive for *S. aureus*. Among the isolates, 6 were identified as methicillin-resistant *Staphylococcus aureus* (MRSA). The prevalence did not vary significantly based on age ($p=0.494$), and sex ($p=0.348$) of pigs. The prevalence was significantly higher in Hampshire than other breeds (41.7%; $p=0.016$). A large proportion 10/15 (66.7%) of the isolates exhibited multi-drug resistance to two or more antibiotics. Resistance to tetracycline was more common (80.0%), with greater proportion of the isolates being susceptible to ciprofloxacin (80.0%) and gentamycin (66.7%). The study highlights nasal carriage of multi-drug-resistant *Staphylococcus aureus* (MDRSA) among pigs which underscores the need for continuous surveillance, prudent antibiotic use and effective biosecurity measures to mitigate public health risk associated with antimicrobial resistance in foods animals.

Keywords: Antibiotic susceptibility, Jos, Multi-drug-resistant, Nasal swab, Pigs, *Staphylococcus aureus*

INTRODUCTION

Antimicrobial resistance (AMR) is a global public health concern that threatens effective treatment of infectious diseases in humans and animals. *Staphylococcus aureus*, a Gram-positive bacterium commonly found on the skin and mucosa, can cause a wide range of diseases from mild skin infections to life-threatening systemic conditions. The particular concern is MRSA, which limits treatment options

and increases morbidity, mortality, and healthcare costs (Lakhundi & Zhang, 2018; Cheung *et al.*, 2021).

While humans remain the principal hosts, increasing evidence indicates that animals, particularly livestock, play a significant role in the epidemiology of MRSA. Pigs have been identified as important reservoirs of MDRSA, with strains such as ST398 reported in both animals and humans, highlighting the zoonotic potential and the occupational risks faced by farmers, veterinarians, and abattoir workers (Wulf *et*

al., 2008; Podkowik *et al.*, 2013). Transmission can occur through direct contact, contaminated environments, or the food chain, underlining the One Health implications of AMR. Prior to the recognition that pigs and other livestock species can be reservoir of *S. aureus*, it was considered a relatively unimportant organism in pigs. Mounting concerns regarding the occupational and public health implications have stimulated research of *S. aureus* in animals and particularly pigs in many countries.

In low- and middle-income countries, including Nigeria, the misuse of antimicrobials in livestock production accelerates the emergence of resistant bacteria (Otaigbe & Elikwu, 2023). Despite growing pig farming practices in Plateau State, data on nasal carriage of MRSA and MDRSA in pigs remain scarce. In addition, pigs are in close contact with humans through farming, trade, and slaughter, creating opportunities for transmission of resistant bacteria. The absence of local data on nasal carriage of multidrug-resistant *S. aureus* in pigs poses a challenge for public health planning. Understanding the prevalence and associated risk factors is therefore essential for guiding interventions, promoting prudent antimicrobial use in animal husbandry, and reducing the risk of zoonotic spread within the community. Consequently, the objectives of the present research were to screen pigs for *Staphylococcus aureus* nasal carriage, to identify risk factors of *Staphylococcus aureus* nasal colonization and to determine the resistance pattern of *Staphylococcus aureus* isolates.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Jos-South Local Government Area, Plateau State, Nigeria. Plateau State is the twelfth largest state in Nigeria, situated roughly in the mid of the country with elevated hills at its boundaries surrounding the capital city of Jos. Jos South Local Government Area has four (4) districts which are; Gyel, Du, Kuru and Vwang. The Jos south has a total area of about 1,037km² with a population of about 306,716 (NPC 2006). The locations specify latitudes as 9.2422°N and 10.1153°N and longitudes as 8.6957°E and 9.5210°E which are 1, 280 m above sea level. The people are predominantly farmers and hunters. The common foods grown are Irish potato, sweet potato, maize, millet, fonio (acha), tomatoes and varieties of vegetables. The average temperature ranges are between 18°C and 22°C which gives the state a temperate climate at the higher altitudes even though it is located in the tropical zone (Chinyere *et al.*, 2020) The location is warmest between the months of March and April, while it sees a lowering in temperatures between December and February.

STUDY DESIGN

A cross-sectional study design was carried out. A purposive non-probability sampling technique was adopted to identify pig farms. A snowball technique where a pig farmer was identified and recommends other farmers to be included in the study was adopted.

SAMPLE COLLECTION

Two hundred and fifty (n=250) nasal swabs were collected from pigs in Jos South Local Government Area. The sample size was determined using the formula:

The sample size was determined using the formula: $N = Z^2pq/12$ (Thrusfield, 2007) Where $z=1.96$, $P=\text{prevalence}=19.4\%$ in pigs in Kebbi (Gaddafi *et al.*, 2021), $q=1-p$, $l=\text{allowable error of } 5\%$ $N= 241.55$. However, 250 samples were collected to increase precision. Information on the age, sex and breed of pigs were obtained and recorded. Samples were labelled and transported on ice to the Central Diagnostic Laboratory; National Veterinary Research Institute Vom, Plateau state, Nigeria.

CULTURE AND ISOLATION OF *STAPHYLOCOCCUS AUREUS*

Nasal swabs were inoculated onto 5% sheep blood agar (Oxoid, UK) and incubated aerobically at 37 °C for 24 hours. Presumptive *S. aureus* colonies were identified based on characteristic morphology, including golden-yellow pigmentation and zones of haemolysis (Figure 1). Representative colonies were sub-cultured for purity and subjected to further phenotypic characterization (Cheesbrough, 2006; Quinn *et al.*, 2011).

GRAM STAINING

Isolates were examined by Gram staining, a differential staining technique that distinguishes Gram-positive cocci from Gram-negative organisms, following standard protocols (Benson, 2002).

BIOCHEMICAL CHARACTERIZATION

CATALASE TEST

Catalase activity was determined by adding a loopful of bacterial colonies to a drop of 3% hydrogen peroxide on a clean glass slide. Immediate effervescence indicated a positive reaction, confirming catalase production typical of *Staphylococcus* species (Cappuccino & Sherman, 2014).

COAGULASE TEST

Coagulase production was tested using the slide agglutination method. A suspension of bacterial colonies was emulsified in sterile normal saline, followed by the addition of a drop of plasma. Visible clumping within 10 seconds indicated a positive result, differentiating *S. aureus* from coagulase-

negative staphylococci (*S. epidermidis*, *S. saprophyticus*) (Koneman et al., 2017).

DETERMINATION OF MRSA

All confirmed *S. aureus* isolates were tested for methicillin resistance using the cefoxitin disc diffusion method (Figure I), as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2011). Briefly, bacterial suspensions were adjusted to 0.5 McFarland turbidity and inoculated on Mueller–Hinton agar. A 30 µg cefoxitin disc (Oxoid, UK) was placed on the agar surface, and plates were incubated at 35–37 °C for 18–24 hours. Zone diameters were measured and interpreted according to CLSI guidelines: isolates with inhibition zones ≤ 21 mm were classified as MRSA, while those with zones ≥ 22 mm were considered methicillin-susceptible *S. aureus* (MSSA)

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility of confirmed *S. aureus* isolates was determined by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2011). Bacterial suspensions were adjusted to 0.5 McFarland turbidity standard and evenly spread on Mueller–Hinton agar plates. Commercial antibiotic discs (Oxoid, UK) were applied using a sterile disc dispenser. Plates were incubated at 37 °C for 18–24 h, and inhibition zone diameters were measured in millimeters. Results were interpreted as susceptible, intermediate, or resistant, according to CLSI breakpoints (Bauer et al., 1966; Murugkar et al., 2004).

DATA ANALYSIS

Descriptive statistics (Frequency, percentage, prevalence) was used to describe the study population. Chi-square test was used to determine the association between *S. aureus* carriage and factors such as age, sex, breed and location. P-value less than 0.05 will be considered significant. The statistical package for the social sciences (SPSS) version 27 was used to analyse the results.

RESULTS

Out of the 250 samples collected and analysed using standard microbiological procedures, 38 (representing a prevalence of 15.2%) were positive for *S. aureus*. Antibiotic susceptibility of the fifteen isolates showed 6 were MRSA (table I). The prevalence did not vary significantly based on age ($p=0.494$), and sex ($p=0.348$) of pigs (table II). The prevalence was found to be higher in Hampshire than other breeds ($p=0.016$). The result showed that greater proportion 10/15 (66.7%) of the isolates were resistant to 2 or more drugs suggesting that they are multi-drug resistant (table III). Four (4) of the isolates were resistant to only one antibiotic while one isolate was not

resistant to any drug (table III). Twelve (12) of the isolates making up 80.0% were resistant to Tetracycline (table III). One isolate was susceptible to 8 antibiotics while 3 were to 6 and 1 was susceptible to one (table III). Ciprofloxacin and gentamycin showed the highest zone of inhibition of the isolates (Figure II). Greater proportion (80.0%) of the isolates were susceptible to ciprofloxacin followed by gentamycin (66.7%) (Table IV).

Table I: Antibiotic susceptibility profile of fifteen *Staphylococcus aureus* isolates against Nine antibacterial agents.

<i>S. aureus</i> isolates	No. of susceptible drugs	Antibiogram (susceptible drugs)	% susceptible
C1	5	CIP, CN, FOX, S, MET	66.7
A5	6	CIP, CN, FOX, OX, S, MET	66.7
A14	4	CIP, CN, OX, S	44.4
B19	3	CIP, C, S	33.3
B2	3	CIP, OX, S	33.3
A13	2	CIP, E	22.2
B1	3	OX, S, MET	33.3
B14	1	CN	11.1
A19	8	CIP, CN, FOX, C, E, OX, S, MET	88.9
C13	3	CIP, CN, C	33.3
C17	5	CIP, CN, FOX, OX, MET	55.6
B5	6	CIP, CN, FOX, C, S, MET	66.7
B9	3	CIP, CN, FOX	33.3
D14	5	CIP, CN, FOX, OX, MET	55.6
D22	1	S	11.1

C= Chloramphenicol, OX = Oxacillin, CIP=Ciprofloxacin, FOX=Cefoxitin, CN=Gentamycin, MET=Metronidazole, E=Erythromycin, S = Streptomycin

Table II: Prevalence of *S. aureus* in pigs based on age, sex and breed

Variable	No. examined	No. positive	% prevalence	P-value
Sex				
Male	108	18	16.7	0.348
Female	142	20	14.1	
Breed				
Landrace	198	25	12.6	0.016
Hampshire	12	5	41.7	
Large white	40	8	20.0	
Age (years)				
>1	173	28	16.2	0.494
1–2	50	5	10.0	
>2	27	5	18.5	

Table III: Antibiotic resistance profile of fifteen (15) *Staphylococcus aureus* isolates against 9 antibacterial agents

<i>S. aureus</i> isolates	No. of drugs to which isolate was resistant	Antibiogram	% resistance
C1	1	TE	11.1
A5	1	TE	11.1
A14	4	FOX, TE, E, MET	44.4
B19	4	FOX, TE, OX, MET	44.4
B2	2	FOX, TE	22.2
A13	5	CN, FOX, C, OX, S	55.6
B1	3	CN, FOX, TE	33.3
B14	5	TE, C, E, OX, MET	55.6
A19	1	TE	11.1
C13	3	FOX, TE, MET	33.3
C17	3	TE, C, E	33.3
B5	1	OX	11.1
B9	3	TE, OX, MET	33.3
D14	0	0	0.0
D22	5	CN, TE, C, OX, MET	55.6

C= Chloramphenicol, OX= Oxacillin, CIP=Ciprofloxacin, FOX= Cefoxitin, CN= Gentamycin, MET=Metronidazole, E= Erythromycin, S= Streptomycin, TE= Tetracycline

worldwide, including Belgium (44%) (Stegger *et al.*, 2011), Germany (52%) (Lyon *et al.*, 1989), and Nigeria (55.5%) (Okunlola *et al.*, 2013). The emergence of livestock-associated MRSA (LA-MRSA) is of particular concern due to its ability to resist multiple antimicrobials (Kadlec *et al.*, 2009; Argudín *et al.*, 2011; Crombé *et al.*, 2013). This resistance has been largely linked to indiscriminate antimicrobial use in animal husbandry, which drives the selection of resistant strains. The present study also revealed no significant association between *S. aureus* carriage and pig age or sex, suggesting that these factors do not influence colonization, consistent with previous observations (Battisti *et al.*, 2010). However, breed-specific differences were noted, with a higher prevalence in Hampshire pigs, which may reflect genetic or management-related susceptibilities. Antimicrobial susceptibility testing showed that a greater proportion of isolates (66.7%) were resistant to two or more drugs, indicating multidrug resistance (MDR). Only one isolate was pan-susceptible, while 80.0% were resistant to tetracycline, a pattern consistent with reports of widespread tetracycline resistance among swine-associated *S. aureus* (Alt *et al.*, 2011; Peters *et al.*, 2015). The high resistance to tetracycline may be linked to its extensive use in veterinary medicine as a growth promoter and therapeutic agent. In contrast, resistance to chloramphenicol (26.7%), erythromycin (20.0%), gentamycin (20.0%), and streptomycin (6.7%) was comparatively lower, while ciprofloxacin resistance was absent. These findings agree with earlier surveys documenting variable but generally

Table IV: Antimicrobial Susceptibility pattern of *Staphylococcus aureus* isolates from nasal cavity of pigs in Jos, Plateau State Nigeria

Antibiotics	S	I	R	(%)
C I P (5 u g)	1 2 / 1 5 (8 0 . 0)	3 / 1 5 (2 0 . 0)	% (0 . 0 0)	
C N (1 0 u g)	1 0 / 1 5 (6 6 . 7)	3 / 1 5 (2 0 . 0)	2 / 1 5 (1 3 . 3)	
F O X (3 0 u g)	5 / 1 5 (3 3 . 3)	2 / 1 5 (1 3 . 3)	8 / 1 5 (5 3 . 3)	
T E (3 0 u g)	0 / 1 5 (0 . 0)	0 / 1 5 (0 . 0)	1 5 / 1 5 (1 0 0 . 0)	
C (3 0 u g)	2 / 1 5 (1 3 . 3)	7 / 1 5 (4 6 . 7)	6 / 1 5 (4 0 . 0)	
E (5 u g)	2 / 1 5 (1 3 . 3)	9 / 1 5 (6 0 . 0)	4 / 1 5 (2 6 . 7)	
O X (1 u g)	0 / 1 5 (0 . 0)	% (0 . 0)	1 5 / 1 5 (1 0 0 . 0)	
S (5 0 u g)	8 / 1 5 (5 3 . 3)	4 / 1 5 (2 6 . 7)	3 / 1 5 (2 0 . 0)	
M E T (1 0 u g)	0 / 1 5 (0 . 0)	0 / 1 5 (0 . 0)	1 5 / 1 5 (1 0 0 . 0)	

C=Chloramphenicol, OX=Oxacillin, CIP=Ciprofloxacin, FOX=Cefoxitin, CN=Gentamycin, MET=Metronidazole, E=Erythromycin, S=Streptomycin; S-Susceptible, I-Intermediate Susceptibility, R-Resistant (CLSI, 2014)

DISCUSSION

In this study, the prevalence of *S. aureus* among pigs in Jos, Plateau State, was 15.2%, with 38 isolates recovered from 250 nasal swabs. This prevalence aligns with earlier findings from a report by Kwaga *et al.* (2018) of 17.0% in pigs from Kogi State, Nigeria. However, this finding is considerably lower than the 82.1% prevalence reported in Italy in a study done by Pirolo *et al.* (2019). Such variations may be attributed to differences in husbandry practices, biosecurity measures, and antimicrobial usage across regions. Methicillin-resistant *S. aureus* (MRSA) accounted for 40.0% of the tested isolates in this study. Comparable studies have reported MRSA prevalence ranging between 21–55% in swine populations

lower resistance rates to fluoroquinolones and aminoglycosides in livestock-associated *S. aureus* (Kadlec *et al.*, 2009; Zarfel *et al.*, 2013; Mutters *et al.*, 2016).

Ciprofloxacin and gentamycin were the most effective antibiotics in this study, with 80.0% and 66.7% susceptibility rates, respectively. These agents demonstrated the largest inhibition zones, underscoring their potential utility in treating *S. aureus* infections in pigs. Nevertheless, the presence of MDR strains remains a significant concern for both animal health and public health, particularly given the zoonotic potential of LA-MRSA and its documented transmission between animals and humans (Bahrami *et al.*, 2021).

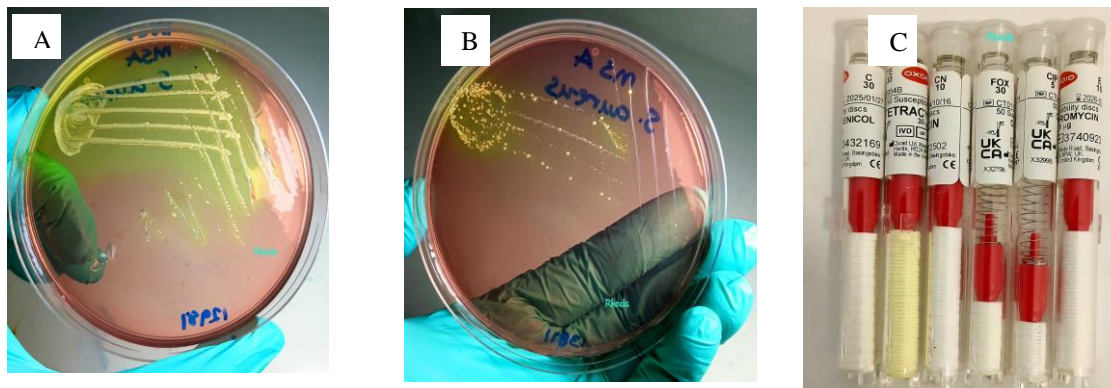


Figure I: A & B: Culture plates for *Staphylococcus aureus*; C: Antimicrobial discs

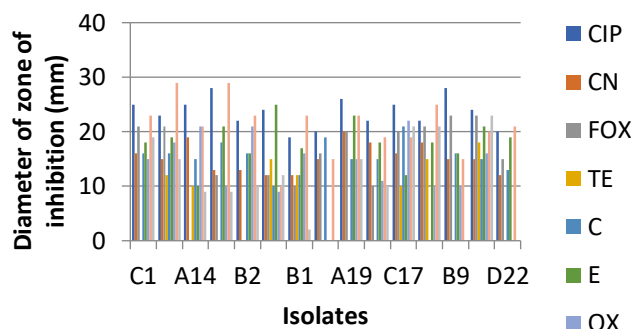


Figure II: Diameter of zone of inhibition by some antibacterial drugs on some *S. aureus* isolates

CONCLUSION

The prevalence of MRSA in pigs in the studied region is moderate. Susceptibility testing revealed extensive resistance to different classes of Antimicrobials, especially those commonly used in pig husbandry. The findings underscore the importance of monitoring the evolution of MRSA in pig farms in order to implement control measures and reduce the risk of spread in the animal and human population. Further research should be done to extensively monitor antimicrobial resistant pattern as well as identify the genes responsible.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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