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Proceeding Paper

Occurrence and Antibiotic Sensitivity Patterns of Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus* in Pigs in Ibadan, Nigeria †

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Abstract: ***Background:*** *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA), has emerged as a leading antimicrobial-resistant pathogen challenging global health systems and antibiotic therapy. Pigs have been identified as important reservoirs for livestock-associated MRSA. The major concern with MRSA occurrence in pigs is the potential for human transmission. Reports on the prevalence and antibiotic sensitivity pattern of methicillin-susceptible *S. aureus* (MSSA) and MRSA strains in pigs in Nigeria are still limited, hence, the need for this study. This study was designed to investigate the prevalence of MRSA and methicillin-sensitive *Staphylococcus aureus* in pig farms in Ibadan, Oyo State, Nigeria, and to determine their antibiotic resistance patterns. ***Methods:*** We collected 93 composites (n = 5; total n = 465) of faecal samples from pigs in twenty-five farms across five local government areas in Ibadan, Nigeria. Isolation of *S. aureus* was conducted using standard procedures. Antibiotic sensitivity testing was conducted using the disc diffusion method. The data obtained were analysed using descriptive statistics and compared with the CLSI and EUCAST standards for sensitivity and resistance. ***Results:*** The prevalence of *S. aureus* was 31.2%, with the proportion of MSSA and MRSA isolates being 23.7% and 7.5%, respectively. The antibiotic susceptibility profiles revealed a high multidrug resistance prevalence among both MSSA (86.4%) and MRSA (100%). All MRSA isolates and 40.9% (9/22) of MSSA were found to be resistant to at least five different sub-classes of antibiotics. ***Conclusions:*** This study supports the existing reports on pigs being an important reservoir of highly resistant *S. aureus* strains. The high multidrug resistance and the occurrence of MRSA may be evidence of continuous antimicrobial exposure and substandard hygienic practices on these farms. This is undesirable because it constitutes a health hazard for farmers, veterinarians, abattoir workers, and pork consumers, who may further disseminate these highly resistant strains to their families and society. There is a need for further surveillance and a multisectoral approach involving policymakers, farmers, health practitioners, and the public in implementing good infection control practices and safe antibiotic usage from the grassroots level on farms in line with the vision of the one health approach.

Keywords: AMR; antimicrobial resistance; *Staphylococcus aureus*; MRSA; methicillin; pigs

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1. Introduction

Staphylococcus aureus (S. aureus) occurs naturally as part of the flora found in the nares and on the skin of humans and animals [1]. According to the global priority pathogens list

of antibiotic-resistant bacteria, *S. aureus* is one of the most clinically significant multi-drug-resistant concerns globally due to its resistance to methicillin and other antibiotics [2].

Methicillin was introduced into medicine as an antibiotic against *S. aureus* in the year 1959 as a replacement for penicillin which was formerly the most effective beta-lactam antibiotic against *S. aureus* infections up until the 1940s, when *S. aureus* became resistant to it by producing plasmid-encoded beta-lactamase enzyme (penicillinase). This enzymatically cleaved the beta-lactam ring of penicillin, rendering the antibiotic inactive [3].

Methicillin, a semi-synthetic penicillin, acted as a better substitute for penicillin because it could resist the enzymatic breakdown by *S. aureus* penicillinase; however, methicillin resistance in *S. aureus* isolates started to become a challenge for antibiotic therapy in less than two years and, by 1961, the first MRSA isolate was reported in the United Kingdom [4].

Methicillin resistance in *S. aureus* results from the production of an additional penicillin-binding protein (PBP) called *PBP2a*, which has a decreased affinity for penicillin and beta-lactam antibiotics [5]. Beta-lactam antibiotics usually act against *S. aureus* by rendering its four native PBPs inactive, thereby hindering their roles in the synthesis of the peptidoglycan chains that make up the bacterial cell wall [6].

PBP2a can replace the inactive PBPs of *S. aureus* in peptidoglycan cell wall synthesis because it is not inhibited by beta-lactams, resulting in the pathogen's resistance [5]. PBP2a is produced by a gene known as *mecA* and its regulatory genes, *mecI* and *mecR1* collectively designated *mecA* complex. A 30–60 kb chromosomal element known as the staphylococcal chromosomal cassette mec (SCCmec) carries the *mecA* complex [6].

Pigs have been identified to be asymptomatic carriers of MRSA, although infections are usually infrequent in them [7]. Both asymptomatic carriers and infected pigs can transmit *S. aureus* to other animals and humans. The risk of contracting MRSA infections by humans is further enhanced in livestock production facilities where animals are often reared closely with human habitations, with poor or non-existent biosecurity procedures, farm waste being dispersed carelessly, and antibiotics repeatedly abused or misused. This is often the situation in developing nations, including Nigeria and most African countries [8].

The risk of transmission to humans is the primary concern with the occurrence of MRSA in pigs [9]. Studies on the prevalence of *S. aureus*, including methicillin-susceptible (MSSA) and methicillin-resistant strains, in animals, particularly pigs in Nigeria, are limited and have only been conducted in a few regions [10–12], and this study was therefore designed to fill the knowledge gap.

2. Methodology

2.1. Study Location

The field survey was conducted on selected pig farms across five local government areas in Ibadan, Oyo state, and the laboratory aspect at the Food and Meat Hygiene Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Oyo state in Nigeria. The climate is equatorial, with notably dry and wet seasons and relatively high humidity. The dry season lasts from November to March while the wet season starts in April and ends in October. The average daily temperature ranges between 25 °C (77.0 °F) and 35 °C (95.0 °F), almost throughout the year. The study area's agricultural production system is a mixed crop and livestock farming system.

2.2. Sampling

Sample size (N) was estimated to be 384 using the Thrusfield formula.

$$N = \frac{Z^2 \times P(1-P)}{d^2}$$

with 95% confidence interval (Z), 50% expected prevalence (P), and 5% absolute precision (d) [13].

Twenty-five farms located across five local government areas (Akinyele, Ibadan North, Ido, Lagelu, and Ona-ara) in Ibadan, Oyo State were surveyed. Pigs of varying age groups, including weaners, growers, and finishers, on the farms were sampled for early morning faecal droppings. Ninety-three composite faecal samples were collected across all the farms. Each composite sample was made of fresh faecal samples (about 5 g each) from 5 separate pigs, giving a total sample size of 465 pigs. Faecal samples were collected by handpicking them from the floors of each pen using sterile hand gloves and then transported to the laboratory with labelled Ziploc bags in an icebox. Samples were enriched by incubating them overnight at 37 °C with Buffered Peptone water.

2.3. Bacteriological Examination

Following enrichment, the inoculum was streaked directly on Mannitol Salt agar and incubated at 37 °C for 24–48 h. The primary culture yielded a variety of bacteria colonies, most being yellow colonies surrounded by yellow zones, presumptive for *S. aureus* colonies. Sub-culturing of the individual colony was performed on Mannitol Salt agar to obtain pure cultures. Various biochemical tests were carried out to identify the isolates, including catalase, oxidase, and coagulase tests. Gram staining of the pure presumptive *S. aureus* colonies and microscopic examination using an oil immersion objective (100×) lens was conducted to determine the Gram-staining reaction and morphology, respectively.

2.4. Phenotypic Identification of MRSA and In-Vitro Antibiotic Susceptibility Test

The antimicrobial sensitivity testing was conducted using the Kirky Bauer disc diffusion method using nine antibiotics namely, ampicillin (AMP; 10 µg), erythromycin (ERY; 15 µg), ciprofloxacin (CIP; 5 µg), penicillin (PEN; 10 µg), tetracycline (TE; 30 µg), amoxicillin-clavulanic acid (AMC; 30 µg), neomycin (N; 30 µg), enrofloxacin (ENR; 5 µg), and cefoxitin (FOX; 30 µg). A colony of each of the pure isolates was diluted with normal saline to prepare a bacterial suspension until the suspension's turbidity matched a 0.5 McFarland turbidity standard equivalent to approximately 150 million cells per mL [14]. This standardised suspension was streaked over the Mueller-Hinton agar to obtain a smooth homogenous lawn. The discs were placed gently to make full contact with the media and microbial lawn using an antimicrobial susceptibility disc dispenser. Plates were incubated at 37 °C for 18 to 24 h. The inhibition zones of the different antibiotics were measured and recorded in millimetres (mm) and results were expressed in terms of sensitivity (S), resistance (R), and intermediate (I) based on interpretations obtained from CLSI standards [15]. Data were analysed using descriptive statistics.

3. Results

Staphylococcus aureus was recovered from pigs raised in four local government areas and 29/93 (31.2%) samples, with Akinyele having the highest number of isolates (Table 1). More isolates were recovered from the finisher pigs than other age groups, although more samples were collected from the finishers than the others (Figure 1).

Table 1. Sample distribution and rate of isolation of *Staphylococcus aureus*.

Location (Number of Samples Collected)	Isolates of *Staphylococcus aureus*	
	Number	%
Akinyele (n = 19)	10	10.8
Ido (n = 19)	0	0
Ibadan North (n = 17)	9	9.7
Lagelu (n = 19)	6	6.4
Ona ara (n = 19)	4	4.3
Total (N = 93)	29	31.2

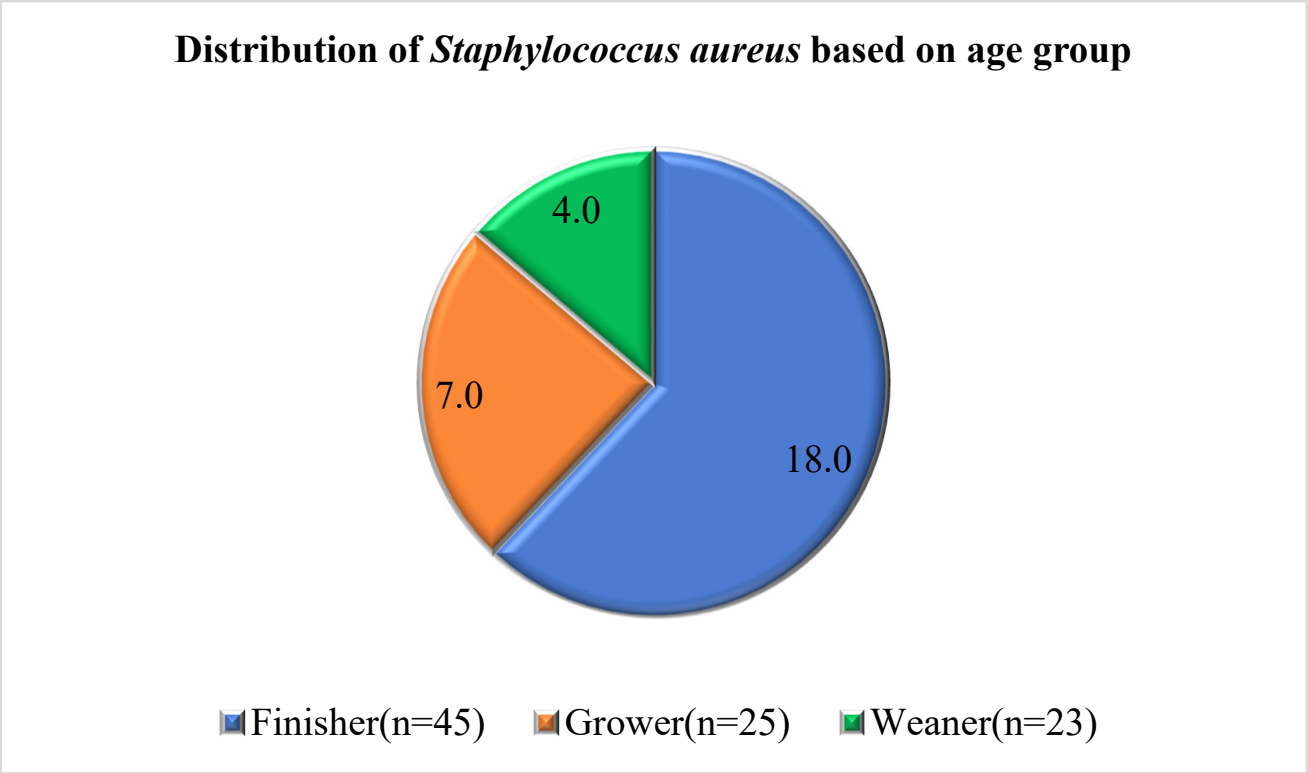

Figure 1. Distribution of *Staphylococcus aureus* based on Age Group.

Antimicrobial susceptibility tests (AST) revealed an absolute resistance of 100% by isolates to ampicillin, penicillin, and amoxicillin-clavulanic acid. A high level of resistance was also demonstrated by isolates to tetracycline (79.3%) and erythromycin (75.9%). AST phenotypically revealed the presence of MRSA through cefoxitin resistance in 7.5% (7/93) of the samples collected which amounted to 24.1% (7/29) of the recovered isolates, while the remaining isolates (75.9%; 22/29) were methicillin-sensitive (Figure 2).

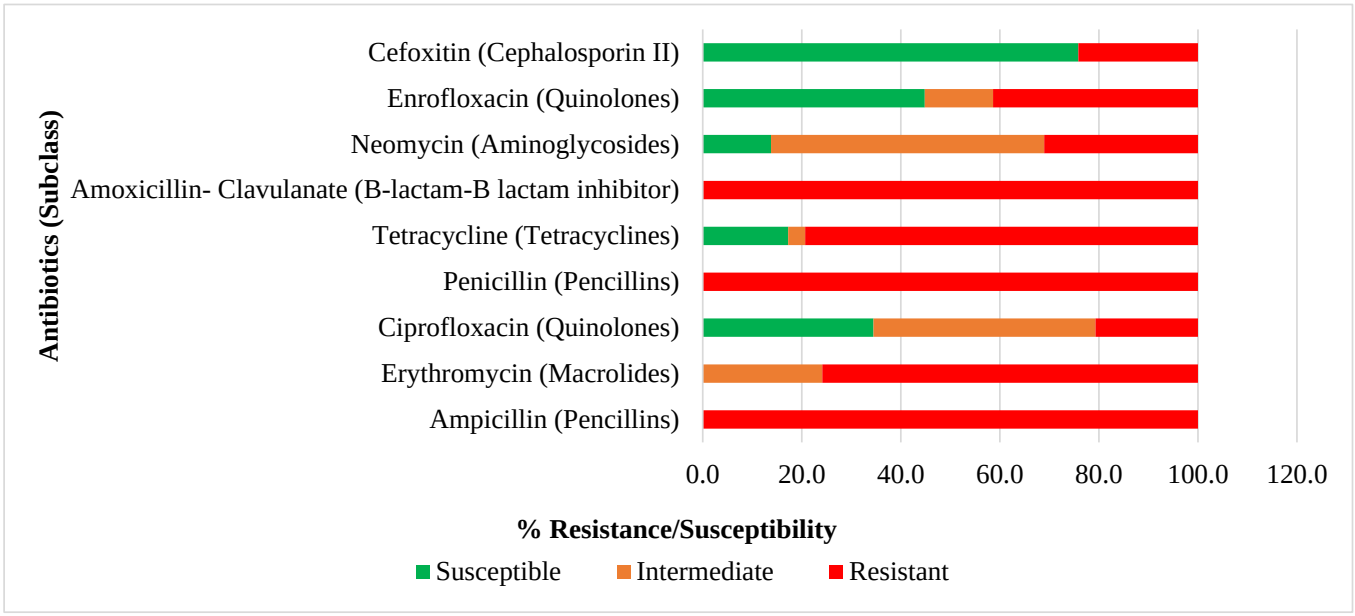

Figure 2. Antimicrobial Resistance Profile of *S. aureus* isolated from pigs within Ibadan, Nigeria.

MRSA isolates demonstrated a higher prevalence of antibiotic resistance than MSSA isolates (Figure 3).

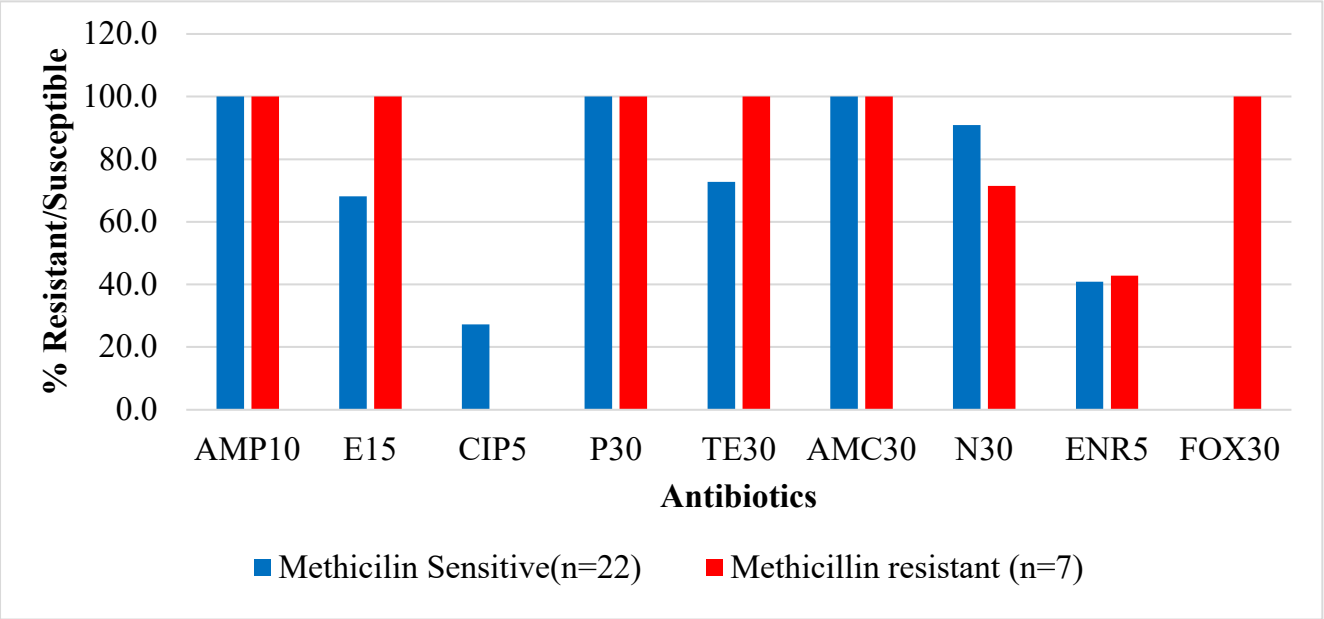

Figure 3. Comparison of the Resistance Patterns of Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus*.

The highest prevalence of MRSA was recorded in Ibadan-North despite having the least number of samples collected (Table 2). On the other hand, finishers had a higher prevalence of MRSA among the three age groups (Table 3).

Table 2. Distribution of MRSA isolates from pigs across five local government areas in Ibadan.

	MRSA Isolates Recovered	
Location and Number of Samples Collected	**Number**	**%**
Akinyele (n = 19)	1	1.1
Ido (n = 19)	0	0
Ibadan North (n = 17)	5	5.3
Lagelu (n = 19)	1	1.1
Ona ara (n = 19)	0	0
Total (N = 93)	7	7.5

Table 3. Distribution of MRSA isolated from different age groups of pigs in Ibadan.

	Number of MRSA Isolates Recovered	
Age Group (Number of Samples)	**Number**	**%**
Finisher (n = 45)	3.0	3.2
Grower (n = 25)	2.0	2.1
Weaner (n = 23)	2.0	2.1
Total (N = 93)	7.0	7.5

Findings also revealed a high prevalence of multidrug resistance, MDR (89.6%; 26/29), and extra drug resistance, XDR (55.2%; 16/29), observed among all *S. aureus* isolates. Among MRSA and MSSA, there was MDR prevalence of 100% (7/7) and 86.4% (19/22), respectively, and XDR prevalence of 100% (7/7) and 40.9% (9/22), respectively.

4. Discussion

This investigation provides information on the characteristics and antibiotic sensitivity patterns of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* isolated from pigs raised intensively in Ibadan, Oyo State. A prevalence of 31.2% (29/93) was obtained which was significantly higher than the 6.5% reported in a previous cross-sectional study conducted by Odetokun et al. [16], who used phenotypic and molecular techniques to identify *S. aureus* from the nasal swabs of slaughtered pigs, goats, cattle, and abattoir workers in Ibadan and Ilorin. Other recent studies that have reported a level of occurrence of *Staphylococcus aureus* similar to our findings include Mamfe et al. [17], who reported a prevalence of 19.1% from the nasal swabs of pigs in Makurdi and Sineke et al. [18], reported a prevalence of 29.1% from a combination of samples, particularly pig faecal samples, farm slurry samples, and human nasal swabs in pig production settings in South Africa. The relatively high prevalence observed in the various recent studies points towards the fact that pigs are important carriers of the pathogen and may contribute to the propagation of *S. aureus* to humans.

Our investigation on methicillin resistance, using Cefoxitin antibiotics, revealed a prevalence of 7.5% (7/93) of MRSA, while MSSA was 23.7% (22/93). This finding is comparable with a previous study in Ilora, Oyo State by Okunlola and Ayandele [10], who reported a 9% MRSA prevalence from the nasal swabs of pigs and higher than the report of 1.2% in Ibadan and Ilorin [16], and 4.7% in Kogi [11]. Ref. [19] combined nasal and rectal sampling and they reported a prevalence of 14.9% in pigs in Benin City, Nigeria. Meanwhile, Dweba et al. [20] reported an MRSA prevalence of 55% in pigs in South Africa. This inconsistency with respect to MRSA prevalence in the different studies may be attributed to the types of samples collected, the varying isolation and characterisation techniques employed, and the different geographical settings where the studies were carried out.

From the view of the five local government areas sampled, *S. aureus* was isolated from four of them, namely, Akinyele, Ibadan North, Lagelu and Ona ara, with the least number of isolates recovered from Ona ara, while Akinyele and Ibadan North contributed the highest number of isolates. MRSA was isolated from three out of the four local governments where *S. aureus* was found. Ona ara local government had no MRSA. 71.4% (5/7) of the MRSA isolates were from pigs in Ibadan North alone, thus identifying Ibadan North Local Government as a potential hotspot for MRSA. While the reason for the higher prevalence from Ibadan North was not fully elucidated, it may be related to substandard hygienic practices and the improper antimicrobial usage culture observed on the farms in this region.

Similarly, among the three age groups, MRSA isolates were found to be higher among the finishers. These findings may be related to the fact that they had been around on the farms for a longer time and therefore, may have accumulated more pathogens than the younger animals in addition to the fact that more samples were obtained from this age group.

Antimicrobial susceptibility testing (AST) revealed that the *S. aureus* isolates, both MRSA and MSSA, demonstrated high levels of resistance (100%) to β-lactam antibiotics including penicillin, ampicillin, and amoxicillin-clavulanic acid. This resistance pattern is similar to the findings reported by Mamfe et al. [17], who observed a 100% resistance to β-lactam antibiotics by *Staphylococci* isolates in Makurdi, Nigeria, and this may be attributed to the possibility that the isolates may have acquired the ability to produce β-lactamase due to prolonged antibiotic misuse, thereby, neutralising the antibiotic effects of β-lactams.

Varying resistance rates were also reported against other antibiotics from as low as 20.7% against ciprofloxacin to 79.3% against tetracycline. The high resistance rates of 75.9% and 79.3% recorded against erythromycin and tetracycline, respectively agree with the findings reported by Lim et al. [21], who also reported a high prevalence of erythromycin and tetracycline-resistant *S. aureus* strains in a study conducted in Malaysia. This finding is disturbing as it implies that there would soon be a limit in the choices of antibiotic drugs available for treatment against infectious diseases. Erythromycin and tetracycline resistance

are always attributed to the presence of resistance genes, and this may be because these drugs form part of the most readily available antibiotics that are continuously abused by farmers; hence, the tendency of the organisms to acquire resistant genes to them with time.

A comparison of the antibiotic resistance patterns of both MRSA and MSSA isolates revealed that MRSA isolates manifested a higher level of resistance to antibiotics than MSSA. Apart from Ampicillin, Penicillin, and Amoxicillin-Clavulanic acid, which both MRSA and MSSA were resistant to, all the MRSA isolates were resistant to Tetracycline, Erythromycin, and Cefoxitin. Bhatta et al. [22] reported a similar finding where MRSA isolates were more resistant to antibiotics than MSSA. This finding is consistent with the usual behaviour of MRSA isolates which are naturally more resistant because they possess genes that give them the ability to readily acquire resistance to most antibiotics.

The high prevalence of multidrug and extra-drug resistance observed among all *S. aureus* isolates reflects prolonged antibiotic misuse and abuse on the farms.

5. Conclusions

This study supports the existing reports on pigs being a potential reservoir of highly resistant *S. aureus* isolates. The high multidrug resistance and the occurrence of MRSA may be evidence of continuous antimicrobial exposure and poor biosecurity measures on these farms, potentially leading to the acquisition of resistant genes by the resistant strains. There is the possibility of a horizontal transfer of resistant genes to other staphylococcal species such as *S. hyicus* also implicated in exudative epidermitis of pigs.

Some of the concerns related to the transmission of these resistant strains include limitations in choices of drugs available for treatment, difficulty in treating infections, prolonged hospitalization and medication, increased morbidity and mortality rates, increased healthcare costs, and economic losses.

There is a need for further studies to conduct molecular characterisation of the isolates from this study in a bid to identify the genes responsible for resistance. To address the increasing prevalence of antimicrobial resistance, further surveillance, and a multisectoral approach involving policymakers, farmers, health practitioners, and the public should be implemented.

The risks of widespread transmission of these resistant strains and a persistent MRSA prevalence exist if unhealthy practices ranging from substandard hygienic culture to antibiotic misuse or abuse are not checked. The adoption of good infection control measures, safe antibiotic usage, proper livestock management, and the use of alternative antibiotics and non-antibiotic therapies across all levels is strongly recommended to achieve better public health outcomes.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to the fact that the samples used in this study were not obtained directly from the bodies of the animals. The faecal samples collected were early morning fresh faeces which had just been dropped on the floors and environment within the pens by the pig in each pen.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author, (O.S.F.).

Conflicts of Interest: The authors declare no conflict of interest.

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