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RESEARCH ARTICLE

CHARACTERIZATION OF MULTI-DRUG RESISTANCE LIVESTOCK ACQUIRED METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (LA-MRSA) ISOLATES FROM DIFFERENT SWINE FARMS IN ENUGU METROPOLIS, NIGERIA

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Abstract

Aim and Objective: Globally, livestock animals, particularly swine, calves and poultry are colonized by Livestock acquired methicillin resistant *Staphylococcus aureus* (*S. aureus*) (LA-MRSA). This study was aimed at screening for multi-drug resistant in LA-MRSA strains isolated from swine from selected swine farm within Enugu metropolis.
Methods: A total of 307 pig nasal swabs samples were collected, from farm A (76), farm B (116) and from C (108) in Enugu metropolis. Isolated *S. aureus* and LA-MRSA strain were phenol-typing screened and identified for MRSA using Kirby-Bauer disc diffusion method with 1 µg oxacillin/30 µg cefoxitin antibiotic Disc and Tetracycline Disk Test respectively. Antibiogram studies of LA-MRSA against several antibiotic discs and multiple antibiotic resistance indexes were determined.
Results: Results showed overall isolation rate of 76.2% *S. aureus* comprising of 90.5%, 80.3% and 62.9% in Farm B, Farm A and Farm C respectively, total MRSA detection rate of 125 (40.7%) comprising Farm A 50.0%, Farm B 46.6%, Farm C 30.6%. LA-MRSA were identified in 84(27.4%) of swine with high proportion of 29(38.2%) in Farm A followed by Farm C30(27.8%) and Farm B 25(21.6%). LA-MRSA from Farm A Nursery: 27.3%, Weaning 0.0%, Grower 77.0% while Farm B Nursery 24.1%, Weaning 0.0%, Grower 7.3%, finisher 100% and Farm C Nursery 28.0%, Grower 34.5% and finisher 53.3%. LA-MRSA isolates exhibited a significantly ($p \leq 0.05$) high% resistance within the range of 50-100% against tetracycline, erythromycin, cefotaxime, clindamycin, Trimethoprim-Sulfamethoxazole and exhibit MDR with MARI value ≥0.3 but were susceptible to ciprofloxacin 77.8%, amikacin 100% and imipenem 100%.
Conclusion: LA-MRSA strains increased levels of MDR phenotype suggest that the emergence of LA-MRSA in swine up keeping could be promoted through veterinary antibiotics. Thus, to prevent antimicrobial resistance in animals and humans, joint cross-examination of multi-resistant livestock acquired *S. aureus*, with an incorporated 'One Health' advancement is needed for effective curbing and control measures for LA-MRSA infections.
Keywords: Antibiotics, Bacteriological, Isolation, Livestock, Multi-drug resistance, *Staphylococcus aureus*.

INTRODUCTION

As a facultative anaerobe, when viewed under a microscope, *Staphylococcus aureus* are clustered in grape-like form, as large, round, golden-yellow colonies, and when grown on blood agar, it presents regular hemolysis. *S. aureus* is a pathogenic food borne agent to both animal and human that causes skin infection, pneumonia and septicemia[1]. The genus of Staphyloccocal consists of over fifty species known as commensals microorganisms as well as opportunists of mammals and birds, responsible for diverse clinical

issues. *S. aureus* is tagged environmental pollutants found on the skin as normal flora with no disease incidence. However, contamination of food both at pre and post-preparation is commonly attributed to *S aureus*. This could buttress the fact why food products such as fish products, dairy products, farm animals and products, and milk are common sources from which *S aureus* is isolated[2]. Owing to the eruption of increasing resistant strains, capable of resisting and outliving new therapeutic antibiotic, a global public health concern is shifted to antibiotic resistance[3]. *S. aureus* resistant strains are reported to be resistant against dyes, antibiotics, heavy metals and disinfectants, and these traits of genetic variation show that *S. aureus* is capable of spreading due to its adaptive and evolutionary abilities[4].

The most significant resistance mechanism acquired by *S. aureus* against β-lactam antibiotics, is the staphylococcal cassette chromosome *mec* (*SCCmec*), a moving genetic element, consisting of the genes, mec A and C. Obviously, the widely reported strains of *S. aureus* species with this resistance trait are known as methicillin-resistant *S. aureus* (MRSA). Globally, the possession of this resistance mechanism by this bacterium transforms its epidemiological understanding. Several animal species are reported to be associated with LA-MRSA since 2005, affecting humans as a result of infections[4,5]. Emergence of LA-MRSA have been reported in calves, swine and among humans who have occupational relation with livestock in European countries[6,7] and in Nigeria in 2022[8]. In humans, MRSA prevalence range from 0.8-1.3%[9] and report from[10] stated that among veterinarian, its prevalence rate is 0 and 50%. The most commonly reported and dominant strains of LA-MRSA in Europe is CC398 (*spa* t011) and in Asia is CC5 (*spa* type t002). However, the classical strain for LA-MRSA is reported to be CC398, and it is said to be methicillin-susceptible *S. aureus* and causing infections in human[10,11,12]. In Italy, CC97 strains of MRSA was reported to be transmitted from swine to cattle, and could affect several species of livestock. Thus, the strain of MRSA, how long it stays on animals and the age of animals are factors to be considered for MRSA infection, and baby swine are the higher risk group and should be given greater attention[13-15]. In 2012, the 1st CC398 MRSA case was among veterinarian and farmers in Irish[16], and among swine farmers, veterinarians and slaughter workers, in Switzerland in 2009[17].

In 2013, CC398 LA-MRSA strain was 1st isolated in a Turkish poultry farm[18], with the isolates showing that the poultry farm was invaded[19]. While in the United Kingdom, and in 2014, CC398 LA-MRSA was 1st isolated and confirmed in a piglet with wasting and pneumonia, from a swine farm by the Omagh disease surveillance laboratory, AFBI[20], and APHA veterinary center[21], was also isolated from non-diseased pig caecal remain in England at an abattoir by APHA laboratory research[22]. The use of antibiotics promotes the dissemination of MRSA in a non supportive environment for the survival of non-resistant microorganism. Hypothetically, disallowing the use of antibiotics, strains of bacterial such as *S. aureus* would overwhelm MRSA owing to dominant resistant genes[23]. It was reported that decreased administration of antibiotics in a controlled hospital environmental resulted in an improved reduction of MRSA[4], but not completely eliminated resistance of bacterial[24]. An effective strategic potential of eliminating the bacterium is thought of changing the antibiotic class of drug more frequently. However, this could develop the chance for multi-drug resistance with susceptible strains that are treated, creating new resistance with every change in antibiotic[10,25]. Attempt to treat and prevent MRSA employed the use of probiotics as antibiotics alternatives, where *Lactobacillus* spp., a lactic acid bacteria presented an inverse correlation to *S. aureus*, inferring that such bacteria could prevent the growth of LA-MRSA[10,25]. Also, vaginal lactobacilli, which produces H_2O_2 is thought to possess anti-bacterium effect against *S. aureus*[10]. Owing to the emerging concern of LA-MRSA, implementation of control measures, to monitor the evolution and reduction of the risk of spread in pig farms and population of animals in Nigeria is imperative. Therefore, it became imperative to determine the spread and multi-drug resistant model of Live-stock related methicillin resistant *S. aureus* isolates from swine farms within Enugu metropolis.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used are of high analytical grade from Mark scientific and Chemical Company England, AFB Biochemicals, Nigeria and Damazo Nigeria Limited, Nigeria.

Media and Antibiotic Discs

All media and antibiotic discs used were purchased from Oxoid limited (Oxoid Ltd, Basingstoke Hamsphire, Uk).

Study Area

Samples were collected from Farm A, Farm B and Farm C, as the study area in Enugu State. This study area is found in the metropolitan city of Enugu State. As a state in the South East of Nigeria, situated at the Udi Upland, to the south, Enugu has border with Imo and Abia State. To the east, it shares border with Ebonyi state, to the northeast, with Benue State, to the west, with Anambra State and to the northwest, to Kogi State. The metropolitan city is characterized with an averagely hot temperature of 87.16 ^oF (30.64^oC) in the warm weather period in the month of February and mild temperature of 60.54^oF (15.86 ^oC) in the cooler weather period in the month of November. The climate and soil/land conditions are favourable with approximately 733 ft (224 m) higher than the level of the sea. The state experiences the lowest rainfall (0.16 cm^3) in February and highest of about (35.7 cm^3) in July.

Determination of Sample Size

Sample size was calculated using the formula

$$n=(Z_1\text{-}\alpha)2(p(1\text{-}p)\backslash d^3$$

Where n=sample size; *p*=estimated proportion; d=expected precision. With a prevalence rate

approximated to 63.6%, 5% precision and 95% interval of confidence, the sample size was calculated; and 1.96 was calculated as the value of Z_1-α at significance level of 5%. Finally, a random selection from 3 different farms (A, B and C), gave a sample size of 300 swine by the application of the formula with 10% attrition.

Study Consent

This study employed the use of both written and verbal consent from the management of the various swine farm A, B and C within Enugu metropolis selected for the study.

Collection of Samples

From farms A, B, and C; a total 307 samples of nasal swab were collected from swine, consisting of 76, 116 and 108 respectively, across board that is from nursery, weaning and finisher swine in each farm, according to the size of the flock. In order to improve the rate of isolation of *S. aureus*, plastic swabs were pre-moistened in sterile 0.9% NaCl as described by Lahuerta-Marin *et al.*,[26]. The sample swabs were collected, after labeling, and in an ice park, were transported to Microbiology laboratory unit of Caritas University for bacteriological analysis within one hour as described by Lahuerta-Marin *et al.*,[26].

Phenotypic Detection of Methicillin Resistance *S. aureus* (MRSA)

Brilliance MRSA II Agar

Suspension of isolates of *S. aureus* was adjusted to 0.5 McFarland turbidity equivalent standard (MFTES). Seeded on plate of sterilized Brilliance agar was a sterile swab dipped in each suspension. Positive for MRSA was confirmed by the presence of blue colony growth after overnight incubation at 35^{O}C and absence of blue colony is indicative of MRSA positive strain[27].

Oxacillin Resistance Disk Test

Oxacillin resistance was tested using 1 µg oxacillin disc diffusion. Inoculation of 10 µL of 0.5 McFarland (10^6 CFU/ml) suspension of the isolate with Mueller-Hinton agar (MHA) plates containing 4% NaCl. Was done by smearing and then incubated for 24 hours at 35^{o}C. Resistance for oxacillin was seen as a zone diameter of ≤ 10 mm according to the CLSI[29].

Cefoxitin Disk Diffusion Test

Using the Kirby-Bauer disk diffusion method, MRSA strains were detected. Briefly, 0.5 MFTES of test bacteria were aseptically smeared on MHA plates and 30 µg Cefoxitin disk was place on the plate(s) and incubated for 24 hours at 37^{o}C. Test isolate phenol-typing of MRSA are indicated with zones of inhibition ≤ 21 mm for the Cefoxitin antibiotic against the test isolate and >21 mm shows MSSA.

Phenotypic Detection of Livestock Acquired Methicillin resistance *S. aureus* (LA-MRSA)

Erythromycin and Tetracycline Disk Diffusion Test

Employing the Kirby-Bauer disk diffusion method, detection of LA-MRSA strains was achieved. On a MHA plate was 0.5 MFTES of test bacteria aseptically smeared. Then on the plate, was 15 µg of Erythromycin and 30 µg Tetracycline disk laid and for 24 hours was incubated at 37^{o}C. Pheno-typing indication of LA-MRSA was taken as Inhibition zone≤ 14 mm for the 30 µg Tetracycline and zone of inhibition ≤ 17 mm for 15 µg Erythromycin.

Antimicrobial Susceptibility Test

This was carried out following the standard method of the CLSI[29]. Adjusting the bacterial suspension of $1x10^6$ (cfu/ml) to 0.5 MFTES and then swabbing onto Petri dishes containing solidified MHA, the inoculated organisms were allowed to pre-diffuse after standing for 15 minutes. By aseptically laying the prepared antibiotics on the surface of the solidified MHA plates, using a sterile forceps, to guarantee complete contact. The antibiotics used include; Erythromycin (15 µg), Amikacin (30 µg), Imipenem (10 µg), Piperacillin – Tazobactam (110 µg), Cefotaxime (30 µg), Clindamycin (15 µg), Vancomycin (30 µg), Tetracycline (10 µg) and Trimethoprim-Sulfamethoxazole (30 µg). For 18–24 hours, the plates were incubated at 37°C and inhibition zone was taken after 24 hours; isolates of LA-MRSA were categorized as susceptible, resistance and intermediate to the antibiotics tested.

Multiple Antibiotic Resistance (MAR) index Determination

Using the formula MAR=x/y, MAR index was determined. Where x=number of antibiotics to which test isolate displayed resistance and y=total number of antibiotics to which the test organism has been evaluated for sensitivity.

Statistical Analysis

Data were calculated using frequency distribution, and with the aid of the statistical package for social sciences (SPSS) software, IBM (Version 25), USA, the Statistical analyses were carried out. Comparison between definite variables was done by T-test. Statistically significant results were marked at ($p \leq 0.05$).

RESULTS

Distribution of *S. aureus* isolated from nasal swab sample of pig from different farms within Enugu metropolis

The distribution of *S. aureus* recorded high proportion of 105(90.5%) in Farm B followed by Farm A 61(80.3%) and Farm C 68(63.0%) with the least isolation rate (Table 1). Total frequency of *S. aureus* was 234(76.2%) from nasal swab sample of pig from different farms within Enugu metropolis.

Distribution of Methicillin Resistant *S. aureus* isolates from nasal swab sample of pig from different farms within Enugu metropolis

Methicillin Resistant *S. aureus* were highly predominant in Farm A resulting in 38(50.0%) followed by 54(46.6%) and 33(30.6%) against Farm B and Farm C respectively (Table 3). MRSA in grower swine gave 20(77.0%) nursery gave 13(39.4%) and weaning 5(29.4%) from Farm A. The prevalence of MRSA from Farm B consists of 8(6100%), 18(44.0%), 25(43.1%), 3(33.3%) against finishers, grower, nursery and weaning respectively while MRSA in Farm C finishers gave 9(60.0%), nursery 14(32.6%) grower 10(34.5%), and weaning 0(0.0%).

Table 1: Distribution of *S. aureus* isolated from nasal swab sample of pig from different farms within Enugu metropolis.

Farm Location	Categories of Swine	Age (weeks)	No. of sample	No. of *S. aureus* (%)
Farm A	Nursery	0-3	33	27(81.8)
	Weaning	>3 to ≤10	17	10(58.8)
	Grower	>10 to ≤ 18	26	24(92.3)
Total			76	61(80.3)
Farm B	Nursery	0-3	58	51(88.0)
	Weaning	>3 to ≤10	9	9(100)
	Grower	>10 to ≤ 18	41	37(90.2)
	Finisher		8	8(100)
Total			116	105(90.5)
Farm C	Nursery	0-3	43	32(74.4)
	Weaning	>3 to ≤10	21	12(57.1)
	Grower	>10 to ≤ 18	29	15(51.7)
	Finisher	>18 to ≤ 26	15	9(63.0)
Total			108	68(62.9)
Overall			307	234(76.2)

Distribution of Livestock acquired Methicillin Resistant *S. aureus* isolated from nasal swab sample of pig from different farms within Enugu metropolis.

In Table 3, the LA-MRSA showed highest rate of 30(27.8%) in Farm C compared with Farm A 29(38.2%) and Farm B 25(21.6%). Total frequency of LA-MRSA was 84(27.4%) from nasal swab sample of pig from different farms within Enugu metropolis.

Antibiotic susceptibility profile of LA-MRSA isolated from nursery Swine at Farm A

Table 4 shows that LA-MRSA isolate from nursery swine were highly resistant to 100% Erythromycin, 100% Trimethoprim- Sulfamethoxazole, 100% Tetracycline, 77.8% Vancomycin but were susceptible to 100% amikacin, 100% Imipenem and 77.8% Ciprofloxacin

Antibiotic susceptibility profile of LA-MRSA isolated from grower Swine at Farm A.

Table 5 shows the antibiotic resistant profile of LA-MRSA isolated from grower swine, which revealed 75.0%, 65.0%, 60.0%, 55.5%, 50.0% resistant to Cefotaxime, Erythromycin, Piperacillin–Tazobactam, Vancomycin and Ciprofloxacin respectively but were 100% sensitive to Amikacin and imipenem.

Table 2: Distribution of Methicillin resistant *S. aureus* isolates from nasal swab sample of pig from different farms within Enugu metropolis.

Farm Location	Categories of Swine	Age (weeks)	No. of sample	No. of *S. aureus* (%)	MRSA (%)	MSSA (%)
Farm A	Nursery	0-3	33	27(81.8)	13(39.4)	14(42.3)
	Weaning	>3 to ≤10	17	10(58.8)	5(29.4)	5(29.4)
	Grower	>10 to ≤ 18	26	24(92.3)	20(77.0)	4(15.4)
Total			76	61(80.3)	38(50.0)	23(30.3)
Farm B	Nursery	0-3	58	51(88.0)	25(43.1)	26(44.8)
	Weaning	>3 to ≤10	9	9(100)	3(33.3)	6(66.7)
	Grower	>10 to ≤ 18	41	37(90.2)	18(44.0)	19(46.3)
	Finisher	>18 to ≤ 26	8	8(100)	8(100)	0(0.0)
Total			116	105(90.5)	54(46.6)	51(44.9)
Farm C	Nursery	0-3	43	32(74.4)	14(32.6)	18(41.9)
	Weaning	>3 to ≤10	21	12(57.1)	0(0.0)	12(57.1)
	Grower	>10 to ≤ 18	29	15(51.7)	10(34.5)	5(17.2)
	Finisher	>18 to ≤ 26	15	9(60.0)	9(60)	0(0.0)
Total			108	68(63.0)	33(30.6)	35(32.4)
Overall			307	234(76.2)	125(40.7)	109 (35.5)

MRSA-Methicillin Resistant *S. aureus*, MSSA- Methicillin Susceptible *S. aureus*

Antibiotic susceptibility profile of LA-MRSA isolated from nursery Swine at Farm B

LA-MRSA isolates are shown in Table 6, which demonstrate resistant to 92.9% Clindamycin, 100% Tetracycline, 57.1% Vancomycin and 78.6% Cefotaxime but were 100% susceptible to Erythromycin, amikacin and Imipenem. In Table 7, LA-MRSA demonstrated high rate of resistance to 100% Trimethoprim-Sulfamethoxazole, Cefotaxime and Erythromycin; and 66.7% Clindamycin but susceptible to 33.3% Piperacillin –Tazobactam, 100% imipenem, 100% Amikacin.

Antibiotic susceptibility profile of LA-MRSA isolated from finishers Swine at Farm B

Table 8 shows that LA_MRSA was 100% resistance to Clindamycin and Erythromycin 62.5% resistant to Cefotaxime and Piperacillin–Tazobactam but was very susceptible to 100% Imipenem, 75% Ciprofloxacin and 62.5% vancomycin.

Table 3: Distribution of Livestock acquired Methicillin resistant *S. aureus* isolated from nasal swab sample of pig from different farms within Enugu metropolis.

Farm Location	Categories of Swine	Age (weeks)	No. of sample	MRSA (%)	LAMRSA (%)	LAMSSA (%)
Farm A	Nursery	0-3	33	13(39.4)	9(27.3)	4(12.1)
	Weaning	>3 to ≤10	17	5(29.4)	0(0.0)	5(29.4)
	Grower	>10 to ≤ 18	26	20(77.0)	20(77.0)	0(0.0)
Total			76	38(50.0)	29(38.2)	9(11.8)
Farm B	Nursery	0-3	58	25(43.1)	14(24.1)	11(19.0)
	Weaning	>3 to ≤10	9	3(33.3)	0(0.0)	3(33.3)
	Grower	>10 to ≤ 18	41	18(44.0)	3(7.3)	15(36.6)
	Finisher	>18 to ≤ 26	8	8(100)	8(100)	0(0.0)
Total			116	54(46.6)	25(21.6)	29(25.0)
Farm C	Nursery	0-3	43	14(32.6)	12(28.0)	2(4.7)
	Grower	>3 to ≤10	29	10(34.5)	10(34.5)	0(0.0)
	Finisher	>18 to ≤ 26	15	9(60.0)	8(53.3)	1(6.7)
Total			108	33(30.6)	30(27.8)	3(2.8)
Overall			307	125(40.7)	84(27.4)	41(13.4)

MRSA-Methicillin Resistant *S. aureus*, LAMRSA- Livestock Acquired Methicillin Resistant *S. aureus*, LAMSSA- Livestock Acquired Methicillin susceptible *S. aureus*.

Antibiotic susceptibility profile of LA-MRSA isolated from nursery Swine at Farm C

In Table 9, antibiotic resistant profile of LA-MRSA indicated 100% resistance to Trimethoprim-Sulfamethoxazole, Tetracycline, Clindamycin, Erythromycin, and was susceptible to 50% Cefotaxime, 58.3% Ciprofloxacin, 75% Piperacillin–Tazobactam and 58.3% vancomycin.

Antibiotic susceptibility profile of LA-MRSA isolated from Grower Swine at Farm C

Antibiotic susceptibility profile of LA-MRSA isolated from Grower Swine at Farm C livestock production showed that all isolate were 100% susceptible to imipenem, ciprofloxacin and amikacin and that LA-MRSA exhibited 100% resistant to tetracycline, Trimethoprim-Sulfamethoxazole, 90.0% to Erythromycin and 40.0% to Vancomycin as shown in Table 10**.**

Antibiotic susceptibility profile of LA-MRSA isolated from finishers Swine at Farm C

Table 11 revealed that LA-MRSA possessed 100% resistant to Erythromycin and clindamycin Piperacillin –Tazobactam and 62.5% to Vancomycin while it was 62.5% susceptible to Cefotaxime 75.0% susceptible to Ciprofloxacin and 100% susceptible to amikacin.

Multiple Antibiotic Resistant Index (MARI) of Livestock acquired Methicillin Resistant *S. aureus* isolated from nasal swab sample of pig from different farms within Enugu metropolis

LA-MRSA strain demonstrated multidrug resistant with MARI value of 0.4 and 0.6 from Farm A, 0.3, 0.5, 0.7 from Farm B and 0.3, 0.5, 0.6 Farm C exhibited by different category of pig as shown in Table 12.

Table 4: Antibiotic susceptibility profile of LA-MRSA isolated from nursery Swine at Farm A.

Antibiotics (µg)	Resistance (%) (n=9)	Susceptible (%) (n=9)
Amikacin (30)	0(0.0)	9(100)
Cefotaxime (30)	5(55.6)	4(44.4)
Imipenem (10)	0(0.0)	9(100)
Erythromycin (15)	9(100)	0(0.0)
Clindamycin (15)	6(66.7)	3(33.3)
Ciprofloxacin (5)	2(22.2)	7(77.8)
Piperacillin –Tazobactam (110)	6(66.7)	3(33.3)
Trimethoprim-Sulfamethoxazole (30 µg)	9(100)	0(0.0)
Tetracycline	9(100)	0(0.0)
Vancomycin (30)	7(77.8)	2(22.2)

n=Number of isolate

Table 5: Antibiotic susceptibility profile of LA-MRSA isolated from grower Swine at Farm A.

Antibiotics (µg)	Resistance (%) (n=20)	Susceptible (%) (n=20)
Amikacin (30)	0(0.0)	20(100)
Cefotaxime (30)	15(75.0)	5(25.0)
Imipenem (10)	0(0.0)	20(100)
Erythromycin (15)	13(65.0)	7(35.0)
Clindamycin (15)	20(100)	0(0.0)
Ciprofloxacin (5)	10(50)	10(50)
Piperacillin–Tazobactam (110)	12(60)	8(40)
Trimethoprim-Sulfamethoxazole (30 µg)	20(100)	0(0.0)
Tetracycline	20(100)	0(0.0)
Vancomycin (30)	11(55.0)	9(45.0)

Table 6: Antibiotic susceptibility profile of LA-MRSA isolated from nursery Swine at Farm B.

Antibiotics (µg)	Resistance (%) (n=14)	Susceptible (%) (n=14)
Amikacin (30)	0(0.0)	14(100)
Cefotaxime (30)	11(78.6)	3(21.4)
Imipenem (10)	0(0.0)	14(100)
Erythromycin (15)	0(0.0)	14(100)
Clindamycin (15)	13(92.9)	1(7.1)
Ciprofloxacin (5)	6(42.9)	8(57.1)
Piperacillin –Tazobactam (110)	10(71.4)	4(28.6)
Trimethoprim-Sulfamethoxazole (30 µg)	14(100)	0(0.0)
Tetracycline	14(100)	0(0.0)
Vancomycin (30)	8(57.1)	6(42.9)

Table 7: Antibiotic susceptibility profile of LA-MRSA isolated from Grower Swine at Farm B.

Antibiotics (µg)	Resistance (%) (n=3)	Susceptible (%) (n=3)
Amikacin (30)	0(0.0)	3(100)
Cefotaxime (30)	3(100)	0(0.0)
Imipenem (10)	0(0.0)	3(100)
Erythromycin (15)	3(100)	0(0.0)
Clindamycin (15)	2(66.7)	1(33.3)
Ciprofloxacin (5)	1(33.3)	2(66.7)
Piperacillin –Tazobactam (110)	2(66.7)	1(33.3)
Trimethoprim-Sulfamethoxazole (30 µg)	3(100)	0(0.0)
Tetracycline	3(100)	0(0.0)
Vancomycin (30)	2(66.7)	1(33.3)

DISCUSSION

In this study, *S. aureus* in swine had 76.2% as the total prevalence rates and this was in agreement with the report of Köck *et al.*,[30], in Germany with 70%, and in comparison with that of 37.8% in Belgium, 39.0% in the Netherlands, it was higher[2,31]. Denis *et al.*,[31] reported that in Africa, *S. aureus* rate was said to be between 25.0-55%. Thus there seems to be difference in the various strains reported, which could be attributed to factors such as; the subject used in the study, sample size and targeted species of bacteria studied, which might result in increased prevalence[31].

Essentially, *S. aureus* bacteria are clinically significant in colonizing and inhabiting animals and humans, through their genetic and ubiquitous nature, to incur diseases such as toxic shock syndrome, diseases of soft tissue and skin and endocarditis[2]. In swine, the total prevalence of MRSA in this study was 40.7% and swine are reported to be often inhabited by *S. aureus*, making them MRSA reservoir[32].

This study showed LA-MRSA had a total prevalence that was higher than those of 3.4% and 4.4% in Korea[6,7] but was closely agreed with 20.7% in Spain[32], 49% in Germany[33], 38% in the Netherlands[2] and 43.8% in England[34].

Table 8: Antibiotic susceptibility profile of LA MRSA isolated from finishers Swine at Farm B.

Antibiotics (µg)	Resistance (%) (n=8)	Susceptible (%) (n=8)
Amikacin (30)	0(0.0)	8(100)
Cefotaxime (30)	5(62.5)	3(37.5)
Imipenem (10)	0(0.0)	8(100)
Erythromycin (15)	8(100)	0(0.0)
Clindamycin (15)	8(100)	0(0.0)
Ciprofloxacin (5)	2(25.0)	6(75.0)
Piperacillin–Tazobactam (110)	5(62.5)	3(37.5)
Trimethoprim-Sulfamethoxazole (30 µg)	8(100)	0(0.0)
Tetracycline	8(100)	0(0.0)
Vancomycin (30)	3(37.5)	5(62.5)

Thus, the observed differences across these countries could be due to factors relating to geographical region, farm management, number of pig in a pen, collection of sample and method of isolation[34]. Farm A of this study, among the three different farms sampled had the highest rate of MRSA of 50%, yet with smallest sample size. A possible cause of higher rate of MRSA in swine of farm A could have come from contamination of batches of sample, high use of microbial growth promoter (such as antibiotics), other contamination source in the farm, contamination at transport of sample and striving of MRSA within farm[6]. LA-MRSA in swine in this study was found to be 27.4%, this is in agreement with the report from UK, Italy, Korea and England, giving the range of LA-MRSA to be 0.1–30.7%[3,6,34,35]. In Nigeria, La-MRSA was reported for the first time in 2022 from a poultry farm in Abakaliki[8].

Table 9: Antibiotic susceptibility profile of LA-MRSA isolated from nursery Swine at Farm C.

Antibiotics (µg)	Resistance (%)(n=12)	Susceptible (%) (n=12)
Amikacin (30)	0(0.0)	12(100)
Cefotaxime (30)	6(50)	6(50)
Imipenem (10)	0(0.0)	12(100)
Erythromycin (15)	12(100)	0(0.0)
Clindamycin (15)	12(100)	0(0.0)
Ciprofloxacin (5)	5(41.6)	7(58.3)
Piperacillin –Tazobactam (110)	3(25.0)	9(75.0)
Trimethoprim-Sulfamethoxazole (30 µg)	12(100)	0(0.0)
Tetracycline	12(100)	0(0.0)
Vancomycin (30)	5(41.6)	7(58.3)

Table 10: Antibiotic susceptibility profile of LA-MRSA isolated from Grower Swine at Farm C.

Antibiotics (µg)	Resistance (%) (n=10)	Susceptible (%) (n=10)
Amikacin (30)	0(0.0)	10(100)
Cefotaxime (30)	6(60)	4(40)
Imipenem (10)	0(0.0)	10(100)
Erythromycin (15)	9(90.0)	1(10)
Clindamycin (15)	8(80)	2(20)
Ciprofloxacin (5)	0(0.0)	10(100)
Piperacillin –Tazobactam (110)	3(30)	7(70)
Trimethoprim-Sulfamethoxazole (30 µg)	10(100)	0(0.0)
Tetracycline	10(100)	0(0.0)
Vancomycin (30)	4(40)	6(60)

This draws a concern to it as an emerging issue, suggesting that it possibility of becoming an outbreak in the future life of livestock is not far fetched[6]. LA-MRSA is reported to majorly infect livestock such as swine and poultry birds[6,19]. However, humans with occupational contact with livestock animals have been reported to be infected by LA-MRSA[6,8,36]. Similarly, humans without occupational contact with livestock animals may be susceptibly affected from the environment because LA-MRSA was detected in pig holdings dust, making the environment a channel for the strains of LA-MRSA transmission from livestock animals to man[35,37]. In Africa, much has not been reported as per factors relating to the transmission of *S. aureus* to humans from swine or other livestock animals[38]. However, rise in MRSA infections in humans was reported to be associated with the dissemination of LA-MRSA CC398 (ST398 and ST541) in production of pork and swine[38]. LA-MRSA isolates ability for genetic variation and possession of PVL genes, *TSST*-1, and *ET* genes (*eta*, *etb*, and *etd*) and *SE* genes opens a serious health risk to the public[39]. For instance, in the Lombardy region in Italy, pig and dairy farmers were said to develop pyomiositis of the buttock, cellulitis and necrotizing fasciitis of the neck, due to serious LA-MRSA infection[40]. LA-MRSA was not found in farms A and B in weaning swine but was detected in farm C in this study.

Table 11: Antibiotic susceptibility profile of LA-MRSA isolated from finishers Swine at Farm C.

Antibiotics (µg)	Resistance (%) (n=8)	Susceptible (%) (n=8)
Amikacin (30)	0(0.0)	8(100)
Cefotaxime (30)	3(37.5)	5(62.5)
Imipenem (10)	0(0.0)	8(100)
Erythromycin (15)	8(100)	0(0.0)
Clindamycin (15)	8(100)	0(0.0)
Ciprofloxacin (5)	2(25)	6(75)
Piperacillin –Tazobactam (110)	5(62.5)	3(37.5)
Trimethoprim-Sulfamethoxazole (30 µg)	8(100)	0(0.0)
Tetracycline	8(100)	0(0.0)
Vancomycin (30)	4(50)	4(50)

Thus this study revealed the incidence of LA-MRSA could be increased in weaning pigs depending on the following factors; hygiene of the farm environment, number of pigs in the pen, contamination within the farm and LA-MRSA persistence in different swine batches[3]. Thus, it becomes glaring that functional and effectual sanitation plan should be put in place to decrease the incidence of LA-MRSA from cross–contamination. It was reported that the status of LA-MRSA in a swine detected to be positive might change as the swine may only be transiently and not permanently infected[6]. In this study, 100% resistance was demonstrated by most isolates to the antibiotics (antimicrobials) used such as tetracycline (TET) and trimethoprim-sulfamethoxazole. This results are in agreement with report from Korea, Nigeria, Italy and Norway[6,8,25,35]. The use of TET in the treatment of respiratory diseases in swine has been reported, stating

that continues use of TET could become an advantage to LA-MRSA isolates. Therefore, it could be inferred that in this study, the isolated LA-MRSA strains may selected TET antimicrobials among others, with respect to multi-drug resistance phenotype[8]. Similarly, LA-MRSA resistance to TET was reported that its elevated prevalence in swine was related with zinc resistance aided by *czrC* gene[6,43,44]. Thus, preparing weaning swine feed with zinc as an additive to prevent diarrhea might increase the choice of *czrC* in CC 398 LA-MRSA and could promote LAMRSA prevalence in swine[6]. It is important for more findings to be conducted, handling larger strains of MRSA in order to explain the significance of zinc and TET associated resistance in livestock[41,45,46]. In this study, swine's finishers' and grower isolates had 90-100% resistance of LA-MRSA to erythromycin. This is consistent with the report of Camoez *et al*.,[42], where 85% resistance of LA-MRSA isolates and methylase *erm* (*C*) gene in LA-MRSA strain to erythromycin was reported. Similarly, farm B showed isolate from nursery swine to be sensitive to erythromycin, supporting the reports of 90.2% and 86.7% LAMRSA isolate to erythromycin[8].

Table 12: Multiple Antibiotic Resistant Index (MARI) of Livestock acquired Methicillin Resistant *S. aureus* isolated from nasal swab sample of pig from different farms within Enugu metropolis.

Farm Location	Categories of Swine	MARI
Farm A	Nursery	0.4
	Grower	0.6
Farm B	Nursery	0.3
	Grower	0.5
	Finisher	0.7
Farm C	Nursery	0.3
	Grower	0.5
	Finisher	0.6

MRSA-Methicillin Resistant *S. aureus*, LA-MRSA- LivestockAcquired Methicillin Resistant *S. aureus*, LAMSSA- Livestock Acquired Methicillin Susceptible *S. aureus*.

This could point that erythromycin seems not to have been frequently used in farm B nursery swine and erythromycin sub-lethal dose had no effect on LA-MRSA isolates. In this study, the isolated strain of LA-MRSA had resistance to clindamycin. This was in agreement with the fact that clindamycin has become well accepted for patient use in Spain and for poultry use in Nigeria[8,42].

The rate of multi-drug resistance of 0.3-0.7, shown by LA-MRSA in this study and its resistance to the antibiotics used might be attributed to the large commercial farms sampled, population of pig in a farm and availability of antimicrobial resistance[30]. Several studies have reported that humans could become infected when potentially virulent *S. aureus* isolates get into humans from livestock related contact[6]. LA-MRSA in infected asymptomatic carrier humans may last as long as several months without being tested positive for LA-MRSA[25]. In this study, the use of imipenem and amikacin demonstrated improved effect against LA-MRSA isolates and such antimicrobials could be administered when needed. These drugs are known globally to have significant place in human and animal medicine as important antibiotics[8].

CONCLUSIONS

A strain of LA-MRSA without known animal-specific adaptations may have the ability to reproduce and spread in other livestock. Meanwhile, the incidence of LA-MRSA was variable, specifically among farm pigs 38.2%, 27.8% and 21.6% for farm A, farm C and farm B respectively, indicating that a number of factors may influence the incidence of LA-MRSA in pigs at the farm level. Notably, the MDR range of 0.3-0.7 underscores the need for veterinarians to prescribe all antimicrobial agents used for food-producing animals in Nigeria.

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AUTHOR'S CONTRIBUTION

Okolie SO: writing original draft, designed the study, literature searches. **Iroha IR:** statistical analysis, formal analysis. **Ikpe VPO:** conceptualization, methodology. **Peter UO:** methodology, data collection. **Victor EJ:** editing, methodology. **Alexander I:** research design, data collection. All the authors reviewed the results and approved the final version of the manuscript.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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