UMYU Scientifica, Vol. 3 NO. 4, December 2024, Pp 148 – 161
https://doi.org/10.56919/usci.2434.013
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Prevalence, Molecular Characterization and Antibiogram Pattern of Livestock Associated Methicillin Resistant *Staphylococcus aureus* in Livebird Markets Gusau, Zamfara State, Nigeria

Victor Chijioke Ukwaja[1], Nurudeen Salisu[2], Murtala Aliyu[3] and Naibi Mohammed[4]
[1,2&4]Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria
[3]Department of Biological Science, Federal University Gusau, Zamfara State, Nigeria

ABSTRACT
Poultry farms have been identified as hotspots for the emergence and spread of antibiotic-resistant bacteria, especially livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA). In this study, a total of 320 swab samples were randomly collected from fecal litter, Hands of poultry handlers, Soil, Feed, and Water from five different poultry markets in the Gusau metropolis. The LA-MRSA isolates involved were detected culturally and identified phenotypically by the use of PCR assay. The pooled prevalence of the LAMRSA isolates in the samples was 22(100%). The chi-square test was used to compare the prevalence of LAMRSA among the samples collected at 95% confidence interval and 0.05 level of significance. There is no statistically significant difference in the prevalence of LAMRSA among the samples. Findings from the antibiogram study conducted revealed that all the 22(100%) LAMRSA isolates were resistant to Penicillin antibiotics, with resistance to other antibiotics following the order: Nitrofurantoin, 17(77.3%), Sulphamethoxazole, 13(59.1%), Chloramphenicol 6(27.28%), Ciprofloxacin 5(22.7%) and Erythromycin 4(18.2%). A total of 14 multidrug resistant phenotypes were identified from the 22 LAMRSA isolates with 16(72.2%) demonstrating cross resistant to ≥3 of the six different classes of antibiotics used. The MDR rate in soil samples was the highest (27.3%) ($p<0.05$) compared to other MDR isolates from the other sources. Of all the isolates, 22(100%) demonstrated a MAR Index ranging from 0.2-0.8. These are disturbing trends with significant risks to humans and animals. Suggesting a potential for inoculation of self and transmission to others, particularly through those who sell and make daily contact with live birds. Therefore, the government should awaken the consciousness of poultry farmers/handlers by way of sensitization on the need to improve water quality and practice routine personal and environmental hygiene as it relates to poultry management, with a focus on antibiotic stewardship within the one health framework so as to lower endemicity and equally break the chain of transmission of LA- MRSA across the farm to fork continuum.

ARTICLE HISTORY
Received August 10, 2024
Accepted October 20, 2024
Published mmmm 28, 2024

KEYWORDS
Antibiotics, Prevalence, LA-MRSA, Antibiogram Pattern

INTRODUCTION
Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is an evolving category of methicillin-resistant *Staphylococcus aureus* (MRSA) reported across the globe (Anjum *et al.,* 2019). It is a type of bacteria traced back to livestock and an increasing cause of human infections, which does not respond to treatment with the antibiotic, methicillin. MRSA has a global impact as a threat to public health by contributing significantly to morbidity, mortality, and socio-economic costs estimated at 3 billion dollars annually (Saenhhom *et al.,* 2022; Shoaib *et al.,* 2023). In 2019, multi-drug resistant bacterial pathogens, to which LA-MRSA was implicated, contributed to a staggering 4.95 million deaths globally, making it the third leading cause of death that year (Matuszewska *et al*., 2022).

Three distinct strains of MRSA are of global public health concern, which causes infections associated with hospitals (HA-MRSA), general communities (CA-MRSA), and livestock (LA-MRSA). Despite the efforts in classifying the different MRSA strains, the epidemiology of MRSA colonization is changing. Strains of CA-MRSA can share genes between both LA- and HA-MRSA, and some clones are present in more than one classification group, blurring the distinction between strains (Crespo-Piazuelo & Lawlor, 2021). The wide application of antibiotics in human medicine and agriculture, especially in plant and

Correspondence: Victor Chijioke Ukwaja. Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria.
✉ ukwajavictor@fugusau.edu.ng
How to cite: Ukwaja, V. C., Salisu, N., Aliyu, M., & Muhammed, N. (2024). Prevalence, Molecular Characterization and Antibiogram Pattern of Livestock Associated Methicillin Resistant *Staphylococcus aureus* in Livebird Markets Gusau, Zamfara State, Nigeria. *UMYU Scientifica*, 3(4), 148 – 161. https://doi.org/10.56919/usci.2434.013

animal production, has caused a significant increase in their concentration in the environment, which, together with their often-inappropriate prescription and use, has resulted in tremendous selection pressure promoting their antibiotic resistance (WHO, 2024).

The pathogen is one of the most important pathogenic members of the genus Staphylococci and a leading cause of nosocomial, community, and livestock-associated infections (implicated in dermatitis, arthritis, osteomyelitis, synovitis, tenosynovitis, femoral head necrosis, bumble-foot, and omphalitis) (Bitrus *et al.,* 2018; Abd El-Ghany, 2021). Matuszewska *et al.* (2022) in their studies, detected the most common type of LA-MRSA in Europe (clonal-complex 398 [CC398]), bearing two mobile genetic elements (Tn916 transposon and SCCmec) and carrying antibiotic-resistance genes (tetM and mecA) respectively. The φSa3 prophages carrying a human immune evasion gene cluster are often gained when the pathogen infects humans. This leads to highly drug-resistant human MRSA infections (Bloemendaal *et al.,* 2010).

Livestock has been considered to be a reservoir of MRSA with high potential for zoonotic transmission to humans (veterinarians and farm workers). It has been observed that the spread of LA-MRSA from animals to humans occurs frequently and that livestock workers present a high risk for LAMRSA colonization and subsequent infections (Ceballos *et al.,* 2022). The transmission of the LA-MRSA strain from animals to humans was considered difficult, as this strain was initially thought of as non-disease-causing in humans. However, this paradigm is changing as fatal cases with humans have been reported (Crespo-Piazuelo and Lawlor, 2021). Treatment due to this pathogen remains challenging globally due to the emergence of multi-drug resistant strains, informing the WHO decision to categorize MRSA in group two (high) of the global priority list of antibiotic-resistant bacteria (Saenhhom *et al.,* 2022).

There is no vaccine against MRSA due to its non-protective immune imprint (Chih-Ming *et al.,* 2022). Its resistance to multiple classes of antibiotics, including antibiotics of last resort (vancomycin and linezolid), has been reported across the globe. Antibiotic resistance is one of the biggest challenges that has bedeviled the health sector worldwide, and this medical predicament threatens our ability to effectively manage and treat some infectious diseases (Sineke *et al.,* 2021). Regrettably, the slow pace in the discovery and development of novel antibiotics has not actually kept pace with the emergence and rate at which bacteria develop and mount resistance to some available antibiotics (Garga, 2017).

Nigeria places a premium on the national public health agenda. The Nigerian response to antimicrobial resistance led the Nigeria Centre for Disease Control (NCDC) in 2016 to undertake a situational analysis of common antimicrobial-resistant pathogens within the framework of one health. These efforts informed the development of the National Action Plan (NAP) on Antimicrobial Resistance. Overall, Nigeria's NAP seeks to address five key pillars in consonance with the WHO Global Action Plan on AMR, among which are building a One-Health surveillance system, intensifying infection prevention, control, and biosecurity, promoting rational use of antimicrobials and access to quality medications and research into alternatives to antimicrobials, new diagnostics, and therapeutics (Federal Ministry of Agriculture Environment and Health, FMAEH 2017).

Despite the increasing evidence about antibiotic resistance and zoonotic disease transmission caused by LA-MRSA, its prevalence and transmission dynamics remain poorly understood especially in the study area, deterring the use of effective therapeutic strategies to mitigate the spread of this important public health threat. This study, therefore, aims to address the growing concern of LA-MRSA in poultry markets by undertaking a comprehensive study required to provide evidence-based data on the prevalence, antibiotic-resistant genes (mecA and tetM), antibiogram pattern, and multiple antibiotic-resistant index of LA-MRSA isolates crucial to control its endemicity and dissemination as well as guide clinicians in the appropriate selection of the most effective treatment options for the management of LA-MRSA infections.

MATERIALS AND METHODS

Study Design

Both the cross-sectional and experimental study design was used for data collection and analysis. The study was conducted from January 2024 to August 2024.

Study Area

This study was conducted in the Microbiology Department, Federal University Gusau Zamfara State. The state is mainly populated by Hausa/Fulani people and other ethnic minorities. The state has an average precipitate of 61.41mm and an average annual temperature of 30.22^{o}C. The state has an area of 39,762 km^{2} with an estimated population of 9,278,873 (National Population Commission, NPC 2017).

Ethical Consideration

Ethical clearance was obtained from the Directorate of Animal Health, Rugga and Livestock Development Gusau, Zamfara state, with reference number ZAN/RU/0021/2023.

Inclusion Criterion

Only individuals who trade in and make daily contact with live birds were recruited to participate in this study.

Informed Consent Form

Informed consent was obtained from participating subjects with assurance that information obtained from them shall be held in confidence and used for research purposes only.

Sample Size

The sample size was determined using the formula described in Igbinosa *et al.* (2023). The value of the population proportion used in this study was maintained at 0.5 since the previous study on this subject was scanty within the study area. Accordingly, the calculated value for sample size equaled 267 samples. The relevant formula for sample size determination used was $N = \frac{Z^2 \times P(1-P)}{e^2}$. Where: N= population size, Z= Z-Score Standard normal variant at 5% type I error = 1.96 (at 95% confidence limit), P = Population proportion (0.5), e= margin of error= 0.05. $N = \frac{1.96^2 \times 0.5(1-0.5)}{0.06^2} = 267$. To increase the robustness of the data set, the number of samples was increased to 320, made up of 64 samples each from fecal droppings, hand swabs, soil, feed, and water, as described in Heston (2023).

Media Preparation

All microbiological media used were prepared according to the manufacturer's instructions accompanying the label. The media used include Mannitol Salt Agar, Nutrient Agar, Nutrient Broth, and Mueller Hinton Agar.

Sample Collection

The method described in Peter *et al.* (2022) was used for sample collection. A total of Three Hundred and Twenty (320) samples were randomly collected aseptically from six (6) different live bird markets in the state, made up of Sixty-Four (64) samples each from fecal droppings of bird (F), feed (Fe), water (W), hands of handlers (H) and from soil in poultry environment (S). Samples were serialized and coded according to the type of sample collected. The collected samples were immediately transported to the Microbiology laboratory of the Federal University Gusau for further analysis.

Primary Isolation

The collected swab samples were suspended in a sterile Nutrient broth for enrichment and incubated at 37°C for 24 hours. A loopful of the turbid bacterial growth was seeded onto Mannitol salt agar medium (Oxoid, Cambridge, UK) since it was quicker, more economical, and the culture was easier to read and then incubated at 37°C for 24 hours. The appearance of golden yellow colonies in the culture plates indicated the presence of *Staphylococcus aureus* (Garga, 2017). The bacteria isolated were kept on a nutrient agar slant for further analysis.

Standardization of the Inoculum

Standardization of inoculum was carried out according to the method of Garga, (2017). The isolates were sub-cultured onto fresh nutrient agar plates and incubated at 37°C for 24 hours. After the incubation, 5 ml of sterile distilled water was placed into different universal bottles and was used to prepare the size of the inoculums. The McFarland scale of 0.5, whose equivalence is 1.5 x 10^8cfu/ml, was used.

Characterization of *Staphylococcus aureus*

The Gram staining reaction, catalase, and coagulase test were used as markers for the phenotypic identification of *Staphylococcus aureus* as described in (Garga, 2017).

Grams Staining and Microscopy

This was carried out as described by Garga, (2017). A drop of water was placed on a clean, grease-free glass slide and emulsified with a colony of bacteria from an overnight culture (24-hour culture) to make a thin smear. The smear was allowed to air dry and was flooded with crystal violet for 60 seconds before being washed with tap water. Lugol's iodine was added for 60 seconds and washed with tap water. It was decolorized with 95% ethanol for 15 seconds. The smear was finally flooded with safranin solution for 1 minute and then washed with water and allowed to air dry. It was viewed under oil immersion at (x100). Cells that stain purple were reported as Gram-positive, while Gram-negative cells stain red.

Catalase test

The catalase test was performed as described in Garga (2017). A few colonies of bacterial culture were transferred with a sterile wire loop to a drop of hydrogen peroxide on a slide and emulsified. This was observed for the formation of bubbles (oxygen), which indicates a positive test, while lack of bubbles indicates a negative test.

Coagulase test

A coagulase test was performed as described in Garga (2017). A drop of distilled water was placed on two slides; a colony of bacteria was emulsified in the drop of water to make a thick suspension. A loop full of plasma was added to the thick suspension on one slide, mixed gently, and observed within ten minutes. Clumping indicated a positive test, while the absence of clumping indicated a negative test. The other slide was used as a control.

Preparation of McFarland Turbidity Standard

McFarland Turbidity Standards are a set of tubes with increasing concentrations of barium sulfate suspension. A 0.5 McFarland Standard was prepared by mixing 0.05 ml of 1% anhydrous barium chloride ($BaCl_2$) with 9.95 mL of 1% Sulphuric acid (H_2SO_4) which forms barium sulfate precipitate (turbid solution). The tube was tightly sealed and kept until required. The standard is used to determine approximate bacterial density in a broth culture (Garga, 2017).

Detection of LA-MRSA Isolates

The standardized test bacterial isolates were aseptically inoculated on the surface of the Mueller Hinton Agar plates, spread evenly using a sterile bent glass rod, and allowed to dry for 5 minutes. Antibiotic discs were placed on the surface of the media by means of sterile forceps and incubated at 37°c for 24 hours. The antibiotics, cefoxitin (30µg) and Tetracycline (30µg), were used as surrogate markers for the detection of MRSA and LA-MRSA,

respectively, as described by Chung *et al.* (2021). Isolates with zones of inhibition (≤21mm) due to cefoxitin (30µg) antibiotics were reported as MRSA isolates, while those MRSA with zones of inhibition (≤14mm) due to Tetracycline (30µg) antibiotics were reported as LA-MRSA isolates as described in CLSI, (2020).

Molecular Characterization of LA-MRSA Isolates

The method described by Beshiru *et al.* (2024) was used to characterize LAMRSA molecularly with slight modification. Antibiotic-resistant genes (mecA and tetM) in LAMRSA isolates involved in this study were detected using PCR assay, which was performed with DNA amplification instrument, Mastercycler gradient (Nexus gradient, 115 V/50 – 60 Hz (USA)). Cellular DNA was obtained from *Staphylococci* colonies initially grown on mannitol salt agar plates and revived overnight on nutrient agar medium using DNA Extraction Kit (Bioneer Co., Korea). The *mecA and tetM*- specific primer pairs used for amplification of 533 base pair (bp) and 417 bp fragments were Forward: 5'-TCCAGATTACAACTTCACCAGG-3', - Reverse: 5'-CCAATTCCACATTGTTTCGGTCTA-3' and- Forward: 5'-GGAGATCATCCCTGATTGG-3' and - Reverse: 5'-TCTAGTTCAGGTCGAAGTG-3' respectively. Each of the PCR mixtures was prepared by mixing volume of 2µL of prepared DNA template to a final volume of 25 µL mixture containing10 µL of 2× Master Mix (Ampliqon, Denmark), including 1× PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTP, 1.25 µm *Taq* DNA polymerase, 0.7 µL of 1µmol/L each primer and 12.6 µL of sterile distilled water. The thermal cycling protocol for PCR was comprised 94 °C for 5minute, followed by 33 cycles of 94 °C for 30 seconds, 58 °C for 30 s, and 35 cycles at 72 °C for 1 minute, with a final extension at 72 °C for 5 min. The amplified products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide. This procedure was repeated to detect each of the antibiotic-resistant genes.

Determination of Prevalence of Occurrence of LA-MRSA

The method of Peter *et al.* (2022) was used to determine the prevalence/ detection rate of LA-MRSA isolates from the samples collected and calculated using the formula: Prevalence = Number of Isolate / Total population) x 100%. Twenty-two (22) LA-MRSA isolates were screened from the Thirty-Four (34) isolates of MRSA, which occurred in the Three Hundred and Twenty (320) samples collected, and the pooled prevalence was calculated. The prevalence of colonization of LA-MRSA in each sample collected was computed and recorded. The prevalence of LAMRSA at 95% confidence limits from poultry samples was further determined and recorded.

Determination of Antibiogram Pattern of the Test Isolates

The test bacterial isolates were sensitized with a panel of six antibiotics. The antibiotics selected were used for livestock management in Zamfara state and were also recommended for use in a surveillance study by the World Health Organization recommendations, as cited in Mudenda *et al.* (2023). Antibiogram study was performed using the Kirby Bauer disc diffusion method with sterile plates of Muellar Hinton agar. Six antibiotics discs (Oxoid Ltd, Basingstoke, UK) were used as: Penicillin (10µg), Sulphamethoxazole (23.75µg), Nitrofurantoin (300µg), Chloramphenicol (30µg), Ciprofloxacin (5µg) and Erythromycin (15µg). Two antibiotic discs from each of the antibiotics used were placed on the surface of Mueller Hinton agar plates already seeded with the test bacterial isolates and were incubated overnight at 37°C. The antibiotics impregnated in the disc diffused in the medium, causing zones of inhibition in the plates. The mean zones of inhibition were measured and recorded in millimeters. Interpretation of the zones of inhibition was carried out according to the guidelines of the Clinical Laboratory Standard Institute- CLSI (2020), and the test isolates were classified as either resistant or susceptible. The intermediate breakpoint was included in the susceptibility profile result to allow for a broader or more comprehensive understanding of the isolates susceptibility to the various antibiotics used as described in Aguilera-Alonso (2022).

Determination of Multiple Antibiotic Resistance Index (MARI)

The method described by Egwu *et al.* (2021) was used to determine the Multiple Antibiotic Resistant Index (MARI) of the LA-MRSA Isolates. The MARI was determined by dividing the total number of antibiotics to which a test isolate displayed resistance to the total number of antibiotics to which the test organism has been evaluated for sensitivity. Computation of MARI was done using the formula a/b, where a is the total number of antibiotics to which the isolate displayed resistance while b is the total number of antibiotics to which the isolate was exposed. Isolates having MARI index ≥ 0.2 were reported as originating from a high-risk source of contamination where several antibiotics were used (Afunwa *et al.,* 2020; Peter *et al.*, 2022). Isolates showing resistance to ≥3 antibiotics from different classes were reported as multi-drug resistant (MDR), as described in Akambi *et al.* (2017).

Statistical Analysis

The results obtained from this study were summarized as percentages in the form of tables and charts for descriptive purposes. Statistical packages such as SPSS (IBM Windows, Version 20 IBM Corp, Armonk, N. Y. USA) and R (programming Language, Vienna, Austria) were used to analyze the data set, while comparison between definite variables was done using ANOVA and Chi-Square test at 95% confidence interval and at 0.05 level of significance. A probability value below 0.05 was regarded as indicative of statistical significance.

RESULT

Table 1 shows the source distribution of *Staphylococcus aureus* isolates in samples collected from poultry farms.

The pooled prevalence of *Staphylococcus aureus* isolated was 114(100%). The highest and least source prevalence of the bacterial isolates were 27(23.68%) and 14(12.29%) representing isolates from hands and fecal droppings, respectively.

Percentage

Table 2 shows the pooled prevalence, 34(100%) of MRSA isolated from different samples (Fecal droppings, hand swap, soil, feed and water) in poultry farms within the study area. The MRSA isolates were detected using cefoxitin (30µg) disk antibiotics as the surrogate marker of the mecA gene (CLSI, 2020). The highest prevalence of MRSA, 9(26.47%), occurred in water samples, while the least occurrence of the test bacterial isolates, 6(17.64%), was each detected in feed, fecal droppings, and hand swab samples.

Table 3 shows the pooled prevalence or detection rate, 100% (95% CI: 97.72%-100%) and the prevalence of LAMRSA isolates from the various samples studied (Fecal 13.64% (95% CI: 10.93%-16.36%), hand swap, 18.18% (95% CI: 14.83%-21.53%), soil, 22.73% (95% CI: 19.43%-26.03%), feed 18.18% (95% CI: 14.83%-21.53%) and water, 27.27% (95% CI: 23.43%-31.11%). The highest prevalence of LA-MRSA, 6(27.27%), occurred in water samples, while the least prevalence of colonization of the test bacterial isolates, 3(13.64%), was detected in fecal samples. The prevalence of LAMRSA was categorized based on the Prevalence Cutoffs of LAMRSA in poultry farms as cited in the document of the European Food Safety Authority-UFSA (2017) document as Low <5%, Moderate=5-20%, High>20%, Very High>40%. The chi-Square test was used to compare the prevalence of LAMRSA across the various sources at 95% confidence interval and 0.05 level of Significance. Since the p-Value obtained (0.17) is greater than the level of significance (0.05), there is no statistically significant difference in the prevalence of LAMRSA among the samples at 95% confidence interval and 0.05 level of significance. The Prevalence of LA-MRSA in each sample studied is presented in Charts 1 and 2, respectively.

Table 4 shows the Antibiogram profile of the test bacterial isolates to the panel of antibiotics used in this study, while Table 5 indicates the CLSI (2020) interpretative category and diameter breakpoints to the nearest whole millimeter (mm), from which the susceptibility profile of the test isolates was determined and recorded. The susceptibility profile data was reported as dual variable (susceptible (S) and Resistance (R)) for each antibiotic tested. The intermediate breakpoint was included in the susceptibility profile result to allow for a broader and more comprehensive understanding of the isolates susceptibility to the various antibiotics used as described in Aguilera-Alonso (2022). The LA-MRSA isolates were 81.8% (S) and 18.2% (R) to Erythromycin (15µg), 77.3% (S), and 22.7% (R) to Ciprofloxacin (30µg), 72.7% (S) and 17.3% (R) to Chloramphenicol (30µg), 40.9% (S) and 59.1% (R) to Sulphamethoxazole (23.75µg), 22.7% (S) and 87.3% (R) to Nitrofurantoin and 0% (S) and 100% (R) to Penicillin (10µg) antibiotics. Isolates were further categorized as demonstrating high-level resistance (≥25%), resistance to antibiotics, moderate-level resistance (=15-24%), and low-level resistant (<14%), as cited in the CLSI (2020). ANOVA was used to compare the resistant profile of the test isolates to the panel of antibiotics at 95% confidence interval and α=0.05 level of significance. The P-value <0.05 indicates that there is a statistically significant difference in the resistant profile of the LAMRSA isolates.

Table 6 shows the multiple antibiotic resistance index (MARI) of the LA-MRSA isolates. A total of 16(72.2%) LAMRSA isolates (F34, F37, F48, H8, H15, H51, S11, S22, S25, S43, Fe14, Fe28, Fe54, W1, W3, and W31) phenotypically demonstrated cross-resistance to ≥3 classes of antibiotics used in this study and were accordingly reported as multi-drug resistant (MDR) LA-MRSA as described in Akambi *et al.,* (2017), while this trend was absent in 6 of the remaining isolates (H18, S33, Fe48, W9, W23 and W25) representing 27.3%. The detection rate of LAMRSA isolates showing MDR phenotype in the studied samples showed 13.6% of the isolates each in fecal droppings, hand, feed, and water samples, while 27.3% of the isolates were detected in water samples. All the test isolates showed MAR Index≥ 0.2 and were reported as originated from high-risk sources of contamination where several antibiotics were used for therapeutic purposes (Afunwa *et al.,* 2020; Peter *et al.*, 2022). The Chi Square test was used to compare MARI of the LA-MRSA isolates at 95% confidence interval and 0.05 level of significance. Since the P- value obtained was =0.9999 and greater than 0.05, signifying that there is no statistically significant difference in the MARI of the LA-MRSA isolates studied.

Table 1: Source Distribution of *Staphylococcus aureus* from Samples Collected

Sources of Sample Collected	Number of Samples	Number of *Staphylococcus aureus* +ve Isolates	Prevalence of Occurrence of *Staphylococcus aureus* (%)
Feces	64	14	12.28
Hand Swab	64	27	23.68
Soil	64	26	22.80
Feed	64	21	18.42
Water	64	26	22.81
Total	**320**	**114**	**100%**

Foot Note: (+): Positive; (%): Percentage

Table 2: Distribution of Methicillin Resistance *Staphylococcus aureus* (MRSA) Detected using Cefoxitin (30µg) Antibiotic Disk

Sources of Sample Collected	*Number of S. aureus* (+ve) Isolates	Number of MRSA (+ve) Isolates	Prevalence of MRSA (%)
Fecal Droppings	14	6	17.64
Hand swab	27	6	17.64
Soil	26	7	20.59
Feed	21	6	17.64
Water	26	9	26.47
Total	**114**	**34**	**100%**

Foot Note: MRSA: Methicillin Resistance *Staphylococcus aureus* (+): Positive; (%): Percentage. Isolates with Zones of Inhibition (≤21mm) due to Cefoxitin (30µg) Antibiotics were reported as MRSA as described in CLSI (2020)**.**

Table 3: Prevalence of LA-MRSA Isolates from Samples Collected

Source of Samples Collected	Number of MRSA Isolates	Number of LA-MRSA +ve Isolates and Prevalence (%)	Prevalence Range of LAMRSA at 95% Confidence Interval	P-Value
Fecal	6	3(13.64)	10.93-16.36	
Hand swab	6	4(18.18)	14.83-21.53	
Soil	7	5(22.73)	19.43-26.03	0.17
Feed	6	4(18.18)	14.83-21.53	
Water	9	6(27.27)	23.43-31.11	
Total	**34**	**22(100%)**	**97.72-100%**	

Foot Note: MRSA: Methicillin Resistant *Staphylococcus aureus;* LA-MRSA: Livestock Associated Methicillin Resistant *Staphylococcus aureus;* (+ve): Positive, Isolates of MRSA with a zone of inhibition (≤14mm) due to Tetracycline (30µg) antibiotics were reported as LA-MRSA isolates as described in CLSI, (2020)**. Prevalence of LAMRSA was reported based on the Prevalence Cutoffs of LAMRSA as cited in the document of the European Food Safety Authority-**UFSA (2017)**-Low <5%, Moderate=5-20%, High>20%, Very High>40%.**

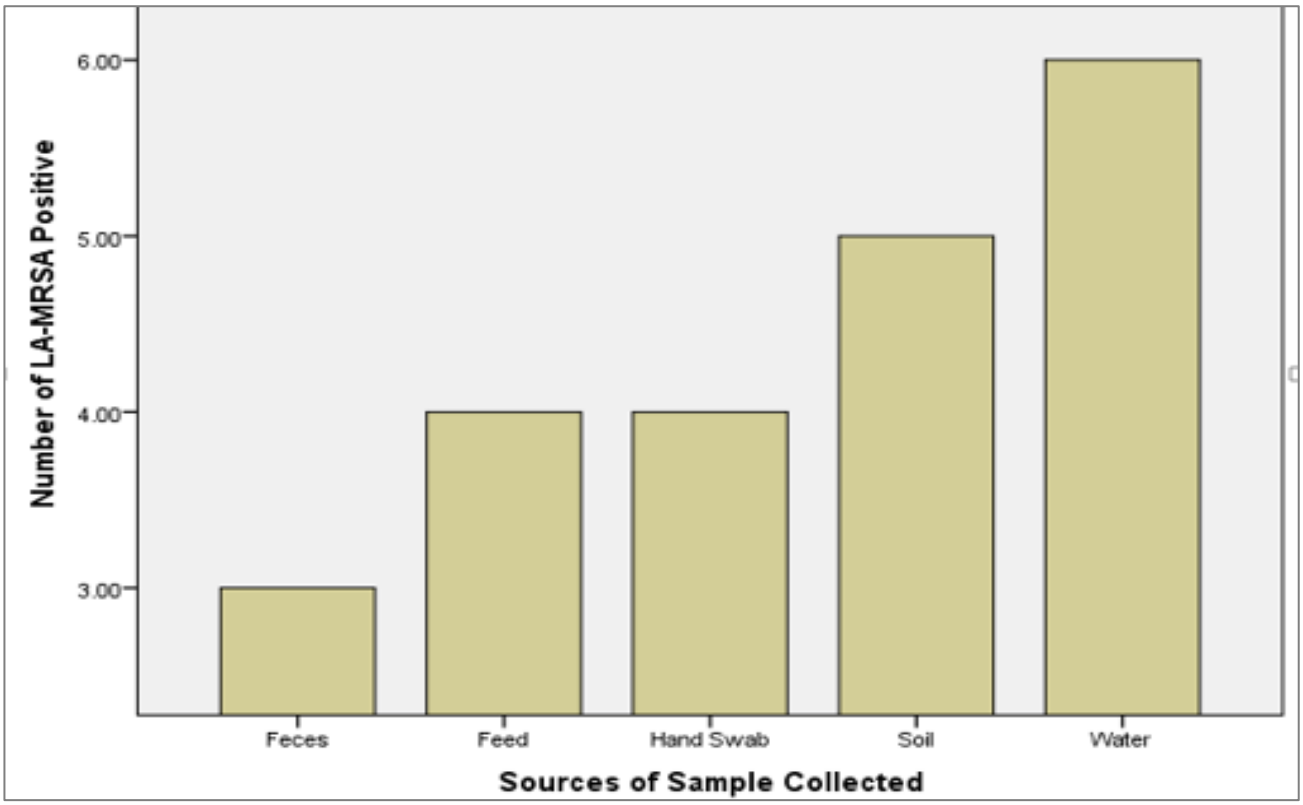

Chart 1: Bar Chart Showing Source Distribution of LA-MRSA Isolates from Samples Collection

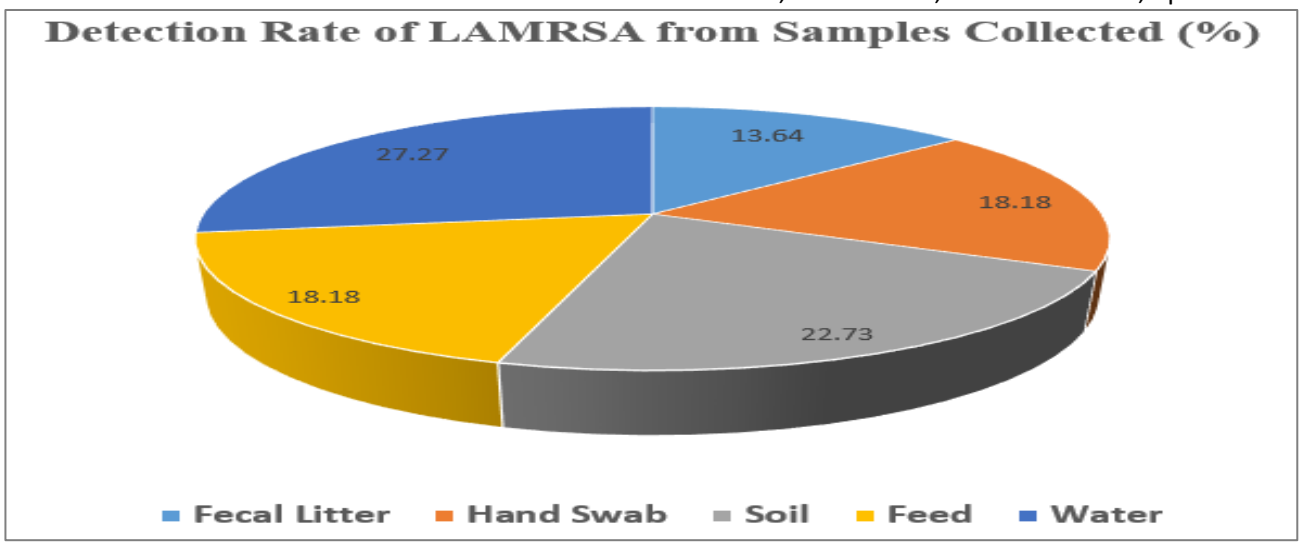

Chart 2: Pie Chart Showing the Detection Rate of LA-MRSA Isolates from Samples Collected

Table 4: Antibiotics Susceptibility Profile of LA-MRSA Isolates

Antibiotics	Conc. (µg)	Class	Antibiogram Profile		P-value
			Susceptible (%)	Resistant (%)	
Chloramphenicol	30	Amphenicol	16(72.7)	6(27.28)	
Erythromycin	15	Macrolide	18(81.8)	4(18.2)	
Sulphamethoxazole	23.75	Isoxazole	9(40.9)	13(59.1)	0.01545
Penicillin	10	β-Lactam	0(0.00)	22(100)	
Nitrofurantoin	300	Nitrofuran	5(22.7)	17(77.3)	
Ciprofloxacin	5	Fluoroquinolone	17(77.3)	5(22.7)	

Foot Note: Conc.- Concentration, Suscept.- Susceptibility. ANOVA was used to compare the susceptibility profile of the test isolates to the panel of antibiotics at 95% confidence interval and α=0.05 level of significance. The P-value obtained =0.01545 is α<0.05. Therefore, there is a statistically significant difference in the susceptibility profile of the isolates to the panel of antibiotics used.

Table 5: CLSI, 2020 Interpretative Category and Diameter Breakpoints to the nearest whole Millimeter (mm) of the Panel of Antibiotics used in this Study

Panel of Antibiotics	Interpretation Category and Zone Diameter Breakpoints to the Nearest Whole Millimeter (mm)		
	Susceptible (S)	Intermediate (I)	Resistant (R)
Ciprofloxacin (5µg)	≥21	16-20	≤15
Erythromycin (15µg)	≥23	14-22	≤13
Nitrofurantoin (300µg)	≥17	15-16	≤14
Sulphamethoxazole (23.75µg)	≥16	11-15	≤10
Penicillin (10µg)	≥29	—	≤28
Chloramphenicol (30µg)	≥18	13-17	≤12

Foot Note: Clinical Laboratory Standard Institute-CLSI, (2020)

Table 6: Multi-drug Drug Resistance & Multiple Antibiotic Resistance Index LA-MRSA Isolates

LA-MRSA Isolates Code	Antibiotics Resistant Phenotype	Percentage LAMRSA Isolates with MDR Phenotype in each Sample (%)	Multiple Antibiotic Resistance Index MARI=a/b	P-Value
F34	C, SXT, P and F/M		0.7	
F37	C, SXT, P and F/M	13.6	0.7	
F48	SXT, P and F/M		0.5	
H8	C, P and F/M		0.5	
H15	SXT, P and F/M		0.5	
H18	P and F/M	13.6	0.3	
H51	C, P and F/M		0.5	

To be continued next page

Table 6 Continued

LA-MRSA Isolates Code	Antibiotics Resistant Phenotype	Percentage LAMRSA Isolates with MDR Phenotype in each Sample (%)	Multiple Antibiotic Resistance Index MARI=a/b	P-Value
S11	SXT, P and F/M		0.5	0.9999
S22	SXT, P and F/M	27.3	0.5	
S25	E, P and F/M		0.5	
S33	P		0.2	
S43	SXT, P and F/M		0.5	
Fe14	E, SXT, P and F/M		0.7	
Fe28	SXT, F/M and CIP	13.6	0.5	
Fe48	P and F/M		0.3	
Fe54	C, SXT, P and CIP		0.7	
W1	E, SXT, P, CIP,F/M		0.8	
W3	E, SXT, P and F/M	13.6	0.7	
W9	P		0.2	
W23	SXT and P,		0.3	
W25	SXT and CIP		0.3	
W31	P, FM and CIP		0.5	

Foot Note: a= total number of antibiotics to which an isolate was resistant; b= number of antibiotics to which an isolate was exposed to. C (30µg) = Chloramphenicol; E15µg= Erythromycin; SXT23.75µg= Sulphamethoxazole; P10µg= Penicillin; F/M300µg= Nitrofurantoin; CIP5µg= Ciprofloxacin. F-Fecal; H- Hand swab; S- Soil; Fe- Feed and W- Water. Isolates showing MARI index ≥ 0.2 were reported as originated from high-risk sources of contamination where several antibiotics were used for therapeutic purposes (Afunwa *et al.,* 2020; Peter *et al.*, 2022). Isolates showing resistance to ≥3 antibiotics from different classes were reported as multi-drug resistant (MDR) LA-MRSA (Akambi *et al.,* 2017). Chi Square test was used to compare the MDR rate of the LA-MRSA isolates at 95% confidence interval and 0.05 level of significance. Since the P-value obtained (0.9999) is greater than 0.05, there is no significant difference in the MDR rate of the LA-MRSA isolates studied.

DISCUSSION

Livestock associated methicillin resistant *Staphylococcus aureus* remains one of the evolving clones of *Staphylococcus aureus* of global public health concern. In this study, screening for LA-MRSA was done culturally, biochemically, and through the use of cefoxitin (30µg) (≤21mm zone diameter) and tetracycline (30µg) (≤14mm zone diameter) antibiotics discs as surrogate makers of the mecA and tetM genes respectively. However, the use of molecular methods remains the gold standard. This corroborates the knowledge put forward by Pramodhini *et al.* (2012), CLSI (2020), and Ceballos *et al.* (2022), who adduced to the use of cefoxitin and tetracycline disc test as the proven and accurate substitutes that indicate the presence of the mecA and tetM genes in LA-MRSA. Cefoxitin antibiotic is used as a potent inducer for the expression of the *mec*A gene because it can increase the expression of penicillin-binding protein (PBP2a), which is encoded by the *mec*A gene. The protein confers resistance to most of the β-lactam antibiotics. Equally, ribosomal protection, which is mediated by transposon or chromosomal *tet*M determinants, is induced by tetracycline antibiotics. Using PCR assay for LAMRSA detection allays the risk of false-negative results common with phenotypic methods. In this study, we identified LA-MRSA isolates molecularly by determining the 533bp amplicon size to mecA gene and the 417 bp amplicon size to tetM gene using PCR assay. The two genes are the definitive indicator for LAMRSA detection. All the staphylococcal strains were mecA- and tetM positive and indicated universal resistance to all β-lactam and tetracycline antibiotics. This is consistent with the findings of Silva *et al.* (2021) in Portugal as well as Gaddaf *et al.* (2023) in Nigeria, who used PCR assay as a definitive tool in the identification of their LAMRSA isolates as cited in Beshiru *et al.,* (2024).

The detection rate of LAMRSA isolates remains a veritable tool for providing snapshots of disease burden, distribution pattern, and risk factors underlying its spread. The pooled prevalence of LAMRSA documented in this study was 22(100%) (Table 3). With the exception of the study conducted by Bernier-Lachance *et al.* (2020), who reported lower LAMRSA detection rate of 15(1.3%) in Canada, another study conducted by AbdTawab *et al.* (2017) in Egypt, Peter *et al.* (2022) in Nigeria and Sawadogo *et al.,* (2023) in Burkina Faso, documented a high prevalence of 53(66%), 77(66.9%) and 29(51.78%) respectively which are similar to our findings. These differences underscore the difference in the level of hygiene and quality of avian farm administration, especially with regard to environmental sanitation, the number of poultry in the market, methods used for sample collection, and methods of isolation of test bacteria in these studies. However, based on the prevalence cutoff

and assessment matrix of LAMRSA adduced by the UFSA (2017), the pooled prevalence of LAMRSA obtained in this study was high (>20%) similar to that obtained in soil, 5(22.73%) (95% CI: 19.43%-26.03%), and water, 6(27.27%) (95% CI: 23.43%-31.11%) samples, implying potential for significant LAMRSA transmission to humans through these sources. The moderate detection rate of the test isolates was shown in Fecal, 13.64% (95% CI: 10.93%-16.36%), feed, 18.18% (95% CI: 14.83%-21.53%) and Hand swab, 18.18% (95% CI: 14.83%-21.53%) samples, all of which were within the moderate prevalence cut off (=5-20%), specified by the UFSA, (2017), suggesting potential for moderate transmission of LAMRSA. The chi-square test was used to compare the prevalence of LAMRSA among the sample sources. There is no statistically significant difference in the prevalence of the LAMRSA isolates at 95% confidence interval and P<0.05 level of significance. Highlighting variability in sample collection and processing. However, the higher detection rate of LAMRSA in soil samples is attributable to poor sanitation and hygiene practices, particularly due to inadequate animal waste (hot spot for the exchange of antibiotics-resistant genes) disposal system, which could potentially contaminate soil. Nasal carriage caused by predisposal to bioaerosols is possible due to suspended dust particles from soil carrying LAMRSA isolates and could account for the high prevalence of LAMRSA in the soil. The influence of contaminated water in cup drinkers from which birds drink, due to their prolonged exposure without washing, provides a suitable environment for biofilm production by the LAMRSA isolates and is linked to the resistance to cefoxitin and tetracycline antibiotics used for the identification of the LAMRSA isolates and the reduced uptake of some of the antibiotics (high level resistant) observed in this study. This has far-reaching public health and economic implications, particularly increased risk of LAMRSA transmission to poultry handlers and dissemination to others, spread to other livestock, increased financial burden on veterinary care and economic losses due to decreased poultry sales, contamination of poultry products, decreased poultry productivity with morbidity and mortality,

In spite of efforts to minimize the antimicrobial-resistant burden through judicious antibiotic prescriptions by veterinarians, poor antibiotic stewardship persists in poultry markets due to their excessive use as prophylaxis and growth enhancers. Our findings demonstrated that all the 22(100%) LAMRSA isolates exhibited resistance to Penicillin antibiotics (Table 5), which is consistent with the findings of Bakheet *et al.* (2018) in Egypt, Kim *et al.* (2020) in Korea and Igbinosa *et al.* (2023) in Nigeria, each of whom reported 100% resistance to Penicillin antibiotics in their respective study. Similarly, Bounar-Kechih *et al.* (2018) in Algeria however reported 79% and 92% resistance in layer and broiler farms, respectively. These emphasize the rising resistance to β-lactam antibiotics reported across the globe, which underscores their overuse in livestock production, leading to persistent selection pressure that favored the emergence of antibiotic-resistant strains. Equally, our studies identified high resistant levels (≥25% resistant among the LAMRSA isolates) to Chloramphenicol 6(27.28%), Sulphamethoxazole, 13(59.1%), and Nitrofurantoin, 17(77.3%), which is consistent with the findings of Sawadogo *et al.,* (2023) in Burkina Faso who reported 54 (100%) resistant to Chloramphenicol antibiotics but varies with the findings of Zaman *et al.,* (2020) in Bangladesh who reported a lower resistant level, 15(6%) to Chloramphenicol antibiotics in their study on MRSA strains isolated from ornamental birds. Rao *et al.* (2022) in the United States and Bounar-Kechih *et al.* (2018) in Algeria reported a lower 4(9.1%) and 94(21%) resistant to Sulphamethoxazole respectively, among MRSA strains isolated from livestock, which are within the intermediate resistant threshold. A lower resistance pattern was observed in *S. aureus* strains against Ciprofloxacin 2 (2.8%) and Erythromycin 1(1.8%) in a study carried out by Geta and Kibret (2022) at Bahir Dar City, Ethiopia. Among the LAMRSA isolates in this study, 4(18.2%) were resistant to Erythromycin, while 5(22.7%) demonstrated resistance to Ciprofloxacin, both of which were within the moderate resistant threshold (=15-24%). This is at variance with the study conducted by Hermana *et al.* (2021) in Indonesia. The researchers demonstrated that 95% of *Staphylococcus aureus* strains were resistant to Erythromycin, while 56% demonstrated resistance to Ciprofloxacin. Gaddafi *et al.* (2022) in Kebbi State, Nigeria, and Beshiru *et al.* (2024) in Nigeria also reported 20(40%) and 10(26.3%) were resistant to Erythromycin in their respective study. Similarly, Igbinosa *et al.* (2023), in their study in Nigeria, reported high resistant levels, 71(64.6%) to Ciprofloxacin which is also consistent with the study of Beshiru *et al.* (2024) in Nigeria, who also reported elevated resistant level, 15(39.5%) to Ciprofloxacin among the LAMRSA isolates in their study. The resistance to Nitrofurantoin reported in this study is in contrast with the findings of Amoako *et al.* (2019) in South Africa, who reported that 120(100%) isolates of *Staphylococcus aureus* were susceptible to Nitrofurantoin which contradicts the findings of Igbinosa *et al.* (2023) in Edo State who reported 110(100%) resistance of Nitrofurantoin. The high resistance levels to Penicillin, Sulphamethoxazole, Chloramphenicol, and Nitrofurantoin demonstrated by the isolates in this study are connected to their excessive and inappropriate use for therapeutic purposes in poultry management. Conversely, the susceptibility/ moderate resistant levels demonstrated by the isolates, particularly to Ciprofloxacin Erythromycin Sulphamethoxazole, Chloramphenicol, and Nitrofurantoin, is linked to their more prudent and rational use in livestock production in the study area. However, the moderate resistant levels suggest that the potential for the emergence of resistant strains in the future is possible if appropriate measures are not taken.

Our study also identified 14 multi-drug resistant phenotypes among the LAMRSA Isolates (Table 6), with 16(72.2%) of the isolates demonstrating cross resistant to ≥3 different classes of antibiotics. This compares with the

findings of Igbinosa *et al.* (2023) in Edo State, who reported 57% resistant phenotypic pattern and 96.0% phenotype in another study in Iran. Igbinosa *et al.* (2023) and Bala *et al.* (2019) in Zaria, Nigeria, also reported higher MDR rates of 89(80.9%) and 82(83.3%) among MRSA strains in their respective study compared with the 16(72.2%) of MDR revealed in our study. Similarly, our study also compares with the lower MDR threshold, 82(20%), reported by Bounar-Kechih *et al.* (2018) in Algeria. The MDR rate in soil samples was the highest (27.3%) ($p<0.05$) compared with other MDR isolates from the other sources. The high MDR rate among LAMRSA isolates from various samples in this study is suggestive of the co-selection pressure arising from the prolong overuse and misuse of antibiotics, improper disposal of unused or expired antibiotics, and the presence of antibiotics in litters from treated birds containing poorly absorbed antibiotics from the poultry gut. All the isolates, 22(100%) demonstrated MAR Index ranging from 0.2-0.8, which is comparable to the findings of Igbinosa *et al.* (2023), who reported 93.6% and cited another study in Iran, which revealed that 96% of the isolates were resistant to ≥2 antibiotics. The result from this study suggests that all the isolates originated from high-risk sources of contamination where antibiotics were used excessively for therapeutic purposes. This is similar to the finding of Geta and Kebret (2022) in Ethiopia who reported that most of their LAMRSA isolates demonstrated MAR Index ≥0.2 in their study. Igbinosa *et al.* (2023) reported a MARI of up to 0.81 in their study. All of which are consistent with our findings. Impliedly, the soil, water, fecal droppings, feed, and hands of poultry handlers in this study contain antibiotics that are sourced from runoffs, poorly disposed or expired antibiotics, and antibiotics in litters that were poorly absorbed in the gut of treated birds. These antibiotics were either misused or overused in poultry management and therefore created a co-selection pressure leading to the emergence of LAMRSA isolates with multiple antibiotics resistant index. This, therefore, underlines the need for routine environmental sanitation, personal hygiene, vaccination, and antibiotic stewardship within the one health framework so as to minimize the emergence and spread of LA-MRSA across the farm-to-fork continuum.

CONCLUSION AND RECOMMENDATION

In this study, a total of 320 swab samples were randomly collected from fecal litter, Hands of poultry handlers, Soil, Feed, and Water from five different poultry markets in the Gusau metropolis. The LA-MRSA isolates involved were detected culturally and identified phenotypically and the use of PCR assay. The pooled prevalence of the LAMRSA isolates in the samples was 22(100%). The chi-square test was used to compare the prevalence of LAMRSA among the samples collected at 95% confidence interval and 0.05 level of significance. There is no statistically significant difference in the prevalence of LAMRSA among the samples. Findings from the antibiogram study conducted revealed that all the 22(100%) LAMRSA isolates were resistant to Penicillin antibiotics, with resistance to other antibiotics following the order: Nitrofurantoin, 17(77.3%), Sulphamethoxazole, 13(59.1%), Chloramphenicol 6(27.28%), Ciprofloxacin 5(22.7%) and Erythromycin 4(18.2%). A total of 14 multi-drug phenotypes were identified from the 22 LAMRSA isolates with 16(72.2%) demonstrating cross resistant to ≥3 of the six different classes of antibiotics used. The MDR rate in soil samples was the highest (27.3%) ($p<0.05$) compared to other MDR isolates from the other sources. All the isolates, 22(100%) demonstrated MAR Index ranging from 0.2-0.8. These are disturbing trends with significant risks to humans and animals. Suggesting a potential for inoculation of self and transmission to others, particularly through those who sell and make daily contact with live birds. Therefore, the government should awaken the consciousness of poultry farmers/handlers by way of sensitization on the need to practice routine personal and environmental hygiene as it relates to poultry management, with a focus on antibiotic stewardship within the one health framework so as to lower endemicity and equally break the chain of transmission of LA- MRSA across the farm to fork continuum.

ACKNOWLEDGMENTS

The researchers appreciate Tetfund for their generosity in funding this research through the institutional-based research grant (IBR) with grant allocation number TETF/DR&D/CE/UNI/GUSAU/IBR/2023/VOL.1. We further extend our gratitude to Maimuna Abubakar for contributing in sample collection and the bench work. The authors extend their profound gratitude to the management of Federal University Gusau for providing the enabling environment to conduct this study.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

DISCLOSURE

The authors declare no competing interests in this work.

REFERENCES

Abd El Tawab, A. A., Hofy, F. I., Mohamed, S. R. and Amin, S. H. (2017). Characterization of Methicillin Resistance *Staphylococcus aureus* Isolated from Chicken and Human. *Benha Journal of Veterinary Medicine*. 32(1): 132-137. **[Crossref]**

Abd El-Ghany, A.W. (2021). *Staphylococcus aureus* in Poultry, with Special emphasis on Methicillin-resistant Strain Infection: A Comprehensive Review from One Health Perspective. *International Journal of One Health*. 7(2): 257-261. **[Crossref]**

Achi, R. C., Ayobami, O., Mark, G., Egwuenu, A., Ogbolu, D. and Kabir, J. (2021). Operationalizing One Health in Nigeria: Reflection from a High Level Expert Panel Discussion Commemorating the 2020 World Antibiotics Awareness Week. *Frontiers in Public Health*. 1-7. **[Crossref]**

Afunwa, R. A., Ezeanyinka, J., Afunwa, E. C., Udeh, A. S., Oli, N. A. and Unachukwu, M. (2020). Multiple Antibiotic Resistant Index of Gram-Negative Bacteria from Bird Droppings in Two Commercial Poultries in Enugu, Nigeria. *Open Journal of Medical Microbiology.* 10: 171-181. **[Crossref]**

Aguilera-Alonso, D., Campos, L. M., Fernández, C. M., Calvo, C. L. and Baquero-Artigao, F. (2021). New Antibiotic Susceptibility Testing Definitions: «I» No Longer Means Intermediate Susceptibility. *Asociacion Espanola de Pediatria.* 96(2):157-158. **[Crossref]**

Akanbi, O. E., Njom, H. A., Fri, J., Otigbu, A. C. and Clarke, A. M. (2017). Antimicrobial Susceptibility of *Staphylococcus aureus* Isolated from Recreational Waters and Beach Sand in Eastern Cape Province of South Africa. *International Journal of Environmental Research and Public Health.*14 (9):1-15. **[Crossref]**

Amoako, D. G., Somboro, A. M., Abia, A. L. K., Molechan, C., Perrett, K., Bester, L. A., & Essack, S. Y. (2020). Antibiotic Resistance in *Staphylococcus aureus* from Poultry and Poultry Products in uMgungundlovu District, South Africa, Using the "Farm to Fork" Approach. *Microbial Drug Resistance (Larchmont, N.Y.)*, *26*(4), 402–411. (2): 25-33. **[Crossref]**

Anjum, M. F., Marco-Jimenez, F., Duncan, D., Marin, C., Smith, R. P. and Evans, S. J. (2019). Livestock Associated methicillin Resistant *Staphylococcus aureus* from Animals and Animal Products in the UK. *Frontiers in Microbiology.* 10(2136):1-7. **[Crossref]**

Bakheet, A. A., Amen, O., Sayed H. A., Habaty, S., Darwish, F. (2018). Prevalence of *Staphylococcus aureus* in Broiler Chickens with Special Reference to Beta-Lactam Resistance Genes in the Isolated Strains. *Alexandria Journal of Veterinary Sciences.* 57. **[Crossref]**

Bala, H. K., Igwe, J. C. and Onaolapo, J. A. (2019). Molecular Characterization of Staphylococcus aureus from Poultry Farms in Kano State Nigeria. Bayero Journal of Pure and Applied Science. 12(1):359-365. **[Crossref]**

Bale, M., Kolawole, D. O., Babatunde, S. K., Ajiboye, E. O., Adedayo, R. M., Adetumbi, M. and Ajao, A. T. (2018). Methicillin Resistant *Staphylococcus aureus* in Commercial Layers and Poultry Farm Attendants in North Central, Nigeria. *FUTA Journal of Research in Science.* 14(1):112-120. https://journals.futa.edu.ng/home/paperd/653/47/index.html

Bernier-Lachance, J., Arsenault, J., Usongo, V., Parent, É., Labrie, J., Jacques, M. (2020) Prevalence and characteristics of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) Isolated from Chicken Meat in the Province of Quebec, Canada. *PLoS ONE* 15(1): 1-6e0227183. **[Crossref]**

Beshiru, A., Igbinosa, I. H., Akinnibosun, O., Ogofure, A. G., Dunkwu-Okafor, A., Uwhuba, K. E. & Igbinosa, E. O. (2024). Characterization of Resistance and Virulence Factors in Livestock-Associated Methicillin-Resistant *Staphylococcus aureus*. *Science Report* **14**, 13235. **[Crossref]**

Bitrus, A. A., Peter, O. M., Abbas, M. A. and Goni, M. D. (2018). *Staphylococcus aureus*: A Review of Antimicrobial Resistance Mechanisms. *Veterinary Sciences: Research and Reviews.* 4(2): 43-54. **[Crossref]**

Bitrus, A. A., Zunita, Z., Goni, M. D., Mshelia, I. T. (2018). Dissemination of Resistance and Virulence Determinants in Methicillin-resistant *Staphylococcus aureus* during Colonization and Disease. *Advances Animal and Veterinary Science.* 6(1): 44—54. **[Crossref]**

Bloemendaal, A. L., Brouwer, E. C. and Fluit, A. C. (2010). Methicillin Resistance Transfer from *Staphylocccus epidermidis* to methicillin-susceptible *Staphylococcus aureus* in a patient during Antibiotic Therapy. *PLOS One Journal.* 5(7): 1-7 **[Crossref]**

Bounar-Kechih, S., Hamdi, T. M., Aggad, H., Meguenni, N. and Cantekin, Z. (2018). Carriage of Methicillin Resistant Staphylococcus aureus in poultry and Cattle in Northern Algeria. Veterinary Medicine International. 2018:1-5. . **[Crossref]**

Ceballos, S., Lozano, C., Aspiroz, C., Ruiz-Ripa, L., Eguizábal, P., Campaña-Burguet, A., Cercenado, E., López-Calleja, A. I., Castillo, J., Azcona-Gutiérrez, J. M., Torres, L., Calvo, J., Martin, C., Navarro, M., Zarazaga, M., Torres, C., & The Study Group Of Clinical LA-MRSA (2022). Beyond CC398: Characterisation of Other Tetracycline and Methicillin-Resistant *Staphylococcus aureus* Genetic Lineages Circulating in Spanish Hospitals. *Pathogens (Basel, Switzerland)*, 11(3):1-15. **[Crossref]**

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press. Pg. 46-70. **[Crossref]**

Chih-Ming, T., Caldera, J. R., Irshad, A. H., Austine, T. W., Chih-Hsiung, T., Haining, L., Maria, L. D., Cesia, G., Desmond, T., Glislaine, A. M., David, M. Underhill, M. A., Nathan, E. L., George, Y. L. (2022). Non-protective Immune Imprint Underlines Failure of *Staphylococcus aureus* isdB vaccine. *Cell, Host and Microbe.* **30**(8): 1-10.

Clinical and Laboratory Standards Institute (CLSI) (2012). Performance Standards for Antimicrobial Susceptibility Testing. 22nd Information Supplement. M100-S22. Sourced online from: http//antimicrobianos.com

Clinical and Laboratory Standards Institute (CLSI) (2020). Performance Standards for Antimicrobial Susceptibility Testing.22nd Information Supplement.M100-S22. Pg 49-80. Sourced

online from: http//antimicrobianos.com on 15th March, 2024

Crespo-Piazuelo, D. and Lawlor, G. P. (2021). Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA). Prevalence in Humans in Close Contact with Animals and Measures to Reduce On-Farm Colonization. *Irish Veterinary Journal*.74 (21):1-12. **[Crossref]**

Crespo-Piazuelo, D., & Lawlor, P. G. (2021). Livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA) Prevalence in Humans in close Contact with Animals and Measures to Reduce on-farm Colonization. *Irish Veterinary Journal*, *74*(1): 21. **[Crossref]**

Egwu, I. H., Iroha, I. R., Egwu-Ikechukwu, M. M., Peter, I. U., Nnabugwu, C. C., Ali, C. M., Elom, E. E., Ogene, L. N., Edemekong, C. I. and Mohammed, I. D. (2021) "Antimicrobial Susceptibility Pattern and Molecular Identification of Acinetobacter baumannii in Alex Ekwueme-Federal University Teaching Hospital Abakaliki, Nigeria. *Journal of Pharmaceutical Research International*, 33(44):409–419. **[Crossref]**

Environments in Bahir Dar City, Northwestern Ethiopia. *Journal of Multidisciplinary healthcare*, *15*: 1403–1414. **[Crossref]**

European Food Safety Authority-EFSA. (2017). Guidance on the Monitoring of MRSA in Poultry. EFSA Journal. 15(10):15-60. **[Crossref]**

Federal Ministry of Agriculture Environment and Health (2017). Antimicrobial use and Resistance in Nigeria, Situation Analysis and Recommendations. Pg. 45-100. Sourced Online from: https://ncdc.gov.ng/themes/common/docs/protocols/56_1510840387.pdf

Frank-peterside N. and Mukoro, M. (2010). The Current Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* to Antibiotics in Port-Harcourt. Global Journal of Pure and Applied Sciences. 16(4): 375- 379. **[Crossref]**

Gaddafi, M. S., Yakubu, Y., Bello, M. B., Bitrus, A. A., Musawa, A. I., Garba, B. , Lawal, H., Aliyu, M. A., Barka, S. A. , Emeka, A. J. (2022). Occurrence and Antibiotic Resistance Profiles of Methicillin-resistant Staphylococcus aureus (MRSA) in layer chickens in Kebbi, Nigeria. *Folia Veterinaria.* 66(2): 37-45. **[Crossref]**

Gaddafi, M. S., Yakubu, Y., Junaidu, A. U., Bello, M. B., Bitrus, A. A., Musawa, A. I., Garba, B., Lawal, H. (2023). Occurrence of Methicillin-resistant Staphylococcus aureus (MRSA) From Dairy Cows in Kebbi, Nigeria. *Iranian Journal of Veterinary Medicine*. 17(1): 19-26. **[Crossref]**

Garga, M. A. (2017). Antibacterial Activity and Phytochemical Screening of *Moringa oleifera* Leaves and Seeds extract on some Selected Clinical Bacterial Isolates. A Master of Science (M.Sc.) Degree Dissertation of the Department of Microbiology, Usmanu Danfodiyo University, Sokoto. Pg. 26-28

Geta, K. and Kibret, M. (2022). Antibiotic Resistance Profiles of Bacteria Isolated from Hotspot Environments in Bahir Dar City, Northwestern Ethiopia. Journal of Multidisciplinary Healthcare 15: 1403–1414. **[Crossref]**

Hermana, N., Safika, S., Indrawati, A., Wibawan, I. and Pasaribu, F. (2021). Identification of Antibiotic Resistance Pattern and Antibiotic Resistance Gene of *Staphylococcus aureus* from Broiler Chicken Farm in Sukabumi and Cianjur Regency, West Java Province, Indonesia. May 2023 IOP Conference Series Earth and Environmental Science. 1174(1):2-7. **[Crossref]**

Heston, T. F. (2023). The Robustness Index: Going Beyond Statistical Significance by Quantifying Fragility. *Cureus*, 15(8), e44397. **[Crossref]**

IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA

Igbinosa, E. O., Beshiru, A., Igbinosa, I. H., Ogofure, A. G., Ekundayo, T. C., & Okoh, A. I. (2023). Prevalence, Multiple Antibiotic Resistance and Virulence Profile of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Retail Poultry Meat from Edo, Nigeria. *Frontiers in Cellular and Infection Microbiology*, 13: 1122059. **[Crossref]**

Kim, S. Y., Kuppusamy, S., Kim, J. H., Yoon, Y.-E., Kim, K.-R., & Lee, Y. B. (2016). Occurrence and Diversity of Tetracycline Resistance Genes in the Agricultural Soils of South Korea. *Environmental Science and Pollution Research.* 23: 22190-22196. **[Crossref]**

Kim, Y. H., Kim, H. S., Kim, S., Kim, M., & Kwak, H. S. (2020). Prevalence and Characteristics of Antimicrobial-Resistant *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* from Retail Meat in Korea. *Food Science of Animal Resources*, *40*(5): 758–771. **[Crossref]**

Losito, P., Vergara, A., Muscariello, T. and Ianieri, A. (2005). Antimicrobial Susceptibility of Environmental *Staphylococcus aureus* Strains Isolated from a Pigeon Slaughterhouse in Italy. *Journal of Poultry Science.* 84:1802–1807. **[Crossref]**

Matuszewska, M., Murray, G. R., ***B***, X., W, Holmes, A. M. and Weinert, L. A. (2022). Stable Antibiotics Resistance and Rapid Human Adaptation in Live Stock Associated Methicillin Resistance *Staphylococcus aureus*. *Life Journal.* 11(3): 1-22. **[Crossref]**

Mudenda S, Bumbangi, F. N., Yamba, K., Munyeme, M., Malama, S., Mukosha, M., Hadunka, M. A., Daka, V., Matafwali, S. K., Siluchali, G., Mainda, G., Mukuma, M., Hang'ombe, B. M., and Muma, J. B. (2023). Drivers of Antimicrobial Resistance in Layer Poultry Farming: Evidence from high

Prevalence of Multidrug-resistant *Escherichia coli* and enterococci in Zambia. *Veterinary World.* 16(9): 1803-1814. **[Crossref]**

National population commission (2017). Knowledge of Family Planning Method and 2006 population and housing Census of the Federal Republic of Nigeria. Zamfara State. Priority Table volume 1. Abuja, NPC. Pg. 22-34. Sourced online from: https://nationalpopulation.gov.ng/

Okolie, S. O., Iroha, R. I., Ikpe, V. P., Peter, U. O., Victor, E. J. and Alexander, I. (2023). Characterization of Multi-drug Resistance Livestock Acquired Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) Isolates from Different Swine Farms in Enugu Metropolis, Nigeria. *Universal Journal of Pharmaceutical Research.* 7(5): 1-10. **[Crossref]**

Peter, I. U, Ngwu, J. N., Edemekong, C., Ugwueke, I. V., Uzoeto, H. O., Joseph, O. V., Mohammed, I. D., Mbong, E. O., Nomeh, O. L., Ikusika, B. A., Ubom, I. J., Inyogu, J. C., Ntekpe, M. E., Obodoechi, I. F., NseAbasi, P. L., Ogbonna, I. P., Didiugwu, C. M., Akpu, P. O.,Alagba, E. E., Ogba, R. C., Iroha, I. R. (2022). First Report Prevalence of Livestock Acquired Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) Strain in South Eastern, Nigeria. *IOSR Journal of Nursing and Health Science.* 11(1): 50-56. **[Crossref]**

Pramodhini S., Thenmozhivalli P. R., Selvi R., Dillirani, V., Vasumathi, A. and Agatha D. (2011). Comparison of Various Phenotypic Methods and mecA Based PCR for the Detection of MRSA. *Journal of Clinical and Diagnostic Research.* 5(7): 1359-1362. **[Crossref]**

R Core Team (2021). R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project/.

Rao, S., Linke, L.,Magnoson, R., Jauch, L. and Hyatt, D. R. (2022). Antimicrobial Resistant and Genetic Diversity of *Staphylococcus aureus* collected from Livestock, Poultry and Humans. One Health. 15(2022):3-5. **[Crossref]**

Saenhom, N., Kansan, R., Chopjitt, P., Boueroy, P., Hatrongjit, R. and Kerdsin, A. (2022). Evaluation of In-house Cefoxitin Screening Broth to Determine Methicillin-resistant Staphylococci. *Heliyon.* 8(3):1-8. **[Crossref]**

Sawadogo, A., Kagambèga, A., Soro, D., Bognini, H., Dione, M., Cissé, H., Dieni, I. and Barro, N. (2023). Prevalence, Antibiotic Resistance of *Staphylococcus aureus* Isolated from Layer farms (eggs, droppings/litter) in the Outskirts of the City of Ouagadougou, Burkina Faso. *African Journal of Microbiology Research.* 17(12): 306-313

Sharma, V., Sharma, S., Dahiya, D. K., Khan, A., Mathur, M and Sahrma, A. (2017). Coagulase Gene Polymorphism, Enterotoxigenicity, Biofilm Production and Antibiotics Resistance of *Staphylococcus aureus* Isolated from Bovine Raw Milk In Northwest India. *Annals of Clinical Microbiology and Antimicrobials.* 16(65):1-10. **[Crossref]**

Shoaib, M., Aqib, A. I., Muzammil, I., Majeed, N., Bhutta, Z.A., Kulyar, MF-e-A., Fatima, M., Zaheer, C-NF., Muneer, A., Murtaza, M., Kashif, M., Shafqat, F. and Pu, W. (2023). MRSA Compendium of Epidemiology, Transmission, Pathophysiology, Treatment, and Prevention within one Health Framework. *Frontiers in Microbiology.* 13(1067284):1-30. **[Crossref]**

Silva, V., Vieira-Pinto, M., Saraiva, C., Manageiro, V., Reis, L., Ferreira, E., Caniça, M., Capelo, J. L., Igrejas, G., & Poeta, P. (2021). Prevalence and Characteristics of Multidrug-Resistant Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) CC398 Isolated from Quails (*Coturnix Coturnix Japonica*) Slaughtered for Human Consumption. *Animals: An Open Access Journal from MDP.* 11(7): 2038. **[Crossref]**

Sineke, N., Asante, J., Amoako, D. G., Abia, A. L. K.,, Perrett, K., Bester, L. A. and Essack, S. Y. (2021). *Staphylococcus aureus* in Intensive Pig Production in South Africa: Antibiotic Resistance, Virulence Determinants and Clonality. *Pathogens.* 10 (317):1-19. **[Crossref]**

Suleiman, A., Zaria, L.T., Grama, H. A. and Ahmed, P. (2013). Antimicrobial Resistant Coagulase Positive *Staphylococcus aureus* from Chicken in Maiduguri Nigeria. *Sokoto Journal of Veterinary Science.* 11(1):51-55. **[Crossref]**

Thrusfield, M. (2007).Veterinary Epidemiology.3rd Edition. Blackwell Science Limited. Pg. 1-12 Sourced online from: www.blackwellpublishing.com.

Tsai, C., Caldera, J. R., Hajam, A. I., Chiang, T. W. A, Tsai, C., Li, H., Díez, M. L., Gonzalez, C., Trieu, D., Martins, G. A., Underhill, M. D., Arditi, M., Lewis, N. E. and Liu, G. Y. (2022). Non-protective Immune Imprint Underlines Failure of *Staphylococcus aureus* IsdB Vaccine. *Journal Host Microbe.* 30(8):1-10. **[Crossref]**

Tumbarski, Y. D. (2020). Food Borne Zoonotic agents and their Food Bioterrorism Potential: A Review. *Bulgarian Journal of Veterinary Medicine.* **23**(2): 147–159. **[Crossref]**

VAN DEN Broek, I. V., VAN Cleef, B. A., Haenen, A., Broens, E. M., VAN DER Wolf, P. J., VAN DEN Broek, M. J., Huijsdens, X. W., Kluytmans, J. A., VAN DE Giessen, A. W., & Tiemersma, E. W. (2009). Methicillin-resistant *Staphylococcus aureus* in People Living and Working in Pig Farms. *Epidemiology and Infection.* 137(5): 700–708. **[Crossref]**

Vestergaard, M., Frees, D. and Ingmer, H. (2019). Antibiotic Resistance and the MRSA Problem. *Microbiology Spectrum.* 7(2):1-13. **[Crossref]**

World Health Organization (2024). Antimicrobial Resistant. Sourced online from: https://www.who.int/news-room/fact-

sheets/detail/antimicrobial-resistance, on 14th April, 2024

World Health Organization [WHO] (2014). Antimicrobial Resistance Global Report on Surveillance. World Health Organization Technical Report. Pg. 14-28. Sourced online from: https://apps.who.int/iris/handle/10665/112642 on 20th February, 2024

Zaman, S. B. ., Sobur, M. A. ., Hossain, M. J. ., Pondit, A. ., Khatun, M. M. ., Tawyabur, M. ., & Rahman, M. T. (2020). Molecular detection of methicillin-resistant Staphylococcus aureus (MRSA) in ornamental birds having public health significance. *Journal of the Bangladesh Agricultural University*, *18*(2), 415–420. **[Crossref]**