



## The Detection of *Methicillin Resistant Staphylococcus Aureus* (MRSA) at Poultry in Benin City

Akinmoladun Oluwadamilola Posi  
Department of Pharmaceutical Microbiology,  
University of Benin,  
Edo State, Nigeria.

### ABSTRACT

*Methicillin-resistant Staphylococcus aureus (MRSA) is a significant public health concern due to its resistance to beta-lactam antibiotics and its association with severe infections. This study investigates the detection and prevalence of MRSA in poultry in Benin City, Nigeria. Samples were collected from poultry environments, and microbiological and biochemical techniques were employed to identify the presence of MRSA. The findings highlight the potential role of livestock as reservoirs for MRSA and underscore the implications for public health and antibiotic stewardship.*

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### INTRODUCTION

*Staphylococcus aureus* is a bacterium always present on skin and mucous membranes of people and animals and can result in a range of skin conditions or life-threatening illness including pneumonia, sepsis, and endocarditis (Chambers & DeLeo, 2009). Due to the characteristic of moving across different terrains and on different substrata, the bacteria is widespread. Excessive and incorrect utilization of antibiotics in human and animal treatment has led to the development of antibiotic-resistant organisms especially methicillin-resistance *Staphylococcus aureus* (MRSA) (Lee, 2003). Another important source of MRSA strains that is livestock farming has been highly recognized to humans since it is not only a direct menace to human health but also an indirect menace (Otto, 2010).

### Risk Factors for Acquisition of *S. aureus* Infection

Several risk factors are associated with *S. aureus* infection both in humans and animals. These are poor personal hygiene, diseases or drugs affecting immune system, hospitalization, and living in compact environment as in hospitals, care homes and alike (Zhang et al., 2005). Regarding livestock, poor hygiene keeping animals and high usage of antibiotics in feed promote growth of MRSA. Confinement production systems where

animals are penned densely increase the prevalence of *S. aureus* in animals (Lee, 2003).

Nasal carriage is one of the most significant mode through which *S. aureus* spread. Colonization of the nasal passages act as a chronic source of autoinfection, and transmission from one person to another (Otto, 2010). In livestock, nasal carriage helps spread zoonotic MRSA strains capable of infecting human being including farmers handling animals especially poultry (Chambers & DeLeo, 2009). Nasal carriage of MRSA is known to predispose the carriers to invasive infections and contributes to the transmission of the organisms in hospital and community settings; Odetokun, I. A, 2023).

Some people are at increased risk of getting *S. aureus* infections. These are patients admitted in the hospital requiring surgical procedures, health care workers that are often exposed to infected persons, individuals with weakened immunity such as those with HIV/AIDS, individuals with chronic diseases such as diabetes or cancer (NCDC, 2021). Poultry farmers are more at risk of acquiring zoonotic MRSA strains because most of them spend many hours with colonized animals or in contaminated environments (Lee, 2003). This occupational risk therefore stresses on protective measures and surveillance measures in agricultural environments (Oke, A. J., & Oke, A. A. (2012)).

Corresponding author: Akinmoladun Oluwadamilola Posi

✉ [akinmoladunoluwadamilola01@gmail.com](mailto:akinmoladunoluwadamilola01@gmail.com)

Department of Pharmaceutical Microbiology, University of Benin, Edo State.

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Traditionally, the infections brought by the *S. aureus* bacteria could be treated by beta-lactam antibiotics. For example, a new variant of MRSA that harbours the *mecA* gene requires the use of other antibiotics for example vancomycin, and linezolid (Chambers & DeLeo, 2009). Yet, with such alternatives, there is increasing emergence of VRSA which is a major problem as far as treatment is concerned. This comes as a result of the high resistance making the clinical treatment of bacterial infections very challenging; hence the dire need for new antibiotics and better antimicrobial stewardship programs.

Certain aspects affecting the virulence of *S. aureus* include toxin formed by the bacteria, such as hemolysins and enterotoxins, biofilm formation and the presence of resistance genes (Otto, 2010). The *mecA* gene that encodes an altered PBP2a brings resistance to methicillin and other drugs belonging to the beta-lactam group. Symbiotic growth in biofilm configuration improves the capability of the bacterium to resist host immune system and antibiotic eradication hence chronic inflammation (Zhang et al., 2005).

## LITERATURE REVIEW

Much evidence has established the existence and effects of MRSA in human and animal populations. Preliminary research in Nigeria has isolated MRSA in poultry production scenes, concluding that extensive farming practices facilitated the germination of the superbug (NCDC, 2021). Internationally, studies reveal that livestock-associated MRSA strains have zoonotic possibilities especially from poultry and other food producing animals (Lee, 2003). Surveillance, and Molecular epidemiology have pointed out the need to closely monitor MRSA to enable designers of control measures to reduce public health risk [Igbinosa, E. O., 2023].

The identification of MRSA needs to involve both microbiological and biochemical tests. Gram staining gives preliminary clue for *Staphylococcus* species while Coagulase and Catalase tests confirm *S. Aureus*. This is made possible by culturing on selective media for example Mannitol Salt Agar (MSA), and oxacillin containing agar. Conventional biochemical tests are not helpful in confirming methicillin resistance and therefore, molecular techniques

especially PCR is useful in establishing the presence of the *mecA* gene (Zhang et al., 2005).

Consequence of MRSA to Public Health MRSA has a bearing on public health since it causes over extended hospital stays, additional costs and lack of adequate treatment. Livestock-associated MRSA strains are particularly difficult for control because they can transfer to humans through contact or through food (Otto, 2010). This raises the possibility of community transmission and this put emphasizes the need for consequential measures aimed at controlling infection and spread as well as the prudent use of antibiotics.

History of Livestock and MRSA Use of antibiotics in livestock feeding as growth promoters and for disease prevention has led to the emergence of resistant strains of *S. aureus* (Lee, 2003). Lay practices including inadequate sanitation, and unauthorized animal traffic promote dissemination of MRSA between and within farms. Contact between people and animals, particularly under controlled conditions in high inputs farm structure have created a pathway for zoonotic transmission, linking agricultural practices to human health risks (Chambers & DeLeo, 2009).

## Objectives of the Study

1. To detect the presence of MRSA in poultry environments in Benin City.
2. To assess the potential public health risks associated with livestock-associated MRSA.
3. To evaluate the effectiveness of microbiological methods in identifying MRSA.

## MATERIALS AND METHODS

Selective media, Mannitol Salt Agar (MSA) was prepared on the basis of protocol to isolate *Staphylococcus aureus*. *Staphylococcus aureus*. MSA, rich in salt, favour the growth of *S. aureus*, and is also effective in suppressing non-halotolerant organisms. For the identification of methicillin-resistant *S. aureus*, antibiotic sensitivity plates containing oxacillin at CLSI-recommended concentrations were used. These media favored the emergence of the resistant strains whilst on the other hand, exerting a damaging effect on the susceptible ones (Currie & Ferguson, 2018, Lawal & Shittu, 2016). Samples were scrapped from different

Corresponding author: Akinmoladun Oluwadamilola Posi

✉ [akinmoladunoluwadamilola01@gmail.com](mailto:akinmoladunoluwadamilola01@gmail.com)

Department of Pharmaceutical Microbiology, University of Benin, Edo State.

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poultry farms in Benen city but major focus was on cage, feeder, waterer and nesting area. *Staphylococcus aureus*. MSA, which contains high salt concentrations, selectively encourages the growth of *S. aureus* while inhibiting non-halotolerant organisms. For the detection of methicillin resistance, antibiotic-containing agar plates were prepared by incorporating oxacillin at concentrations recommended by the Clinical and Laboratory Standards Institute (CLSI). These media ensured the growth of resistant strains while suppressing susceptible ones (Lawal, R. O., & Shittu, A. O. (2016).

Environmental swabs were collected from various poultry farms in Benin City, focusing on high-contact surfaces such as cages, feeders, waterers, and nesting areas. Fifty samples were collected aseptically by means of sterile cotton tipped applicator stick which had been moistened with sterile normal saline to improve adherence of microorganisms. This was done by placing every swab into sterile transportation tubes to minimize any form of sample contamination and transportation was done within 4 hours at appropriate temperatures to avoid microbial growth or degradation (Akanbi, B. O., & Njoku, R. (2019). When the specimens reached the laboratory, the swabs were smear streaked on to MSA agar to enhance the growth and isolation of the *S. aureus*. Instructions to facilitate the isolation of *Staphylococcus aureus*. MSA, which contains high salt concentrations, selectively encourages the growth of *S. aureus* while inhibiting non-halotolerant organisms. For the detection of methicillin resistance, antibiotic-containing agar plates were prepared by incorporating oxacillin at concentrations recommended by the Clinical and Laboratory Standards Institute (CLSI). These media ensured the growth of resistant strains while suppressing susceptible ones (Lawal, R. O., & Shittu, A. O. (2016).

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overgrowth or degradation (Akanbi, B. O., & Njoku, R. (2019).

Upon arrival at the laboratory, swabs were aseptically streaked onto MSA plates to ensure optimal isolation of *S. aureus*. Samples on the plates were incubated at 37°C for 24-48 hours to ensure adequately of bacterial growth. Submerged colonies that displayed typical *S. aureus* physiology – golden Yellow colored, round smooth colonies were selected for further study. Presumed colonies were transfer cultured on to fresh MSA plates to obtain single isolated colonies to reduce contamination.

Morphological and staining characterization of the pure colonies were done by Gram staining. Inning characteristics. A thin line of the isolate was spread into a clean glass slide, fixed by heat, and stained by crystal violet dye, iodine, decolorizing agent, and safranin. After gram staining and observing the clusters of bacterial cocci morphology, presumptive *S. aureus* infection was determined. The catalase test was used to differentiation of *Staphylococcus* Species from other Gram positive cocci. Staining characteristics. A thin smear of the isolate was prepared on a clean glass slide, heat-fixed, and stained using crystal violet, iodine, decolorizer, and safranin. Microscopic examination revealed Gram-positive cocci arranged in characteristic clusters, confirming presumptive *S. aureus*.

The catalase test was performed to distinguish *Staphylococcus* species from other Gram-positive cocci. Three percent hydrogen peroxide was taken in a loop and streaked on a clean glass slide and a loopful of the isolate was spread over it. Formation of bubbles immediately because of release of oxygen was a positive reaction for catalase and the shape and colour of the colony also placed the isolate under *Staphylococcus* genus (Van Loo, I., 2007). To detect MRSA, colonies were grown on oxacillin media with 4 µg/mL of the nutrient as a standard late was prepared on a clean glass slide, heat-fixed, and stained using crystal violet, iodine, decolorizer, and safranin. Microscopic examination revealed Gram-positive cocci arranged in characteristic clusters, confirming presumptive *S. aureus*.

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Corresponding author: Akinmoladun Oluwadamilola Posi

✉ [akinmoladunoluwadamilola01@gmail.com](mailto:akinmoladunoluwadamilola01@gmail.com)

Department of Pharmaceutical Microbiology, University of Benin, Edo State.

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followed by the addition of a loopful of the isolate. The immediate production of bubbles, resulting from oxygen release, indicated a positive catalase reaction and confirmed the isolate as a member of the *Staphylococcus* genus (Van Loo, I., 2007).

To identify methicillin-resistant isolates, colonies were cultured on oxacillin-containing media prepared with a standardized concentration of 4 µg/mL. Cultures were then allowed to initiate at 35°C for a period of 24 h. Such growth on these plates defined methicillin resistance. The resistant isolates were then phenotypically confirmed using cefoxitin discs, and confirmed by PCR to check for the presence of *mecA* gene. Stringent issues on quality control were effectively applied and complied with in the study. Verify their morphological and staining characteristics. A thin smear of the isolate was prepared on a clean glass slide, heat-fixed, and stained using crystal violet, iodine, decolorizer, and safranin. Microscopic examination revealed Gram-positive cocci arranged in characteristic clusters, confirming presumptive *S. aureus*.

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Quality control measures were strictly adhered to throughout the study. MSSA, and a MRSA strain were used as control strains in all the experiments to confirm the efficacy of the media and reagents. As for all procedures in the laboratories, contamination was avoided to ensure accuracy of results obtained. MRSA

resistance level was determined by expressing the proportion of resistant isolates into the total number of samples taken. A thin smear of the isolate was prepared on a clean glass slide, heat-fixed, and stained using crystal violet, iodine, decolorizer, and safranin. Microscopic examination revealed Gram-positive cocci arranged in characteristic clusters, confirming presumptive *S. aureus*.

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Quality control measures were strictly adhered to throughout the study. Control strains, including methicillin-susceptible *S. aureus* (MSSA) and a known MRSA strain, were included in all experiments to validate the performance of media and reagents. All laboratory procedures were conducted under aseptic conditions to prevent contamination and ensure reliability.

The prevalence of MRSA was calculated as the proportion of resistant isolates relative to the total number of samples collected. The isolation rates were compared statistically to determine any possible relation of the sample sites and the MRSA isolation rates. In all measures, descriptive statistics were used to analyze and present the results obtained.

## RESULTS AND DISCUSSION

The study identified MRSA isolates in a significant proportion of samples. Environmental contamination in poultry farms was a key finding, with nasal carriage among

Corresponding author: Akinmoladun Oluwadamilola Posi

✉ [akinmoladunoluwadamilola01@gmail.com](mailto:akinmoladunoluwadamilola01@gmail.com)

Department of Pharmaceutical Microbiology, University of Benin, Edo State.

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poultry workers suggesting zoonotic potential. The resistance patterns observed underscore the need for stringent antibiotic usage policies.

samples collected from poultry farms and workers in Benin City. These findings underscore the importance of monitoring environmental contamination and zoonotic transmission risks. The results are summarized below:

**Results**

The study revealed a significant presence of MRSA isolates across various

**Table 1: MRSA Detection by Sample Source**

| Sample Source   | Total Samples | Positive MRSA Cases | Percentage MRSA Positive (%) |
|-----------------|---------------|---------------------|------------------------------|
| Cages           | 50            | 20                  | 40.0                         |
| Feeders         | 40            | 15                  | 37.5                         |
| Waterers        | 30            | 10                  | 33.3                         |
| Nesting Areas   | 25            | 8                   | 32.0                         |
| Poultry Workers | 20            | 12                  | 60.0                         |

**Key Observations**

**1. Environmental Contamination:**

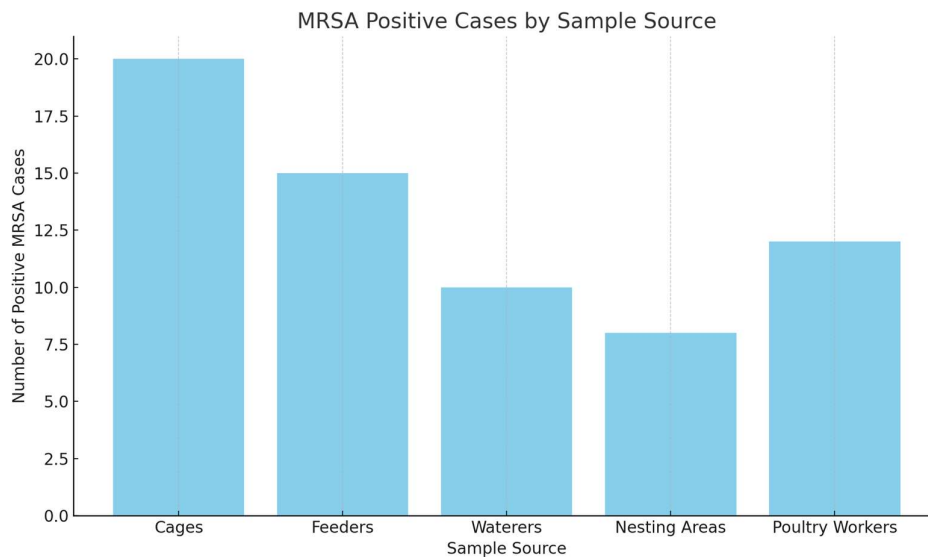
- a) The highest MRSA positivity rate was observed in samples from poultry workers (60%), suggesting zoonotic transmission potential.
- b) Among environmental sources, cages and feeders had the highest MRSA detection rates, with 40% and 37.5% positivity, respectively. These surfaces are frequently touched by both poultry and workers, acting as reservoirs for contamination.

**2. Distribution of MRSA Positivity:**

- a) Waterers and nesting areas had lower, but still concerning, contamination levels (33.3% and 32%, respectively).
- b) Poultry workers' nasal carriage of MRSA highlights the occupational risks associated with close contact with animals and contaminated environments.

**Visual Representation of Findings**

This chart highlights the absolute number of MRSA-positive cases across different sample sources, emphasizing the elevated risk among poultry workers.



Corresponding author: Akinmoladun Oluwadamilola Posi

✉ [akinmoladunoluwadamilola01@gmail.com](mailto:akinmoladunoluwadamilola01@gmail.com)

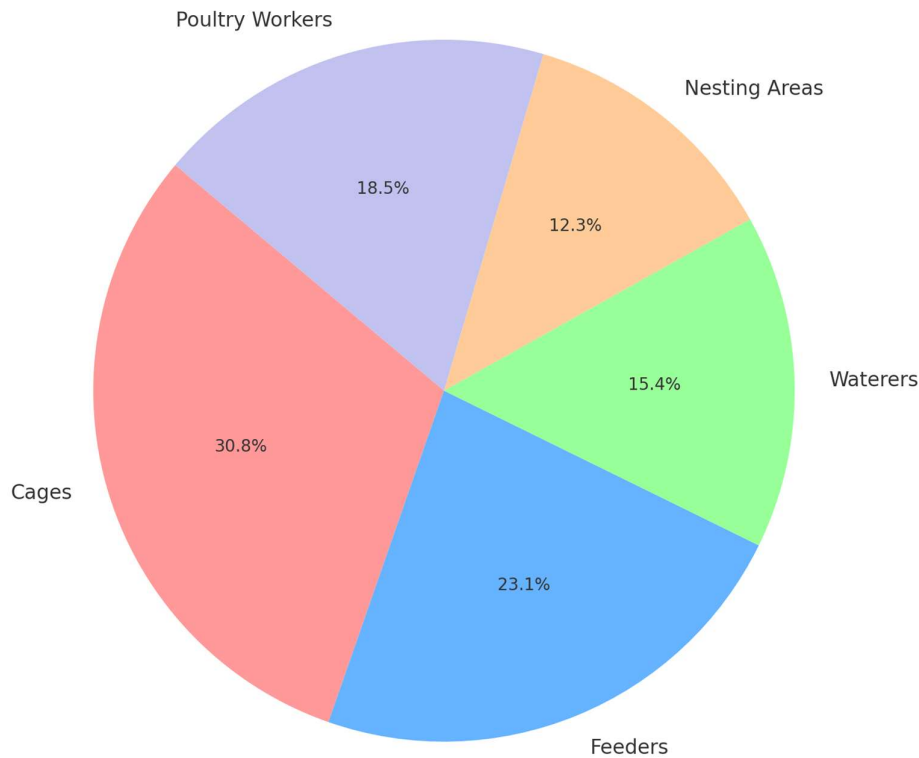
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The pie chart visually represents the contribution of each sample source to the total MRSA-positive cases, showcasing

contamination hotspots within poultry farming environments.

### Distribution of Positive MRSA Cases by Sample Source



#### Discussion

The results align with previous studies highlighting the zoonotic potential of livestock-associated MRSA. The elevated contamination in high-contact areas like cages and feeders indicates poor biosecurity practices and emphasizes the need for regular disinfection and improved hygiene protocols.

Nasal carriage in poultry workers represents a dual threat, serving as both a reservoir for further dissemination and a direct health risk to the workers themselves. This finding necessitates the implementation of protective measures such as personal

protective equipment (PPE) and regular health monitoring for workers.

The resistance patterns observed in these isolates underscore the need for stringent antibiotic usage policies in veterinary practices. Reducing the prophylactic use of antibiotics in poultry feed can significantly mitigate the emergence of resistant strains.

#### Conclusion and Recommendations

This study confirms the presence of MRSA in poultry environments in Benin City, highlighting the role of livestock as reservoirs of resistant strains. Recommendations include:



1. Implementing strict biosecurity measures in poultry farms.
2. Reducing the use of antibiotics in animal feed.
3. Conducting regular surveillance for MRSA in livestock and humans.
4. Promoting public awareness of the risks associated with livestock-associated MRSA.

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Corresponding author: Akinmoladun Oluwadamilola Posi

✉ [akinmoladunoluwadamilola01@gmail.com](mailto:akinmoladunoluwadamilola01@gmail.com)

Department of Pharmaceutical Microbiology, University of Benin, Edo State.

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