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Antibiotic resistance and heavy metal tolerance of livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in selected livestock settings in Zaria, Nigeria

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Abstract

The inappropriate antibiotic use in animal husbandry raises concerns about possible reservoirs and transmission pathways of methicillin-resistant Staphylococcus aureus (MRSA) in livestock environments. This study assessed the antibiotic resistance and heavy metal tolerance of S. aureus isolated from livestock farms in Zaria, Nigeria. Samples were collected, and heavy metal concentrations were analyzed. Staphylococcus aureus was isolated, followed by antibiotic susceptibility testing and heavy metal tolerance assays. Copper and Zinc concentrations varied in the water and animal wastes (cow dung and poultry litter) samples. Copper concentrations were higher in cow dung (13.5-16.0 mg/L) compared to poultry litter (7.8-12.2mg/L). Zinc concentrations of poultry litter (77.7-99.9 mg/L) were greater than cow dung (44.1-66.4 mg/L). Out of the total S. aureus isolates, 65.8% (50/76) were MRSA, with prevalence rates of 77.8% (28/36) in abattoir samples and 55% (22/40) in cattle ranch and poultry farm samples. 75% and 50% of S. aureus from the cattle environment (abattoir and cattle ranch), and poultry farm, respectively, had MAR index ≥0.2, with most isolates exhibiting beta-lactam resistance. MRSA isolates from poultry farm workers (27.3%) were greater than those from the abattoir/cattle ranch workers (7.1%). S. aureus isolates exhibited varying Copper and Zinc tolerance limits irrespective of their source. There was a significant positive correlation (r(38) = .348, p = .028) between the MAR index and copper tolerance limits of S. aureus isolates from the poultry farms. Proper implementation of biosecurity measures, antimicrobial resistance (AMR) surveillance, and policies are required to decrease the transmission of MRSA and AMR dissemination in livestock settings.

Keywords: Antimicrobial resistance, S. aureus, heavy metal tolerance, MRSA, poultry farm, abattoir.

INTRODUCTION

Staphylococcus aureus (*S. aureus*), a commensal typically found on the skin and mucosa of people and animals, is facultatively pathogenic, producing moderate to severe life-threatening infections (Crespo-Piazuelo and Lawlor 2021). MRSA is a big threat to global public health. Clinically relevant MRSA strains are classified into three types based on their source: health-care-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA). LA-MRSA has a higher resistance to antimicrobial drugs than clinical strains, raising concerns regarding antimicrobial resistance (AMR) spread in the environment (Beshiru et al., 2024). Occupational livestock exposure raises the risk of MRSA colonization and carriage in livestock-related workers compared to the general population (Crespo-Piazuelo and Lawlor 2021). Direct and indirect livestock contact increases the risk of MRSA interspecies transmission, underscoring the importance of a one-health strategy to address AMR in various sectors globally (Umair *et al.*, 2023).

The improper use of antimicrobial agents, particularly antibiotics and heavy metals, as growth promoters in water and feeds to fulfill the expanding demand for food-animal products promotes the establishment of AMR in livestock settings. Consequently, animal farms, abattoirs, and food production systems are frequently polluted with antibiotics, heavy metals, and resistant microbes.

This has resulted in antibiotic co-selection in pathogens, including LA-MRSA isolates with heavy metal and antibiotic resistance (Graveland *et al*., 2011; Dweba *et al*., 2018). LA-MRSA's development of new virulence and resistance factors, particularly metal resistance, is expanding the MRSA burden in humans (Fetsch et al., 2021). Previous studies have shown that LA-MRSA is more resistant to antimicrobial drugs than clinical strains, raising concerns regarding AMR spread in the environment (Beshiru et al., 2024). Also, at least one metal-resistance gene was found in 76.2% (413/542) of LA-MRSA isolates from farm animals and/or food items, demonstrating that metal tolerance genes are widely distributed among LA-MRSA (Argudin et al., 2016). Furthermore, heavy metal tolerance in HA-MRSA (Mohamed et al., 2020) and LA-MRSA may complicate the treatment of MRSA infections (Argudin *et al*., 2016; Dweba *et al*., 2018; Biswas *et al*., 2021).

According to the World Health Organization (WHO), there is little research and data on antimicrobial resistance, including heavy metal resistance of LA-MRSA, in African and other low- and middle-income countries (LMICs) (Dweba et al. 2018). Previous research on MRSA in livestock settings in Nigeria has focused on analyzing antibiotic resistance patterns (Beshiru et al., 2024), with a few reporting heavy metal tolerance and coresistance (Nwobi *et al*., 2023). In addition, a few studies in Nigeria have detected MRSA from various sources in livestock settings, such as waste, animals, people, and food products (Adekanmbi and Falodun, 2015; Beshiru *et al*., 2024). This study assessed the antibiotic susceptibility pattern and heavy metal tolerance of MRSA and MSSA isolated from different samples collected from selected livestock settings (abattoir, cattle ranch, and poultry farms) in Zaria, Nigeria. Some information on the sanitary farm practices and habits of livestock workers in those settings was also gathered.

MATERIALS AND METHODS

Ethics

The management of the selected farms and abattoir provided informed consent for access and participation in the research. The Ahmadu Bello University, Zaria ethics committee granted ethical approval for using human subjects in the study.

Study area, sample collection, and processing

Eight sampling sites included *Zangon Shanu* abattoir in Zaria, a cattle ranch located at Shika, Zaria, and six randomly selected commercial poultry farms within Samaru, Zaria, Kaduna state, Nigeria. Questionnaires were administered to 18 consenting workers, including 4 from the abattoir, 2 from the cattle ranch, and 12 from poultry farms, to gather data on sanitary practices and safety habits in these livestock facilities. A total of 24 samples (18 environmental samples and 6 hand swabs) were collected from the abattoir and cattle ranch, while 30 samples (18 environmental samples and 6 hand swabs) were collected from the poultry farms. Composite environmental samples, including water from drinking troughs and wastewater (100 ml), poultry litter (10 g), and cow dung (10 g), were collected from three different sampling points per site in sterile containers for microbiological and heavy metal analysis. The samples were collected in duplicate from all the sampling sites. Hand swabs were also collected in duplicate from consenting livestock farm workers. All samples for microbiological analysis were transported in an insulated box containing ice packs to the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. Composite samples of water, litter, and cow dung were assessed for their Copper and Zinc concentration by Atomic Absorption Spectroscopy (AAS) technique following acid digestion at the Multi-User Laboratory, Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

Isolation and characterization of *S. aureus*

1 mL of each water sample was inoculated into 9 mL of tryptic soya broth (TSB) (Merck, Darmstadt, Germany), while for the litter and cow dung, 1 ml of suspension prepared with 1g of each and 2 mL of normal saline was added to 9 mL of TSB. All swab samples were inoculated in nutrient broth (containing 6.5% NaCl) and incubated for 24 hours at 37^0C, followed by subculturing a loopful of culture onto mannitol salt agar (MSA) using the streak plate method. After incubation, presumptive *Staphylococcus* isolates on MSA characterized by a distinct yellow colour were sub-cultured on nutrient agar to obtain a pure culture. Two presumptive *S. aureus* colonies on MSA plates per sample were selected for characterization and further analysis. *S. aureus* isolates were characterized based on colonial morphology, Gram staining, and biochemical tests (catalase, coagulase, and DNase) (Arumugam *et al*., 2017; Arjyal *et al*., 2020)

Antibiotic susceptibility testing

Standardized inocula of the identified *S. aureus* isolates were subjected to antibiotic susceptibility tests by the Kirby-Bauer disc diffusion method as recommended by CLSI guidelines.

The antibiotics used were gentamicin (10 μg), erythromycin (30 μg), ciprofloxacin (10 μg), norfloxacin (10 μg), amoxil (20 μg), streptomycin (30 μg), rifampicin (20 μg), chloramphenicol (30 μg), ampiclox (20 μg) and levofloxacin (20 μg). Methicillin resistance was also assessed by screening standardized inocula with cefoxitin (30μg) disks. All inoculated Muller Hinton agar plates with the selected antibiotic discs were incubated at 37°C for 24 hours. Inhibition zones were measured, and results were interpreted according to the CLSI guideline. Isolates with a zone of inhibition ≤ 21 mm around the cefoxitin disc were interpreted as MRSA strains (CLSI, 2021).

Heavy metal tolerance test

Nutrient agar was prepared according to the manufacturer's instruction and then amended separately with $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ at different concentrations (0, 2, 4, 8, and 16 mM). The plates were divided into quadrants, spot inoculated with the standardized inocula of *S. aureus* isolates, and incubated at 37°C for 24-48 hours. After incubation, the plates were observed for growth on the media (Amalesh *et al.*, 2012).

Data analysis

The data obtained were analyzed using descriptive statistics using Microsoft Word Excel 2013. Using SPSS software version 26, Pearson correlation was conducted to assess if there is a correlation between the multiple antibiotic index (MARi) and heavy metal tolerance limits of *S. aureus* isolates from the selected livestock settings.

RESULTS

Sanitary practice and habits of livestock workers

The frequency of cleaning varied in the different sites: abattoir (twice a week), cattle ranch (daily), and poultry farms (weekly). Antibiotics were not administered to the cattle at the abattoir but administered to the livestock at the cattle ranch and poultry farms (33%). In all sites, animal wastes (poultry litter and cow dung) were collected for use as organic manure on farms. Based on the data gathered, 50% (2/4) of abattoir workers never use gloves, 100% (2/2) of the cattle ranch workers sometimes use gloves, and 100% (12/12) of the poultry workers never use gloves while working. 50% (2/4) of abattoir workers sometimes use face masks, 50% (1/2) of the cattle ranch workers sometimes use face masks, and 100% (12/12) of the poultry workers never use facemasks while working. 50% (2/4) of abattoir workers sometimes use boots, 50% (1/2) of the cattle ranch workers sometimes use boots, and 100% (12/12) of the poultry workers never use boots while working. All the workers claimed to wash their hands after handling animals on the farms and abattoirs.

Copper and Zinc concentration

Copper concentration ranged from 8.4±0.6 - 10.2±0.85 mg/L, 13.5±0.2 - 16.0±0.6 mg/L, 1.0±0.2-2.0±0.1 and 7.8±0.1- 12.2±0.75 for water from the abattoir and cattle ranch, cow dung, poultry water, poultry litter samples respectively. For the Zinc concentration, this ranged from 9.2±0.85-21.2±0.85, 44.1±3.10-66.4±1.40, 14.8±1.30- 26.3±0.45 and 77.7±0.45-99.9±0.14 mg/L for water sample from the abattoir, water sample from cattle ranch, cow dung, poultry water, poultry litter samples respectively (Table 1).

Table 1: Concentration of Cu and Zn in different samples collected from the selected study sites

Samples	Sample ID	Cu (mg/L)	Zn (mg/L)
Water	WCA1	10.2±0.85	18.6±0.45
	WTR1	9.3±0.70	21.2±0.85
	SWA1	8.4±0.60	9.2±0.85
Cow dung	CDA1	16.0±0.60	66.4±1.40
	CDR2	13.5±0.20	44.1±3.10
Poultry water	PW1	1.8±0.70	14.8±1.30
	PW2	1.6±0.10	15.4±1.20
	PW3	1.5±0.50	19.1±0.20
	PW4	1.4±0.10	26.3±0.45
	PW5	2.0±0.10	16.2±1.05
	PW6	1.0±0.20	22.8±0.15
Poultry litter	PL1	9.2±0.10	99.9±0.14
	PL2	7.8±0.10	83.3±0.77
	PL3	8.9±0.30	77.7±0.45
	PL4	11.5±0.10	86.7±2.29
	PL5	12.2±0.75	81.6±2.56
	PL6	11.5±0.55	84.5±0.73

Key: WCA: Water from open channels at abattoir; WTR: Water from drinking trough at cattle ranch; SWA: Surface waste water abattoir; CDA1: Cow dung from abattoir; CDR2: Cow dung from cattle ranch; PW: Poultry water; PL; Poultry litter, Cu: Copper; Zn: Zinc.

Antibiogram and MAR index of *S. aureus* isolates

A total of 36 and 40 *S. aureus* isolates were characterized from the abattoir, cattle ranch, and poultry farms, respectively, based on the colonial characteristics on MSA agar yellow colonies, gram reaction, Gram-positive cocci in clusters, and biochemical tests. Antibiotic susceptibility testing of *S. aureus* isolates from abattoir and cattle ranch revealed that more isolates exhibited resistance to beta-lactam antibiotics than other antibiotic classes screened: 41.7% (15/36) for amoxil and 58.3% (21/36) for ampiclox. Antibiotic resistance amongst *S. aureus* was lowest for quinolones: 0% (0/36) for ciprofloxacin, 2.8% (1/36) for levofloxacin and 5.5% (2/36) for norfloxacin. Similarly, for *S. aureus* isolates from the poultry farms, the highest resistance was observed with beta-lactams: 42.5% (17/40) and methicillin, 55% (22/40), and norfloxacin (quinolones), 50% (20/40). However, the resistance was lowest with other quinolone antibiotics: 5% (2/40) for ciprofloxacin, levofloxacin, and macrolide, and 7.5% (3/40) for erythromycin. The MAR index with the highest percentage of isolates were 0.1 and 0.6 for abattoir and cattle ranch (25% each) and 0.0 for the poultry farms (32.5%) (Figure 1). 75% (27/36) and 50% (20/40) of *S. aureus* from the abattoir cattle ranch and poultry farms, respectively, and had a MAR index ≥0.2.

Distribution of MRSA based on sampling site

A total of 65.8% (50/76) of *S. aureus* were methicillin-resistant *S. aureus*, out of which 77.8% (28/36) and 55% (22/40) of the MRSA were from abattoir and cattle ranch and poultry farms respectively as shown in Figure 2. Out of MRSA isolates from abattoir and cattle ranch, 39.30% (11/28) were MDR-MRSA, while 59.1% (13/22) of those from the poultry farms were MDR-MRSA. MRSA distribution by sample type collected from the abattoir and cattle ranch revealed that the highest MRSA isolates were from the water samples (35.7%), while the hand swabs (7.1%) had the lowest. For the poultry farm, the highest MRSA isolates were from the water samples (45.5%), while the poultry litter (9.1%) had the lowest (Figure 3). From the *S. aureus* isolates with MAR index ≥ 0.2, 77.7% (21/27) and 90% (18/20) from the cattle environments (abattoir and cattle ranch) and poultry farms, respectively, were MRSA.

Copper and Zinc tolerance of *S. aureus*

Irrespective of the methicillin resistance patterns, *S. aureus* from the abattoir and cattle ranch had a Cu and Zn tolerance limit of 2-8 mM and 2-8 mM, respectively. For the poultry farms, *S. aureus* isolates had a Cu and Zn tolerance limit of 0-8 mM and 0-8 mM, respectively. Most *S. aureus* isolates from the abattoir and cattle ranch had a Zn tolerance limit of 8 mM (83.4%) and a Cu tolerance limit of 4 mM (89%), while *S. aureus* isolates from poultry farms had a Zn tolerance limit of 4 mM (47.5%) and a Cu tolerance limit of 8 mM (62.5%) (Figure 4).

Correlation between MARi and heavy metal tolerance limits of *S. aureus*

For *S. aureus* isolates from abattoir and cattle ranch, Pearson correlation indicated that there is a non-significant small positive relationship between MARi and Cu tolerance limit, (r(34 p = 0.118) and non-significant very small negative relationship between MARi and Zn tolerance limit, (r(34) = 0.0332, p = 0.848). For the *S. aureus* isolates from the poultry farms, Pearson correlation indicated that there is a significant medium positive relationship between MARi and Cu tolerance limit (r(38) = 0.348, p = 0.028) and a non-significant very small positive relationship between MARi and Zn tolerance limit, (r(38) = 0.0483, p = 0.767).

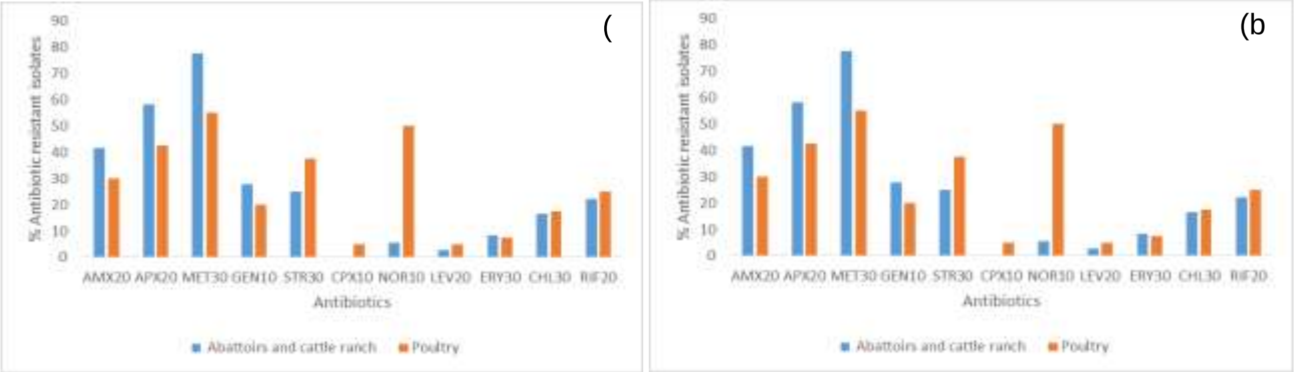

Figure 1: Antibiotic susceptibility and MAR index of *S. aureus* isolates from abattoir and cattle ranch, and poultry farms. (a) Antibiogram; (b) MAR index. The total number of *S. aureus* isolates from abattoirs and cattle ranch, and poultry farms was 36 and 40, respectively. The antibiotics tested were gentamicin (GEN), erythromycin (ERY), ciprofloxacin (CPX), norfloxacin (NOR), Amoxil (AMX), streptomycin (STR), rifampicin (RIF), chloramphenicol (CHL), ampiclox (APX), levofloxacin (LEV), and methicillin (MET).

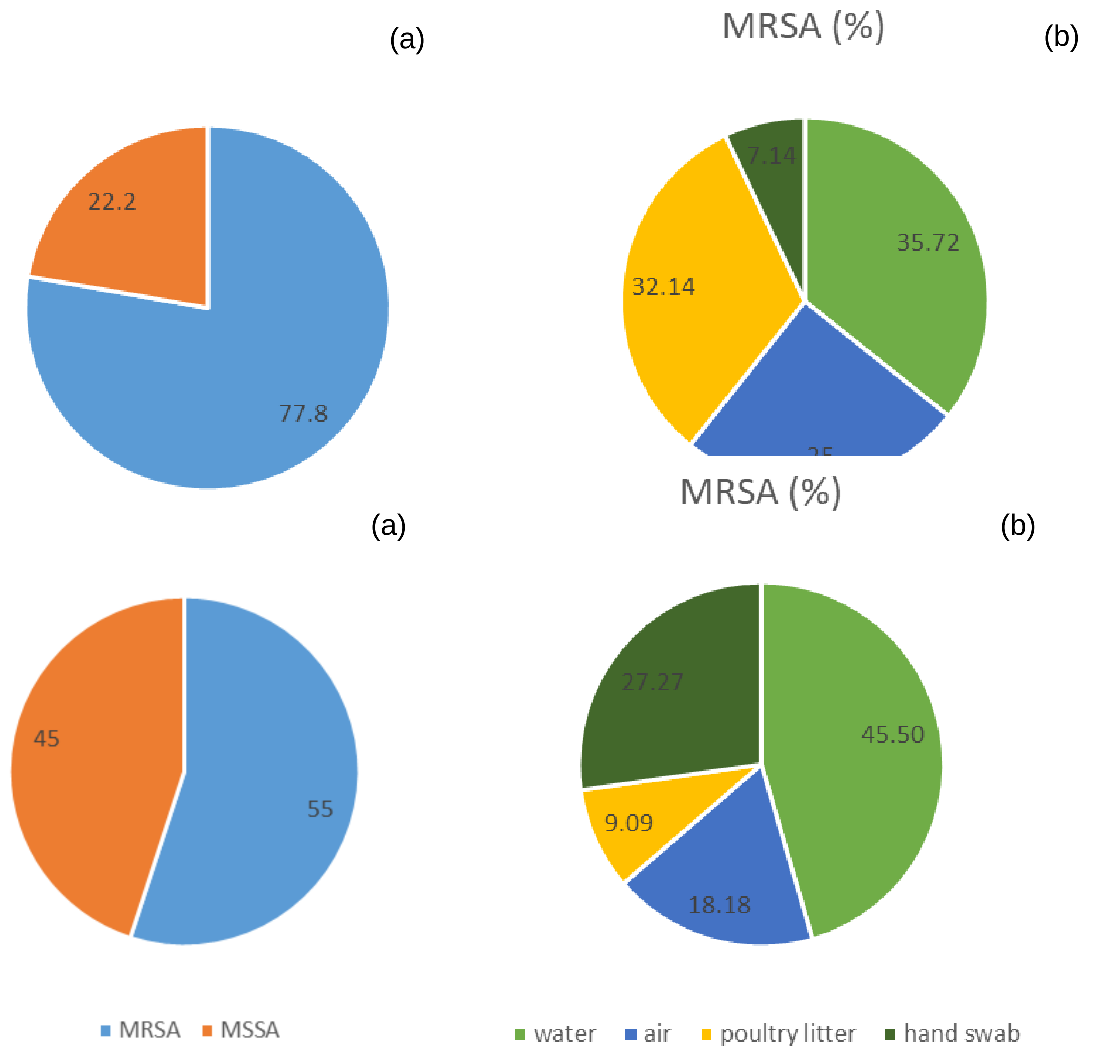

Figure 3: Percentage distribution of MSSA and MRSA isolates from poultry farms based on the sample types.

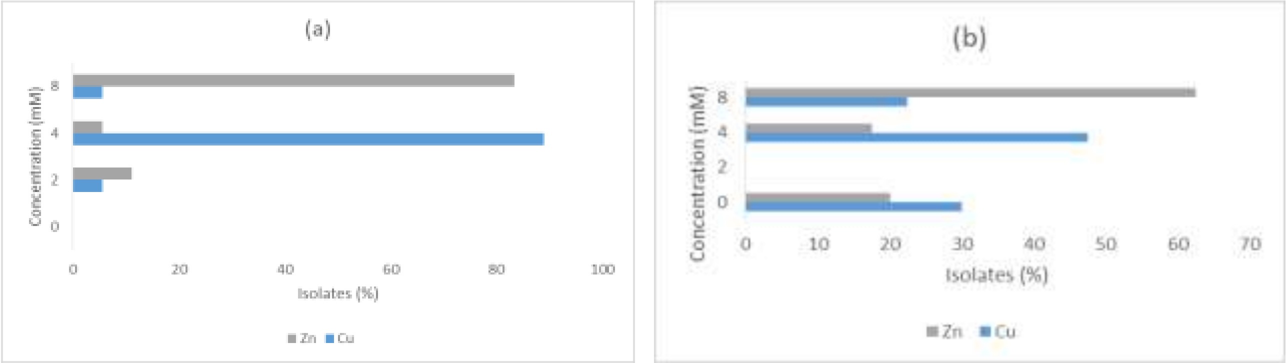

Figure 4: Copper and Zinc tolerance limit of *S. aureus* isolated from (a) Abattoir and cattle ranch (b) Poultry.

DISCUSSION

This study assessed the heavy metal concentration of some samples, antibiotic resistance, and heavy metal tolerance of *S. aureus* isolated from different samples in Our finding of periodical cleaning of livestock farms agrees with the previous report of the periodic cleaning of 77% of poultry pens in Lagos (Akinnusi *et al.*, 2018). Poor compliance in the use of protective gear (gloves, boots, and face masks) by all livestock workers is supported by previous reports (Crespo-Piazuelo and Lawlor, 2021; Fetsch *et al.*, 2021; Nwobi *et al.*, 2023). In agreement with our findings, several studies have reported the presence of heavy metals in various samples such as feeds, animal wastes, carcasses, and animal food products collected from livestock settings. The Zinc concentration of poultry litter from the selected farms (77.7±0.45-99.9±0.14 mg/L) is similar to 99.4-134.8 mg/L in poultry litter samples in Saudi Arabia reported by Korish and Attia (2020). However, the Cu concentration of the litter samples (7.8±0.1- 12.2±0.75 mg/L) from this study was lower compared to 23.3-28.5 mg/L reported by Korish and Attia (2020). For cow dung, the copper (13.5±0.2 - 16.0±0.6mg/L) and Zinc concentration (44.1±3.10-66.4±1.40 mg/L) were similar to the Cu (19.48-66.58) and Zn (65.27-319.27mg/kg) were reported in cattle manure from farms with 100-300 cattle in Northeast China (Zhang *et al.*, 2012). The detection of 65.8% (50/76) of MRSA with 77.8% (28/36) and 55% (22/40) from the different samples collected from abattoir, cattle ranch, and poultry farms, respectively, is supported by previous studies (Adekanmbi and Falodun 2015; Beshiru *et al.*, 2024). MRSA has been isolated from water, soil, air, wastes, food products, livestock, and livestock handlers, posing an increasing health threat globally (Graveland *et al.*, 2011; Dweba *et al.*, 2018). Beshiru *et al.* (2024) reported a prevalence of 63.5% (n= 254) for *Staphylococcus aureus* and 55% (n= 220) for MRSA from 400 samples (200 rectal and 200 nasal swabs) from cattle in Edo State, Nigeria, Contrary to our finding, a significantly higher MRSA colonization in poultry compared to bovine animals in Algeria has been reported (Bounar-Kechih *et al.*, 2018). The detection of MRSA strains amongst livestock workers in both settings is supported by the finding of highly similar clones of MRSA strains detected in slaughter animals, abattoir workers, and abattoir environments in Nigeria (Odetokun *et al.*, 2022). Previous studies have shown that *S. aureus* is a multi-host bacterial pathogen with a pool of genes for host adaptation (Kasela *et al.*, 2023). The selected livestock farming settings: abattoir, cattle ranch, and poultry farms in Zaria, Nigeria. Information on some farm practices and habits of livestock workers was also gathered.

presence of MRSA in air samples is supported by previous studies on airborne MRSA and MRSA from nasal swabs collected from livestock settings (Samutela *et al.*, 2021; Beshiru *et al.*, 2024). In agreement with our findings, Fasiku *et al.* (2024) reported high multidrug resistance to at least five sub-classes of antibiotics amongst MRSA and MSSA isolated from faecal samples from pigs in twenty-five pig farms in Ibadan, Nigeria. Furthermore, high antibiotic resistance by *S. aureus* to ß-lactam antibiotics, including penicillin, ampicillin, and amoxicillin-clavulanic acid, has been previously reported (Mamfe *et al.*, 2021; Fasiku *et al.*, 2024). The low resistance of *S. aureus* strains to erythromycin observed in our study disagrees with previous findings reported by Lim *et al.* (2013) in Malaysia and Fasiku *et al.* (2024) in Nigeria. A higher multiple antibiotic resistance pattern of MRSA compared to MSSA is supported by a number of studies (Bhatta *et al.*, 2018; Fasiku *et al.*, 2024). The higher tolerance of *S. aureus* isolates to Zn at 8 mM and Cu at 4 mM is supported by the findings of Nwobi *et al.* (2023) and may be attributed to the use of Zn and Cu as livestock feed supplements in addition to their presence in the environment. Similar to our findings, Adekanmbi and Falodun (2015) reported multiple heavy metal tolerance to six metals (lead, copper, zinc, chromium, cadmium, and nickel) by 41% of *S. aureus* isolated from Bodija Abattoir in Ibadan, Oyo State, Nigeria. The positive correlation between the MAR index and copper tolerance of *S. aureus* isolates from the selected poultry farms agrees with findings on heavy metal and antibiotic coresistance in previous studies (Icgen and Yilmaz, 2014; Oves and Hussain, 2016; Jardine *et al.*, 2019; Edet *et al.*, 2023). Furthermore, recent studies, mostly in developing countries, have reported ß-lactam antibiotics and heavy metals (Zn, Cu, and Hg) coresistance in MRSA isolates from livestock, a correlation between heavy metal susceptibility and MRSA, and the presence of a least one metal resistance gene (Dweba *et al.*, 2018).

Animal feeds and water are usually supplemented with heavy metals to boost immunity and facilitate growth, development, and reproduction. For poultry, Zn also aids feather and skeletal development, while Cu supports immunity and enhances feed efficiency, gut health, and growth rates (Zhang *et al.*, 2012). Heavy metals in the collected waste samples, including poultry litter and cow dung, reflect the animals' feed composition and conversion efficiency (Zhang *et al.*, 2012; Korish and Attia, 2020). Unhygienic practices and habits in livestock settings promote the transmission of pathogens, including LA-MRSA from livestock to livestock handlers, and the Animals are a major source of MRSA in the environment and can serve as a reservoir for drug-resistance genes (Kasela *et al.*, 2023). The detection of airborne MRSA in livestock settings indicates a risk of nasal carriage of LA-MRSA even with short-time exposure by the livestock workers who did not use face masks in both settings. Poor sanitary and management practices on livestock farms and production systems in Nigeria have been associated with the high prevalence of diseases which are increasingly being managed by the indiscriminate use of antimicrobials rather than improving sanitary and biosecurity measures (Kwaghe *et al.*, 2016). As the widespread use of antibiotics in livestock farming expands, this raises significant concerns about the spread of pathogens, including LA-MRSA, among humans, livestock, and the environment. A systematic review of LA-MRSA in Africa revealed that risk factors associated with *S. aureus* carriage amongst livestock farmers include gender, occupational exposure, and working in an abattoir (Samutela *et al.*, 2021). The occupational exposure of livestock workers creates a significant route of LA-MRSA transmission, causing a high tendency for host switching with changes in mobile genetic elements and posing a threat to public health (Wang *et al.*, 2022).

The antibiotic resistance pattern of *S. aureus* isolates, and the higher MRSA from the abattoir and cattle ranch compared to the poultry farms may be attributed to antibiotic usage and other practices. This is also indicated by the large proportion of *S. aureus* with MAR index ≥ 0.2, which infers that the isolates originate from sources with high antibiotic usage (Tchounwou *et al.*, 2012). The high multidrug resistance amongst MRSA compared to MSSA has been attributed to the presence of more antibiotic-resistance genes. Further, β-lactam resistance exhibited by *S. aureus* isolates may be due to the acquired resistance mechanisms, such as β-lactamase production encoded by *mec* genes, following prolonged antibiotic exposure. The *mec* genes, including *mecA*, *mecB*, and *mecC*, are located in the staphylococcal chromosomal cassette (*SCCmec*) (Kasela *et al.*, 2023; Fasiku *et al.*, 2024). The common practice of using antimicrobials, including heavy metals as growth promoters in contamination of food animal products and the environment (Agu *et al.*, 2021; Odetokun *et al.*, 2022). Differences in antibiotic use and farm practice, including biosecurity measures and waste management, may be responsible for the variation in the MRSA distribution relative to the livestock settings and sample type. sub-therapeutic concentrations, facilitates the emergence of antimicrobial resistance (Seiler and Berendonk, 2012; Jayaweera and Kumbukgolla, 2017; Mittapally *et al.*, 2018). The molecular mechanisms for developing antibiotic and heavy metal resistance are similar and may result in coselection either by co-resistance or cross-resistance (Cheng *et al.*, 2019). The co-selection of antibiotic and metal resistance in bacteria is significant as it can drive antimicrobial resistance in bacterial populations even in the absence of antibiotics. Co-selection involves two mechanisms; cross-resistance occurs due to a single mechanism, while coresistance occurs when two or more distinct resistance genes are co-located on the same genetic material (Grenni and Corno 2019). This study is one of the few studies that have assessed the heavy metal tolerance and antibiotic resistance pattern of *S. aureus* in different environmental samples in livestock settings in Nigeria. Due to financial constraints, molecular characterization of MRSA to determine the spa types, clonal complex, and virulence factors was not conducted. Only a few livestock workers consented to participating in this study hence the small number of livestock farmers sampled.

CONCLUSION

The presence of drug-resistant and heavy metal-tolerant *S. aureus*, including MRSA strains from various environmental samples and hand swabs in the selected livestock settings, raises public health concerns about AMR dissemination in the environment and transmission from the workers to the community. Consequently, there is a need to properly implement biosecurity measures/control strategies and monitoring programs in livestock settings in Nigeria. Subsequent studies should collect more data on livestock workers' knowledge, attitudes, and practices and assess the molecular epidemiology of LA-MRSA in different livestock settings to provide information on the genotypic and virulence characteristics of LA-MRSA and gain insights on host switching. Future studies should also focus on assessing the role of livestock biosecurity measures in mitigating antibiotic resistance. These will be relevant for the implementation of AMR-National Action Programs in Nigeria.

Author contributions

All authors contributed to the study conception and design, sample collection and analysis, data analysis, and interpretation. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest: Authors declared that there is no conflict of interests directly or indirectly related to the work submitted for publication.

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