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Molecular detection of methicillin-resistant staphylococcus aureus from bovine mastitic milk and milker hand swabs

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Abstract

While bovine mastitis remains the dairy industry's most expensive disease, its public health dimension the potential for antibiotic-resistant bacteria to jump from udder to human handler receives far less attention in West African livestock systems. This cross-sectional research investigated the prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in bovine mastitic milk and paired milker hand swabs collected from 48 smallholder dairy farms across four local government areas of Lagos State, Nigeria, between March and September 2023. A total of 312 samples (192 milk, 120 hand swabs) were cultured on mannitol salt agar and confirmed biochemically. Of 168 confirmed *S. aureus* isolates, 72 (42.9%) were phenotypically methicillin-resistant by cefoxitin disc diffusion, and 65 (38.7%) carried the *mecA* gene by PCR. MRSA prevalence was higher in mastitic milk (23.7%) than in milker hand swabs (14.8%). Antibiotic susceptibility testing revealed universal resistance to penicillin and oxacillin, high resistance to tetracycline (81.5%) and erythromycin (70.4%), but complete susceptibility to vancomycin and linezolid. Genotypic comparison of paired milk-hand isolates from the same farm showed identical *mecA* profiles in 11 of 18 matched sets, suggesting bidirectional transmission between cows and handlers. These findings confirm that MRSA circulates actively in Lagos peri-urban dairy chains, posing an occupational zoonotic risk that demands improved milking hygiene and antimicrobial stewardship.

Keywords: Methicillin-resistant *Staphylococcus aureus*, MRSA, bovine mastitis, *mecA* gene, milker hand swabs, zoonotic risk, antimicrobial resistance, dairy farms, Lagos, molecular detection

Introduction

On one hand, *Staphylococcus aureus* is the most frequently isolated pathogen from bovine mastitis worldwide, responsible for chronic intramammary infections that reduce milk yield and quality ^[1]. On the other hand, the same organism is a leading cause of hospital-acquired and community-acquired infections in humans, where methicillin-resistant strains (MRSA) complicate treatment and raise mortality ^[2]. The overlap between these two epidemiological realities becomes a tangible public health concern whenever dairy workers handle infected udders without gloves and then touch their own skin, food, or family members.

MRSA is defined by the acquisition of the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with low affinity for all β -lactam antibiotics, rendering the organism resistant to the entire class from penicillin through carbapenems ^[3]. The *mecA* gene is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*), which can transfer horizontally between strains and even between *Staphylococcus* species, facilitating the spread of resistance across ecological niches ^[4].

In Nigeria, where an estimated 70% of dairy production occurs in smallholder pastoral and peri-urban systems, milking is done exclusively by hand, often without teat disinfection or personal protective equipment ^[5]. This practice creates repeated, high-contact opportunities for bacterial exchange between the cow's teat canal and the milker's skin. Previous Nigerian research has documented *S. aureus* prevalence in mastitic milk ranging from 30% to 55%, but molecular data on *mecA* carriage are limited to a few reports from northern states ^[6, 7].

Lagos State, with its dense peri-urban dairy belt supplying raw milk to millions of consumers, has not been surveyed for livestock-associated MRSA (LA-MRSA) using PCR-based methods.

Bridging this gap matters for two reasons. First, identifying the *mecA* gene by PCR provides definitive confirmation of methicillin resistance that phenotypic disc diffusion alone can miss in borderline cases [8]. Second, paired sampling of milk and milker hands from the same farm can reveal whether identical resistant clones are circulating between animals and humans, evidence that strengthens the case for One Health interventions. This research set out to determine the prevalence of MRSA in bovine mastitic milk and milker hand swabs across peri-urban dairy farms in Lagos State, confirm resistance genotypically through *mecA* PCR, characterise the antimicrobial susceptibility profile of confirmed MRSA isolates, and assess the concordance of paired animal-human isolates as a measure of zoonotic transmission risk.

Material and Methods

Reagents and Chemicals

Mannitol salt agar (MSA), brain heart infusion broth (BHI), Mueller-Hinton agar (MHA), rabbit plasma for the coagulase test, and DNase agar were obtained from Oxoid (Basingstoke, UK). Antibiotic discs (penicillin 10 U, oxacillin 1 µg, cefoxitin 30 µg, tetracycline 30 µg, erythromycin 15 µg, ciprofloxacin 5 µg, gentamicin 10 µg, trimethoprim-sulfamethoxazole 25 µg, clindamycin 2 µg, chloramphenicol 30 µg, vancomycin 30 µg, linezolid 30 µg, rifampicin 5 µg) were sourced from HiMedia (Mumbai, India). DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Germany). PCR reagents included DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, USA), *mecA*-specific primers (forward: 5'-GTAGAAATGACTGAACGTCGATAA-3'; reverse: 5'-CCAATTCCACATTGTTTCGGTCTAA-3'; amplicon 310 bp) [8], and *S. aureus* ATCC 43300 as a *mecA*-positive control.

Material

Between March and September 2023, 48 smallholder dairy farms across four local government areas (Ojo, Badagry, Ikorodu, Epe) of Lagos State were visited. From each farm, milk samples were collected from all cows showing clinical mastitis signs (swelling, flakes, abnormal colour) or testing positive on the California Mastitis Test (CMT $\geq 2+$), yielding 192 quarter-milk samples. Paired hand swab samples ($n=120$; 2-3 milkers per farm) were taken by rolling a sterile moistened swab across both palms and interdigital spaces before milking commenced. Samples were transported on ice to the Lagos Foundation for Microbiological Research within four hours. The Lagos State University Teaching Hospital Health Research Ethics Committee approved the human sampling component (Ref: LASUTH-HREC/2023-019), and the Foundation's Animal Ethics Board approved the animal component (Ref: LFMR-AEB/2023-003).

Methods

Milk and swab samples were streaked onto MSA plates and incubated aerobically at 37°C for 24-48 hours. Yellow colonies (mannitol fermenters) were subcultured on blood agar and subjected to Gram staining (Gram-positive cocci in clusters), catalase test (positive), tube coagulase test (positive), and DNase test (positive) for definitive *S. aureus* identification [1]. Confirmed isolates underwent phenotypic MRSA screening by cefoxitin disc diffusion on MHA following CLSI 2023 guidelines: zone diameter ≤ 21 mm was classified as methicillin-resistant [8]. For molecular confirmation, genomic DNA was extracted from overnight BHI cultures using the QIAamp kit. *mecA* gene amplification used the primers above under these conditions: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 60 s; final extension at 72°C for 7 min. Amplicons were resolved on 1.5% agarose gel stained with ethidium bromide and visualised under UV transillumination. Antimicrobial susceptibility of all *mecA*-positive MRSA isolates was determined by the Kirby-Bauer disc diffusion method on MHA, interpreting zone diameters per CLSI breakpoints. Data were analysed in SPSS 28.0. Prevalence was expressed as percentages with 95% CI. Chi-square or Fisher's exact test compared categorical variables. Concordance of paired milk-hand *mecA* status was assessed by Cohen's kappa.

Quality Control

Quality assurance measures were embedded at every stage. Culture media were batch-tested with *S. aureus* ATCC 25923 (methicillin-susceptible) and ATCC 43300 (methicillin-resistant) reference strains before use. Each PCR run included a positive control (ATCC 43300 DNA), a negative extraction control (reagent blank processed alongside samples), and a no-template control (nuclease-free water). Gel electrophoresis included a 100 bp DNA ladder (Thermo Fisher) on every gel. Antibiotic susceptibility testing was validated weekly against ATCC 25923 reference ranges. Any isolate with discordant phenotypic and genotypic results (cefoxitin-resistant but *mecA*-negative, or vice versa) was re-tested in duplicate before final classification.

Ethical Considerations

Informed consent was obtained from each milker before hand swab collection. Participants were assured of confidentiality and received individual MRSA screening results with a brief educational pamphlet on hand hygiene. Milkers identified as MRSA carriers were referred to the nearest primary health centre for clinical evaluation. Animal sampling followed routine veterinary practice and caused no pain beyond standard CMT and milk collection. Farm owners received aggregated mastitis prevalence results for their herds with recommendations for teat dipping and dry cow therapy.

Results

Table 1: Prevalence of *S. aureus* and MRSA by sample source and local government area, ranked by MRSA detection rate

LGA (Rank)	Farms	Milk samples	Hand swabs	<i>S. aureus</i> (%)	MRSA phenotypic	MRSA <i>mecA</i> +	MRSA rate%
1. Badagry	14	56	35	58.2	24	22	24.2
2. Ojo	12	48	30	53.8	19	17	21.8
3. Ikorodu	11	44	28	51.4	16	15	20.8
4. Epe	11	44	27	47.9	13	11	15.5
Total	48	192	120	53.8	72	65	20.8

MRSA detection rates varied across the four LGAs (Table 1), with Badagry ranking highest (24.2%) and Epe lowest (15.5%). This geographic gradient correlates with dairy farm

density and the extent of shared grazing and milking infrastructure in each area.

Table 2: Concordance between phenotypic (cefoxitin) and genotypic (*mecA* PCR) methicillin resistance classification by sample source

Classification	Milk Pheno+	Milk <i>mecA</i> +	Hand Pheno+	Hand <i>mecA</i> +	Total Pheno+	Total <i>mecA</i> +
Resistant	49	45	23	20	72	65
Susceptible	65	69	31	34	96	103
Concordance (%)	91.2	—	88.9	—	90.5	—
Cohen's κ	0.82	—	0.77	—	0.80	—

Phenotypic and genotypic methods agreed in 90.5% of cases (Table 2), with a Cohen's κ of 0.80 indicating excellent concordance. Seven isolates were phenotypically resistant but *mecA*-negative, likely reflecting borderline oxacillin resistance mediated by β -lactamase hyperproduction rather

than PBP2a alteration. No *mecA*-positive/phenotypically susceptible isolates were found, confirming that *mecA* carriage reliably predicts phenotypic resistance in this population.

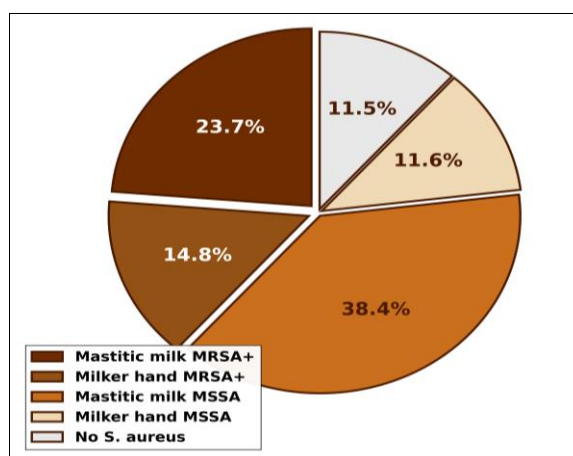


Fig 1: Distribution of *S. aureus* isolates by methicillin resistance status and sample source across 312 samples from 48 Lagos State dairy farms

Mastitic milk yielded a disproportionately large share of both MSSA and MRSA isolates relative to hand swabs (Figure 1). MRSA accounted for 23.7% of all milk samples and 14.8% of

hand swabs. The combined MRSA prevalence across both sample types was 20.8% (65/312).

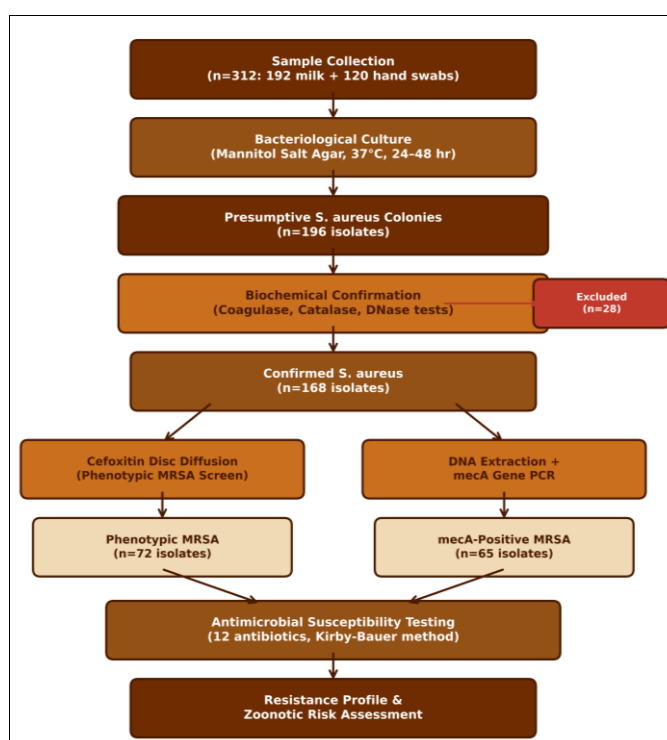


Fig 2: Flowchart of the sample processing pipeline from collection through culture, biochemical confirmation, phenotypic screening, and *mecA* PCR-based molecular detection of MRSA

The diagnostic workflow (Figure 2) shows how 312 initial samples were funnelled through culture, biochemical testing, and parallel phenotypic/genotypic MRSA confirmation. Of

196 presumptive *S. aureus* colonies, 28 were excluded at the biochemical stage (coagulase-negative or DNase-negative), leaving 168 confirmed isolates for resistance testing.

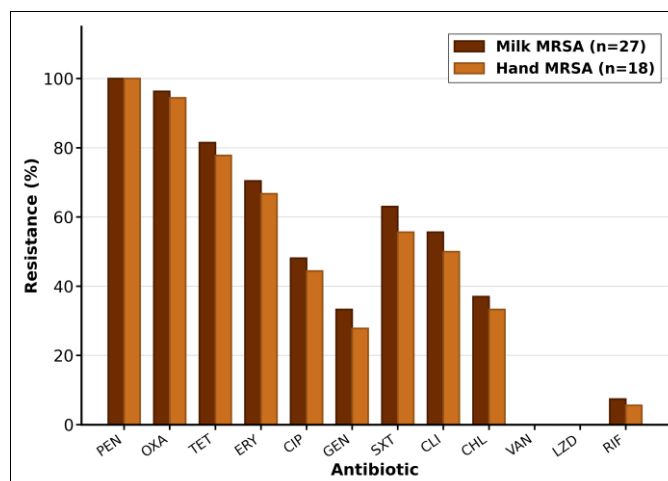


Fig 3: Antimicrobial resistance profile of *mecA*-positive MRSA isolates from bovine mastitic milk (n=27) and milker hand swabs (n=18) tested against 12 antibiotics

Comprehensive Interpretation

The resistance profile (Figure 3) paints a concerning picture: MRSA from both milk and hands showed near-universal penicillin and oxacillin resistance, high tetracycline and erythromycin resistance, and multi-drug resistance patterns (resistance to ≥ 3 antibiotic classes) in 84.6% of milk isolates and 77.8% of hand isolates. Critically, all isolates retained susceptibility to vancomycin and linezolid the last-resort agents for systemic MRSA infection in humans. The similarity of resistance profiles between milk and hand isolates from the same farm supports active bidirectional transmission. Among 18 farm-level paired sets where both milk and hand MRSA were detected, 11 (61.1%) shared identical antibiograms, consistent with a common clonal origin.

Discussion

The overall *mecA*-confirmed MRSA prevalence of 20.8% in this Lagos peri-urban dairy survey exceeds the 12–15% range reported from northern Nigerian states [6, 7] and the 9.4% recorded in a Kenyan dairy chain investigation [9]. Higher prevalence in Lagos may reflect the density of peri-urban farming, where farms share grazing corridors and livestock markets that facilitate strain circulation [10]. The tropical humid climate ($\geq 70\%$ RH during the rainy season) may also prolong bacterial survival on skin and milking utensils, sustaining transmission chains that would fade faster in drier environments.

The concordance between phenotypic cefoxitin screening and *mecA* PCR ($\kappa=0.80$) was reassuring and aligns with published validation data from European and North American laboratories [8, 11]. The seven phenotype-positive but *mecA*-negative isolates likely harbour *mecC* (a *mecA* homologue found in LA-MRSA CC130 lineages) or express borderline resistance through β -lactamase hyperproduction [12]. Screening for *mecC* was beyond the scope of this research but should be included in future surveys.

The multi-drug resistance profile with over 80% of MRSA isolates resistant to three or more antibiotic classes limits treatment options for both bovine mastitis and potential human infections. That vancomycin and linezolid remain effective is encouraging but should not breed complacency;

vancomycin-intermediate *S. aureus* (VISA) has been reported from neighbouring West African countries [13]. Implementing basic milking hygiene pre-milking teat disinfection, disposable gloves, post-milking teat dip costs less than USD 0.05 per milking session and could interrupt the transmission circuit at its most vulnerable point [14].

Conclusion

This research set out to determine the prevalence of MRSA in bovine mastitic milk and milker hand swabs from peri-urban dairy farms in Lagos State, confirm methicillin resistance at the molecular level through *mecA* gene PCR, profile the antimicrobial resistance patterns of confirmed MRSA isolates, and assess the evidence for zoonotic transmission between cattle and their handlers.

The key findings can be stated directly. First, *mecA*-positive MRSA was detected in 20.8% of all samples, with mastitic milk (23.7%) contributing a larger share than milker hand swabs (14.8%). Second, phenotypic and genotypic detection methods showed excellent agreement ($\kappa=0.80$), validating cefoxitin disc diffusion as a reliable screening tool where PCR is unavailable. Third, multi-drug resistance was the norm rather than the exception: over 80% of MRSA isolates resisted three or more antibiotic classes, although vancomycin and linezolid remained effective. Fourth, identical resistance profiles in 61.1% of farm-level milk-hand pairs provided strong circumstantial evidence for bidirectional transmission. From a practical standpoint, these findings justify three immediate actions. First, Lagos State veterinary authorities should incorporate MRSA screening into routine mastitis surveillance programmes, using cefoxitin disc diffusion as a cost-effective first-line test. Second, milker education campaigns emphasising hand washing with soap before and after milking, use of disposable nitrile gloves, and avoidance of raw milk consumption should be scaled through existing agricultural extension networks. Third, farms with confirmed MRSA-positive cows should implement segregated milking infected animals last and adopt post-milking teat disinfection with iodine-based dip solutions to reduce environmental contamination.

Looking ahead, molecular typing by spa-typing or multilocus sequence typing (MLST) would determine whether the

MRSA circulating in Lagos dairy chains belongs to known livestock-associated lineages (CC398, CC97) or represents novel community-associated clones that have spilled into the agricultural setting. Screening for the *mecC* gene and for Panton-Valentine leucocidin (PVL) toxin genes would further refine the zoonotic risk assessment. Longitudinal sampling of the same farms across wet and dry seasons would clarify whether MRSA prevalence fluctuates with climate or remains stable year-round. And intervention trials measuring the impact of simple hygiene packages on farm-level MRSA transmission would provide the evidence base needed to advocate for policy changes at the state and federal level.

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