integrity Research Journals

Journal of Bioscience and Biotechnology Discovery
Volume 10(4), pages 86-92, October 2025
Article Number: E0BC38C32
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2025.239
https://integrityresjournals.org/journal/JBBD

Full Length Research

Isolation, characterisation and plasmid profiling of methicillin-resistant *Staphylococcus aureus* isolated from mothers and neonates attending postnatal clinics in Auchi, Edo State, Nigeria

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Received 27th July 2025; Accepted 19th October 2025

ABSTRACT: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of neonatal infections in both hospital and community settings. Identifying its sources and mechanisms of resistance is essential for effective control. This study investigated MRSA colonisation in mothers and their newborns and assessed whether resistance was plasmid-mediated. A total of 201 swab specimens were collected from 100 mothers and 101 babies using sterile swabs. Samples were cultured on mannitol salt agar and blood agar, and incubated at 37°C for 24 hours. Colonies were confirmed as *S. aureus* through standard bacteriological and biochemical tests. Antibiotic susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method. Plasmid profiling was carried out on a 0.8% agarose gel for 10 methicillin-resistant isolates, and plasmid curing was attempted with sodium deodecyl sulphate. Seventy-four *S. aureus* isolates were recovered, of which 19 (25.7%) were resistant to methicillin. Eleven of these originated from neonates. All MRSA isolates demonstrated multidrug resistance. Plasmid analysis revealed differences between isolates from mothers and babies, suggesting both vertical and horizontal transmission. After curing, seven plasmid-bearing isolates showed altered antibiotic susceptibility, confirming a plasmid role in resistance. Two-way analysis of variance compared MRSA from neonates only, mothers only, and mother–baby pairs. At $p>0.05$, differences were insignificant; however, at $P<0.05$, MRSA from mother–baby pairs showed significant variation. This study highlights the high prevalence and multidrug resistance of MRSA in mothers and neonates. The findings emphasise the need for coordinated national surveillance, robust infection control measures, and strengthened antibiotic stewardship to reduce the growing burden of MRSA infections in Nigeria.

Keywords: Multidrug resistance, babies, horizontal transmission, vertical transmission.

INTRODUCTION

Current trends in the spread of infectious diseases and disease-causing agents have become a cause for global health concern. Ranging from malaria to Lassa fever and, most recently, COVID-19 disease, it has become important to trace sources of infections as a means of curtailing their spread and then administering an appropriate treatment regimen. If efforts are not engaged in this direction, even with the development of drugs, the issue of drug resistance will remain intractable as a result of the abuse of drugs and other factors. One of such global challenges of drug resistance is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA).

Methicillin-resistant *Staphylococcus aureus* (MRSA) refers to a group of Gram-positive bacteria that are genetically distinct from other strains of *Staphylococcus aureus.* MRSA is any strain of *S. aureus* that has developed,

through horizontal gene transfer and natural selection, multiple drug resistance to beta-lactam antibiotics (Gurusamy *et al.,* 2013). According to the Centres for Disease Control and Prevention, the definition of MRSA spans resistance of *S. aureus* not only against methicillin but also other related, more commonly used antibiotics such as oxacillin and amoxicillin (CDC, 2024). The Global Burden of Antimicrobial Resistance study placed MRSA among the leading pathogens associated with AMR-related mortality worldwide (Naghavi et al., 2024).

MRSA is a significant antimicrobial-resistant bacterium capable of producing severe and often invasive infections, including bloodstream infections, lower respiratory tract disease, and skin or soft tissue involvement (Pantosti *et al.*, 2007). Shoaib *et al.* (2022) explained that MRSA originated from MSSA following the acquisition of the SCCmec element through gene transfer. This element carries the mecA gene, which encodes the PBP-2α protein, making the strain resistant to the majority of β-lactam antibiotics.

Neonatal Intensive Care Unit (NICU) outbreaks remain a concern, with MRSA being one of the leading causes of healthcare-associated infections in neonates (National Healthcare Safety Network (NHSN), 2023). Bravo-Queipo-de-Llano *et al*. (2025) reported that Methicillin-resistant Staphylococcus aureus (MRSA) poses a major public health concern, as it is responsible for skin and soft tissue infections (SSTIs) and can lead to serious complications, particularly in children. Neonates are particularly vulnerable to S. aureus infections due to their underdeveloped skin and mucosal defenses, as well as the frequent invasive medical procedures they undergo while in the hospital (Wei *et al*., 2022).

Humans harbour *Staphylococcus aureus* as normal flora in some parts of the body, so neonates have a very high likelihood of exposure soon after birth. Most common sites of colonisation with *S. aureus* include the umbilical cord, skin, nasopharynx, and gastrointestinal tract. For MRSA, the nares and umbilicus are the most common sites of initial colonisation (Bizzarro and Gallagher, 2007). Neonates can become colonised by MRSA in a number of different ways. Traditionally, MRSA has been demonstrated to spread horizontally by healthcare-associated transmission, i.e. via contact with healthcare workers or the hospital environment (Cimolai, 2003). Additional factors, such as overcrowding and understaffing in the neonatal intensive care unit (NICU), have been associated with increased risk of healthcare-associated transmission and colonisation, which may lead to epidemics of MRSA infection. Vertical transmission of MRSA from mothers to their infants has also been described (Shiojima *et al*., 2003). Neonatal MRSA infections often present as pustular skin lesions, omphalitis, or invasive diseases such as bacteremia, pneumonia, and meningitis (Sharma *et al*., 2024).

As noted by Obeng (2025), neonates are predisposed to colonisation and infection by *S. aureus* and MRSA, largely because of their weakened immune systems. Treating such infections has become more difficult since resistance to first-line antibiotics is on the rise, thereby narrowing effective treatment choices. Recent surveillance reports show that although some regions have reduced MRSA rates through targeted prevention programs, sporadic outbreaks continue to emerge globally (ECDC, 2024).

This study, therefore, is aimed at isolating MRSA from mothers and their babies as well as comparing the susceptibility patterns of isolates before and after plasmid curing to determine if their resistance was plasmid-mediated or not.

MATERIALS AND METHODS

Collection, isolation and identification of bacteria isolates

Sterile swab sticks (Sterilin) were used to swab the palms, skin and nares of 100 consenting mothers and their 100 babies and specimens were streak-inoculated onto sterile solidified blood and mannitol salt agar media (Oxoid) and then incubated at 37°C for 24 hours. Discrete colonies from overnight cultures were subcultured in freshly prepared nutrient agar to obtain pure cultures for further tests. Colonial characteristics of isolates were determined using parameters such as size, elevation, pigment, margin and shape. The bacterial isolates were identified using the Gram staining technique and biochemical tests, including motility, catalase, coagulase tests and mannitol utilisation.

Antibiotic susceptibility testing

Antibiotic susceptibility testing with Mueller-Hinton agar was carried out using the modified Kirby-Bauer disc diffusion technique (Cheesbrough, 2005). The following antibiotic discs (Oxoid, Ltd) were tested: Methicillin (1µg) [Oxacillin], Ceftazidime (30 µg), Cefuroxine (30 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (5 µg), Ofloxacin (5µg), and Augmentin (30 µg). The discs were aseptically placed on the plate using sterilised forceps. The methicillin antibiotic disc was then placed at the centre of the plate. Media containing antibiotics were incubated for 18-24 hours at 35^{o}C. A control plate was also prepared using *Staphylococcus aureus* ATCC 25923. The diameters (in millimetres) of the zones of inhibition were thereafter measured following the Clinical Laboratory and Standards Institute guidelines.

Extraction of plasmid DNA

Plasmid DNA of MRSA isolates were extracted using the

ZymoPURETM Plasmid Miniprep Kit, following the manufacturer's instructions, and analysed by electrophoresis on a 0.8% agarose gel and then visualised under a UV trans-illuminator. The DNA bands were photographed using a digital camera and compared with those for the lambda DNA Hind III digest molecular weight marker, and the results were recorded.

Plasmid curing

Curing of plasmid was done using sodium dodecyl sulphate (SDS) on isolates that possessed plasmid gene. Briefly, 1ml of the cured culture was inoculated into 9ml of freshly prepared nutrient broth and incubated for 24hrs at 37^{o}C. A 1ml suspension of each bacterial isolate from the nutrient broth growth equivalent to 0.5 MacFarland turbidity standard was aseptically seeded into a Mueller-Hinton agar plate. The antibiotic discs containing Ceptazidine (CAZ), Cefuroxine (CRX), Gentamicin (GEN), Cetriaxone (CTR), Erythromycin (ERY), Cloxacillin (CXC), Ofloxacin (OFL) and Augmentin (AUG) were placed on the agar plate using sterilised forceps. Methicillin (OX) antibiotic disc was then placed at the centre of the plate and incubated at 37°C for 18-24 hours. The diameters (in mm) of the zones of inhibition were thereafter measured and recorded.

Ethical approval

Ethical approval for this study was obtained from Ambrose Alli University, Ekpoma Health Research Ethics Committee, with Reference number 016/18

RESULTS

Seventy-four (74) isolates of *Staphylococcus aureus* were recovered from the 201 samples cultured (100 from mothers (M) and 101 babies (B)), following their cultural, morphological and biochemical characteristics. Nineteen (25.7%) of the *Staphylococcus aureus* isolates were methicillin-resistant (Table 1).

From Table 1, it is observed that two mothers (10M,12M) and their babies (10B,12B) harboured MRSA. 12M was from mother of twins, with only one of the babies (12B) infected with MRSA. All methicillin-resistant *Staphylococcus aureus* were also multidrug resistant as they were resistant to at least five other antibiotics.

Plate 1 shows that 7 out of the 10 randomly picked and profiled isolates contain plasmid genes. Isolates 12m is positive for plasmid genes with double band at 2kb and 20kb, 1solates 12b, 28b, 3b and 10m are positive for plasmid genes with band at 20kb, 1solates 31b and 9m are positive for plasmid genes with band at 48.5kb while isolates 40m, 10b and 39b are negative for plasmid gene.

Table 1. Antibiogram of Methicillin - resistant *Staphylococcus aureus* (N= 19).

No.	Source	Resistant pattern
1	3B	OX,CAZ,CRX,CXC,AUG.
2	9M	OX,CAZ,CRX,CTR,CXC,AUG.
3	10B	OX,CAZ,CRX,CTR,CXC,AUG.
4	10M	OX,CAZ,CRX,CTR,CXC,AUG.
5	12B	OX,CAZ,CRX,CTR,CXC,AUG.
6	12M	OX,CRX,GEN,CTR,ERY,CXC,AUG.
7	15B	OX,CAZ,CRX,CTR,CXC,AUG.
8	16M	OX,CAZ,CRX,CTR,CXC,AUG.
9	28B	OX,CAZ,CRX,CTR,CXC,AUG.
10	31B	OX,CAZ,CRX,CTR,ERY,CXC,AUG.
11	38B	OX,CAZ,CRX,CTR,CXC,AUG.
12	39B	OX,CAZ,CRX,CTR,CXC,OFL,AUG.
13	40M	OX,CAZ,CRX,CTR,CXC,AUG
14	41B	OX,CAZ,CRX,CTR,CXC,AUG.
15	45M	OX, CAZ,CRX,CTR,CXC.
16	47M	OX,CAZ,CRX,CTR,ERY,CXC,AUG.
17	50B	OX,CAZ,CRX,CXC,AUG.
18	52B	OX,CAZ,CRX,CTR,CXC,AUG.
19	76M	OX,CAZ,CRX,CTR,CXC,AUG.

Keys: B= Baby, M= Mother, S= Sensitive, R= Resistant, OX= Methicillin (Oxacillin), CAZ= Ceptazidime, CRX= Cefuroxine, CTR= Ceftriaxone, ERY= Erythromycin, CXC= Cloxacillin, AUG= Augmentin.

DISCUSSION

Staphylococcus aureus has become one of the most frequently encountered microorganisms in microbiology laboratories in Nigeria. Since the 1960s, when methicillin resistance began to challenge effective treatment regimens for infections caused by *Staphylococcus aureus*, the latter has become a major health concern worldwide, with varying prevalence rates. The purpose of this study was to determine the prevalence rate, as well as the plasmid profile of MRSA isolated from mothers and neonates attending postnatal clinics in Auchi, Nigeria.

A prevalence rate of 25.7 % was recorded for MRSA in this study; this rate is closely similar to rates recorded in Abuja (26.9%) (Abdullahi and Iregbu, 2018), Kano (28.6%) (Nwankwo *et al*., 2010) and 28% in Bauchi (Ghamba *et al.,* 2012). The MRSA resistance rate of 79% was reported in Benin (Onemu and Ophori, 2013). Varying prevalence rates have also been observed in other climes outside Nigeria (Cheddie *et al.*, 2020; Fritz *et al.,* 2009)

The highest number of isolates of MRSA (11, 57.9%) were recovered from neonates. This rate is lower than that recorded by Iroha *et al*. (2012), where 73.3% of MRSA isolates were recovered from patients between the ages 0-6 years. It is explainable because not only babies were examined in his research. In a study carried out in Israel (Regev-Yochay *et al*., 2000), the highest *Staph aureus*

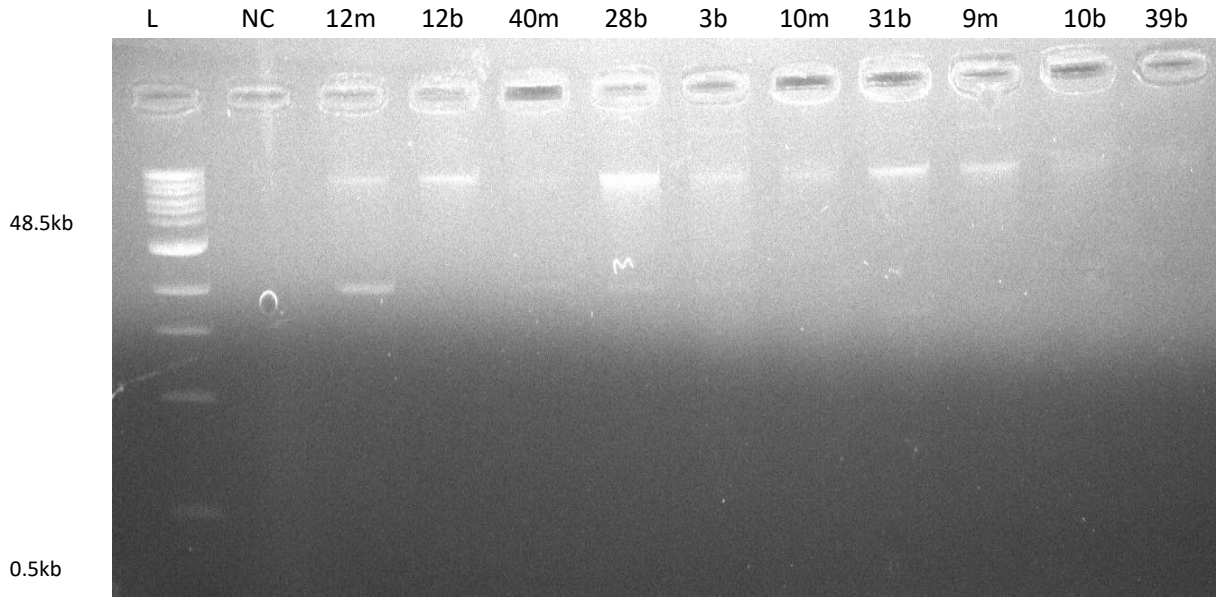

Plate 1. Plasmid profile of methicillin-resistant *Staphylococcus aureus* isolated from mothers and neonates analysed with 0.8% agarose gel electrophoresis stained with ethidium bromide. L is a 0.5kb-48.5kb DNA ladder (molecular marker). Isolates 12m is positive for plasmid genes with double band at 2kb and 20kb, 1solates 12b, 28b, 3b and 10m are positive for plasmid genes with band at 20kb, 1solates 31b and 9m are positive for plasmid genes with band at 48.5kb while isolates 40m, 10b and 39b are negative for plasmid gene. NC: negative control.

carriage rate was observed in infants aged 3 months or younger. From this present study, Isolates 10M,10B and 12M,12B were from two mothers and their babies, whereas isolates 9M,16M,40M,45M,47M and 76M were from mothers whose babies were negative for MRSA. Isolates 3B, 15B, 28B, 31B, 38B, 38B, 39B, 41B, 50B and 52B were from babies whose mothers' specimens yielded no MRSA.

A high rate of Methicillin-Resistant *Staphylococcus aureus* and Multidrug Resistant strains have also been recorded in Ido-Ekiti by Olowe *et al*. (2011). As shown in the present study, as many as 12 of the 19 isolates displayed multidrug resistance to 6 of the 9 antibiotics used for the susceptibility studies (Table 1). This underscores the need to engage in proper antibiotic treatment policy, rather than self-medication, in order to curtail the rising trend of multidrug resistance in medical treatment. The presence of insertion sites for plasmids and transposons in the mecA complex of MRSA, which carry antibiotic resistance genes, accounts for the resistance to several classes of antibiotics (Abdullahi and Iregbu, 2018). James *et al.* (2025) reported that antimicrobial resistance accounts for an estimated 2.4% loss of Nigeria's GDP, with the management of resistant infections costing nearly three times more than treating non-resistant cases. The study attributed this burden to limited public awareness, inappropriate antibiotic use, and inadequate waste disposal practices across both healthcare and agricultural settings.

The multiple antibiotic resistance (MAR) indices ranged from 0.5 to 0.7 (Table 2). It has been noted that MAR indices above 0.2 indicate that such isolates originate from an environment where antimicrobial agents are freely available and accessible with high potential for abuse (Krumperman, 1983). This is true of most of the cities in Edo State where people assess antibiotics over -the -counter, almost without restrictions. This poses a serious public health concern. Eleven out of the 19 isolates (57.9%) were recovered from neonates. According to Leshem *et al*. (2012), newborns of carrier mothers are at risk of acquiring *S. aureus* colonisation. Most newborns of carrier mothers are colonised within the first month of life, and horizontal transmission from mother to child is probably the major source fo*r S. aureus* carriage in newborns. Conditions such as household crowding, frequent visits to the hospital environment or hospitalisation may promote nasal carriage of MDR and MRSA.

The susceptibility testing of the MRSA strains subjected to plasmid DNA curing with SDS in Table 3 showed that some of the MRSA strains previously showing resistance to methicillin became susceptible to the same antibiotic after the curing. For instance, isolates 9M, 10M, 12B, 28B and 31B that were resistant to Ceftriaxone and Gentamicin (12M) before curing became sensitive to the antibiotics after curing, implying that their resistance was plasmid-mediated. This is in agreement with the study of Ojo *et al.*

Table 2. Number of antibiotics to which *Staphylococcus* isolates showed resistance and corresponding multiple antibiotic resistance indices.

Number of Antibiotics to which isolates are resistant	No. of isolates with MAR	*MAR Index
5	3	0.5
6	12	0.6
7	4	0.7

$* \text{MAR Index} = \frac{x}{y} = \frac{\text{No.of antibiotics isolate is resistant to}}{\text{No.of antibiotics tested (9)}}$. MAR is defined here as joint resistance of *S. aureus* isolates to more than two antibiotics and was determined using the formula MAR=x/y, where x is the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Akinjogunla and Enabulele, 2010).

Table 3. Comparison of the antibiogram of the MRSA isolates before and after curing with sodium dodecyl sulphate (SDS).

Source		OX	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG
3B	PRE	R	R	R	S	I	S	R	S	R
	POST	S	R	R	S	S	S	R	S	R
9M	PRE	R	R	R	S	R	S	R	S	R
	POST	S	R	R	S	S	S	R	S	R
10M	PRE	R	S	R	S	R	S	R	S	R
	POST	S	S	R	S	S	S	R	S	R
12B	PRE	R	R	R	S	R	S	R	S	R
	POST	S	R	R	S	S	S	R	S	R
12M	PRE	R	R	R	R	S	R	R	S	R
	POST	S	R	R	S	S	R	R	S	R
28B	PRE	R	R	R	S	R	S	R	S	R
	POST	S	R	R	S	S	S	R	S	R
31B	PRE	R	R	R	R	R	R	R	S	R
	POST	S	R	R	R	S	R	R	S	R

Key: B= Baby, M = Mother, PRE = before curing, POST = after curing, S = Sensitive, R = Resistant, I = Intermediate, OX = Methicillin (Oxacillin), CAZ = Ceftazidime, CRX = Cefuroxine, CTR = Ceftriaxone, ERY = Erythromycin, CXC = Cloxacillin, AUG= Augmentin.

(2014). On the other hand, some strains, e.g. 3B, 9M, 10M, 12B, 12M, 28B, 31B were resistant to Augmentin, Cefuroxine and Ceftazidime before and after plasmid curing, an indication that their resistance may have been due to mechanisms other than plasmid mediation. Plasmid genes demonstrated bands ranging from 2kb to 48.5kb. In this study, however, we did not investigate the presence of methicillin resistance genes mecA or mecC. Also, there is a possibility of potential sampling bias.

Conclusion

The prevalence of methicillin-resistant *Staphylococcus aureus* in Nigeria poses a significant and escalating public health threat, particularly for the highly vulnerable population of neonates. The evidence indicates that this is not merely an isolated clinical issue but a systemic problem rooted in a high burden of hospital-acquired infections, the widespread multidrug resistance of circulating strains, and a complex and diverse genetic landscape. The available data suggest that while maternal colonisation can be a source of infection, the hospital environment itself represents the most critical reservoir for MRSA acquisition in newborns. The path forward requires a coordinated and multifaceted approach. Nigeria must establish a unified national surveillance system to accurately map the epidemiology of this pathogen. This must be complemented by the rigorous implementation of robust infection control measures in all healthcare facilities and the adoption of comprehensive antibiotic stewardship programs. By prioritising these actions, healthcare authorities can begin to turn the tide against this formidable pathogen, protecting the health and future of its most vulnerable citizens.

CONFLICT OF INTEREST

The authors declare no conflict of interest exists.

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