

Current Prevalence and Antimicrobial Susceptibility Pattern of Staphylococcus Aureus and Methicillin-Resistant Staphylococcus Aureus Among Pregnant Women at Mile 4 Hospital, Ebonyi State Nigeria

Chimaobi, Charity Chinyere*¹, Okoronkwo Chimaobi², Egwu-Ikechukwu Modesta Mmaduabuchi¹, Herbert Ikechukwu Egwu³, Nومه Onyinyechi Lovette¹ and Ikwuakor Chinyere Geraldine¹

¹Department of Microbiology, Alex-Ekwueme Federal University Ndufu Alike, P.M.B 1010, Abakaliki, Ebonyi State, Nigeria

²Department of Environmental, Ministry of Environment, Ebonyi State, Nigeria

³Department of Applied Microbiology, Ebonyi State University, Abakaliki, P.M.B 053, Abakaliki, Ebonyi State, Nigeria

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Corresponding Author: **Chimaobi Charity Chinyere**

E-Mail: nnabugwu27@gmail.com

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is widely recognized as a significant pathogen responsible for numerous community-acquired infections across the globe. This study aimed to investigate the prevalence of *Staphylococcus aureus*, including MRSA strains, as well as their antimicrobial susceptibility patterns among pregnant women attending antenatal clinics at Mile Four Hospital and Maternity in Ebonyi State. A total of 150 urine specimens were collected from the participants and delivered to the Microbiology Laboratory of Ebonyi State University within two hours for laboratory examination. Upon arrival, the samples were initially inoculated into nutrient broth and incubated overnight to encourage bacterial growth. The enriched cultures were then subcultured on mannitol salt agar (MSA) plates and incubated aerobically at 37 °C for 24 hours to facilitate the isolation of *S. aureus*. The bacterial isolates were enumerated in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), and their antimicrobial susceptibility was determined using the standard disc diffusion method. Of the 150 urine samples analyzed, 61 (40.7%) yielded *Staphylococcus aureus*. Among these isolates, 22 (36.0%) were identified as methicillin-resistant strains. The occurrence of both *S. aureus* and MRSA infections was most

prominent among pregnant women aged 33–37 years and 38–42 years, with prevalence rates of 66% and 100%, respectively. Among occupational groups, civil servants showed the highest *S. aureus* prevalence (53%), while self-employed women had the highest MRSA prevalence (37%). Educational level also influenced prevalence, with *S. aureus* highest in women with secondary education (51%) and MRSA highest in those with primary education (45%). Third-trimester pregnant women showed a prevalence of 57% for both *S. aureus* and MRSA. The antimicrobial susceptibility analysis indicated that the *Staphylococcus aureus* isolates demonstrated substantial resistance to the majority of the antibiotics evaluated. High resistance rates were observed against oxacillin (97%), ceftriaxone (100%), gentamicin (97%), clindamycin (100%), tetracycline (92%), cefotaxime (100%), erythromycin (100%), vancomycin (100%), and ciprofloxacin (95%). In contrast, imipenem exhibited comparatively better effectiveness, with approximately 61% of the isolates showing susceptibility to this antibiotic. These findings are concerning, as most of these resistant antibiotics are widely used in hospitals and available on the market, highlighting an urgent need for monitoring and effective antibiotic stewardship

Keywords: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, antibiotics, urine and Pregnant women..

INTRODUCTION

Staphylococcus aureus is a widely recognized bacterial pathogen that colonizes humans and is capable of causing a broad range of infections, including those affecting pregnant women. It is commonly associated with skin and soft tissue infections, osteoarticular infections, and severe systemic conditions such as septicemia, endocarditis, pneumonia, as well as infections involving the eye and the central nervous system [1,2]. The pathogenic success of *S. aureus* is largely attributed to its numerous virulence factors, which enable the organism to

invade host tissues and evade immune defenses. One of these factors is the enzyme catalase, which promotes bacterial survival within host cells by neutralizing hydrogen peroxide produced during immune responses. Surface-associated proteins such as coagulase and clumping factors also play important roles by facilitating clot formation and promoting bacterial adhesion to host tissues. In addition, *S. aureus* produces a variety of toxins that contribute significantly to disease progression.

These toxins include hemolysins, leukocidins, exfoliative toxins, and enterotoxins B and C, all of which can cause tissue damage and trigger inflammatory reactions. Pantone–Valentine leukocidin (PVL) is another potent toxin that destroys leukocytes and promotes the formation of abscesses, thereby increasing the severity of infection. The bacterium can also acquire a new genetic element, and environmental stressors such as low pH, hypoxia, nutrient limitation, extreme temperatures, and antibiotic exposure can modulate gene expression, enhancing its survival and pathogenicity [3]. Transmission of *Staphylococcus* species including *Staphylococcus aureus* between humans and animals is of clinical importance. Methicillin-resistant and other antibiotic-resistant strains can spread between domestic animals and their owners, veterinary staff and patients, and among hospitalized individuals. This study aims to isolate and identify *Staphylococcus* spp. from healthy human pregnant women who visited hospital for ante-natal clinics.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as an important hospital-associated pathogen that contributes substantially to global morbidity and mortality. The prevalence of MRSA infections varies considerably across different regions of the world and remains a major public health concern. In many healthcare facilities and long-term care institutions, endemic MRSA strains have been reported to account for approximately 29–35% of clinical isolates. The growing incidence of MRSA infections has highlighted the need for continuous surveillance in both healthy and infected populations, particularly in high-risk hospital settings such as intensive care units. Although severe infections such as pneumonia and bacteremia are among the most frequently reported complications, MRSA is also associated with a wide range of other conditions including intra-abdominal infections, osteomyelitis, toxic shock syndrome, staphylococcal food poisoning, and deep tissue infections. Glycopeptide antibiotics, particularly vancomycin, continue to be commonly used for the treatment of MRSA infections. In addition, newer antimicrobial agents such as linezolid and quinupristin/dalfopristin have broadened available therapeutic options, however, their high cost, potential adverse effects, and the emergence of resistance limit their routine clinical application. Therefore, the present study aims to evaluate the current prevalence and antibiotic susceptibility patterns of *Staphylococcus aureus*, including MRSA strains, among pregnant women using clinical isolates.

2. Methods

2.1 Study Setting and Period of Study

The study was conducted among one hundred and fifty (150) pregnant women attending ante-natal clinic in Mile four hospital Ebonyi State from March–July, 2024. Mile four hospital is located at Ohatekwe Agalegu Ishieke community in Ebonyi local Government Area of Ebonyi, South eastern part of Nigeria.

Sample collection and processing and Antimicrobial Susceptibility Testing (AST)

Clean-catch midstream urine samples were collected from the pregnant women volunteers who were not on antibiotics treatment at the time of sampling and analyzed using microbiological standard procedures. All samples collected were transported to microbiology laboratory Ebonyi State University (EBSU) immediately using a cold ice bag for culturing and antimicrobial susceptibility tests [4,7,8]. The samples were first inoculated unto broth for the purpose of enrichment.

Mannitol salt agar (MSA) was used for *Staphylococcus aureus* identification, guidelines from American Society for Microbiology (ASM) were followed for morphological and biochemical tests. Using Kirby-Bauer disc diffusion method, antimicrobial susceptibility testing (AST) was carried out [9,10].

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of the bacterial isolates was evaluated using the standard disc diffusion method originally described by Bauer et al. (1966). Antibiotic discs used in this investigation were sourced from Oxoid (Hampshire, England). The antibiotics tested included methicillin (ME, 5 µg), ceftriaxone (CRO, 30 µg), cefotaxime (CTX, 30 µg), oxacillin (OX, 30 µg), erythromycin (E, 15 µg), ciprofloxacin (CIP, 5 µg), gentamicin (CN, 10 µg), tetracycline (TE, 30 µg), clindamycin (DA, 2 µg), vancomycin (VA, 30 µg), and imipenem (IPM, 30 µg). Bacterial inocula were prepared from overnight cultures grown on Mueller–Hinton (MH) agar plates obtained from Oxoid (Hampshire, England). The turbidity of each bacterial suspension was adjusted to correspond with the 0.5 McFarland turbidity standard, representing approximately 1.5×10^8 colony-forming units per milliliter (CFU/mL). The standardized inoculum was then uniformly spread across the surface of Mueller–Hinton agar plates using sterile cotton swabs to ensure even bacterial distribution.

After the inoculated plates were allowed to dry briefly, the antibiotic discs were aseptically positioned on the agar surface with the aid of sterile forceps. The plates were subsequently incubated aerobically at 37°C for a period of 18–24 hours. Following incubation, the diameters of the inhibition zones around each antibiotic disc were measured in millimeters. The susceptibility results were interpreted based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) [10].

Statistical Analysis

All data collected in this research was analyzed and interpreted statistically using the chisquare test to determine the significant difference between the two genders. The Microsoft excel program was used to enter the data collected. Data were analyzed using the statistical package for the social science (SPSS).

Ethical approval

This study was approved by the Ethical committee of Mile four hospital and Maternity, Ebonyi State Nigeria, with ethical identification number MHM/2021-009

RESULTS

Isolation and Identification of *Staphylococcus aureus*

150 urine samples collected from the pregnant women were analyzed in this study, from which bacterial isolates were obtained as presented in Table 1. Preliminary identification of *Staphylococcus aureus* was carried out by microscopic examination the gram staining. The isolates as gram-positive cocci typically arranged in clusters resembling grape-like bunches. Further confirmation of the isolates was performed through standard biochemical tests. The results of these biochemical characterizations, which supported the identification of *S. aureus*, are summarized in Table 2. These tests helped distinguish *S. aureus* from other closely related bacterial species.

Table 1: Source and number of *S. aureus* isolates

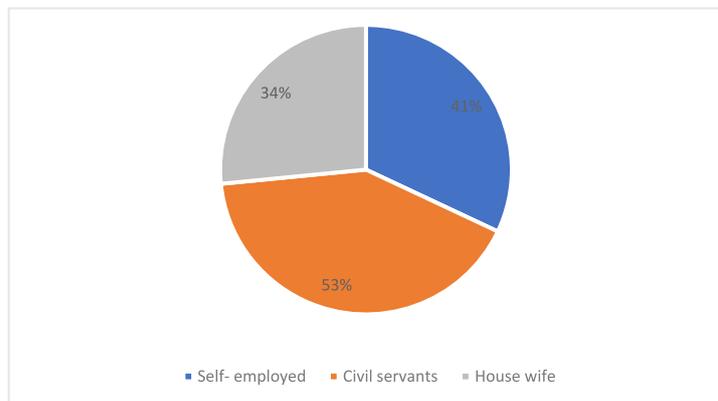
Test	Result
Test on nutrient agar	Yellow golden colony
Catalase	+
Coagulase	+
Oxidase	+
Mannitol fermentattion	Mannitol fermentor

Table 2: The occurrence of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) among pregnant women attending ante natal clinic, according to age group

Age (years)	No. examined	No. positive for <i>S. aureus</i>	No. positive for MRSA
18-22	20	8(40%)	2(25%)
23-27	59	20(34%)	6(30%)
28-32	36	18(50%)	7(39%)
33-37	18	12(66%)	4(33%)
38-42	17	3(17%)	3(100%)
Total	150	61(40%)	22(36%)

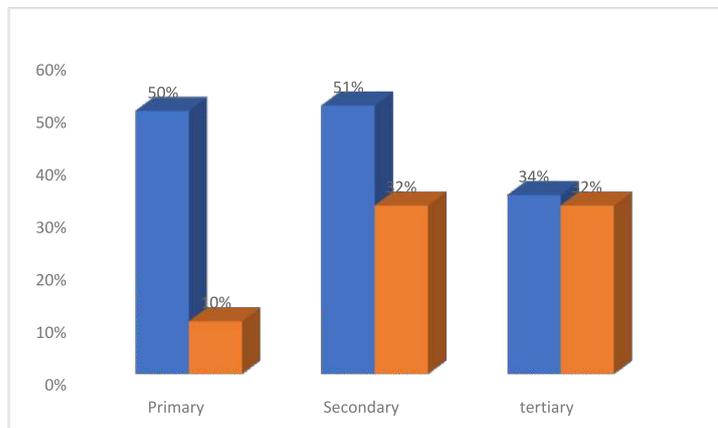
The prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) was observed to be higher among pregnant women within the age groups of 33–37 years and 38–42 years, with occurrence rates of 66% and 100%, respectively.

Table 3: Presents the distribution of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) among pregnant women attending antenatal clinics, categorized according to their occupational status.



The occurrence of *Staphylococcus aureus* was highest among pregnant women who were civil servants, accounting for 53% of the cases. In contrast, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) within the same occupational group was comparatively lower, with a rate of 33%.

Table 4: illustrates the distribution of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) among pregnant women attending antenatal clinics based on their educational background.



The prevalence of *Staphylococcus aureus* was highest among pregnant women who had attained secondary-level education, accounting for 51% of the cases. In contrast, the lowest occurrence was observed among those with primary-level education, where only 10% of the participants were affected.

Table 5: presents the distribution of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) among pregnant women attending antenatal clinics based on the trimester stage of their pregnancy.

Level of education	No. Examined	No. positive for <i>S. aureus</i>	No. positive for MRSA
first	22	5(27%)	2(40%)
Second	89	16(25%)	7(43%)
third	39	40(57%)	13(32%)
Total	150	61(40%)	22(36%)

The prevalence of *Staphylococcus aureus* was highest among pregnant women in their third trimester, accounting for 57% of the cases, the occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) was relatively lower among women in the third trimester of pregnancy.

Table 6: Antibiotics Resistance and Susceptibility pattern of 50 MRSA isolates

S/N	Organism Name	Resistant	Susceptibility
1.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE,
2.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
3.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
4.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
5.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
6.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
7.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
8.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
9.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
10.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
11.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
12.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
13.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
14.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
15.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
16.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
17.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
18.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
19.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
20.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
21.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
22.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
23.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
24.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
25.	<i>S.aureus</i>	CRO,CIP,VA,CN,E	OX,C,MY,IPM,TE
26.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
27.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
28.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
29.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
30.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
31.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
32.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
33.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
34.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
35.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
36.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
37.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
38.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
39.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
40.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
41.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
42.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
43.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
44.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
45.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
46.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
47.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
48.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
49.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE
50.	<i>S.aureus</i>	OX,CRO,CIP,VA	CN,E,C,MY,IPM,TE

OX-Oxacilin, CRO-Ceftriaxone, CIP-Ciprofloxacin, VA-Vancomycin, CN- clindamycin, E-Erythromycin, C-Cefotaxin, MY-Gentamycin, IPM-Imipenem, TE-Tetracycline.

DISCUSSION

Staphylococcus aureus is distributed bacterium known to cause infections in humans, including pregnant women. It is associated with skin and soft tissue infections (STIs) [11] and is also recognized as a significant contributor to foodborne illnesses worldwide [12]. Due to physiological and immunological changes during pregnancy, pregnant women may be more susceptible to bacterial infections, making them an important population for epidemiological investigations. This study was designed to the prevalence of *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in urine samples obtained from pregnant women in Ebonyi State. To the best of our knowledge, there has been limited information regarding the occurrence of these pathogens among this population group in the study area, despite pregnant women being considered a high-risk group globally. The findings of this study revealed that out of the total samples analyzed, 61 isolates (40%) were identified as *Staphylococcus aureus*. Among these isolates, 22 (36%) were confirmed to be methicillin-resistant *Staphylococcus aureus* (MRSA), indicating a notable presence of antibiotic-resistant strains within the study population.

S. aureus and MRSA was higher than reported in studies among pregnant women in other countries for *S. aureus*, and almost the same as reported by [16], which was 22.1% and 2%, respectively. The lower prevalence in other studies may relate to differences in age ranges, as age is an important determinant of *S. aureus* urine carriage [13,16]. This study aligns with reports of [1,15], which showed high MRSA prevalence outside Addis Ababa, but contrasts with [17], who reported 17.5% MRSA in a hospital medical college in Addis Ababa.

S. aureus and MRSA were most common among pregnant women aged 33–37 years and 38–42 years, with rates of 66% and 100%, respectively. Similarly, a study at J.N. Medical College, Wardha (Maharashtra), reported an increase in MRSA prevalence from 30.6% in 1997 to 51.8% in 2010 [26]. European studies also noted rising methicillin resistance in *S. aureus* isolates between 1999 and 2002, with Germany, the UK, and Greece showing increases from 9.4%, 30.5%, and 37% to 19.2%, 44.5%, and 48.6%, respectively [17,18].

Occupational and educational factors influenced prevalence. Pregnant women who were civil servants showed high *S. aureus* occurrence (53%) but lower MRSA rates (33%). Civil servants include individuals with varying education levels, those with less education may have limited health knowledge, while educated civil servants may delay seeking care due to busy schedules. Educational level also impacted prevalence: *S. aureus* was higher among women with secondary education (51%) and lower among those with primary education (10%). Lack of knowledge about hygiene and reproductive health may increase susceptibility [21,22]. Previous studies also reported high *S. aureus* and MRSA prevalence among pregnant women attending antenatal clinics in Mater Hospital, Afikpo, Ebonyi State, Nigeria [2,4,23].

Pregnancy stage affected prevalence: women in their third trimester had higher *S. aureus* rates (57%) but lower MRSA rates. Undiagnosed infections can increase risk for both mother and child, including preterm labor and low birth weight. Age-specific findings showed highest *S. aureus* prevalence among women aged 20–24 years (82.5%) and lowest among those aged 25–30 years (57.1%), higher than previous reports of 47% in students aged 20–25 years [2,24] and partially agreeing with [26]. This contrasts with [24,25], who reported higher *S. aureus* isolation in those aged 15–24 years.

Antibiotic susceptibility of 20 *S. aureus* isolates revealed high susceptibility to Ampicillin (90%), Amoxicillin-clavulanic acid (85%), Gentamycin (60%), and Ofloxacin (50%). Resistance was observed for Ceftriaxone (95%), Ciprofloxacin (75%), Tetracycline (75%), Nitrofurantoin (70%), and Cefoxitin (65%). These findings align with [27], who reported high susceptibility to Ampicillin and Gentamycin (90% and 92%). Studies [1,32] reported high resistance to Ciprofloxacin and Tetracycline. Majority of MRSA isolates were resistant to more than two antimicrobial agents.

All MRSA isolates were resistant to Methicillin (100%), consistent with Kadry et al. (2016) and [30], who reported 100% and 94% resistance, respectively, while [30,32] reported lower rates (80.8% and 50%). High resistance to Ceftriaxone and Cefotaxime (92.2%) was observed, similar to [6,30], whereas [3] reported intermediate resistance (31.6%). Resistance to Tetracycline was 63.1%, comparable to rates of 68.6% and 58.4% reported elsewhere, though some studies reported higher (90.3%) or lower (33%) rates [23]. *S. aureus* showed intermediate resistance to Gentamycin (47.5%), Azithromycin (30.1%), and Erythromycin (31.1%) [25], with other studies showing variation. Low resistance was observed for Chloramphenicol (22.3%) and Ciprofloxacin (20.4%) [23]. Multidrug resistance (MDR), defined as resistance to three or more antibiotics, was observed in 64% of *S. aureus* isolates, similar to reports of 65% and 68.6% [25], though other studies reported lower rates (31%) or higher rates (87%) [Fluit et al., 2001].

CONCLUSION

The study demonstrates that *S. aureus* and MRSA are significant pathogens associated with soft tissue infections. The high prevalence of *S. aureus* may reflect its role as normal body flora, while the lower prevalence of MRSA indicates that not all *S. aureus* infections are resistant, and many can still be treated with commonly used antibiotics. The observed antibiotic resistance patterns, especially multidrug resistance (MDR), emphasize the urgent need for continuous monitoring and detailed surveillance of MRSA alongside its antimicrobial susceptibility [25,27]. Effective hospital infection control policies are essential, including routine screening of pregnant women for *S. aureus* and MRSA, proper hygiene education, and adherence to antimicrobial stewardship programs. Such measures will help reduce the risk of transmission, prevent complications like preterm labor and low birth weight, and support clinicians in providing timely and effective care. Furthermore, public health awareness campaigns should be implemented to educate pregnant women on hygiene, early detection, and the importance of completing prescribed treatments. Continuous research is also needed to track evolving resistance patterns and develop updated treatment guidelines that are specific to local populations.

Authors contribution

All the authors made a valuable contribution in data collection, reporting, analysis and interpretation of this research work. They all participated in revising and reviewing of this article for publication and agreed to submit this research in this journal.

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Disclosure

The authors wish to affirm that there is no competing interest in this research.

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