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MOLECULAR IDENTIFICATION AND ANTIBIOGRAM OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM WOUNDS OF BOTH IN- AND OUT-PATIENTS AT UNIVERSITY OF BENIN TEACHING HOSPITAL (UBTH), BENIN CITY, NIGERIA

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) stands as a prominent bacterial pathogen associated with various human infections, characterized by its remarkable ability to rapidly develop resistance to multiple antibiotics. The study aimed to characterize MRSA isolated from wounds among hospital patients. A total of eighty-seven unique *S. aureus* isolates were obtained from wound cultures collected from patients at the University of Benin Teaching Hospital (UBTH) in Benin City, Nigeria. These samples underwent assessment for methicillin resistance through conventional cultural methods, and detection of methicillin resistance (*mec*A) and *S. aureus* specific (*nuc*) genes using PCR amplification. The MRSA isolates underwent assessment for antimicrobial resistance through disc diffusion testing and for virulence factors using phenotypic testing procedures. An overall prevalence of 34/87 (39.1%) MRSA isolates was recorded. Based on the source of the isolate, the distribution of MRSA isolates from in-patients was 22/56 (39.3%) and out-patients was 12/31 (39.7%). Total Panton valentine leucocidin (PVL) screening revealed 11/34 (32.4%). All the isolates were resistant to penicillin 34/34 (100%) and cefepime 34/34 (100%). Lower level of resistance was demonstrated towards ciprofloxacin 3/34 (8.8%). Overall, every isolate displayed resistance to at least three antibiotics, resulting in a multiple antimicrobial resistance index of at least 0.33. The total virulence factors observed were beta-hemolytic activity 22/34 (64.7%), protease activity 14/34 (41.2%), and lipase activity 19/34 (55.9%). This study emphasized the significance of regularly monitoring MRSA prevalence within both the hospital and the local community. Therefore, the need for better intervention and continuous enlightenment on risks associated with the abuse of antibiotics is necessary.

Keywords: antimicrobial resistance, prevalence, virulence, patient, hospital, *Staphylococcus*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant bacterial pathogen associated with a wide range of human infections in diverse regions around the world. MRSA has the potential to induce a range of illnesses in humans, spanning from soft tissue and skin infections to serious, life-threatening invasive infections (Tong *et al*., 2015). MRSA is of immense public health concern due to the consequence of its associated virulence factors and resistance to several clinically relevant antibiotics. During the past decades, MRSA has shown remarkably rapid resistance development to multiple antibiotics. The capability of the pathogen to persist within the communities and hospitals is significant because of its capacity to take up resistance principally against methicillin, making treatment options further complicated. Methicillin resistance is associated with an alteration in the penicillin-binding protein, leading to reduced binding affinity for β-lactam antibiotics, including penicillins, carbapenems, and cephalosporins (Igbinosa *et al*., 2016a). The development of methicillin resistance is facilitated by the *mecA* gene, which is transferred through a mobile genetic element known as the staphylococcal cassette chromosome mec (SCC*mec*) (Campanile *et al*., 2012). Infections from MRSA are frequently treated with antibiotics such as teicoplanin, vancomycin, cephalexin, trimethoprim-sulfamethoxazole, clindamycin, doxycycline, linezolid, and fusidic acid based on susceptibility to the aforementioned antibiotics. Some studies have documented co-vancomycin-clindamycin-resistant MRSA (Guo *et al*., 2020) which further complicates and limits therapeutic options of MRSA strains.

Infections caused by MRSA can originate from healthcare settings (referred to as HA-MRSA), the community (known as CA-MRSA), or even from contact with livestock (referred to as LA-MRSA) (Campanile *et al*., 2012). MRSA was primarily documented in the 1970s as a hospital-acquired infection, colonizing hospitalized patients representing the principal cause of hospital infections. From 1970 to 1990, several HA-MRSA pandemics and epidemics cases transpired (Campanile *et al*., 2012). Since the 1990s, instances of invasive MRSA skin infections have been associated with individuals who were not hospitalized and did not exhibit identifiable traits of HA-MRSA strains (Qu *et al*., 2014). These were grouped as CA-MRSA. Strains of CA-MRSA are potentially pathogenic even in healthy populations. Most infections attributed to CA-MRSA predominantly affect soft tissues and the skin, with some strains producing the PVL toxin (Stefani *et al*., 2012). The primary mode of transmission is likely through the food chain and through contact with infected livestock and their surroundings (Campanile *et al*., 2012). MRSA can move into hospitals from other sources culminating in outbreaks and severe infections. Clonal characterization is crucial to determine the transmission routes and source of MRSA strains (Hadyeh *et al*., 2019).

Molecular characterization is a major tool used in the investigation and detection of circulating clones of bacteria originating from diverse sources. The primary antimicrobial resistance (AMR) gene identified in clinical *S. aureus* strains is *mecA*, which confers resistance to methicillin. MRSA variants can also contain virulent components that play important roles in Staphylococcal pathogenicity, including panton-valentine leukocidin (PVL), exfoliative toxins (*etb*, *eta*), and toxic shock syndrome toxin-1 (TST-1) (Alfatemi *et al*., 2014). The PVL genes are linked with poor prognosis and severe invasive diseases. They are also less likely to be

recovered from hospitals compared to community settings (Stefani *et al*., 2012). Nevertheless, as the epidemiology of CA-MRSA continues to change, there have been associations with outbreaks in hospitals (Lakhundi and Zhang, 2018). Instances of CA-MRSA outbreaks have been documented globally, particularly in hospital departments like obstetrics or pediatrics, where the prevalence of HA-MRSA is minimal (Nelson and Gallagher, 2012). Furthermore, CA-MRSA outbreaks in patients, patient's relatives, hospital staff, and healthcare workers have been reported (Orendi *et al*., 2010). Likewise, CA-MRSA has been documented as a growing factor in hospital-acquired infections (Moon *et al*., 2010). Thus, monitoring the dissemination of MRSA within the healthcare system is of utmost imperative. The study sought to characterize MRSA from wounds in both in-patients and outpatients at the University of Benin Teaching Hospital (UBTH) in Benin City, Nigeria, using both phenotypic and genotypic methods.

MATERIALS AND METHODS

Sample collection

Eighty-seven non-duplicated *S. aureus* isolates, comprising 56 and 31 from in-patients and outpatients, respectively, was obtained from wound cultures of individuals treated at UBTH in Benin City, Nigeria between November 2022 and May 2023. The healthcare personnel at the Medical Microbiology Laboratory Department of the UBTH collected the *S. aureus* strains from patients' wounds. These strains were then preserved on nutrient agar slants and transported to the Department of Microbiology and Biotechnology at Western Delta University for further analysis.

Inclusion and exclusion criteria

The research encompassed suspected *S. aureus* strains isolated from wound samples obtained from patients at the UBTH as of June 2023. No duplicate samples from the same patient were incorporated in this study.

Ethical considerations

Approval to carry out this study was obtained from the State Ministry of Health, Edo State, Nigeria (Reference number Ha.737/5/T1/019). The experimental procedure followed the ethical guidelines and research methods adopted by the Medical Laboratory Unit authority at the UBTH in Benin City, Nigeria. Patient consent was not required since the isolates excluded personal tags and information.

Phenotypic screening of MRSA

The *S. aureus* isolates, initially presumed from wound samples, underwent sub-culturing and was subsequently screened for methicillin resistance using standard cultural techniques upon their arrival in the laboratory. The *S. aureus* samples were introduced into 5 mL of Tryptone Soy Broth (TSB) (Merck, Darmstadt, Germany) that was enriched with 6 µg/mL of oxacillin and 4% NaCl. They were then cultured at a temperature of 37°C for a period of 24 h. Following that, a loopful of the incubated TSB was streaked onto Oxacillin Resistance Screening Agar Base (ORSAB) and allowed to incubate at 37°C for 24 h. Colonies that were observed to be distinct blue were considered to be presumptive MRSA isolates. The culture-based methicillin resistance screening was conducted as previously described (Igbinosa *et al*., 2023). The isolates that were retrieved underwent additional assessments, which included examining their Gram reaction, catalase tests, coagulase tests, indole tests, and oxidase tests. Those isolates demonstrating positive Gram reactions, positive coagulase reactions, positive catalase reactions, negative indole reactions, and negative oxidase reactions were categorized as MRSA.

Genomic DNA extraction

The genomic DNA extraction process followed the method previously outlined by Igbinosa *et al*. (2016b). Initially, colonies were grown on brain heart infusion agar (Lab M, Lancashire, UK), then transferred to 2 mL of brain heart infusion (BHI) broth (Lab M, Lancashire, UK), and agitated on a shaker at 37°C for 48 h. Subsequently, the cultures were centrifuged for 5 min at 4600 rpm, and the resulting pellets were resuspended in a mixture consisting of 15 µL of 20% Sodium Dodecyl Sulfate (SDS), 3 µL of Proteinase K (20 mg/mL), and 520 µL of Tris-EDTA (TE) buffer. After an hour-long incubation at 37°C, 80 µL of a 10% cetyltrimethylammonium bromide (CTAB) solution in 0.7 M NaCl and 100 µL of 5M NaCl were introduced and incubated at 65°C for 10 min, followed by cooling on ice for 15 min. To this mixture, an equal amount of chloroform: isoamyl alcohol was included, followed by a 5-min incubation on ice and subsequent centrifugation for 20 min at 7200 rpm. The aqueous phase was transmitted to a new tube with isopropanol included, and DNA was allowed to precipitate for 16 h at -20°C. The DNA was then recovered through centrifugation for 10 min at 7200 rpm, washed with 500 µL of 70% ethanol, air-dried at ambient temperature for 3 h, and finally dissolved in 50 µL of TE buffer. The extracted DNA was preserved at -20°C until it was used for genotypic analysis.

Confirmation of MRSA and amplification of *mecA*, *nuc* and PVL gene with polymerase chain reaction (PCR)

S. aureus isolates were verified using specific primers targeting the *nuc* gene, as previously outlined (Othman *et al*., 2014). Each reaction mixture of 25.0 µL comprised 7.5 µL of PCR H2O, 12.5 µL of PCR Master mix, 2.0 µL of both *nuc* primers, and 1.0 µL of genomic DNA (*nuc*-F: 5'-GCGA TTGA TGGT GATA CGGTT-3' and *nuc*-R: 5'-AGCC AAGC CTTG ACGA ACTA AAGC-3'), resulting in an amplicon size of 280 bp. *S. aureus* (ATCC 29213) served as the positive control, while nuclease-free water was used as the negative control. Detection of the *mecA* gene followed the procedure described by Ahmed *et al*. (2014), using a 25.0 µL PCR reaction mixture (12.5 µL of PCR Master mix, 1.0 µL of genomic DNA, and 2.0 µL each of *mecA* primers, along with 7.5 µL of PCR H_2O). The *mecA* gene was amplified with the *mecA*-F (5'-TCCA GATT ACAA CTTC ACC AGG-3') and *mecA*-R (5'-CCAC TTCA TATC TTGT AACG-3') primers, producing an amplicon size of 162 bp. Detection of the PVL gene followed the protocol described by McDonald *et al*. (2005), utilizing a 25.0 µL PCR reaction mixture (2.0 µL each of *lukF*-PV and *lukS*-PV primers, 1.0 µL of genomic DNA, 7.5 µL of PCR H_2O, and 12.5 µL of Red Taq Master mix). The primers used for amplification were *luk*-PV-1 (5'-ACAC ACTA TGGC AATA GTTA TTT-3') and *luk*-PV-2 (5'-AAAG CAAT GCAA TTGA TGTA-3'), yielding an amplicon size of 176 bp. Electrophoresis of the amplicons was conducted using a 1% agarose gel containing ethidium bromide (0.5 mg/L) for 1 h at 100 V in 0.5 × TAE buffer (20 mM Na-acetate, 40 mM Tris-HCl, pH 8.5, 1 mM EDTA), and the results were visualized under a UV transilluminator.

Phenotypic screening for AMR

The MRSA isolates underwent AMR screening using the Kirby-Bauer disc diffusion method. To do this, a suspension of the test isolates was prepared at a 0.5 McFarland standard and evenly spread on Mueller-Hinton agar plates (Lab M, Lancashire, United Kingdom). Sterile forceps were used to

aseptically place antibiotic discs on the agar plates, and the plates were then incubated at 37°C for 24 h. The antibiotics included in the testing were penicillin (10 units), clindamycin (2 µg), tetracycline (30 µg), nitrofurantoin (300 µg), cefepime (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), chloramphenicol (30 µg), and gentamicin (10 µg) (Oxoid, Hampshire, United Kingdom). The diameters of the zones of inhibition were measured and interpreted as "R" (Resistant), "I" (Intermediate), or "S" (Sensitive) based on the guidelines set by the Clinical Laboratory Standards Institute (CLSI, 2020).

Determination of multiple AMR (MAR) index

The calculation of the MAR index followed the method outlined by Beshiru *et al*. (2021). The MAR is computed as MAR = a/b, where "a" corresponds to the count of antibiotics to which the test isolate exhibited resistance, and "b" denotes the total number of antibiotics that were tested.

Phenotypic virulence factors determination of MRSA isolates

The assessment of MRSA isolates' phenotypic virulence characteristics followed the procedure previously outlined by Beshiru *et al*. (2018). In summary, hemolytic activity was assessed using sheep blood agar plates. The presence of beta hemolysis was evidenced by clear, colorless zones surrounding the colonies, signifying complete lysis of the red blood cells. Protease activity was determined on Tryptic Soy Agar (TSA) plates containing 1% casein, and a positive result was indicated by the presence of a zone of clearance due to casein hydrolysis. Lipase activity was assessed on TSA, and a positive result was identified by the presence of a clear halo surrounding the areas where the lipase-producing organism had grown on the agar.

Data analysis

The data were presented using numerical counts and percentages. Descriptive statistics were employed for data analysis, and the results were shown as percentages.

RESULTS AND DISCUSSION

Distribution of MRSA and their genomic characteristics

The 87 non-duplicate *S. aureus* isolates obtained from wound cultures of patients were screened using cultural and biochemical techniques in which 34/87 (39.1%) isolates were MRSA positive. Regarding the origin of the isolates, MRSA distribution was 22 out of 56 (39.3%) among in-patients and 12 out of 31 (39.7%) among outpatients, as indicated in Table 1. MRSA confirmation was achieved by detecting the *S. aureus*-specific gene (*nuc* gene) and the gene liable for methicillin resistance (*mecA*) through PCR. Screening for methicillin-resistant genes in the MRSA isolates revealed that all of them contained both the *nuc* and *mecA* genes within their genomic DNA. Additionally, when screening for the PVL gene in the MRSA isolates, it was found that 11 out of 34 (32.4%) isolates possessed this virulent gene. In terms of the source of the isolates, the distribution of the PVL gene among MRSA isolates was 3 out of 22 (13.6%) for in-patients and 8 out of 12 (66.7%) for out-patients, as depicted in Figure 1.

Phenotypic AMR profile of the MRSA isolates

The evaluation of the MRSA isolates for AMR showed the resistance pattern to commercially available antibiotics as shown in Table 2. It was witnessed that all MRSA isolates unveiled resistance to penicillin, with a rate of 34 out of 34 (100%), as well as cefepime, with another 100% rate of resistance among the isolates. The MRSA isolates demonstrated significant resistance towards erythromycin 28/34 (82.4%), chloramphenicol 22/34 (64.7%), nitrofurantoin 21/34 (61.8%), and tetracycline 14/34(41.2%). The lower level of resistance was demonstrated towards ciprofloxacin 3/34(8.8%) while the least resistance was demonstrated towards clindamycin 1/34(2.9%) and gentamicin 1/34 (2.9%).

The resistance profiles of the isolates based on the type of patients' care showed that isolates associated with both in-patients and outpatients were resistant to penicillin, ceftazidime, and erythromycin. The resistance patterns towards all the antibiotics except nitrofurantoin and tetracycline revealed that the isolates from outpatients demonstrated higher resistance when compared to the in-patient isolates (Table 2).

MAR index of the MRSA isolates

Analysis of the MRSA isolates' MAR index indicated that 13 out of 34 (38.2%) were resistant to a minimum of four different antibiotic classes, with a MAR index of ≥0.44. Additionally, 7 out of 34 (20.6%) of the isolates displayed resistance to ≥6 antibiotics from various antimicrobial classes, yielding a MAR index of 0.67. Looking at the resistant profiles, it was observed that all inpatient isolates showed resistance to ≥3 antibiotics, with a MAR index of ≥0.33, while all outpatient-derived isolates demonstrated resistance to at least four antibiotics, also with a MAR index of ≥0.44. In summary, the resistance profiles of all the isolates collectively revealed that they were resistant to ≥3 antibiotics, with a MAR index of ≥0.33, as detailed in Table 3.

Distribution of phenotypic virulence factors

Figure 2 illustrates the distribution of phenotypic virulence among the MRSA isolates. The virulence factors observed were Beta hemolytic activity 22/34 (64.7%), protease activity 14/34 (41.2%), and lipase activity 19/34 (55.9%). The distribution of phenotypic virulence of MRSA based on isolates source is shown in Figure 3. The distribution of phenotypic virulence from in-patient isolates was Beta hemolytic activity 13/22 (59.1%), protease activity 6/22(27.3%), and lipase activity 15/22(68.2%). The distribution of phenotypic virulence from out-patients isolates was Beta hemolytic activity 19/12 (75.0%), protease activity 8/12 (66.7%), and lipase activity 4/12 (33.3%).

Discussion

MRSA continues to pose a significant threat as a life-threatening pathogen, predominantly in the background of healthcare-linked infections. MRSA is known worldwide as a causative agent of hospital-acquired infections (Igbinosa *et al*., 2022). However, the continuous variations in MRSA epidemiology require nonstop surveillance of MRSA strains circulating in healthcare facilities for the application of preventive and suitable infection control measures. In this study, MRSA obtained from patients' wound cultures was found to account for 34 out of 87 (39.1%) based on cultural, biochemical methods, and DNA screening for the *mecA* gene. This is marginally <40% average frequency of MRSA worldwide as previously reported (Diekema *et al*., 2019). Likewise, lower rates have been documented in earlier studies conducted in various regions, including Nigeria (28.6%) (Nwakwo *et al*., 2010), Addis Ababa (13.2%) (Dilnessa and Bitew, 2016), Cameroon (13.16%) (Bissong *et al*., 2016), Ethiopia (19.6%) (Kahsay *et al*., 2014), Brazil (5.6%) (Almeida *et al*., 2014), and Libya (31%) (Buzaid *et al*., 2011). The variance in prevalence among different regions may be

due to different factors such as the type of hospital, the source of the isolates, and the patient characteristics. Consistent with this finding, previous research has indicated that the epidemiology of MRSA undergoes continuous shifts, and the traits and occurrence of MRSA are recognized to differ among hospitals and specific hospital units (Raji *et al*., 2013). In this study, when considering the origin of the isolates, MRSA distribution among inpatients accounted for 22 out of 56 (39.3%), while among outpatients, it was 12 out of 31 (39.7%). The high prevalence in this study was observed in both in-patient and outpatient indicating that MRSA remains a menace in Nigerian hospitals disseminating within and outside the hospital setting. Poor hygienic conditions and non-adherence to clinically important antibiotic policies have been put forward as possible causes for this high prevalence (Imanah *et al*., 2017). The MRSA prevalence detected in this study could be ascribed to the inappropriate use of antimicrobials, either due to cost considerations or due to their ready availability (Beshiru *et al*., 2016; Igbinosa and Beshiru, 2019). On the other hand, a higher prevalence was reported in Uganda (41%) (Ojulong *et al*., 2009). The genomic DNA analysis of the isolates revealed the presence of the *mecA* gene in all of them. This finding differs from a prior study in which only 11 out of 13 (84.6%) MRSA isolates were associated with the occurrence of the *mecA* gene, despite their methicillin resistance (Campanile *et al*., 2012). This agrees with previous reports that stated that the identification of the *mecA* gene remains the gold standard for methicillin resistance detection and the most acceptable means of MRSA identification thereby limiting the phenotypic and cultural identification techniques.

In this study, the screening for the *PVL* gene revealed that 11/34 (32.4%) isolates harbored the virulent gene. Based on the source of isolates, the distribution of *PVL* determinants in the MRSA was in-patients 3/22 (13.6%) and outpatients 8/12 (66.7%). This agrees with a previous finding that detected the *PVL* gene in MRSA isolates (Beshiru *et al*., 2021; Igbinosa *et al*., 2023). The occurrence of the PVL gene, which is a virulence factor known to target polymorphonuclear and mononuclear cells, causing cell lysis, has been consistently associated with CA-MRSA (El-Ashker *et al*., 2020). Nevertheless, the fact that the PVL determinant was found in isolates from hospitalized patients suggests that this gene is not entirely limited to community-acquired infections. This observation may be explained by the admission of numerous patients who were already carrying asymptomatic CA-MRSA into the hospital. This is due to the high possibility of these patients having faced these organisms before being admitted to the hospital. Additionally, this agrees with reports in different health facilities that community-acquired MRSA clones are attaining an increased level of prevalence in hospital settings (Lakhundi and Zhang, 2018). Our results also demonstrated that out of the 22 MRSA isolates from in-patients, 3 (13.6%) were positive for the PVL toxin gene. This observation underscores the growing concern about community-acquired MRSA strains infiltrating healthcare settings. The association of the PVL toxin gene with MRSA clones could potentially render these strains highly virulent, occasionally leading to severe and even fatal infections (Alfatemi *et al*., 2014).

It was noted in this study that MRSA isolates exhibited the highest level of resistance to penicillin, with all 34 out of 34 (100%) isolates showing resistance, as well as to cefepime, with another 100% resistance rate among the isolates. The isolates also demonstrated high resistance towards erythromycin 28/34 (82.4%), chloramphenicol 22/34 (64.7%), nitrofurantoin 21/34 (61.8%), and tetracycline 14/34 (41.2%). This aligns with the results presented by Wolters *et al*. (2020), who also reported a notable resistance rate of 100% to penicillin and 57% to tetracycline in wound isolates. Additionally, other similar studies have documented substantial resistance to antibiotics like tetracycline and penicillin in *S. aureus* (Beshiru *et al*., 2016; Igbinosa and Beshiru, 2019). High penicillin resistance may be attributed to the frequent prescription of penicillin-based antibiotics in the region, as well as their over-the-counter availability without a prescription in Nigeria (Imanah *et al*., 2017; Beshiru *et al*., 2023; Igbinosa *et al*., 2023).

Elevated levels of resistance substantially contribute to therapeutic challenges by diminishing the effectiveness of antibiotics. In our study, reduced resistance was demonstrated towards ciprofloxacin 3/34 (8.8%), clindamycin 1/34 (2.9%), and gentamicin 1/34 (2.9%). This agrees with previous studies that reported high sensitivity patterns demonstrated by gentamicin and clindamycin (Tsige *et al*., 2020). Significant gentamicin susceptibility has been reported in various Sub-Saharan African nations, as evidenced by studies conducted (Beshiru *et al*., 2016; Dilnessa and Bitew, 2016; Beshiru *et al*., 2021). Contrarily, another study has reported considerably high resistance to gentamicin (Shibabaw *et al*., 2014). The differences in resistance levels may be connected to variations in prescription practices of antibiotics across the diverse regions where these studies were carried out (Igbinosa and Beshiru, 2017). Drug resistance disparities from different localities also show that resistance to antibiotics varies based on geographical and regional locations (Igbinosa *et al*., 2020). The resistant profiles of the isolates based on the type of patients' care revealed that the resistant patterns of all the antibiotics except nitrofurantoin and tetracycline demonstrated higher resistance in outpatient isolates when compared to the in-patient isolates. In agreement with this study, penicillins demonstrated higher AMR in isolate from outpatient isolates when compared with in-patients (Akhavizadegan *et al*., 2021). This further agrees with a previous report that self-medication and indiscriminate ingestion of antibiotics are more prevalent in non-hospitalized patients than those on admission to health institutions, hence, they are more likely to abuse antibiotic usage (Uyaguari *et al*., 2018; Beshiru *et al*., 2022). This is because antibiotics ingestion by hospitalized patients is routinely monitored and they are strictly based on prescription by professional personnel. Moreover, all MRSA isolates in our study exhibited MDR, as they demonstrated resistance to at least three different classes of antibiotics with a MAR index of ≥0.33. This discovery is consistent with a prior study where all MRSA strains isolated displayed resistance to ≥3 antibiotics (Kahsay *et al*., 2014).

The elevated MAR index observed in both in-patient and out-patient isolates may result from the dissemination of resistance factors in healthcare facilities and communities. This situation poses a significant public health risk, as a MAR index exceeding 0.2 is associated with high-risk environments characterized by excessive antibiotic misuse (Uyaguari *et al*., 2018; Igbinosa *et al*., 2021). The increased prevalence of MRSA isolates is linked to inappropriate antibiotic use, self-medication, and inadequate infection control measures (Beshiru *et al*., 2021; Igbinosa *et al*., 2023). As observed in this study, the phenotypic virulence distribution of the MRSA isolates included beta-hemolytic activity in 22 out of 34 (64.7%) cases, protease activity in 14 out of 34 (41.2%) cases, and lipase activity in 19 out of 34 (55.9%) cases. Bacterial virulence factors portray public health concern, as reports have shown a substantiated positive relationship between pathogenicity and virulence factors (Beshiru *et al*., 2018).

Similarly, prior research (Mukherji *et al*., 2014) confirmed that gelatinase boosts a microorganism's capacity to breach the cell membrane of potential host cells, resulting in the development of a disease. The high prevalence of multidrug resistance and linkage with virulence potentials are factors that may predispose patients to high-risk infections.

CONCLUSION

The significant influence of MRSA on the treatment of staphylococcal infections and its capacity to host antimicrobial resistance genes enhance its pathogenicity. Our study emphasizes the importance of ongoing surveillance for MRSA strains in both healthcare facilities and the general population. The genetic and phenotypic traits observed in the MRSA isolates suggest the presence of MRSA variants that could potentially lead to infections that are more burdensome. Enhancing healthcare practices and implementing advanced infection control measures are essential for managing and preventing healthcare-associated MRSA infections. Hence, it is essential to implement well-structured surveillance and intervention plans, limit the use of antimicrobials, and provide ongoing education about the risks associated with antibiotic misuse to prevent the dissemination of MRSA-related infections.

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CONFLICT OF INTEREST

The authors disclose that they have no conflicts of interest.

Table 1: Prevalence of MRSA isolates based on cultural and biochemical identification

Isolate Sources	Number of *S. aureus* screened for methicillin resistance	Occurrence of MRSA isolates
In-patients	56	22/56 (39.3%)
Out-patients	31	12/31 (39.7%)
Total	**87**	**34/87 (39.1%)**

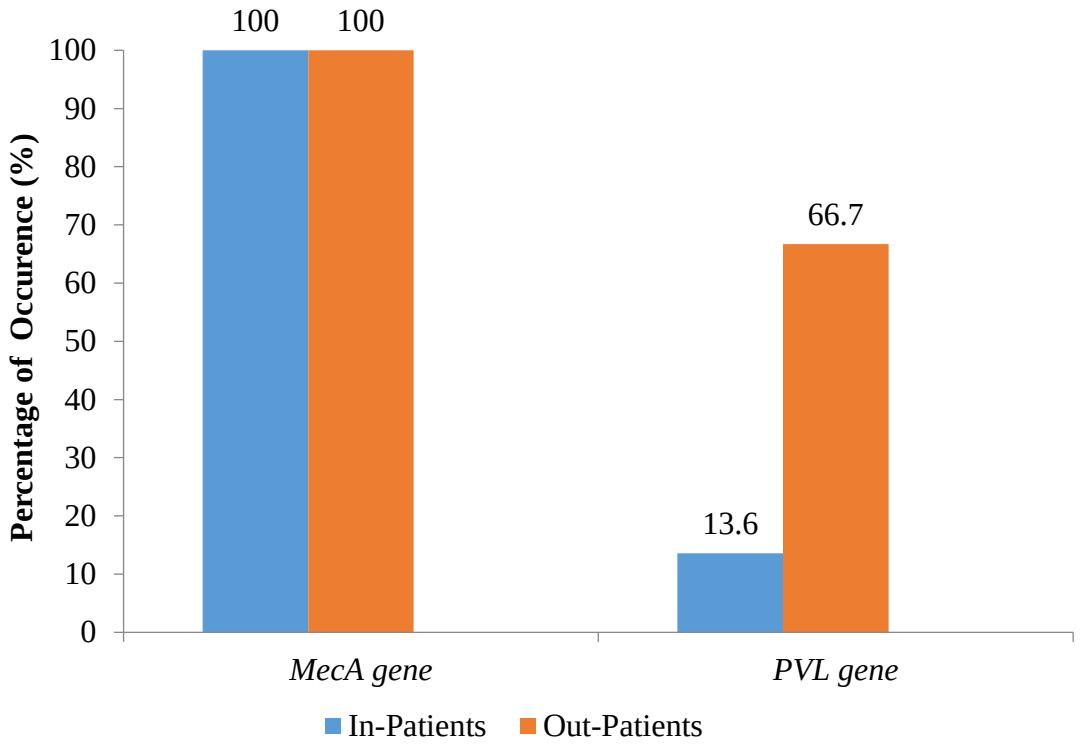

Figure 1: Distribution of *mecA* and *PVL* genes in the MRSA isolates

Table 2: AMR profiles of the MRSA isolates

Antimicrobial Class	Antibiotics	In-patients (*n*=22)			Out-patients (*n*=12)			Total isolates (*n*=34)		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Penicillins	PEN	22 (100)	-	0 (0)	22 (100)	-	0 (0)	34 (100)	-	0 (0)
Lincosamide	CLI	1 (4.5)	0 (0)	21 (95.5)	0 (0)	1 (8.3)	11 (91.7)	1 (2.9)	2 (5.9)	31 (91.2)
Tetracyclines	TET	10 (45.5)	5 (22.7)	7 (31.8)	5 (41.7)	3 (25)	4 (33.3)	14 (41.2)	6 (17.7)	14 (41.2)
Nitrofurans	NIT	16 (72.7)	2 (9.1)	4 (18.2)	5 (41.7)	1 (8.3)	6 (50)	21 (61.8)	3 (8.8)	10 (29.4)
Cephalosporins	CEF	22 (100)	-	0 (0)	12 (100)	-	0 (0)	34 (100)	-	0 (0)
Fluoroquinolone	CIP	1 (4.5)	0 (0)	21 (95.5)	2 (16.7)	1 (8.3)	9 (75)	3 (8.8)	1 (2.9)	30 (88.2)
Phenicols	CHL	13 (59.1)	2 (9.1)	7 (31.8)	9 (75)	1 (8.3)	2 (16.7)	22 (64.7)	3 (8.8)	9 (26.5)
Macrolides	ERY	17 (77.3)	0 (0)	5 (22.7)	11 (91.7)	1 (8.3)	0 (0)	28 (82.4)	1 (2.9)	5 (14.7)
Aminoglycosides	GEN	0 (0)	0 (0)	22 (100)	1 (8.3)	0 (0)	11 (91.7)	1 (2.9)	0 (0)	33 (97.1)

Legend: TET: tetracycline (30 µg), PEN: penicillin (10 units), CLI: clindamycin (2 µg), NIT: nitrofurantoin (300 µg), CEF: cefepime (30 µg), CIP: ciprofloxacin (5 µg), CHL: chloramphenicol (30 µg), ERY: erythromycin (15 µg) and GEN: gentamicin (10 µg).

Table 3: MAR index of the MRSA isolates

Isolates	Number of antibiotics	Resistant phenotypes	Number of resistant isolates (%)	MAR Indexes
In-patient isolates (*n*=22)	6	PEN^R, TET^R, NIT^R, CEP^R, CHL^R, ERY^R	3 (13.7)	0.67
	6	PEN^R, NIT^R, CEP^R, CIP^R, CHL^R, ERY^R	1 (4.5)	0.67
	5	PEN^R, CLI^R, NIT^R, CEP^R, ERY^R	1 (4.5)	0.56
	5	PEN^R, TET^R, NIT^R, CEP^R, ERY^R	5 (22.7)	0.56
	5	PEN^R, TET^R, CEP^R, CHL^R, ERY^R	1 (4.5)	0.56
	5	PEN^R, NIT^R, CEP^R, CHL^R, ERY^R	3 (13.7)	0.56
	4	PEN^R, NIT^R, CEP^R, CHL^R	1 (4.5)	0.44
	4	PEN^R, NIT^R, CEP^R, ERY^R	3 (13.7)	0.44
	3	PEN^R, TET^R, CEP^R	1 (4.5)	0.33
	3	PEN^R, NIT^R, CEP^R	2 (9.1)	0.33
	3	PEN^R, CEP^R, CHL^R	1 (4.5)	0.33
Out-patient isolates (*n*=12)	6	PEN^R, TET^R, NIT^R, CEP^R, CHL^R, ERY^R	1 (8.3)	0.67
	6	PEN^R, TET^R, CEP^R, CIP^R, CHL^R, ERY^R	1 (8.3)	0.67
	6	PEN^R, NIT^R, CEP^R, CIP^R, CHL^R, ERY^R	1 (8.3)	0.67
	5	PEN^R, TET^R, CEP^R, CHL^R, ERY^R	3 (25.0)	0.56
	5	PEN^R, NIT^R, CEP^R, CHL^R, ERY^R	1 (8.3)	0.56
	4	PEN^R, TET^R, CEP^R, ERY^R	1 (8.3)	0.44
	4	PEN^R, NIT^R, CEP^R, ERY^R	1 (8.3)	0.44
	4	PEN^R, CEP^R, CHL^R, ERY^R	3 (25.0)	0.44

Legend: TET: tetracycline (30 µg), PEN: penicillin (10 units), CLI: clindamycin (2 µg), NIT: nitrofurantoin (300 µg), CEF: cefepime (30 µg), CIP: ciprofloxacin (5 µg), CHL: chloramphenicol (30 µg), ERY: erythromycin (15 µg) and GEN: gentamicin (10 µg)

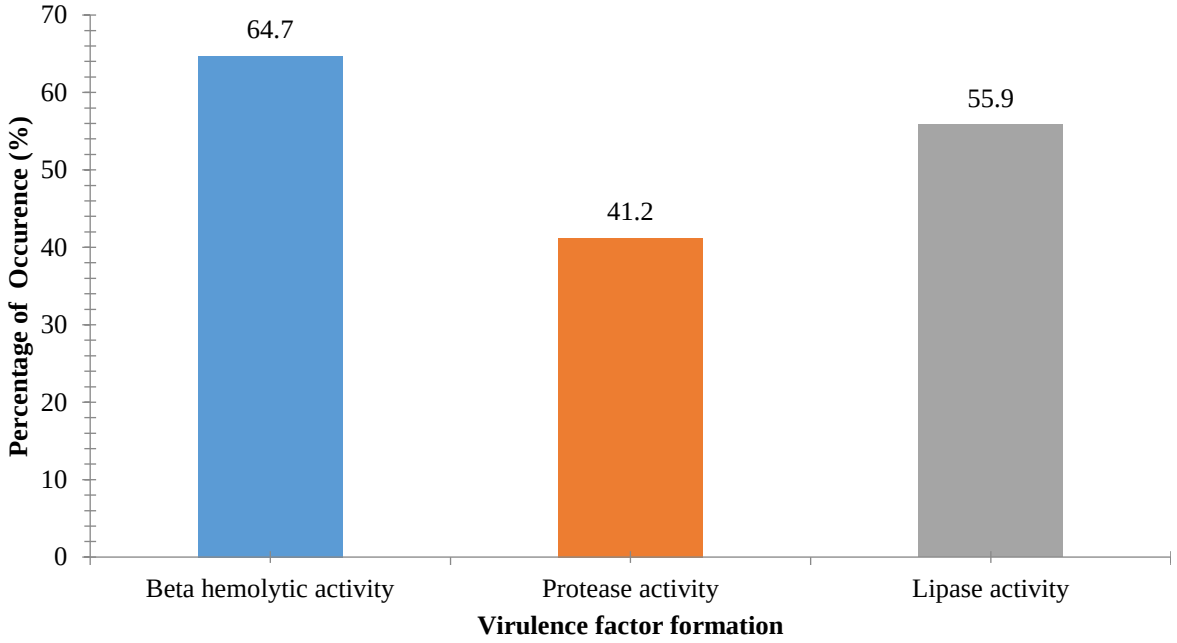

Figure 2: Phenotypic virulence traits among the MRSA isolates

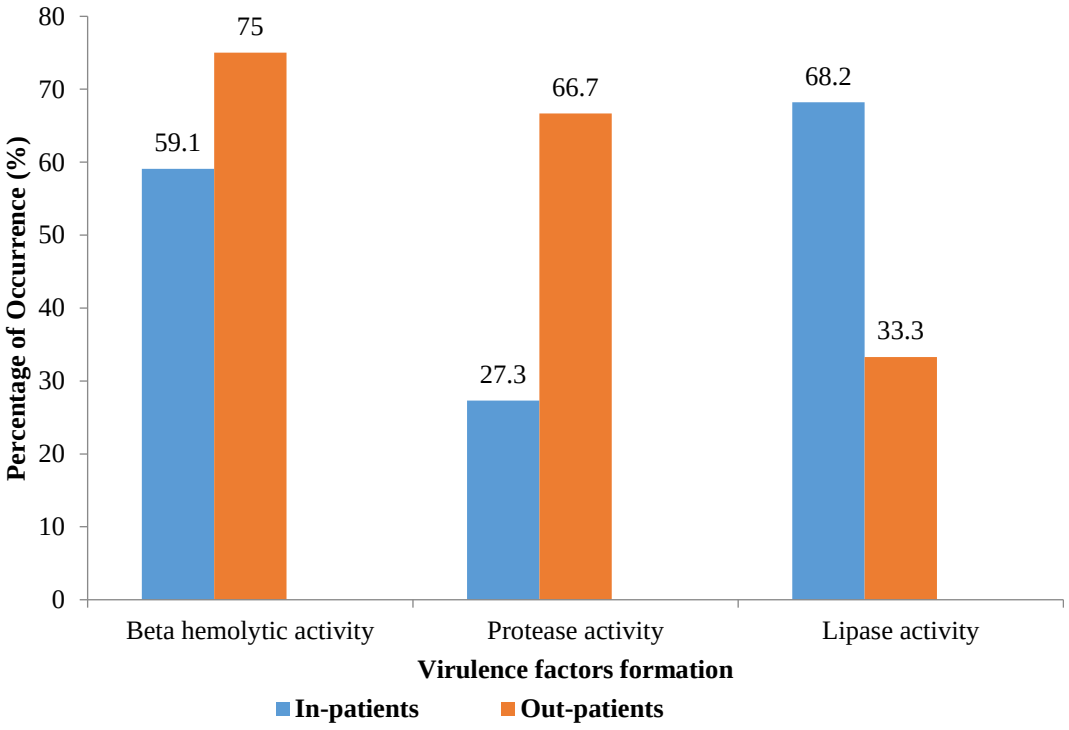

Figure 3: Distribution of phenotypic virulence characteristics of MRSA categorized by the source of isolates

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