First Report of the Emergence of \textit{mecC} Gene and CC8/ST239 Tigecycline-Resistant \textit{Staphylococcus aureus} Clonal Lineage Isolated from Chronic Suppurative Otitis Media

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Abstract

**Background:** \textit{Staphylococcus aureus} is one of the most significant bacteria involved in ear infections. However, insights into the molecular attributes of \textit{S. aureus} collected from patients with chronic otitis media have yet to be reported in Iran.

**Objectives:** The objective of this study was to assess the molecular characteristics of \textit{S. aureus} isolated from patients with chronic otitis media.

**Methods:** A total of 55 \textit{S. aureus} strains retrieved from patients with chronic otitis media were analyzed by the disk diffusion method and polymerase chain reaction (PCR) to identify the \textit{nucA} gene. Isolates were genetically classified using the coagulase typing method. \textit{S. aureus} protein A (\textit{spa}) typing, staphylococcal cassette chromosome \textit{mec} (\textit{SCCmec}) typing, and multilocus sequence typing (MLST) were performed on isolates with resistance to specific antibiotics.

**Results:** Overall, out of 55 \textit{S. aureus} isolates, resistance to mupirocin, fusidic acid, and tigecycline was identified in 12.7%, 5.4%, and 3.6% of isolates, respectively. Fusidic acid-resistant isolates belonged to ST5-\textit{SCCmec}II/t002/\textit{coaII}. Two tigecycline-resistant isolates belonged to CC8/ST239-\textit{SCCmec}III/t234/\textit{coaVIII}. One positive \textit{mecC} isolate belonged to the CC/ST30-\textit{SCCmec}XI/t843/\textit{coaIII} clone. Isolates with the iMLSB phenotype belonged to CC/ST30-\textit{SCCmec}IV/t044/\textit{coaII} (4 isolates), CC8/ST239-\textit{SCCmec}III/t388/\textit{coaVI} (3 isolates), and CC8/ST8-\textit{SCCmec}IV/t008/\textit{coaIII} (1 isolate).

**Conclusions:** Our results indicated that \textit{S. aureus} isolated from patients with chronic otitis media possesses a unique molecular profile with a high percentage of resistance to multiple medications. These findings suggest that resuming the molecular analysis to improve the control and prevention of ear infections related to \textit{S. aureus} is necessary.

**Keywords:** \textit{Staphylococcus aureus}, Otitis Media, Methicillin-Resistant \textit{Staphylococcus aureus}, Multilocus sequence Typing, Multidrug Resistance

1. Background

Chronic suppurative otitis media (CSOM) is characterized by middle ear effusion without symptoms of acute inflammation. According to the evidence, a high incidence of CSOM was reported in the developing countries. The CSOM is mainly caused by bacterial middle ear infection (1). Some studies indicated \textit{Pseudomonas aeruginosa} as the prevalent cause of CSOM, while other researchers displayed \textit{Staphylococcus aureus} as the most important bacteria. In recent decades, simultaneous resistance to multiple drugs in \textit{S. aureus} isolated from CSOM has become a severe threat to global health (2, 3). As methicillin use increases, methicillin-resistant \textit{S. aureus} (MRSA) is increasingly reported. Methicillin resistance is mediated by \textit{mecA} and much less by \textit{mecC} (4). Up to now, several molecular typing methods have been employed for genotyping \textit{S. aureus} strains, including pulsed-field gel electrophoresis, staphylococcal cassette chromosome \textit{mec} (\textit{SCCmec}) typing, \textit{agr} typing, protein A gene (\textit{spa}) typing, multilocus sequence typing (MLST), and coagulase gene (\textit{coa}) typing (4, 5). The \textit{coa} typing is a multiplex polymerase chain reaction (PCR)-based method that is cost-effective,
rapid, easily interpretable, and appropriate for identifying the genetic relationships among S. aureus isolates (5). Only a few studies are available worldwide addressing the genotyping of S. aureus isolated from ear infections (3). In Iran, several studies have focused on the occurrence and phenotypic characteristics of S. aureus isolated from ear infections, but little data has been published on the genetic variability of these isolates (6-8).

2. Objectives

The current research was designed to evaluate the antimicrobial resistance profile and molecular characteristics of S. aureus isolates for CSOM based on coa gene polymorphism analysis.

3. Methods

3.1. S. aureus Identification

This research was carried out during January 2020 to December 2022 in a teaching hospital of Shahid Beheshti University of Medical Sciences, Iran. Informed consent was received from all participants. Purulent discharge was collected from the middle ear via a sterile swab. The patients had not taken any antibiotics for three weeks prior to the visit and had no history of hospitalization. The collected purulent swabs were immediately cultured on blood agar (HiMedia, Mumbai, India) and preliminarily recognized as S. aureus by routine techniques. All phenotypically confirmed S. aureus isolates underwent PCR for the nuc gene detection and final confirmation (9).

3.2. Antibiotic Sensitivity Test

Susceptibility to ten antibiotic disks (Oxoid Ltd, Basingstoke, Hampshire, UK), including gentamicin (GEN), erythromycin (ERY), fusidic acid (FUS), ciprofloxacin (CIP), rifampin (RIF), penicillin (PEN), clindamycin (CLIN), tetracycline (TET), and linezolid (LIN) was carried out by the Kirby-Bauer method. The results were interpreted following the Clinical and Laboratory Standards Institute (CLSI) guideline. The cefoxitin (30 µg) disc diffusion test on Mueller Hinton agar plates was performed for all isolates of S. aureus to screen MRSA isolates. Each run was performed with the reference strains of S. aureus ATCC 25923, ATCC 43300, and ATCC 29213 as control strains. Broth microdilution was used to confirm resistance to vancomycin (VAN), tigecycline (TIG), and mupirocin (MUP) [low-level (LLMUPR) and high-level (HLMUPR) mupirocin resistance] (Sigma-Aldrich, St. Louis, Mo) following the CLSI criteria. D-zone was examined to identify inducible clindamycin resistance phenotype (iMLSg; inducible macrolide-lincosamide-streptogramin B). Multidrug resistance (MDR) in S. aureus strains was defined as resistance to 3 ≥ classes of antibacterial agents, as explained earlier (10, 11).

3.3. Detection of Resistance and Toxin-encoding Determinants

After the genomic DNA extraction using the phenol-chloroform technique, isolates were screened for the existence of the toxin genes, including pvI, eta, etb, and tst, by PCR (10, 12). The mupA, fusA, mecC, and mecA genes were detected by PCR as described elsewhere (4).

3.4. Molecular Typing

A multiplex PCR-based method with four sets (A-D) was used to analyze the coa types (I-X) with specific primers and PCR conditions introduced by Hirose et al. (5). All isolates were characterized by coa typing while S. aureus protein A (spa) typing, staphylococcal cassette chromosome mec (SCCmec) typing, and multilocus sequence typing (MLST) was performed on the mecC-positive, fusidic acid, tigecycline, and mupirocin-resistant isolates according to the same conditions published earlier (11).

4. Results

The current research investigated 55 S. aureus strains obtained from CSOM cases. Of all participants, 21 (38.2%) were male and 34 (61.8%) were female, and the mean age was 28 years (range: 12 - 58 years). All the isolates were confirmed as MRSA. According to the disk diffusion test, the highest levels of resistance were recorded for penicillin (100%) and tetracycline (80%), followed by gentamicin (69.1%), ciprofloxacin (54.5%), erythromycin (50.1%), clindamycin (36.4%), and rifampin (25.5%). All tested strains showed susceptibility to vancomycin and linezolid. In our study, 13 resistance patterns were detected, wherein PEN, GEN, TET, ERY, CLI, CIP, RIF (18.2%; 10/55), PEN, GEN, ERY, TET, CLI (14.5%; 8/55), and PEN, GEN, TET (12.7%; 7/55) were the top three frequently identified profiles. Broth dilution test indicated that 12.7%, 5.4%, and 3.6% of isolates were resistant to mupirocin, fusidic acid, and tigecycline, respectively. Among mupirocin-resistant isolates, HLMUPR and LLMUPR phenotypes were detected at 3.6% and 9.1%, respectively. The susceptibility test showed that 8 isolates (14.5%) were confirmed as iMLSg phenotypes. Moreover, among the tested isolates, 27.3% (15/55) were toxigenic. The pvI gene (18.2%) was recovered the most, followed by the tst gene (9.1%), and according to the multiplex PCR test for coa typing of 55 tested isolates, type III had the highest prevalence, representing 36.4%,
followed by types IVb (18.2%), VIII (14.5%), II (10.9%), X (9.1%), VI (7.3%), and I (3.6%) (Table 1). Two strains of HLMUPR-MRSA were found to harbor the mupA gene and belonged to the CC8/ST239-SCCmecII/t037 lineage. The LLMUPR-MRSA strains belonged to the CC/ST22-SCCmecIV/t790/coaII (60%; 3/5) and ST15-SCCmec IV/t084/coaII (40%; 2/5) clones. All 3 fusidic acid-resistant MRSA isolates exhibited fusidic acid MIC values of ≥ 64 µg/mL, carried the fusA gene, and belonged to the ST5-SCCmec II/t002/coaII clone. Analysis of 2 tigecycline-resistant isolates indicated that they belonged to the CC8/ST239-SCCmecII/t234/coaVII clone. Present data displayed that one isolate belonging to CC/ST130-SCCmecXII/t843/coaII (4 isolates), CC8/ST239-SCCmecIII/t388/coaVI (3 isolates), and CC8/ST8-SCCmecIV/t008/coaIII (1 isolate) clones.

5. Discussion

The present study revealed that the incidence of fusidic acid resistance was 5.4%, harboring fusA, and belonged to the ST5-SCCmec II/t002/coaII clone. This percentage is lower than the reported rates in other countries, such as Ireland (19.9%) (13), Kuwait (9.3%), and Germany (10.3%) (13, 14). In a meta-analysis in 2021, the low prevalence rate of fusidic acid resistance was noted in S. aureus isolates (0.5%). The incidence of fusidic acid-resistant S. aureus isolates was different in the earlier investigations performed in Iran by Zamani et al. (8.3%) (9), Goudarzi et al. (2.5%) (15), Rahimi et al. (3%) (16), and Hasani et al. (3.7%) (17). The higher prevalence rate of fusidic acid resistance in the present study compared to the earlier reports in Iran may be related to the unrestricted prescription of fusidic acid, use of this antibiotic during the initial treatment without susceptibility testing, diverse attitudes towards antimicrobial protocols, and the circulation of fusidic acid-resistant types within the hospitals. Similar to our findings, Chen et al. in Taiwan found that the most prevalent type of fusidic acid-resistant S. aureus strains was found to be ST5-SCCmecII/t002 (29%) and ST239-SCCmecII/t037 (62%) (18). The same research by den Heijer et al. reported the presence of t002 and t005 types carrying fusA in fusidic acid-resistant S. aureus strains from nine European countries (19). It can be concluded that the common lineages of S. aureus strains resistant to fusidic acid may be circulating from country to country. Therefore, a molecular epidemiological map of these isolates should be supervised worldwide.

Resistance to mupirocin among MRSA strains is increasing and is now recognized as a worldwide problem. The results of this research demonstrated the incidence rate of mupirocin resistance in 12.7% of MRSA isolates (HLMUPR 3.6%; LLMUPR 9.1%). The prevalence rate of mupirocin-resistant MRSA varied in different countries, such as 27.8% in South Africa, 12.1% in Canada, 31.3% in the USA, 45.5% in Turkey, and 39.6% in Iran (20). The prevalence of HLMUPR-MRSA in this study was found to be greater than that reported in France (0.8%), Canada (4.3%), and China (7%) (21-23). The present research corroborates the findings of Goudarzi et al. who reported that HLMUPR S. aureus strains belonged to ST8-SCCmecIV (27.4%), ST5-SCCmecIV (9.8%), and ST239-SCCmecIII (7.8%), while the ST22-SCCmecIV/t790 (21.6%), ST239-SCCmecIII/t860 (17.7%), and ST15-SCCmecIV/t084 (15.7%) clones were linked to the LLMUPR phenotype (10). The ST239-SCCmec III clone was also reported in HLMUPR S. aureus strains reported from India and Kuwait (24, 25).

The present study revealed an occurrence rate of mecC in 1.8% of MRSA isolates. In line with findings from previous research, this gene has been detected in S. aureus in Pakistan (26), Austria (27), Slovenia (28), and Switzerland (29). Similar to our findings, CC/ST130-SCCmecXII/t843 carrying the mecC gene was also reported earlier in the UK and Denmark as the most prevalent carrying mecC clone among clinical strains (30). In addition, Dermota et al. in Slovenia reported a prevalence of 1.5% for this gene in MRSA isolates possessing the mecC gene belonging to CC/ST130 (28). In our earlier research, CC/ST599 was reported as a mecC-positive S. aureus isolate (4).

In this study, we found two tigecycline-resistant isolates (3.6%). Different findings were reported in Malaysia (5.5%) (31), Libya (3.6%) (32), and Iran (6.6%) (33). Furthermore, some studies have documented the presence of mecC in MRSA strains recovered from Taiwan, Germany, China, Italy, Canada, France, Nigeria, and Poland (34). It can be inferred that inadequate governance of antibiotic administration strategies, improper policies, and extensive use of antibiotics, which likely increase the chance of genetic variations and acquisition of tigecycline-resistance genes, may be different causes for the emergence of tigecycline-resistant MRSA isolates. For all these reasons, the high prevalence and genetic variability of tigecycline-resistant MRSA isolates might also pose a severe risk to public health, suggesting the need for further attention to the detection and genetic diversity of these isolates. Our results indicated that all MRSA isolates resistant to tigecycline belonged to the CC8/ST239-SCCmecII/t234/coaVIII clone. In a similar study in Brazil, Dabul et al. identified S. aureus strains resistant to tigecycline belonging to the ST105-SCCmecI clone (35). Nonetheless, CC8/ST239 clone resistance to tigecycline has been reported in S. aureus from Switzerland, Spain, UK, Kuwait, Japan, Australia, and China (34).
Table 1. Characteristics of the 55 MRSA Strains Obtained from CSOM Cases

<table>
<thead>
<tr>
<th>coa Type</th>
<th>Toxin Genes, No. (%)</th>
<th>Antibiotic Resistance Profile (No; %)</th>
<th>Total, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>PEN, TET, CIP (1; 50) PEN, GEN, ERY, TET, CIP (1; 50)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>II</td>
<td>pvl (3; 37.5)</td>
<td>PEN, GEN, ERY, TET, CLI (2; 33.3) PEN, GEN, TET, FUS (2; 33.3) PEN, GEN, TET (1; 16.7) PEN, TET, FUS (1; 16.7)</td>
<td>6 (10.9)</td>
</tr>
<tr>
<td>III</td>
<td>pvl (5; 25), tst (2; 10) PEN, GEN, ERY, TET, CLI, RIF, CIP (6; 10) PEN, GEN, ERY, TET, CLI (3; 15) PEN, GEN, ERY, CIP, MUP (3; 15) PEN, GEN, TET, MUP (2; 10) PEN, GEN, ERY, CLI, Rif, CIP, MUP (2; 10) PEN, TET, CIP (4; 20)</td>
<td>20 (36.4)</td>
<td></td>
</tr>
<tr>
<td>IVb</td>
<td>tst (2; 20)</td>
<td>PEN, GEN, ERY, TET, CIP (2; 20) PEN, TET, CIP (4; 40) PEN, GEN, TET (2; 20)</td>
<td>10 (18.2)</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td>PEN (4; 100)</td>
<td>4 (7.3)</td>
</tr>
<tr>
<td>VIII</td>
<td>tst (1; 12.5)</td>
<td>PEN, GEN, TIG, RIF (1; 12.5) PEN, TET, TIC, RIF (1; 12.5) PEN (1; 12.5) PEN, GEN, ERY, TET, CLI, RIF, CIP (2; 25) PEN, GEN, ERY, TET, CLI (2; 25) PEN, GEN, TET (1; 12.5)</td>
<td>8 (14.5)</td>
</tr>
<tr>
<td>X</td>
<td>pvl (2; 40)</td>
<td>PEN, TET, CIP (1; 20) PEN, GEN, TET (1; 20) PEN, GEN, ERY, TET, CLI (1; 20) PEN, GEN, ERY, TET, CLI, Rif, CIP (2; 40)</td>
<td>5 (9.1)</td>
</tr>
</tbody>
</table>

This study found a low to moderate prevalence of isolates with iMLSB phenotype (14.5%). Different rates have also been reported from S. aureus isolates from Iran (10.9%) (H), Jordan (76.7%) (36), Nepal (21%) (37), and Brazil (7.9%) (38), which suggested that it might be a remarkable phenomenon influenced by the excessive usage of macrolides, regional locations of the study population, infection prevention protocols in healthcare facilities, and the prior history of antibiotic usage in patients. In the present study, the iMLSB phenotype (14.5%) belonged to the CC/ST80-SCCmecIV/t044 (4 isolates), CC8/ST239-SCCmecIII/388 (3 isolates), and CC8/ST8-SCCmecIV/1008 (1 isolate) clones. The CC8 clone is described to be a prevalent iMLSB phenotype MRSA in Iran (H). In addition, Goudarzi et al. reported that isolates with iMLSB phenotype were observed in CC88/ST239 (13.3%), CC/ST22 (4%), and CC/ST30 (4%) clonal lineages (12).

5.1. Conclusions

Our study on the molecular characterization of S. aureus obtained from CSOM cases indicates the occurrence of MDR S. aureus, which significantly limits the availability of effective antimicrobial treatments. These findings confirmed the dissemination of specific clonal lineages in mecc-positive, inducible, mupirocin- and tigecycline-resistant S. aureus strains. Further investigation into these emerging clones would improve understanding of the molecular epidemiological map and their resistance profile trends. Future studies that
monitor the genetic diversity of lineages and their prevalence among similar populations are required.

Footnotes

Authors’ Contribution: CH. A. conceived and designed the study and drafted the manuscript. M. G. participated in designing the study, performed parts of the statistical analysis, and helped to draft the manuscript. Z. R. re-evaluated the clinical data, revised the manuscript, performed the statistical analysis, and revised the manuscript. P. B. collected and interpreted the clinical data and revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interests: The authors have no conflict of interest.

Data Reproducibility: The dataset presented in this study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to maintaining confidentiality.

Ethical Approval: This study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences in Tehran, Iran, under the ethical code of IR.SBMU.MSP.REC.1401.366.

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Authors' Contribution:


