

Whole Genome Sequence Analysis of Two Oxacillin-Resistant and *mecA*-Positive Strains of *Staphylococcus haemolyticus* Isolated from Ear Swab Samples of Patients with Otitis Media

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Objective: *Staphylococcus haemolyticus* can cause a series of infections including otitis media (OM), and the oxacillin-resistant *S. haemolyticus* has become a serious health concern. This study aimed to investigate the genomic characteristics of two strains of oxacillin-resistant and *mecA*-positive *S. haemolyticus* isolated from the samples of ear swabs from patients with OM and explore their acquired antibiotic resistance genes (ARGs) and the mobile genetic elements (MGEs).

Methods: Two oxacillin-resistant *S. haemolyticus* strains, isolated from ear swab samples of patients with OM, underwent antimicrobial susceptibility evaluation, followed by whole-genome sequencing. The acquired ARGs and the MGEs carried by the ARGs, harbored by the genomes of two strains of *S. haemolyticus* were identified.

Results: The two strains of oxacillin-resistant *S. haemolyticus* (strain SH1275 and strain SH9361) both carried the genetic contexts of *mecA* with high similarity with the SCC*mec* type V(5C2&5) subtype c. Surprisingly, the chromosomal aminoglycoside resistance gene *aac(6')-aph(2'')* harbored by *S. haemolyticus* strain SH936 was flanked by two copies of IS256, forming the IS256-element (IS256-GNAT-[*aac(6')-aph(2'')*]-IS256), which was widely present in strains of both *Staphylococcus* and *Enterococcus* genus. Furthermore, the two strains of oxacillin-resistant and MDR *S. haemolyticus* were found to harbor antimicrobial resistance plasmids, including one 26.9-kb plasmid (pSH1275-2) containing *msr(A)-mph(C)* and *qacA*, one mobilizable plasmid pSH1275-3 harboring *vga(A)_{LC}*, one plasmid (pSH9361-1) carrying *erm(C)*, and one plasmid (pSH9361-2) carrying *qacJ*.

Conclusion: The systematic analysis of whole-genome sequences provided insights into the mobile genetic elements responsible for multi-drug resistance in these two strains of oxacillin-resistant and *mecA*-positive *S. haemolyticus*, which will assist clinicians in devising precise, personalized, and clinical therapeutic strategies for treating otitis media caused by multi-drug resistant *S. haemolyticus*.

Keywords: *Staphylococcus haemolyticus*, ear swabs, *mecA*, antimicrobial resistance plasmid, whole genome sequence

Introduction

In hospitals, opportunistic infections caused by coagulase-negative staphylococci (CoNS) have attracted considerable attention, especially among neonates, elderly patients, and immunocompromised patients.^{1,2} Among all CoNS, *Staphylococcus haemolyticus* is the second most frequently isolated CoNS in clinical cases (after *Staphylococcus*

epidermidis),³ and *S. haemolyticus* has been considered as the major species of *Staphylococcus* in nosocomial foreign device-related infections.⁴ In addition, *S. haemolyticus* can cause skin or soft tissue infections, bacteremia, septicemia, peritonitis, otitis media (OM), meningitis, and urinary tract infections.⁵ Furthermore, *S. haemolyticus* plays a crucial role in the nosocomial infections caused by multidrug-resistant (MDR) staphylococci,⁶ which result in limited therapeutic options.⁷ Infections caused by oxacillin-resistant staphylococci present a major therapeutic challenge to the health of hospitalized patients.⁸ In staphylococci, methicillin resistance is mainly due to the expression of the *mecA* gene, which encodes penicillin binding protein 2a (PBP2a), a transpeptidase with a low affinity for β -lactams.⁹

S. haemolyticus can be a reservoir for antibiotic resistance genes that are shared with other staphylococci, including *S. aureus*.⁶ Most of the antibiotic resistance genes (ARGs) were thought to be spread via mobile genetic elements (MGEs) across different staphylococci through horizontal gene transfer.¹⁰ The *mecA* gene is carried by an MGE termed the staphylococcal cassette chromosome *mec* (SCC*mec*). To date, 14 types of SCC*mec* have been identified worldwide,¹¹ of which SCC*mec* type V is the most prevalent in methicillin-resistant *S. haemolyticus*.¹² Notably, a wide variety of plasmid-borne genes that mediate resistance to antimicrobial agents have been identified in staphylococci of human and animal origin.¹³ These plasmids have been shown to play a key role as carriers of plasmid-borne resistance genes, as well as being vectors for the dissemination of resistance genes by horizontal gene transfer between members of the same species and between bacteria of different species and genera.¹⁴

In this study, we report two strains of oxacillin-resistant and *mecA*-harboring *S. haemolyticus* isolated from ear swab samples of patients with OM. The antimicrobial susceptibility testing and whole-genome sequencing (WGS) were performed to identify the antimicrobial resistance profiles and genomic characteristics of the two strains of *S. haemolyticus*.

Materials and Methods

Identification of Bacterial Strains

The ear swab samples were collected from the patients with otitis media (OM) in 2023 using the sterilized cotton swabs. The present study complies with the Declaration of Helsinki. The samples were inoculated in blood agar medium for 24 h at 35°C. Two strains were isolated from ear swab samples. The strain identification was performed using a fully automatic VITEK 2 COMPACT system (BioMérieux, France) following the manufacturer's instructions. Identification of the two clinical strains was further confirmed via 16S rRNA gene sequencing using the universal primers 27F and 1492R. The minimum inhibitory concentrations (MICs) of oxacillin, clindamycin, erythromycin, linezolid, penicillin, sulfamethoxazole/trimethoprim, tigecycline, daptomycin, gentamicin, levofloxacin, moxifloxacin, rifampicin, teicoplanin, and vancomycin were determined using the broth microdilution method, and the results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI M100–S32).

Whole Genome Sequencing, Assembly, and Annotation

Whole-genome sequencing of the two strains of *S. haemolyticus* were performed by Genewiz Biotechnology Co. Ltd. (Suzhou, China) using paired-end sequencing with Novaseq 6000 (Illumina, 2×150 bp paired-end reads) and long sequencing with PacBio Sequel IIe (Pacific Biosciences, 10–15 Kb insert whole-genome shotgun libraries). PacBio reads were assembled using the Hifiasm software¹⁵ version 0.13-r308 and Canu¹⁶ version 1.7. The genome assembly was then polished using Pilon software version 1.22¹⁷ using Illumina reads. The assembled genomes of the two strains of *S. haemolyticus* were submitted to the National Center for Biotechnology Information (NCBI) GenBank database and annotated using the NCBI Prokaryotic Annotation Pipeline (PGAP).

Bioinformatics Analysis of the Two Strains of *S. haemolyticus*

The acquired ARGs in the genomes of the two strains of *S. haemolyticus* were identified using ResFinder 4.1,¹⁸ with a minimum coverage of 60% and minimum identity of 90%. Multilocus sequence typing (MLST) of the two strains of *S. haemolyticus* were performed using MLST 2.0,¹⁹ selecting the database as “*Staphylococcus haemolyticus*”. SCC*mec*Finder²⁰ was used to determine the SCC*mec* element type. Replicon types of the plasmids contained by the two strains of *S. haemolyticus* were determined using PlasmidFinder 2.1,²¹ with the database of “Gram Positive”, the

minimum coverage of 60%, and the minimum identity of 95%. The insertion sequences (ISs) adjacent to ARGs in the genomes of the two strains of *S. haemolyticus* were identified using ISfinder.²² A sequence similarity search was performed using MegaBLAST scans against the GenBank nonredundant (nr) database. BLAST Ring Image Generator (BRIG) 0.95²³ and Easyfig 2.2.5²⁴ were used to perform and visualize the sequence comparison.

Nucleotide Sequence Accession Numbers

The genome sequences of *S. haemolyticus* strain SH1275, which contained one chromosome and three plasmids, was submitted to GenBank under the accession numbers CP123979–CP123982. The genome sequences of *S. haemolyticus* strain SH9361, which contained a chromosome and two plasmids, was submitted to GenBank under the accession numbers CP123983–CP123985.

Results

Identification and Antimicrobial Susceptibility of Two Strains of *S. haemolyticus*

Two bacterial strains (SH1275 and SH9361) isolated from ear swab samples of patients with OM were identified as *S. haemolyticus* using the VITEK 2 COMPACT system, with further confirmation by 16S rRNA gene sequencing. Both of the two strains of *S. haemolyticus* showed resistance to oxacillin, clindamycin, erythromycin, penicillin, and levofloxacin (Table 1). Moreover, *S. haemolyticus* SH1275 was shown to be susceptible to sulfamethoxazole/trimethoprim, whereas *S. haemolyticus* SH9361 showed resistance to these same antibiotics (Table 1). In addition, *S. haemolyticus* SH1275 showed intermediate-level resistance to moxifloxacin, and *S. haemolyticus* SH9361 showed resistance to moxifloxacin (Table 1).

Table 1 Minimum Inhibitory Concentration ($\mu\text{g/mL}$) for the Two Strains of *S. haemolyticus*

| Antibiotics | <i>S. haemolyticus</i> SH1275 | | <i>S. haemolyticus</i> SH9361 | |
|-------------------------------|-------------------------------|----------------|-------------------------------|----------------|
| | MIC ($\mu\text{g/mL}$) | Interpretation | MIC ($\mu\text{g/mL}$) | Interpretation |
| Clindamycin | ≥ 4 | R | 0.25 | R |
| Erythromycin | ≥ 8 | R | ≥ 8 | R |
| Linezolid | 2 | S | 2 | S |
| Oxacillin | ≥ 4 | R | ≥ 4 | R |
| Penicillin | ≥ 0.5 | R | ≥ 0.5 | R |
| Sulfamethoxazole/Trimethoprim | ≤ 10 | S | ≥ 320 | R |
| Tigecycline | ≤ 0.12 | S | ≤ 0.12 | S |
| Daptomycin | 0.25 | S | 0.25 | S |
| Gentamicin | ≤ 0.5 | S | 4 | S |
| Levofloxacin | 4 | R | 4 | R |
| Moxifloxacin | I | I | 2 | R |
| Rifampicin | ≤ 0.5 | S | ≤ 0.5 | S |
| Teicoplanin | 2 | S | 2 | S |
| Vancomycin | I | S | ≤ 0.5 | S |

Abbreviations: S, Susceptible; R, Resistant; I, Intermediate.

Genomic Characteristics of the Two Strains of *S. haemolyticus*

Genome sequencing and analysis revealed that the genome of *S. haemolyticus* strain SH1275 comprised a 2.55-Mb chromosome and three plasmids with sizes of 37,857 bp (pSH1275-1), 26,934 bp (pSH1275-2), and 6056 bp (pSH1275-3), and the *S. haemolyticus* strain SH9361 comprised a 2.51-Mb chromosome and two plasmids with sizes of 2473 bp (pSH9361-1) and 2161 bp (pSH9361-2). MLST analysis revealed that strain SH1275 belonged to sequence type (ST) 3 and SH9361 to ST30. Three plasmid replicons, rep20 (pSH1275-1), rep39 (pSH1275-2) and rep5b (pSH1275-3), were identified in the genome of strain SH1275. In the strain SH9361, one of the two plasmids (pSH9361-1) was identified as containing the rep10 replicon, and the other plasmid (pSH9361-2) could not be typed. ResFinder results indicated that *S. haemolyticus* strain SH1275 carried multiple ARGs located on both chromosomes and two plasmids (pSH1275-2 and pSH1275-3). The chromosome of strain SH1275 was found to carry beta-lactam resistance genes *blaZ* and *mecA*. The *S. haemolyticus* genome contained *mecA*, which confers resistance to methicillin. Plasmid pSH1275-2 consisted of two macrolide resistance genes (*mph(C)* and *msr(A)*) and an antiseptic-resistance gene, *qacA*. Plasmid pSH1275-3 contained the gene *vga(A)_{LC}*, which is responsible for significant resistance to both lincosamides and streptogramin A.

ResFinder results indicated that *S. haemolyticus* strain SH9361 contained six ARGs located on both chromosomes and two plasmids (pSH9361-1 and pSH9361-2). The chromosome of strain SH9361 was found to harbor ARGs conferring resistance to beta-lactam antibiotics (*blaZ* and *mecA*), aminoglycosides (*aac(6')-aph(2'')*), as well as trimethoprim (*dfpG*). Plasmid pSH9361-1 carried an erythromycin resistance gene, (*erm(C)*), and plasmid pSH9361-2 carried an antiseptic-resistance gene, *qacJ*.

Genetic Contexts of *mecA* and Other Chromosomal ARG in the Two Strains

With respect to the two *mecA*-positive isolates of *S. haemolyticus* in this study, no SCCmec elements were detected based on the results predicted by SCCmecFinder. However, both SH1275 and SH9361 carried chromosomal fragments containing *mecA* similar to the SCCmec type V(5C2&5) subtype c (GenBank: AB505629) (Figure 1). The methicillin resistance gene *mecA* was bracketed by two copies of IS431.

Notably, the aminoglycoside resistance gene *aac(6')-aph(2'')* and one gene encoding the GNAT family N-acetyltransferase were flanked by two copies of the insertion sequence IS256 in different orientations (Figure 2A) in strain SH9361. Furthermore, we found the IS256-element was widely present in *Staphylococcus* (mainly *S. aureus*, *S. epidermidis*, and *S. haemolyticus*) and *Enterococcus* (*Enterococcus faecalis* and *E. faecium*), not only on chromosomes but also on plasmids (100.00% coverage and 100% identity) (Figure 2B and Table S1).

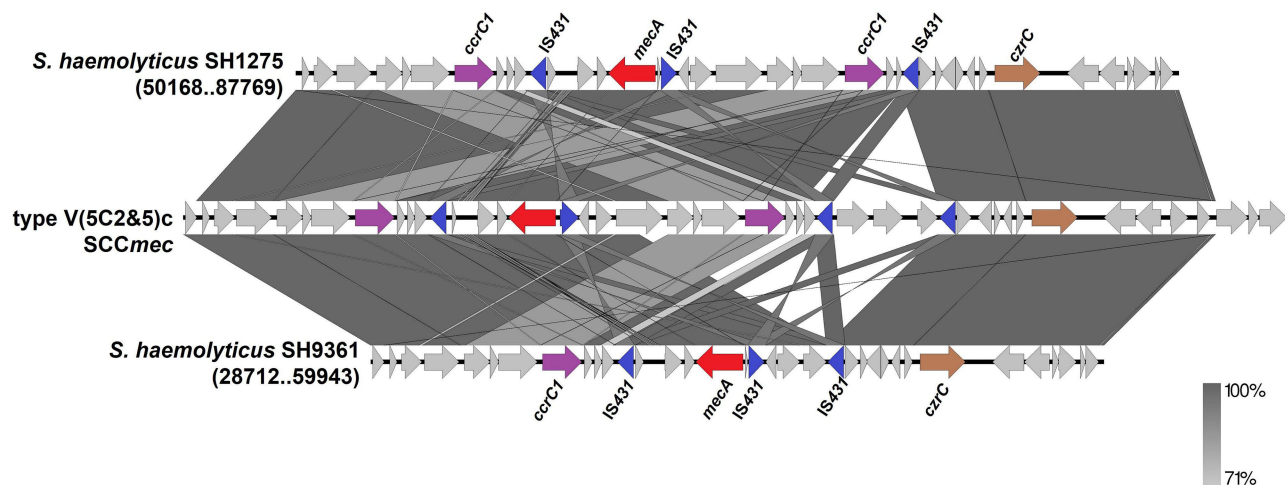


Figure 1 Comparative analysis of genetic contexts of *mecA* in *Staphylococcus haemolyticus* strains SH1275 and SH9361 with SCCmec type V(5C2&5) subtype c (GenBank: AB505629) generated by software Easyfig. Resistance genes, ISs, *ccrC* genes, and *czrC* genes are shown in red, blue, purple, and brown, respectively.

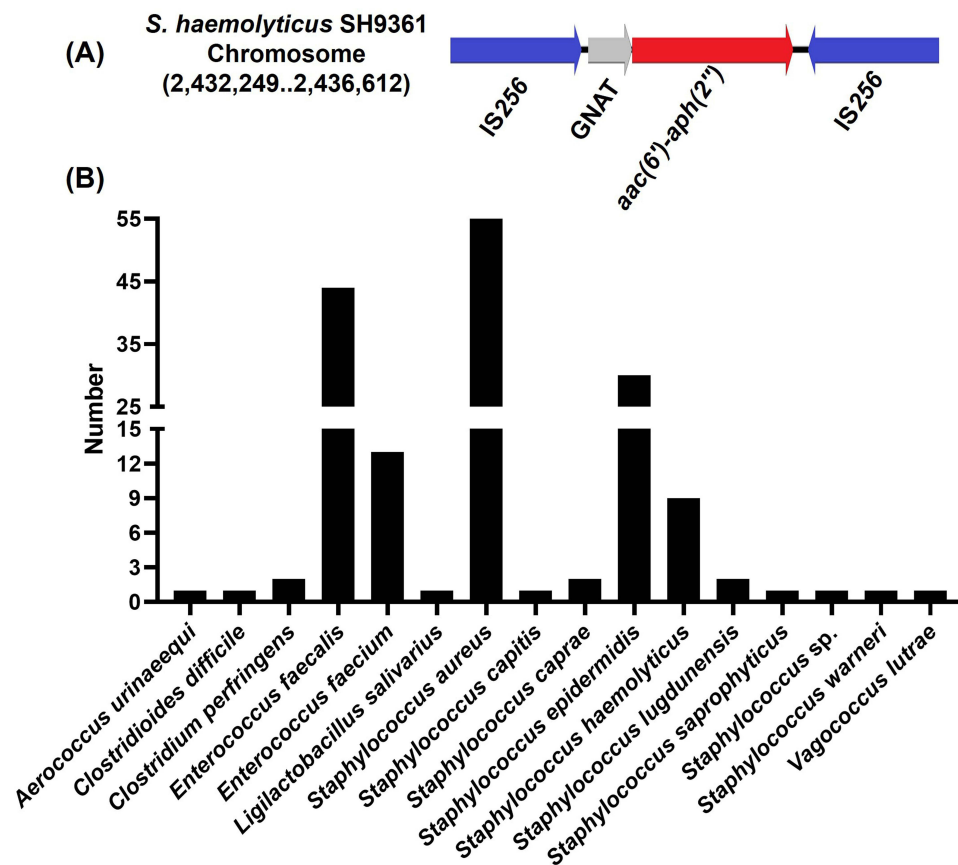


Figure 2 The IS256-element is widely present in *Staphylococcus* and *Enterococcus*. (A) Schematic diagram of the IS256-element found on the chromosome of *S. haemolyticus* SH9361. (B) Histogram about number of the IS256-element distributed in different species.

Genomic Analysis of the Antibiotic-Resistant Plasmids Carried by the Two Strains

In the case of the plasmid pSH1275-2 of *S. haemolyticus* strain SH1275, the genes *msr(A)*–*mph(C)* and the *qacR/qacA* system were located on a ~14.4-kb composite transposon, which was bracketed by two copies of the insertion sequence IS1272 in different orientations (Figure 3). Notably, an insertion sequence, IS431, was found to be located adjacent to *msr(A)*–*mph(C)* macrolide resistance genes. Based on the results of the BLAST search hit from the nr database of GenBank, the 14.4-kb composite transposon of plasmid pSH1275-2 was nearly identical to that of plasmid pSGAir0252 in *S. haemolyticus* strain SGAir0252 (100.00% coverage and 99.91% identity; Figure 3). Moreover, the region within the 14.4-kb composite transposon containing *msr(A)*, *mph(C)*, IS431, and *qacR/qacA* was also found in plasmids from *S. aureus*, *S. epidermidis*, and *S. hominis* (Figure 3). In addition, the BLAST search hit from the nr database of GenBank showed that the structure was present not only in the *Staphylococcus* plasmids, but also in the chromosomes of *Staphylococcus* spp. (Figure 4).

With respect to the 6056-bp plasmid pSH1275-3 containing the gene *vga(A)_{LC}* in *S. haemolyticus* strain SH1275, the results of oriTfinder indicated that the plasmid harbors the origin of the transfer site (*oriT*) and relaxase gene in its genome but lacks the genes coding for type IV coupling protein (T4CP) and type IV secretion system (T4SS); thus it was inferred to be a mobilizable plasmid. The 6-kb potential mobilizable plasmid was also found in another *S. haemolyticus* strain and two *S. hominis* strains (Figure 5 and Table S2). In addition, two plasmids from *S. aureus* (pUR4128, 7567 bp; and pUR2355, 7609 bp) also contained the *vga(A)_{LC}* and a conjugative transfer region (*oriT*, relaxase), similar to those of plasmid pSH1275-3 (Figure 5 and Table S2).

As for the 2.47-kb plasmid pSH9361-1 harboring *erm(C)* in *S. haemolyticus* strain SH9361 only three genes were found in the plasmid genome: the gene encoding replication/maintenance protein RepL, the erythromycin resistance gene *erm(C)*, and the gene encoding the ErmCL peptide. Based on the results of BLAST search hit from the nr database of GenBank, a large number of plasmids with high similarity to the 2.47-kb plasmid (pSH9361-1) were detected in the

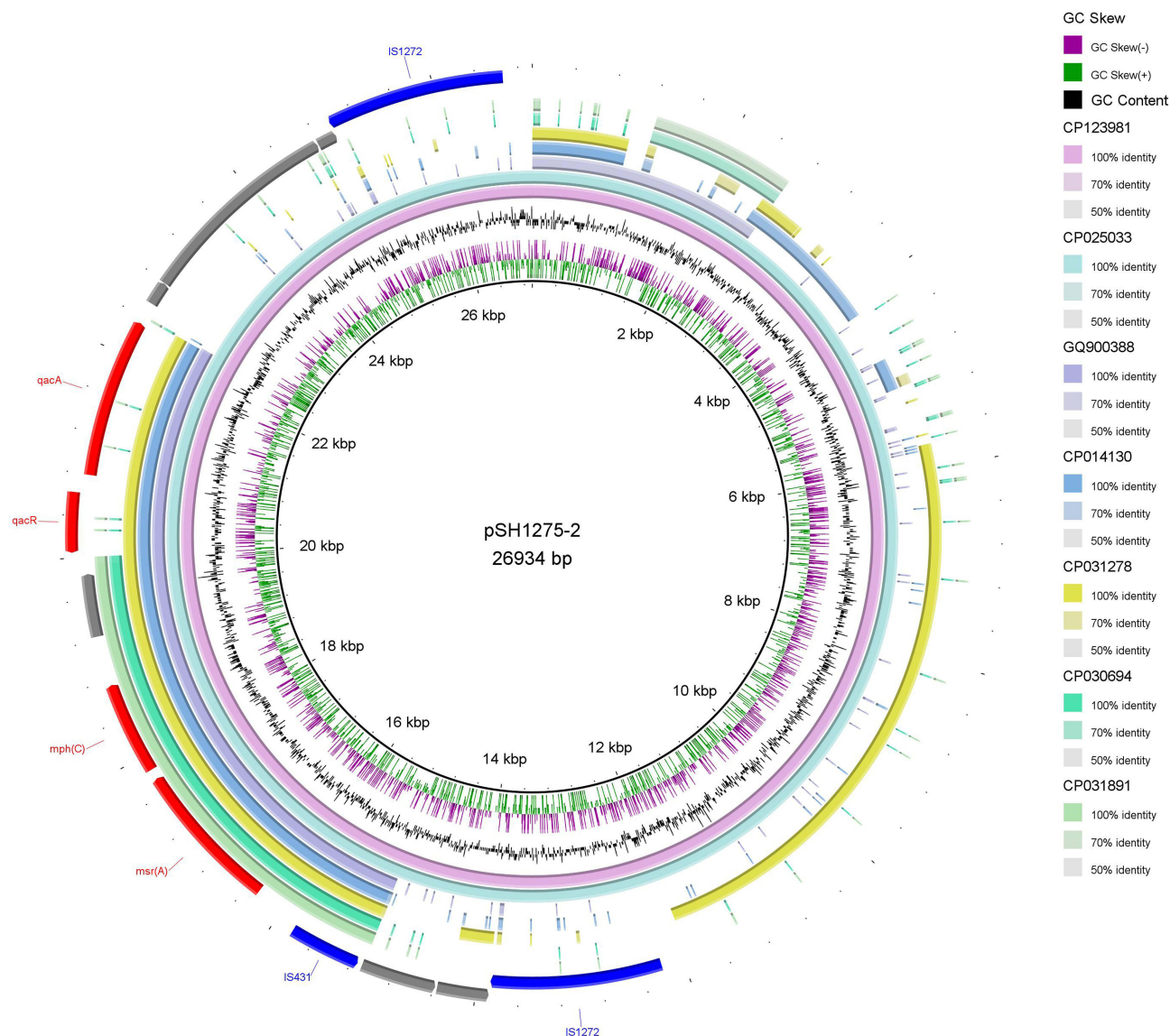


Figure 3 Genetic structures of the 14.4-kb composite transposon (containing *msr(A)*–*mph(C)* and *qacR/qacA*) bracketed by two copies of insertion sequence *IS1272* in the different orientation harbored by plasmid pSH1275-2. The seven plasmids incorporated in the diagram generated by software BRIG denote the *S. haemolyticus* strain SH1275 plasmid pSH1275-2 (CP123981), *S. haemolyticus* strain SGAir0252 plasmid pSGAir0252 (CP025033), *S. aureus* plasmid SAP027A (GQ900388), *S. epidermidis* strain FDAARGOS_161 plasmid unnamed1 (CP014130), *S. hominis* strain 19A plasmid unnamed1 (CP031278), *S. aureus* strain ER01803.3 plasmid unnamed1 (CP030694), and *S. aureus* strain CFSAN082781 plasmid pMRSA_22 (CP031891) from the inside out.

Staphylococcus genus, especially in *S. aureus* (coverage $\geq 99\%$ and identity $\geq 99\%$, [Figure 6](#) and [Table S3](#)). In addition, this 2.47-kb plasmid (pSH9361-1) was highly similar to chromosomal fragments from two strains of *S. aureus* and one strain of *S. haemolyticus* ([Table S3](#)).

The small plasmid pSH9361-2 in *S. haemolyticus* strain SH9361, comprising one *rep* gene and one antiseptic-resistance gene *qacJ*, was found to be highly similar to the 9th plasmid (GenBank: CP027494) of *S. aureus* strain ST2594 ([Figure S1](#)).

Discussion

Among the coagulase-negative staphylococci (CoNS), *S. haemolyticus* is one of the most common pathogens worldwide and is mainly associated with bloodstream and device-associated infections.^{25,26} In the present study, we report the whole genome sequences of two strains of oxacillin-resistant and *mecA*-positive *S. haemolyticus* (ST3 strain SH1275 and ST30 strain SH9361), which were isolated from ear swab samples of patients with OM. Epidemiological surveillance has

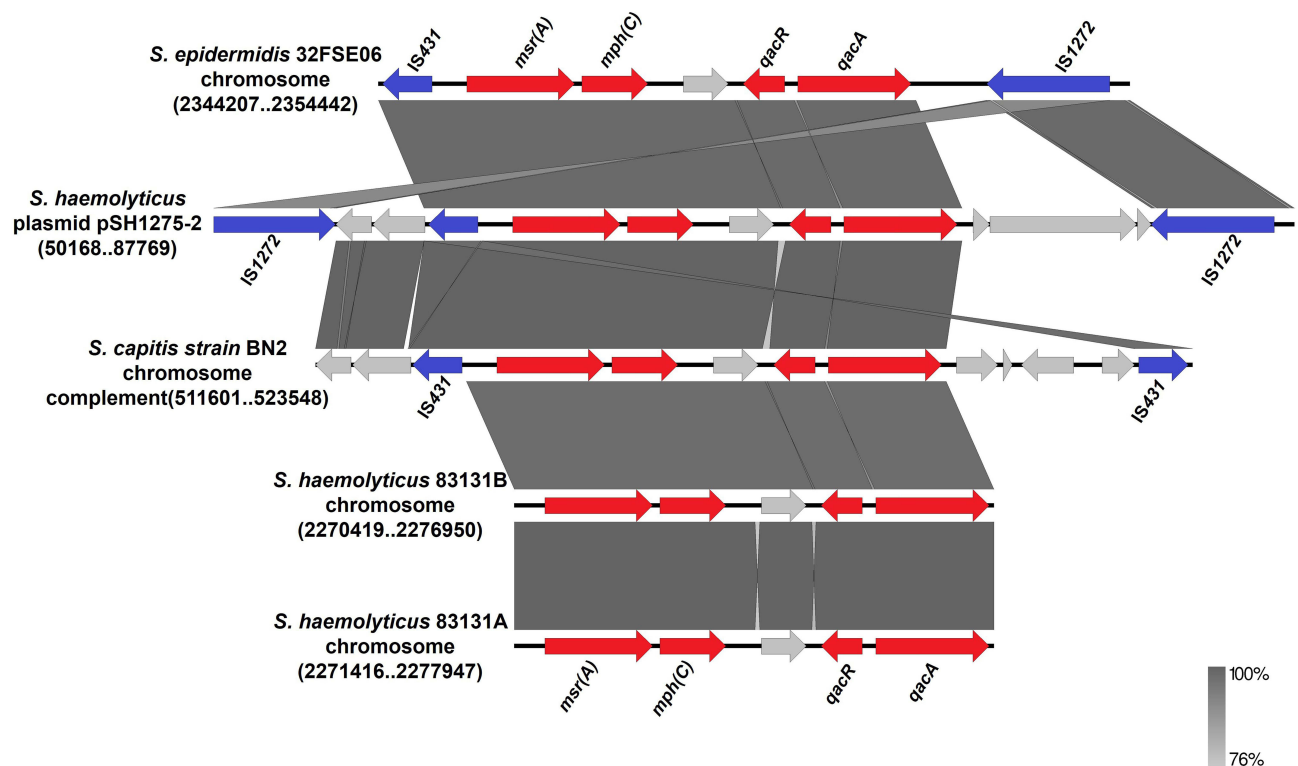


Figure 4 The region of plasmid pSH1275-2 containing *mcr(A)*–*mph(C)*, *IS431*, and *qacR/qacA*, had high similarity with the chromosomal fragments from strains of *Staphylococcus* genus.

shown that *S. haemolyticus* ST3 is the original strain that has evolved into many other molecular types,²⁶ including the emerging ST42 clone disseminated in the hospital environment.^{3,27} However, the prevalence of ST3 has continuously decreased since 2013.²⁶ In addition, *S. haemolyticus* strains belonging to ST30 have been previously detected in different clinical samples of the urinary tract,²⁸ infected eyes,²⁹ nares,³ feces,³ and blood.³⁰

In our study, two strains of oxacillin-resistant *S. haemolyticus* were found to carry the *mecA* gene, which confers methicillin resistance and is usually acquired through a *SCCmec* element in methicillin-resistant strains of staphylococci.³¹ Although no definite *SCCmec* elements were detected in either of the two strains, both carried the genetic context of *mecA* with high similarity to *SCCmec* type V (5C2&5) subtype c, which was first reported in the clonal complex 398 methicillin-resistant *S. aureus* strain JCSC6944.³² Genomic analysis showed that the *mecA* carried by two strains of *S. haemolyticus* in this study was bracketed by *IS431* (*IS431-mecA-ΔmecR1-IS431*). The most prevalent and widely disseminated *mec* complex in *S. aureus* has the structure *mecI-mecR1-mecA-IS431* and is designated as the class A *mecA* gene complex.³³ However, in *S. haemolyticus*, *IS431* was found to be associated with the deletion of *mecI* and *mecR1*, forming the structure *IS431-mecA-ΔmecR1-IS431*, designated as the class C *mecA* gene complex.³⁴ *IS431*, a well-known mobile genetic element in staphylococci, has been implicated in the transfer of ARFs.³⁵

The chromosomal aminoglycoside resistance gene *aac(6′)-aph(2″)* harbored by *S. haemolyticus* strain SH936 is flanked by two copies of *IS256*. *IS256* was first described in strains of *S. aureus* isolated in Australia in 1987³⁶ and was considered the founding member of the *IS256* family of insertion sequence elements,³⁷ and was described as a part of the transposon *Tn4001*, conferring aminoglycoside resistance in *S. aureus*. *IS256* is widely present in MDR staphylococci and enterococci.³⁸ *IS256* is frequently associated with the horizontal spread of ARGs,³⁹ including the *IS256*-element (*IS256*-GNAT-*[aac(6′)-aph(2″)]-IS256*) harbored by *S. haemolyticus* strain SH936, which is widely present in strains of both *Staphylococcus* and *Enterococcus*. Furthermore, *IS256* has been reported to influence antibiotic resistance either by insertion into regulatory genes⁴⁰ or by modulating antibiotic resistance gene

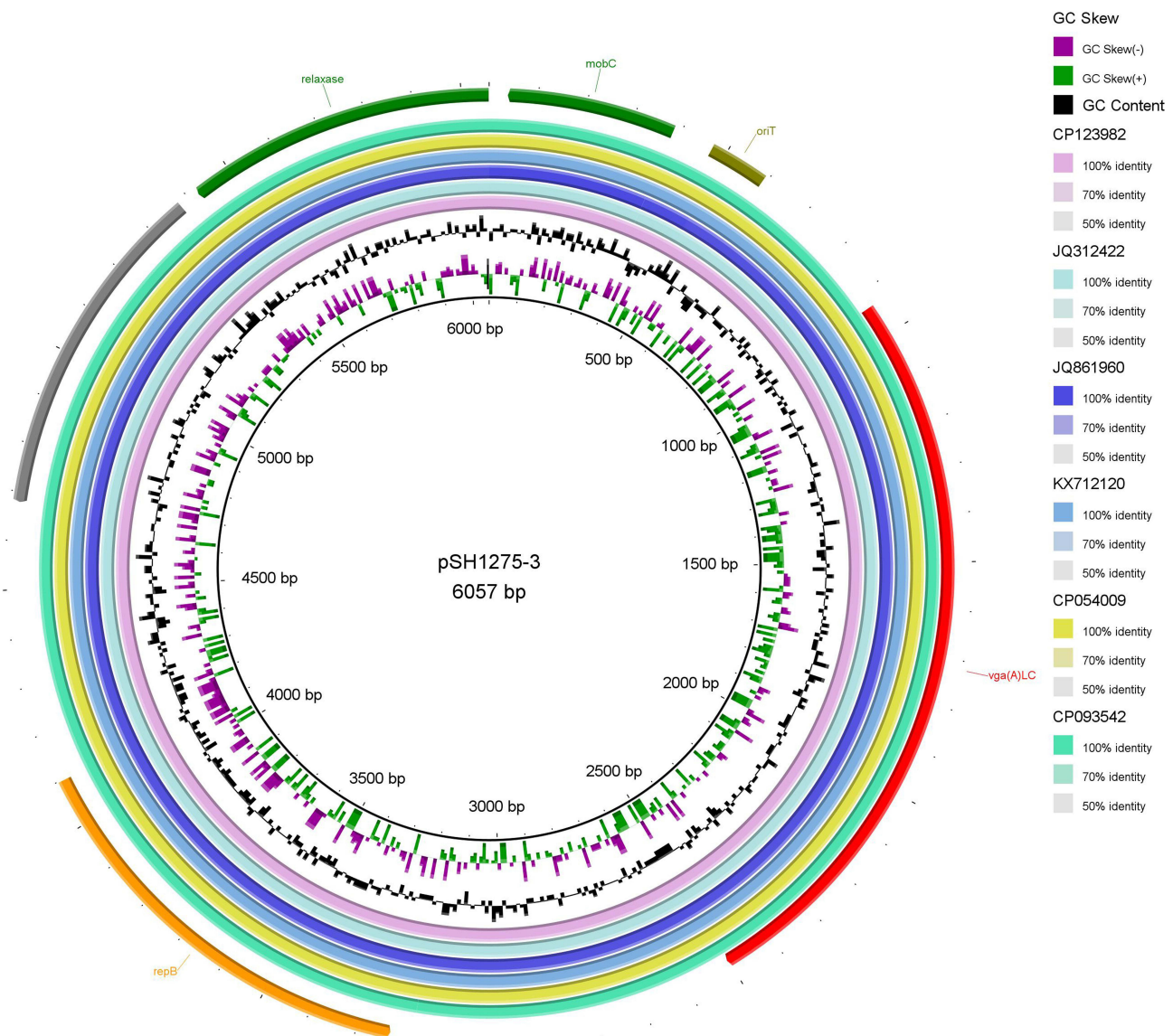


Figure 5 Comparison of plasmid pSH1275-3 of *S. haemolyticus* strain SH1275 and related *Staphylococcus* plasmids generated by the software program BRIG. The seven plasmids incorporated in the diagram generated by BRIG denote the *S. haemolyticus* strain SH1275 plasmid pSH1275-3 (CP123982), *S. aureus* strain C2355 plasmid pUR2355 (JQ312422), *S. aureus* strain C4128 plasmid pUR4128 (JQ861960), *S. haemolyticus* isolate 131A plasmid p131A (KX712120), *S. hominis* strain FDAARGOS_762 plasmid unnamed4 (CP054009), and *S. hominis* strain C5 plasmid unnamed3 (CP093542) from the inside out.

expression through the formation of strong hybrid promoters resulting from transposition into the neighborhood of antibiotic resistance genes.⁴¹ In addition, the presence of IS256 in staphylococci has been reported to be associated with biofilm formation.⁴²

S. haemolyticus can be a reservoir for resistance genes that can be shared with other staphylococci²⁵ via MGEs, including various plasmids.^{10,13} In the 26.9-kb plasmid pSH1275-2 of *S. haemolyticus* strain SH1275, the structure containing *msr(A)*–*mph(C)*, IS431, and *qacR/qacA*, harbored by the *S. haemolyticus* plasmid was also present in *S. aureus*, *S. epidermidis*, and *S. hominis*, both in the plasmids and chromosomes. Staphylococcal antimicrobial resistance plasmids may carry *mob* genes for mobilization, or a *tra* gene complex for conjugative transfer,¹³ similar to the mobilizable plasmid pSH1275-3 containing the gene *vga(A)*_{LC} in *S. haemolyticus* strain SH1275 in our study. Notably, one 2.47-kb plasmid (pSH9361-1) harboring *erm(C)* carried by *S. haemolyticus* strain SH9361 was also widely present in the *Staphylococcus* genus, especially in *S. aureus*.

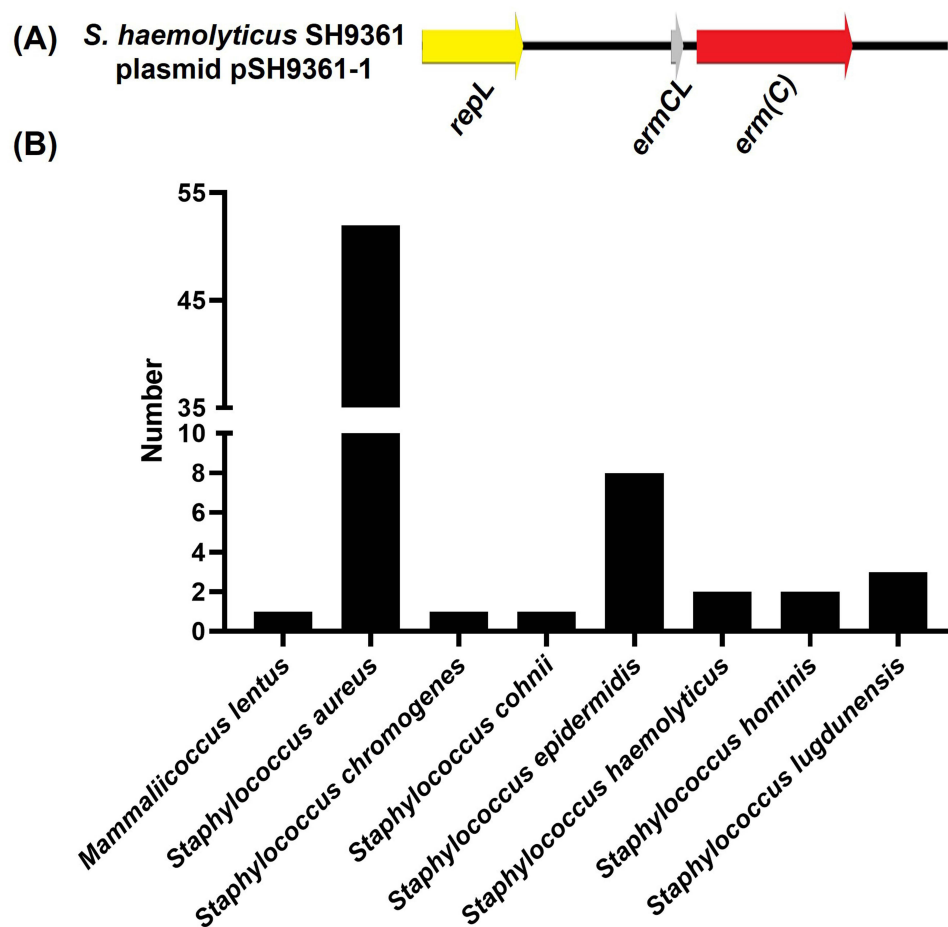


Figure 6 The 2.47-kb plasmid pSH9361-1 in *S. haemolyticus* strain SH9361 is widely present in the *Staphylococcus* genus. (A) Schematic diagram of the genome of pSH9361-1. (B) Histogram showing number of pSH9361-1 distributed in different *Staphylococcus* species (coverage \geq 99% and identity \geq 99%).

Conclusion

In this study, we describe the genomic characteristics of two strains of oxacillin-resistant and *mecA*-positive *S. haemolyticus* (ST3 strain SH1275 and ST30 strain SH9361) isolated from ear swab samples of patients with OM. The two strains of oxacillin-resistant *S. haemolyticus* both carried the genetic contexts of *mecA* (IS431-*mecA*- Δ *mecR1*-IS431) with high similarity with the SCC*mec* type V(5C2&5) subtype c. The chromosomal aminoglycoside resistance gene *aac(6')*-*aph(2'')* harbored by *S. haemolyticus* strain SH9361 was flanked by two copies of IS256 in different orientations, forming the IS256-element (IS256-GNAT-[*aac(6')*-*aph(2'')*]-IS256), which was commonly found in strains of both *Staphylococcus* and *Enterococcus* genus. Furthermore, the two strains of oxacillin-resistant and *mecA*-positive *S. haemolyticus* were found to harbor various antimicrobial resistance plasmids, including one 26.9-kb plasmid (pSH1275-2) containing *msr(A)*-*mph(C)* and *qacA*, one mobilizable plasmid pSH1275-3 harboring *vga(A)*_{LC}, one plasmid (pSH9361-1) carrying *erm(C)*, and one plasmid (pSH9361-2) carrying *qacJ*. These insights can assist clinicians in devising precise, personalized, and clinical therapeutic strategies for treating otitis media caused by multi-drug resistant *S. haemolyticus*.

Ethical Approval Statement

This study has been approved by the Ethics Committee of Zhuhai People's Hospital. The present study was a study focusing on bacteria and did not contain any sensitive personal information. Therefore, informed consent was not required according to "Measures for the Ethical Review of Biomedical Research Involving Humans" (https://www.gov.cn/gongbao/content/2017/content_5227817.htm).

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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