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ORIGINAL ARTICLE

Molecular characteristics and virulence factors in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate *Staphylococcus aureus* from central-southern China

Cailin Liu [a], Zhong-ju Chen [a], Ziyong Sun [a,*], Xianju Feng [b], Mingxiang Zou [c], Wei Cao [d], Shanmei Wang [e], Ji Zeng [f], Yue Wang [a], Mingyue Sun [a]

[a] *Department of Laboratory, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*
[b] *Department of Laboratory, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China*
[c] *Department of Laboratory, Xiangya Hospital of Central South University, Changsha, China*
[d] *Department of Laboratory, The Second Xiangya Hospital of Central South University, Changsha, China*
[e] *Department of Laboratory, Henan Province People's Hospital, Zhengzhou, China*
[f] *Department of Laboratory, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China*

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KEYWORDS
Heterogeneous vancomycin-intermediate *Staphylococcus aureus*;
Methicillin-resistant *Staphylococcus aureus*;
Molecular;

Background: *Staphylococcus aureus* is a leading cause of nosocomial infections. The purpose of this study was to evaluate the prevalence of methicillin-resistant *S. aureus* (MRSA) and heterogeneous vancomycin-intermediate *S. aureus* (hVISA), and compare the antimicrobial susceptibility, molecular characteristic, and virulence factors in methicillin-susceptible *S. aureus* (MSSA), MRSA, and hVISA from central-southern China.
Methods: A total of 184 *S. aureus* were isolated from sterile body fluids. All isolates were subjected to population analysis profiling for the identification of hVISA phenotype and polymerase chain reaction analysis for genotyping and 31 virulence genes.
Results: The prevalence of MRSA isolates was 41.8% in central-southern China. Of 77 MRSA isolates, 17 (22.1%) were identified as hVISA. The most common MRSA and MSSA clones were

* Corresponding author. Department of Laboratory, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 430030 Wuhan, China.
E-mail address: tjszyong@163.com (ZY. Sun).

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Staphylococcus aureus;
Virulence gene

ST239-MRSA-*SCCmec*III-t030-*agr*-I (55.8%) and ST188-MSSA-t189-*agr*-I (20.6%), respectively. The frequency of carriage of *pvl*, hemolysins, *tst*, and staphylococcal enterotoxin genes among MSSA isolates was significantly higher than that for MRSA isolates ($p < 0.05$); 98 MSSA isolates (53.3%) carried ≥10 tested virulence genes simultaneously, which was significantly higher than that of MRSA isolates (33.8%; $p = 0.004$). The 17 hVISA isolates carried a significantly small number of virulence genes; only two hVISA isolates carried ≥10 tested virulence genes simultaneously, and two hVISA isolates harbored only four virulence genes. Compared with other clonal complexes (CCs), CC1 and CC398 isolates harbored a higher frequency of exfoliatin genes, CC1 and CC59 harbored a higher frequency of *pvl* gene, and only CC1 isolates harbored *lukED*.

Conclusion: The prevalence of hVISA was considerably high in central-southern China. Simultaneous carriage of multiple virulence genes was common in *S. aureus* isolates; the virulence genes were more diverse and frequent among MSSA isolates than among MRSA isolates. Furthermore, the distribution of some virulence genes was correlated with the different *S. aureus* CCs.

Introduction

Since Alexander Ogston first isolated *Staphylococcus aureus* from a surgical abscess in 1880 and described its role in localized infection and septicemia,[1,2] *S. aureus* has been recognized as an important cause of human disease for nearly 130 years. The pathogenesis of *S. aureus* infections is related to the expression of a wide variety of virulence factors, including coagulase, hemolysins (which damage cell membrane of various cells), staphylococcal enterotoxins (SEs; which cause food poisoning), toxic shock syndrome toxin-1 (TSST-1; which increases sensitivity to endotoxin), exfoliative toxins (which are implicated in staphylococcal scalded-skin syndrome), staphylococcal protein A (spa), and Panton—Valentine leukocidin (pvl, which has been preferentially linked to furuncles, cutaneous abscesses, and severe necrotic skin infections).[3—5] Generally, serious *S. aureus* infections are caused by the combined actions of several virulence factors; *S. aureus* strains causing infections have variable combinations of virulence genes.[6,7]

Since its emergence in the 1980s, methicillin-resistant *S. aureus* (MRSA) has become a major cause of nosocomial infections worldwide. Vancomycin, a glycopeptide antibiotic introduced 50 years ago, is regarded as the mainstay of treatment for MRSA infections. Since first reported in Japan in 1997,[8] heterogeneous vancomycin-intermediate *S. aureus* (hVISA) have been detected throughout the world, which has threatened the rank of vancomycin as the first-line antibiotic for MRSA infections. The clinical significance of hVISA has been difficult to assess. It is unknown whether these isolates are fully virulent or just more virulent than vancomycin-susceptible *S. aureus* isolates and whether their levels of resistance are responsible for treatment failure.[9] The prevalence of hVISA varied significantly, which can be attributed to many factors, including differences in laboratory definitions, testing strategies, and regional variability.[10,11]

The prevalence of hVISA among MRSA isolates from China in 2007 was 15.7%[12]; however, the hVISA phenotype was determined by a combination of the following factors: (1) measurement by the modified population analysis profile/area under the curve method (PAP/AUC) and (2) estimation based on the measured sensitivity and specificity of a screening method, which was somewhat limited. The aim of this study was to investigate the prevalence of hVISA and compare the antimicrobial susceptibility, molecular characteristics, and virulence-associated gene carriage in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate *S. aureus* from six tertiary teaching hospitals in Hubei, Hunan, and Henan provinces, central-southern China.

Materials and methods

Bacterial isolates

A total of 184 consecutive and nonduplicated *S. aureus* isolates were collected from six tertiary teaching hospitals in central-southern China, from June 2011 to May 2012. The hospitals included Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (57 isolates), the First Affiliated Hospital of Zhengzhou University (45 isolates), Xiangya Hospital of Central South University (39 isolates), the Second Xiangya Hospital of Central South University (24 isolates), Henan Province People's Hospital (12 isolates), and Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (7 isolates). The strains were isolated from blood (141 isolates), cerebrospinal fluid (15 isolates), ascites (11 isolates), pleural effusion (10 isolates), and synovial fluid (7 isolates). All isolates were stored at −80°C until testing.

Antimicrobial susceptibility testing

Identification of *S. aureus* isolates was performed using standard methods and the Vitek 2 compact automated system (bioMérieux, Marcy-l'Étoile, France). Minimum inhibitory concentrations of antibiotics were determined using the agar dilution method, according to the Clinical