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Research article

Whole-genome sequencing analysis of widely disseminated infection caused by ST2631 methicillin-sensitive *Staphylococcus aureus*

Weili Zhang [a,b], Hao Xu [b,c,*]

[a] *Department of Laboratory Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China*
[b] *State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China*
[c] *Research Units of Infectious disease and Microecology, Chinese Academy of Medical Sciences, Beijing, China*

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ABSTRACT

Existing studies revealed high clonal diversity among *Staphylococcus aureus* bacteremia isolates, especially for methicillin-sensitive *S. aureus* (MSSA) strains. A 66-year-old male patient presenting with a widespread methicillin-sensitive *Staphylococcus aureus* (MSSA) infection, accompanied by concurrent carbapenem-resistant *Klebsiella pneumoniae* (CRKP) bloodstream infection.To evaluate the evolution of the present isolate, whole genome sequencing and bioinformatics analysis were performed for all available MSSA isolates. This patient recovered eventually through drainage and antibiotics combination. Therefore, the virulence factors of MSSA, as the primary pathogenicity, led to widely disseminated infection. The appropriate initial treatment is a major concern after culture identification.

1. Introduction

Community- and hospital-acquired Staphylococcus aureus infections could be changed dynamically and geographically, posing a great threat to public health [1]. The incidence of invasive methicillin-sensitive *S. aureus* (MSSA) was 1.8 times higher than methicillin-resistant *S. aureus* (MRSA) in US population, accounted for 60.1 % of deaths [2]. Existing studies revealed high clonal diversity among S. aureus bacteremia isolates, especially for MSSA strains [3]. In addition, clonal complex 30 (CC30), followed by CC45 and CC15 have been prevalent among bloodstream infections [4]. In this report, we described a case of widely disseminated infection caused by ST2631 MSSA, and carbapenem-resistant *Klebsiella pneumoniae* (CRKP) superinfection during the course of MSSA treatment improvement.

* Corresponding author. State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China.
E-mail address: xuhao0523@zju.edu.cn (H. Xu).

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2. Methods

2.1. Bacterial isolates and clinical data

A MSSA strain was isolated from a 66-year-old previously healthy retired man with widely disseminated infection. Antimicrobial susceptibility testing, using VITEK2 automated instrument for ID/AST testing (Bio-Mérieux, France). CRKP was defined as *K. pneumoniae* strain resistant to imipenem, meropenem or ertapenem.

The clinical data were included underlying diseases, comorbidities, invasive procedures, surgical procedures, laboratory examination, treatment history, hospitalization and clinical outcomes.

2.2. Whole genome sequencing (WGS) and comparative genomic analysis

The strain cultured overnight at 37 °C on Blood agar , then genomic DNA was extracted by FastDNA SPIN Kit for Soil (MP Biomedicals, United States) and sequenced using HiSeq 2000 (Illumina, SanDiego, CA, USA). The bioinformatics tools used in this study were available at NCBI (National Center for Biotechnological Information). This Whole Genome Shotgun BioProject for MSSA in our study has been deposited at GenBank under the accession PRJNA555821.

There were 893431 publicly available S. aureus genomes which were used to identify the same Multi-locus sequence typing (MLST) to isolate in our study by mlst software (https://github.com/tseemann/mlst), and then the spa type was predicted by spaTyper (https://cge.food.dtu.dk/services/spaTyper/)Core genome for the genome dataset was calculated by Roary (https://sanger-pathogens.github.io/Roary/), and maximum likelihood-based phylogenetic reconstruction was performed with MEGAX, Phylogenetic tree visualizations were generated by using Interactive Tree Of Life (https://itol.embl.de). Acquired antibiotic resistance and viruence genes in *S. aureus* was identified by abricate (https://github.com/topics/abricate) with Resfinder and VFBD database.

3. Results

3.1. Case description

In 2019, The patient in the present study was referred to Zhejiang Provincial People's Hospital with joint pain for 12 days, and dysuria for 2 days. After 2 days in the department of rheumatology, the patient developed severe oliguria and septic shock, then he was

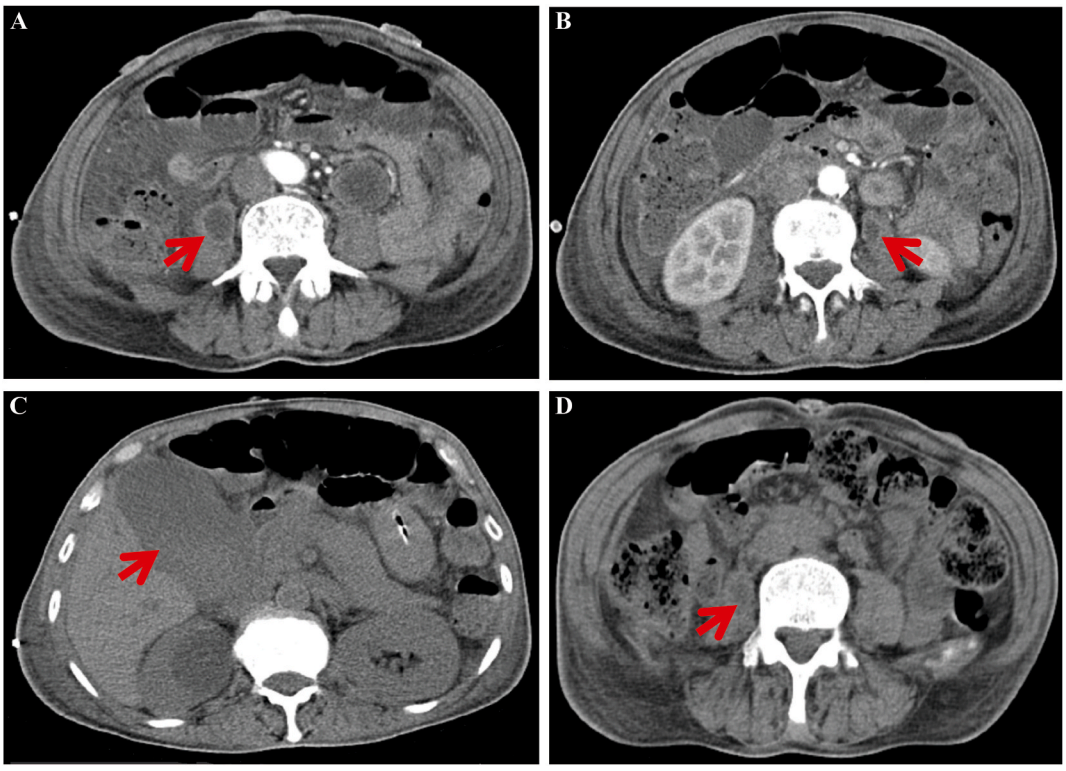

Fig. 1. Abdominal CT scan of the patient. (A) and (B) The arrows indicate the psoas abscess; (C) The arrow indicates the obvious gallbladder enlargement; (D) The arrow indicates the psoas abscess absorption after 34 days.

transferred to department of infectious diseases. The clinical laboratory findings suggested acute kidney injury, and X-ray abdomen film showed intestinal obstruction (Supplementary fig. 1A). In addition, emission computed tomography (ECT) revealed that local bone metabolism was active (Supplementary fig. 1B). It is of note that a 2 cm*2 cm blood blister was seen in the medial dorsum of left foot. A screening test was performed to assess his immunocompetence, and the results showed normal values. After collection of urine, blood blister puncture fluid and blood cultures, antibiotic therapy with meropenem was initiated according to estimated glomerular filtration rate (24.6 mL/min/1.73 m^2).

The culture reports available the following day were positive for MSSA. Antimicrobial susceptibility testing showed resistance to penicillin, erythromycin, clindamycin, and sulfamethoxazole. We subsequently switched over from meropenem to linezolid combined with piperacillin/tazobactam due to widely disseminated infection. The C-reactive protein (CRP) and procalcitonin (PCT) levels decreased, his general condition improved as well (Supplementary fig. 1C). However, this patient suffered from recurrent intestinal obstruction and fever. Computed tomography (CT) scan of the thorax, abdomen, and pelvis showed pleural effusion, obvious gallbladder enlargement, intestinal obstruction and psoas abscess (Fig. 1A–C). Application of endoscope guided transnasal ileus tube placement for the treatment of intestinal obstruction. Antimicrobial susceptibility of the bacteria isolated from the percutaneous chest and gallbladder catheterizing drainage culture still revealed MSSA infections. The antibiotics were changed to linezolid, meropenem and moxifloxacin for 6 days. After the fever was stabilized, and meropenem treatment was stopped.

Unfortunately, on the midnight of the thirty-ninth hospitalization day, his body temperature was 39.7 °C again, and CRKP was identified in the blood cultures. The CRKP isolate was only susceptibility to amikacin and polymyxin B. Then the patient was treated by imipenem, amikacin and moxifloxacin combination. On day 34, second CT image showed a reduction in the size of the psoas abscess (Fig. 1D). The clinical progression was satisfactory, with complete apyrexia and recovery of joint pain and disseminated abscess during the two months follow-up.

3.2. Genomics Characteristics and comparative genomics analysis

Further whole-genome sequence analyses showed the MSSA isolates in this patient was ST2631 and Staphylococcus protein A (spa) type t164. In addition, sequencing also found three exoenzyme genes (*aur*, *splA* and *splE*), two human innate immunomodulatory genes (*sak*, *scn*) and eleven toxin genes (*hlgA*, *hlgB*, *hlgC*, *lukD*, *lukE*, *seg*, *sei*, *sem*, *sen*, *seo*, *seu*). This MSSA isolate and 9 publicly available ST2631 *S. aureus* from 893431 *S. aureus* were selected to determine the evolutionary relationship (Fig. 2, Supplementary Table 1). There were only 10 different bases identified between the present isolate and GCA_023847325 (Supplementary Table 2). It is of note that GCA_023847325 and two strains isolated from the environment and animal clustered into same lineage.

4. Discussion

Previous study provided strong evidence that CC398, containing the *scn* gene, originated from human handling and spread to livestock [5]. The proportion of clonal complex (CC) 20 was showed lower prevalence among livestock and cause of human infections [3,6]. The studies on ST2631 *S. aureus* isolates were limited. As in this case, isolate ST2631 had two locus variant of ST20, belong to CC20 as well. However, this patient had not close contact with livestock. The further comparative genomics analysis based on core genome demonstrated the evolutionary relationship of the isolate in the present study was much closer to GCA_023847325, which had close phylogenetic relationships with strains isolated from the environment and animal. Therefore, it is suggested that this strain may not derive from animals.

Previous studies had demonstrated that the expression of several virulence factors play a pivotal role in pathogenicity, such as enterotoxins, haemolysin, Panton–Valentine leukocidin (PVL), and the toxic shock syndrome toxin 1 (TSST-1) [7]. The identification of *S. aureus* isolates from raw milk harboured several genes encoding virulence factors showed a high risk of spread of food-borne diseases [8]. Genes encoding staphylococcal enterotoxins and SE-like toxins could affect the activation of lymphocytes, eosinophils, macrophages, and mast cells [8,9]. Additional researches would be needed to assess virulence expression regulated by environmental factors and animal preservatives.

5. Conclusion

A significant take away point of this report is to indicate the invasive MSSA contribute significant health burden. In addition, the initial treatment needs to be more appropriate after culture identification. Moreover, these data highlighted the virulence factors

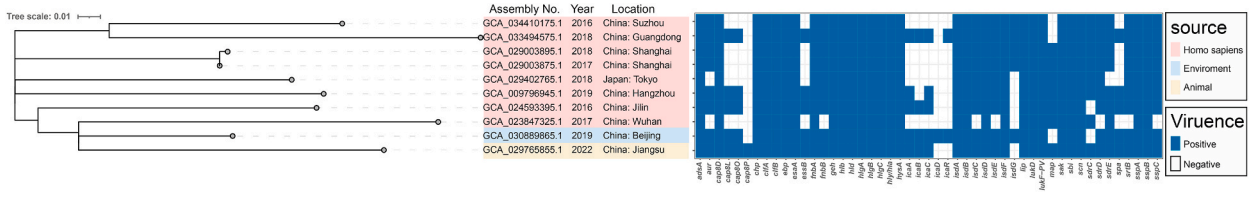

Fig. 2. Core-genome-based phylogenic tree of 10 isolates, including one isolate from this study and 9 strains downloaded from NCBI genome database.

assume the crucial role of pathogenicity.

Declarations

5.1. Ethics statement

This study was reviewed and approved by the clinical research ethics committee of Zhejiang Provincial People's Hospital, with the approval number: 2019KY033.

The patient provided informed consent for the publication of their anonymised case details and images.

5.2. Data availability statement

This Whole Genome Shotgun BioProject for MSSA has been deposited at GenBank under the accession PRJNA555821.

Competing interests

None.

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CRediT authorship contribution statement

Weili Zhang: Writing – original draft, Software, Methodology. **Hao Xu:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29248.

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