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Original Research

fbl-typing and Antimicrobial Resistance Analysis of *Staphylococcus lugdunensis*

Gang Chen[1,†], Yue Hou[2,†], Liyan Ye[1], Qiang Zhao[1], Jie Liu[1,2,*], Zhaoyan Wang[3,*]

[1]Department of Clinical Laboratory, First Medical Center, Chinese People's Liberation Army General Hospital, 100853 Beijing, China
[2]Department of Microbiology Laboratory, Beijing Chaoyang District Center for Disease Control and Prevention, 100021 Beijing, China
[3]Department of Ophthalmology, First Medical Center, Chinese People's Liberation Army General Hospital, 100853 Beijing, China
*Correspondence: jieliu82@126.com (Jie Liu); 2468li@sina.com (Zhaoyan Wang)
[†]These authors contributed equally.

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Abstract

Background: A broad variety of infections, ranging from skin infections to infective endocarditis can be caused by *Staphylococcus lugdunensis*. Bacterial virulence is often related to virulence genes, so we sought to investigate the relationship between virulence genes and the pathogenicity of *S. lugdunensis* and to explore an appropriate typing method to distinguish different pathogenic phenotypes of *S. lugdunensis*. **Methods**: We describe the distribution of several virulence genes in different infection types in an attempt to find the relationship between virulence genes and pathogenicity. Subsequently, we make the Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) dendrogram and *fbl*-typing were performed using BioNumerics software, tried to compare the correlation between different methods and the different infectious diseases, and antimicrobial resistance of the strains, in order to obtain the epidemic characteristics and antimicrobial resistance information of *S. lugdunensis* based on a molecular approach. **Results**: The results of virulence genes showed that the seven virulence genes we have described existed in most strains, and there was no significant correlation between virulence gene distribution and infection type. Compared with the MALDI-TOF MS dendrogram, we found that *fbl*-typing could better correspond to the pathogenic phenotype, with better recognition and reproducibility. In the phylogenetic tree constructed in the *fbl* R-region, we found a tendency for some infection types to be distributed in clusters, new type 3 was the most dominant *fbl*-type, followed by fbl47b. Bone and joint infection isolates and ear infection isolates were significantly clustered together, in addition, all the oxacillin-resistant isolates were concentrated in *fbl*-type fbl45j and fbl47b. **Conclusions**: In this study, we found no significant correlation between virulence genes from *S. lugdunensis* isolates and the site of infection. The *fbl*-typing has the characteristics of convenient operation, low cost, high repeatability, and is preferable to indicate the pathogenic phenotype. Based on *fbl*-typing, we described the epidemiological characteristics of *S. lugdunensis* in a hospital and supplemented the data for *fbl*-typing. We recommend that *fbl*-typing method be extended and supplemented.

Keywords: *Staphylococcus lugdunensis*; *fbl*-typing; virulence genes; antimicrobial resistance

1. Introduction

Coagulase-negative Staphylococci (CoNS) are low-virulence commensal bacteria in human. However, *Staphylococcus lugdunensis*, as a distinctive CoNS, virulence is similar to that of *Staphylococcus aureus* and stronger than other CoNS species [1]. As an opportunistic pathogen, *S. lugdunensis* colonizes skin surfaces with high humidity such as the groin, perineum, breast, and armpit in 30%-50% of patients [2]. The clinical infection characteristics are likely to that of *S.aureus*, including infective endocarditis, bacteremia, meningitis, and bone and joint infections [3,4]. However, the incidence and outcome of *S. lugdunensis* are largely unknown, with only a few small case studies [5]. *S. lugdunensis* was first reported by Freney *et al*. [6] in 1988, which did not attract clinical attention at the beginning. However, with the increasing number of reported cases of *S. lugdunensis* infection, people gradually realized that *S. lugdunensis* was an important cause of serious infection [7]. In the systematic review by Non *et al*. [8], *S. lugdunensis* is associated with a similar proportion of infective endocarditis as *S. aureus*. A study by Shah *et al*. [9] showed that bone and joint infections due to *S. lugdunensis* tend to be more invasive than other CoNS species and are more similar to those due to *S. aureus*. The high pathogenicity of *S. lugdunensis* is closely related to some virulence factors, such as the fibrinogen binding protein (FBL), VWF-binding protein (vWbl) and autolysis (AtlL). Some studies suggest that the virulence factors of *S. lugdunensis* may be related to infectious diseases [10,11]. Therefore, we attempted to describe the relationship between the virulence genes of *S. lugdunensis* and different infectious diseases, so as to explore the relationship between virulence genes and pathogenic phenotypes, more to describe the epidemiological characteristics of *S. lugdunensis* in this hospital using an appropriate molecular biological method.

In addition, the early pathogens typing is decisive to initiate appropriate infection control measures for different pathogenic phenotypes [12]. We searched for suitable typ-

Table 1. Primers used for the amplification and sequence gene identification.

Name	Primer sequence (5′-3′)	References
atlL$_{R3}$	F: GCGTACATTAGCTGTTAAAACAA R: CGTCATAGAAATAGCTATCC	[11]
fbl-R	F: TGGCATGGGATAATGAAGTAG R: CCACTCCCGTATAGTAACT	[14]
slush	F: TTTCGTCTTTGCACACACATTTCCA R: ACAGCACAAAGCCTTAACTATCTCA	[20]
Beta-hemolysin	F: TGGTCAAGGTACAGAAGGTTGGCA R: TATCCCAACTATACGCGTTGCCCT	
Hemolysin III	F: TAATGCTGTTTCGCACGGAGTTGC R: GACGCCTACCCATCCCATTACAA	
vwbl	F: TATATACCATGGCAACAATTCCAGATCGCG R: TTTATACCCGGGTTCTGACTGGATACGTTCATAC	[21]
icaA	F: CGATGGGCTCAAGGTGG R: TTCTTTTCGTAGCGACTGTC	[22]

ing methods to distinguish different pathogenic phenotypes of *S. lugdunensis*, to take effective infection control. At present, the most widely used methods for typing *S. lugdunensis* are pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) [11,13,14]. However, these molecular typing methods have the disadvantages of being time-consuming and high cost. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is an emerging technology that has been increasingly used in clinical laboratories for rapid bacterial identification. Moreover, it has also shown the potential for typing applications among *Staphylococcus* species [15]. Therefore, we attempted to create a MALDI-TOF MS dendrogram to observe whether strains from different infection sites presented cluster distribution. Besides, *fbl*-typing is a special typing method for *S. lugdunensis*. The protein FBL of *S. lugdunensis* is encoded by *fbl* gene and belongs to the Sdr family, which has about 60% homology with clumping factor A (ClfA) of *S. aureus* [16,17]. The *fbl* gene has three regions, namely N1, N2, N3, and a repeat region (R-region). More and more studies have confirmed that the *fbl* gene may be specific in *S. lugdunensis*, and its sequence is relatively conservative. Therefore, the *fbl* gene has been considered as a specific target of *S. lugdunensis* [18,19]. Meanwhile, Dahyot *et al*. [14] proposed that the R-region be used as a genotyping method, which is similar to the *S. aureus spa* molecular typing method. The *fbl*-typing was based on the presence of gene polymorphism in a variable number of the 18 bp repeat sequences in the R-region of the *fbl* gene. Moreover, they confirmed that the *fbl*-typing was highly consistent with the results of MLST typing and had good identification ability [14].

Therefore, this study first described the relationship between the virulence genes of *S. lugdunensis* and different infectious diseases, so as to explore the relationship between virulence genes and pathogenic phenotypes. Subsequently, the MALDI-TOF MS dendrogram and *fbl*-typing were performed using BioNumerics software. We tried to compare the correlation between different methods and the different infectious diseases, antimicrobial resistance of the strains, in order to obtain the epidemic characteristics and antimicrobial resistance information of *S. lugdunensis*.

2. Materials and Methods

2.1 Bacterial Isolates

A total of 65 *S. lugdunensis* were collected from the department of clinical laboratory of the First Medical Center, the Chinese People's Liberation Army General Hospital, Beijing, China. All isolates were identified by VITEK MS automatic rapid microbial mass spectrometry detection system (BioMérieux, Lyon, France), and the quality profiles were obtained in strict accordance with the operation manual and analyzed by VITEK MS database. The standard strain was *Escherichia coli* ATCC 8739, 27 reference sequence of the *fbl* gene of *S. lugdunensis* was downloaded from the website of National Center for Biotechnology Information (NCBI).

2.2 MALDI-TOF MS

For MALDI-TOF MS analyses, isolates were incubated on Columbia blood agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 24 h at 37 °C prior to mass spectral analyses. Single colonies of isolates grown on Columbia agar at 37 °C for 24 h were selected, transferred to target plates, and overlaid with α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution. Colony samples were measured on a VITEK MS RUO system (BioMérieux, Lyon, France), using standard settings for routine identification in a mass range of 2000–20,000 Da. The system was calibrated with a fresh sample of *E. coli* ATCC 8739 that also served as a positive control in a

Table 2. Characteristics of virulence gene of *S. lugdunensis* isolates in relation to infection sites.

	vwbl	*icaA*	s*lush*	hemolysin III	beta-hemolysin	*atlL*$_{R3}$	*fbl*-R
Bone and joint infection, n = 19 (%)	17/19	14/19	19/19	18/19	17/19	19 /19	19/19
Reproductive system infection, n = 14 (%)	13/14	13/14	14/14	14/14	13/14	14/14	14/14
Skin and soft tissue infection, n = 12 (%)	10/12	9/12	11/12	12/12	12/12	12/12	12/12
Urinary system infection, n = 7 (%)	6/7	7/7	7/7	7/7	7/7	7/7	7/7
Ear infection, n = 5 (%)	4/5	4/5	5/5	5/5	5/5	5/5	5/5
Cardiovascular system infection, n = 5 (%)	5/5	4/5	5/5	5/5	5/5	5/5	5/5
Central nervous system infection, n = 2 (%)	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Respiratory system infection, n = 1 (%)	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Total, n = 65	58/65	54/65	64/65	64/65	62/65	65/65	65/65

Table 3. Characteristics of antibiotic resistance of *S. lugdunensis* isolates in relation to infection sites.

	Bone and joint infection, n = 19 (%)	Reproductive system infection, n = 14 (%)	Skin and soft tissue infection, n = 11 (%)	Urinary system infection, n = 7 (%)	Ear infection, n = 5 (%)	Cardiovascular system infection, n = 4 (%)	Central nervous system infection, n = 2 (%)	Respiratory system infection, n = 1 (%)	Total, n = 63
OXC	0/19	1/14	1/11	2/7	3/5	0/4	0/2	0/1	7/63
ERY	9/19	0/14	2/11	1/7	3/5	1/4	0/2	0/1	16/63
CLI	9/19	0/14	2/11	1/7	3/5	1/4	0/2	0/1	16/63
TEC	0/19	0/14	1/11	0/7	0/5	0/4	0/2	0/1	1/63
PEN	19/19	8/14	10/11	7/7	4/5	3/4	2/2	1/1	54/63
MDR	9/19	0/14	2/11	1/7	3/5	1/4	0/2	0/1	16/63

OXC, oxacillin; ERY, erythromycin; CLI, clindamycin; TEC, teicoplanin; PEN, Penicillin; MDR, multi-resistant rate.

second acquisition. As a negative control, matrix-only spots were regularly analyzed. MALDI scores ≥2 were assumed as positive.

2.3 DNA Extraction and PCR Amplification

Isolates cultured overnight on the blood plate were placed in sterilized water and centrifuged at 13,000 rpm for 10 min. The supernatant was taken and heated at 100 °C for 10 min in a metal thermostat. After centrifugation at 13,000 rpm for 10 min, the supernatant was taken and stored at –20 °C for later use. PCRs were performed on a Thermal Cycler in a final volume of 25 µL containing 12.5 µL 2×Taq PCR Master Mix, 0.5 µM of each primer (Table 1, Ref. [11,14,20–22]) and 1 µL of DNA. The PCR conditions were as follows: denaturation at 94 °C for 5 min, followed by 35 cycles of amplification including denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 40 s. A final extension step at 72 °C for 5 min was performed.

2.4 Antimicrobial Resistance Testing

The minimum inhibitory concentration (MIC) of *S. lugdunensis* was detected by the commercialized broth microdilution method (Xingbai Biotechnology, Shanghai, China). The results of sensitivity (S), moderate sensitivity (I) and drug resistance (R) were obtained according to the corresponding standards of the American Clinical Laboratory Standards Institute (CLSI) [23]. Antibiotics include oxacillin (OXC), erythromycin (ERY), clindamycin (CLI), levofloxacin (LEV), tetracycline (TET), gentamicin (GEN), vancomycin (VAN), teicoplanin (TEC), rifampin (RIF), Trimethoprim-sulfamethoxazole (SXT), daptomycin (DAP), Penicillin (PEN), linezolid (LZD), cefoxitin (CFX), nitrofurantoin (NIT). Multidrug resistance is resistance to at least one antimicrobial drug in three or more antimicrobial categories. The control strains were *Enterococcus faecalis* ATCC 29212 and *S. aureus* ATCC 29213. *S. aureus* was used as a reference for oxacillin and cefoxitin, and *Staphylococcus epidermidis* was used as a reference for other antibiotics.

2.5 Sequence Alignment and Homology Analysis

DNA sequences were compared using BLAST program on NCBI website (https://www.ncbi.nlm.nih.gov/). MEGA software (Version 6.0, University of Pennsylvania, Philadelphia, PA, USA) was used for homology analysis, the neighbor-joining method was used for phylogenetic tree construction, and the Bootstraping test was used for reliability analysis. The resulting spectra were downloaded into the BioNumerics software (Version7.6, BioMérieux, Lyon, France) and a dendrogram was constructed using a ranked Pearson correlation as a similarity coefficient. The *fbl* R-regions were analyzed using the plug-in "polymorphic VNTR typing" in Bionumerics software. A typing method of genetic polymorphism is provided by tandemly repeated sequences, known as the variable number of tandem repeats (VNTRs), whose number of repetitions varies at different rates depending on the different loci and even alleles. Statistical analysis was performed using SPSS software (Version 16.0, IBM, Armonk, NY, USA), $p < 0.05$ was considered statistically significant.

3. Results

3.1 Type of Infection Caused by S. lugdunensis

The 65 isolates were isolated from the following infection types: bone and joint infection (n = 19), reproductive system infection (n = 14), skin and soft tissue infection (n = 12), urinary system infection (n = 7), ear infection (n = 5), cardiovascular system infection (n = 5), central nervous system infection (n = 2), respiratory system infection (n = 1).

3.2 Virulence Genes Carried in S. lugdunensis

There was no significant difference in the detection rate of virulence genes in *S. lugdunensis* from different infection sites ($p > 0.05$) (Table 2).

3.3 Analysis of Antimicrobial Resistance of S. lugdunensis

3.3.1 Antimicrobial Resistance Results of *S. lugdunensis*

Isolates in our study were resistant to PEN (85.7%), and were 100% sensitive to LEV, TET, GEN, VAN, RIF, SXT, DAP, LZD and NIT. Oxacillin-resistant *S. lugdunensis* was 11.1% (7/63). The positive rate of CLI induction test was 6.3% (4/63), and the multi-resistant rate (MDR) was 25.4% (16/63). Strains from ear infections had higher rates of drug resistance and multidrug resistance, followed by bone and joint infections. Characteristics of antibiotic resistance of *S. ludunensis* in different infection sites are shown in Table 3.

3.3.2 Antibiotic Resistance Rate of Oxacillin-Resistant *S. lugdunensis* Group and Oxacillin-Sensitive *S. lugdunensis* Group

S. lugdunensis was divided into the oxacillin-resistant group and the oxacillin-sensitive group. LEV, TET, GEN, VAN, RIF, SXT, DAP, LZD, and NIT were all sensitive. The antibiotic resistance rates of ERY, CLI, PEN, and MDR in the oxacillin-resistant *S. lugdunensis* group were higher than those in the oxacillin-sensitive group, but the difference was not statistically significant ($p > 0.05$).

3.4 MALDI-TOF MS Results

In the MALDI-TOF MS dendrogram, isolates were distributed mainly six major clusters (Fig. 1). According to the infection types and clusters, some phenotypic isolates had a tendency to a more homogeneous clustering, such as bone and joint infection and reproductive system infection, but most phenotypes showed a more random distribution across the tree, such as cardiovascular system infection, urinary system infection and ear infection, etc. In general, iso-

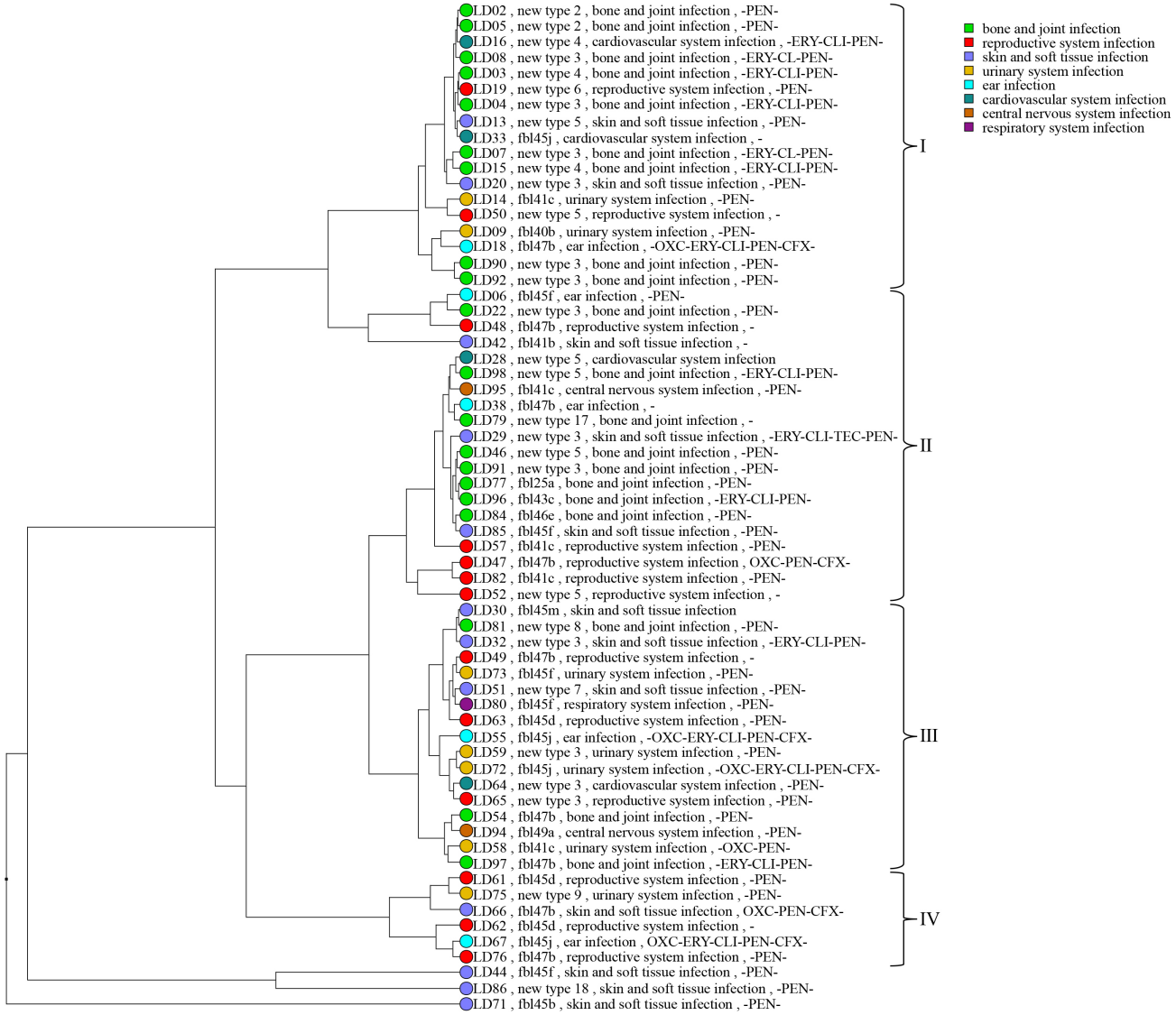

Fig. 1. MALDI-TOF MS dendrogram. Isolates were distributed mainly in six major clusters. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry, MALDI-TOF MS; CFX, cefoxitin.

lates from different infection sites were difficult to have a homogeneous cluster on the MALDI-TOF MS dendrogram.

3.5 fbl-typing Results

The *fbl*-typing was performed on 65 isolates and 27 reference strains by using VNTR method (Fig. 2). A total of 35 genotypes including 3 new repeat sequences (**Supplementary Table 1**) and 18 new genotypes (**Supplementary Table 2**) were identified. The phylogenetic tree of 92 *S. lugdunensis* was constructed in the *fbl* R-region. The phylogenetic tree could be divided into four major evolutionary branches. In this study, new type 3 was the most dominant *fbl*-type, followed by fbl47b.

Bone and joint infections distributed in clusters in branches III, mainly classified as *fbl*-type fbl43c and in several newly discovered types, such as new type 2, new type 3, new type 4, and new type 5. The distribution of bone and joint infection was significantly different between new type 3 and non-new type 3 ($p < 0.05$). In addition, the four strains isolated from ear infection were all distributed in branch I. In branch IV, there was a cluster of strains *fbl*-type fbl47b, in which LD42, reference strains SL55 and FDAARGO_S222 were isolated from skin and soft tissue infection, except reference strain NCTC12217, whose infection site was not found. As the isolates in this study are sensitive to most antibiotics, it has not been observed that the antibiotic resistance of isolates in the evolutionary tree presents a cluster distribution. However, compared with other branches, the MDR in branch I is higher than in other branches, and most of them are oxacillin-resistant isolates. It is noteworthy that all the oxacillin-resistant isolates were concentrated in *fbl*-type fbl45j and fbl47b.

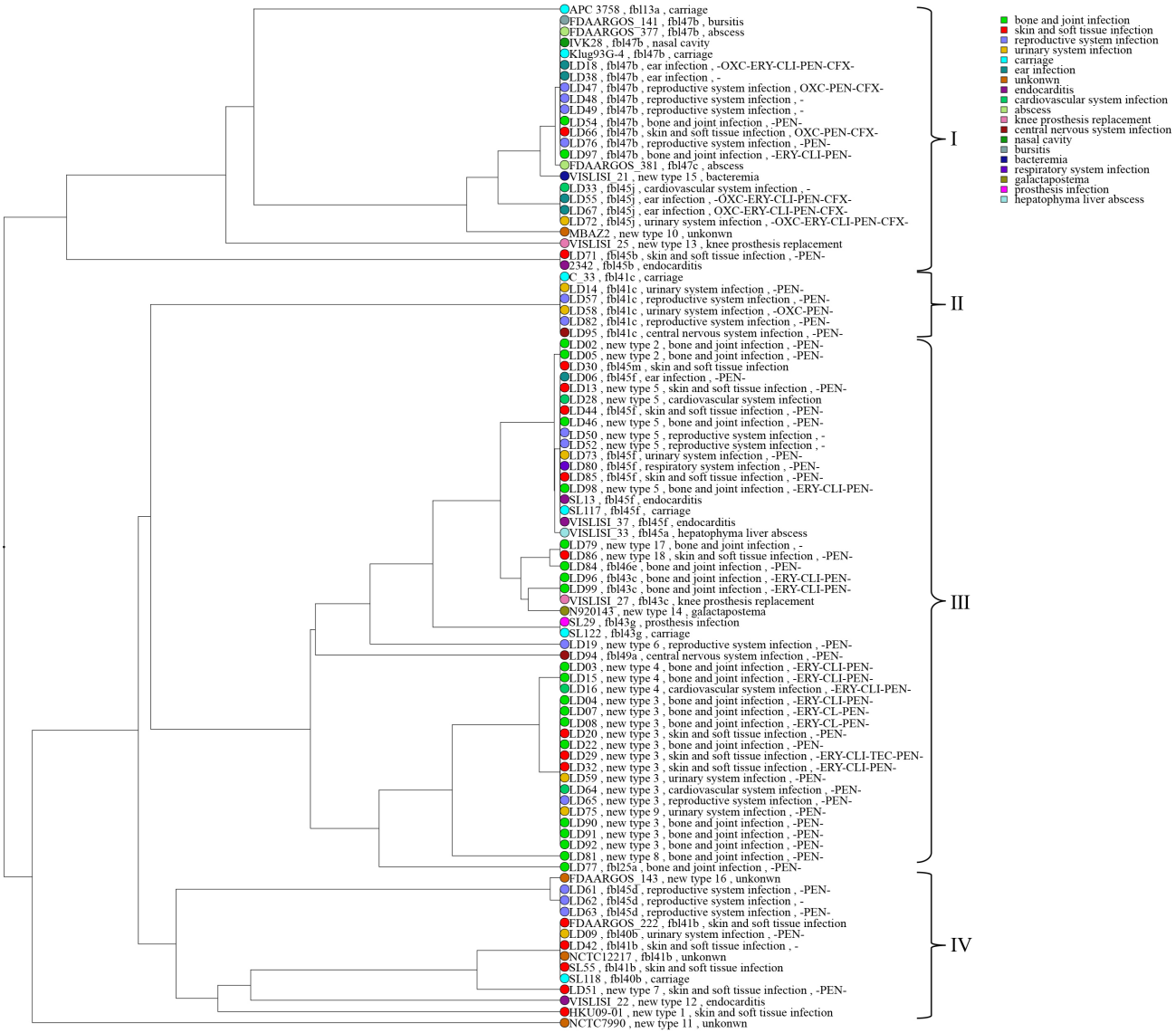

Fig. 2. The phylogenetic tree of 92 *S. lugdunensis* was constructed by using VNTR method. The phylogenetic tree could be divided into four major evolutionary branches. VNTR, variable number of tandem repeats.

4. Discussion

S. lugdunensis is a skin-colonizing bacterium, once the body is damaged, it is more likely to cause skin and soft tissue infection. However, in this hospital, the bone and joint infection type was the common type (25.4%, 19/65), which may be caused by the increase in artificial joint replacement. It is worth noting that reproductive system infection also accounted for a large proportion (21.5%, 14/65), most of which were isolated from semen, cervical or vaginal secretions, suggesting that reproductive system infection was also an important type of infection caused by *S. lugdunensis* in this hospital. The results of virulence genes showed that the seven virulence genes mentioned above existed in most strains, and there was no significant correlation between virulence gene distribution and infection type. The *atlL* gene encodes the major autolysin of *S. lugdunensis*, which plays a key role in cell division, biofilm formation, adhesion, and invasion [24–26]. Some studies have proposed that vWf-binding protein (vWbl) encoded by the *vwbl* gene can directly bind to von Willebrand factor (vWf), which is the key to resisting shear force and cause endocarditis, but other studies have pointed out that vWbl may not be the ligand of vWf [21,27]. The *vwbl* gene is widely present in various infection types, and whether it plays a key role in causing infective endocarditis still needs to be studied which are similar to Giormezis *et al*. [28]. The genes encoding beta-hemolysin and hemolysin III of *S. lugdunensis* were widely present in isolates. In addition, three open reading frames, *slush*-A, *slush*-B and *slush*-C, exist outside the AGR genetic locus, encoding *S. lugdunensis* synergistic haemolysin (SLUSH). Rautenberg *et al*. [29] proposed that SLUSH was a phenol-soluble modulins like peptide (PSM-like peptide), which acted similarly to *S. au-*

reus PSM and can bind to formylpeptide receptor 2 (FPR2) of human leukocytes, which may be associated with its ability to cause invasive infections. The detection rate of *slush* gene was significantly different. Giormezis *et al*. [28] proposed that the *vwbl* and *slush* were more common in isolates of deep infection, but Szabados *et al*. [20] reported that the detection rate of the *slush* gene was low in all infection types, and there was no difference in distribution among all infection types. In this study, the detection rate of *slush* was high, showing no difference in distribution among different infection types. Therefore, the distribution of each virulence gene and its pathogenesis still need to be further studied. Due to the lack of research, it is not clear whether the distribution of virulence genes varies across regions and infection types.

Although the antibiotic resistance of CoNS is serious at present, *S. lugdunensis* in this study is sensitive to most antibiotics in the clinic. There were significant differences in penicillin resistance rates among different reports, and penicillin resistance was serious in this study [30]. The oxacillin-resistant rate of *S. lugdunensis* was 11.1%, and the MDR was 25.4%, which should be paid more attention to clinically. Comparing the antibiotic resistance rates of *S. lugdunensis* isolates from different infection sites, it was found that the antibiotic resistance rates of bone and joint infections and ear infections isolates were generally higher than those of other infection isolates. Three isolates of four ear infections *S. lugdunensis* (antibiotic resistance spectrum: OXC-ERY-CLI---------PEN----CFX--) were oxacillin-resistant isolates, which was higher than the oxacillin-resistance rate for non-ear infections (7.1%, 4/56), and the difference was statistically significant ($p < 0.05$). These results suggest that the majority of ear infections caused by *S. lugdunensis* are multiple resistance isolates, and the antibiotic should be guided by the results of the antibiotic sensitivity test before the empirical use of antibiotics. After the isolates were divided into the oxacillin-resistant group and oxacillin-sensitive group, the antibiotic resistance rates of ERY, CLI and PEN in the oxacillin-resistant group were higher than those in the oxacillin-sensitive group, the difference was not statistically significant ($p > 0.05$). Therefore, although all isolates in this study were sensitive to most antibiotics, the antibiotic resistant isolates of oxacillin still need to be concerned.

In order to explore an appropriate typing method to distinguish different pathogenic phenotypes of *S. lugdunensis*, we created a MALDI-TOF MS dendrogram and performed *fbl*-typing by BioNumerics software. Compared with the two methods, we found that the isolates from different infection sites were difficulty having the tendency of cluster distribution on the MALDI-TOF MS dendrogram, and the recognition and reproducibility were poor. With some reports, MALDI -TOF MS excepts for identification has also been explored for typing of diverse microorganisms (MALDI-typing) [31,32]. But due to the high overall similarity of MS fingerprints within species and the difficulty of reproducibly detecting sufficient numbers of biomarkers below species-level specificity. It was proposed by researchers that MALDI-TOF MS should be established and performed as a robust typing tool under standard culture conditions, i.e., using a single medium type, incubation time and sample preparation procedure [15,33]. In this study, 65 strains of *S. lugdunensis* were classified by *fbl*-typing, among which 30 strains were of novel type and New type3 was the dominant type. The comparison between the two dendrograms showed that the clustering trend of isolates from different infection sites in the two dendrograms had low similarity. The *fbl*-typing of 240 *S. lugdunensis* by Dahyot *et al*. [14], the dominant type was fbl47b. The new type 3 collected in our study was the dominant type, followed by fbl47b, indicating that the epidemic types of *S. lugdunensis* were different in different regions, and it was necessary to continuously monitor it. Phylogenetic tree construction showed that isolates from different infection types had a cluster distribution trend, suggesting that *fbl*-typing could indicate the pathogenicity of *S. lugdunensis* in this hospital.

5. Conclusions

In conclusion, the virulence genes of *S. lugdunensis* in our study were not significantly correlated with the infection sites. And the isolates were sensitive to most antibiotics, but attention should be paid to the oxacillin-resistance isolates. Compared with the MALDI-TOF MS dendrogram, *fbl*-typing could better correspond to the pathogenic phenotype. *fbl*-typing is a method of molecular typing based on the polymorphism of *fbl* gene repeats, which has the advantages of convenient operation, low cost, and reproducide, it is worth promoting and supplementing. Based on *fbl*-typing, the antibiotics resistance and infection sites of 65 *S. lugdunensis* were analyzed, it was found that the antibiotics resistance spectrum and infection sites of isolates had a certain distribution pattern in the phylogenetic tree. Our study provided basic data for the epidemic characteristics of *S. lugdunensis* in this hospital and supplementary data for *fbl*-typing. We concluded that *fbl*-typing is a excellent method for rapid genotyping of *S. lugdunensis* and can be used as an important tool in molecular epidemiological investigation, have been developed to analyze the genetic characteristics and phylogeny of *S. lugdunensis* from different sources.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

GC—Conceptualization, Methodology, Data curation. YH—Conceptualization, Methodology, Writing-Original draft preparation. LY—Methodology, Data curation. QZ—Software, Validation. JL—Conceptualization, Methodology, Writing - Reviewing and Editing. ZW—Conceptualization, Methodology, Writing - Reviewing and Editing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2808165.

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