

Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Hospitalized Patients in Eastern Heilongjiang Province, China

Xueying Yang¹
Jing Zhao¹
Yong Wang¹
Jian Wu²
Xiaohong Wang¹
Yuchao Wang¹
Yiru Zhang¹
Huiling Li¹

¹Department of Microbiology, The First Affiliated Hospital of Jiamusi University, Jiamusi, 154002, Heilongjiang, People's Republic of China; ²Department of Biochemistry, Jiamusi Maternal and Child Health Hospital, Jiamusi, 154002, Heilongjiang, People's Republic of China

Background: Recently, owing to antibiotic resistance, the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization among intensive care unit (ICU) patients has increased rapidly. So far, there are few studies on active screening of MRSA. The purpose of the current study was to verify the effectiveness of active screening and analyze the molecular epidemiological characteristics of MRSA in the region.

Methods: We collected 30 samples of the MRSA strains from a tertiary hospital in the Eastern Heilongjiang Province. Among them, 7 were retrieved through nasal vestibular swabs at the emergency ICU and 23 were obtained from clinical specimens. Additionally, relevant patient medical information was examined retrospectively and molecular epidemiology and risk factor analysis for MRSA were performed.

Results: Molecular epidemiology studies revealed that all strains of bacteria carried the *mecA* resistance gene. The Panton Valentine leukocidin (PVL), for instance, was detected at a rate of 13.33% (4/30). The *Staphylococcus aureus* protein A (*spa*) types, found amongst our samples, were mainly t324, t437, t034, etc., and we discovered a new *spa* type t19702. We also revealed 3 types of SCCmec, namely, SCCmec type II, SCCmec type IVa, and SCCmec type V, with the most prevalent clonotypes being ST72 and ST59. In addition, we also found 7 new ST types, namely, ST6567, ST6568, ST6569, ST6570, ST6571, ST6572, and ST6573. Using risk factor analysis, we also demonstrated that long, invasive procedures used in the ICU, such as tracheal intubation and ventilator usage, along with patients with cerebral infarction and other embolism are more susceptible to developing MRSA colonization and further infections.

Conclusion: We recommend the infection control department within hospitals to actively screen for MRSA and perform risk factor analysis in order to establish accurate preventive measures for controlling MRSA spread.

Keywords: methicillin-resistant *Staphylococcus aureus*, MRSA, active screening, *mecA*, molecular epidemiology, risk factors, Panton Valentine leukocidin

Introduction

In recent years, susceptible strains of *Staphylococcus aureus*, exposed to antibiotics, have become resistant.¹ As a result, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a common pathogen causing infections in hospitalized patients.² It has also been implicated in surgical site infections (SSI).³ MRSA strains produce Panton Valentine leukocidin, an exotoxin that can infect skin and soft tissues.⁴ Around the world, many different countries and regions including Europe, Africa, and China have conducted research on the molecular epidemiology of MRSA.^{5–7}

Correspondence: Huiling Li
Department of Microbiology, The First Affiliated Hospital of Jiamusi University, Jiamusi, 154002, Heilongjiang, People's Republic of China
Tel +86 15694549052
Email lihuilinwujian@163.com

Nevertheless, there is a lack of research on the epidemiological characteristics of MRSA in the Eastern part of Heilongjiang Province.

The principle drug resistance gene in MRSA strains is *mecA*, which is also known to exist in coagulase-negative *Staphylococci* (CoNS).⁸ In addition, MRSA carrying the *mecC* gene has also been reported.⁹ The common colonization sites of MRSA are the nasal vestibule, pharynx, gastrointestinal tract, and axilla. Over the years, the MRSA colonization rate, producing infections, has dramatically increased among different populations, the most susceptible being children and elderly >85 years of age.^{10,11} As such, active screening of MRSA strains has become both necessary and urgent. In this study, we demonstrated that the active screening of nasal vestibular swabs in ICU patients can successfully achieve early detection, early intervention, and early treatment of hospitalized patients. This protocol can, therefore, effectively prevent the extensive spread of MRSA strains among hospitalized patients.

Materials and Methods

Strain Source and Ethical Statement

Seven MRSA strains were obtained from active screening of the nasal vestibular swabs and 23 MRSA strains were collected during clinical work. The *Staphylococcus aureus* strain ATCC25923 was employed as quality control. For patients who actively screened, we obtained oral consent from the patients. The study protocol was approved by the Ethics Committee of Jiamusi University Clinical Medical College (Approval number 0326).

Active Screening Methods

We utilized a bilateral collection of nasal vestibular swabs. The specific protocol of sample retrieval was as follows: a sterile cotton swab was moistened with sterile sodium chloride before slowly inserting it into the patient's nasal vestibule, followed by gentle rotation of the swab for 5 seconds to collect as much of the nasal specimen as possible. The same swab was used to collect specimens from both nostrils. The retrieved specimen was immediately inoculated in a special medium like Mannitol Salt Agar. *Staphylococcus aureus* formed a yellow-colored colony on Mannitol Salt Agar.¹²

DNA Extraction

We used the boiling procedure of bacterial DNA extraction. In brief, 5 hemolytic colonies, cultured overnight on

the blood agar, were selected and mixed with 1 mL of sterile sodium chloride, followed by centrifugation, removal of supernatant, addition of 100 μ L of DNA extract to the pellet, gentle mixing of the pellet in the DNA extract, and heating at 100°C for 10 min. Subsequently, the sample was centrifuged again at 13000 rpm for 1 min and the supernatant, carrying the bacterial DNA, was maintained at -20°C until needed.

Antimicrobial Susceptibility Test (AST)

The antibiotic minimum inhibitory concentrations (MICs) of all MRSA strains were detected using the Siemens Walk Away 40 plus drug susceptibility machine. The MIC results were further confirmed using the disk diffusion method. All drug susceptibility data were based on the criteria developed by the Clinical and Laboratory Standard Institutes (CLSI-2020). The *Staphylococcus aureus* strain ATCC25923 was employed as the control.

Polymerase Chain Reaction (PCR) to Detect the Resistance Gene and Virulence Gene

The drug resistance gene *mecA* and the virulence gene PVL of MRSA strains were identified using PCR. The primer sequences used for the *mecA* gene are as follows: MecA1 (5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3') and MecA2 (5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'), as described previously.¹³ Alternately, the PVL gene was identified using previously reported primers,¹⁴ specifically, luk-PV-1 (5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3') and luk-PV-2 (5'-GCA TCA AST GTA TTG GAT AGC AAA AGC-3'). The sequencing results were confirmed using GenBank database BLAST search.

Spa Typing and SCCmec Typing

The *Staphylococcus aureus* spa gene X region was amplified using ordinary PCR. The primer sequences of the spa gene used were 1095F (5'-AGACGATCCTTCGGTGAGC-3') and 1517R (5'-GCTTTTGCAATGTCATTTACTG-3'), as described previously.¹⁵ Subsequently, the sequencing results were compared and analyzed using the spa dedicated website (<https://www.spaserver.ridom.de/>). Unlike traditional PCR procedure, the current SCCmec typing used a multiplex PCR method, involving nine pairs of primers and with strict reaction parameters requirements. The sequence and reaction parameters were set, according

to published literature.¹⁶ Lastly, SCCmec amplified products were analyzed using 2% agarose gel electrophoresis.

Homology Analysis

When the drug susceptibility spectrum of pathogens from the same department is the same or similar, the homology is highly suspected, and then the MLST genotyping method is used to confirm. In order to better reflect the molecular epidemiological characteristics of the region, we have done homology analysis of all 30 MRSA strains.⁷ Housekeeping genes of *Staphylococcus aureus* were selectively amplified using PCR, sequenced, and compared to the PubMLST database (<http://mlst.zoo.ox.ac.uk>) to obtain ST typing. The primers used were based on published literature.¹⁷

Statistical Analysis

MRSA risk factor analysis used SPSS 22.0 for binary logistic regression analysis. Significance threshold was set at p-value of ≤ 0.05 .

Results

Antimicrobial Susceptibility

MRSA strains drug susceptibility was evaluated using the Siemens Walk Away 40 plus drug susceptibility machine. Based on our results, all examined MRSA strains contained multi-drug resistance (Table 1). In fact, the resistance rate of amoxicillin-clavulanic acid, ampicillin-sulbactam, penicillin, ceftriaxone, ceftiofloxacin, and oxacillin was 100%, whereas the resistance rate of clindamycin and erythromycin was 83.3%, levofloxacin was 10%, and ciprofloxacin was 16.7%. We also demonstrated that among the effective drugs for MRSA strains were gentamicin, vancomycin, and linezolid. Our specimens did not contain any Vancomycin-resistant *Staphylococcus aureus*.

MRSA Resistance Gene and Virulence Gene

All 30 MRSA strains examined contained the *mecA* gene, whereas only 4 MRSA (13.3%) carried the virulence gene PVL. For further confirmation, both *mecA* and PVL genes were sequenced and compared to the GenBank database, namely to the *mecA* gene (GenBank accession number AB236888) and the PVL genes (GenBank accession numbers X72700 and AB006796) respectively. Our results showed that none of the 7 actively screened MRSA strains carried the PVL gene.

Spa Typing and SCCmec Typing

We further amplified the variable region of the *spa* gene, using PCR, and found polymorphism or fragments with varying sizes. The product was about 300 bp. Upon sequencing and comparing to the GenBank database, we found multiple *spa* types; mainly t324, followed by t437 and t034, along with smaller amounts of t9353, t2465, t8347, t664, t091, etc. We also discovered a new *spa* type among the actively screened strains, namely t19702 (Table 2). We, next, performed SCCmec typing using multiplex PCR, which, unlike traditional PCR, requires multiple pairs of primers and has strict reaction parameters requirements. The sequencing results were further confirmed with GenBank database BLAST search. We discovered a total of 3 SCCmec typings, namely, SCCmec type II, SCCmec type IVa and SCCmec type V (GenBank accession numbers D86934, AB063172 and AB12121), of which the latter two types were prevalent among community-related MRSA. Internal standard gene *MecA147* (GenBank accession number X52593) was used as the control.

Homology Analysis

Among the 7 actively screened MRSA strains, the most common type was ST59, followed by ST72, ST7, ST398, and ST2690. Among other MRSA strains, ST861 was also found. We, also, discovered 7 new ST types, namely, ST6567, ST6568, ST6569, ST6570, ST6571, ST6572, and ST6573. The strain sequences were further confirmed with MLST sequence typing reference database (<http://mlst.zoo.ox.ac.uk>). The genetic relationship between the strains is summarized in Figure 1. This figure is uploaded separately as an attached file.

Risk Factor Analysis for MRSA Colonization and Infection

We performed binary logistic regression analysis on 30 experimental MRSA strains and 52 methicillin-sensitive *Staphylococcus aureus* (MSSA) strains. Based on our results, ICU hospitalization history, cerebral infarction and other embolism, tracheal intubation, ventilator usage, and other invasive procedures provide a high risk for MRSA colonization (Table 3).

Discussion

The principle antibiotic resistance pathway used by MRSA involves the *mecA* gene, which produces a low-affinity

Table 1 Antimicrobial Susceptibility, Molecular Characteristics, and Outcomes of MRSA

Isolate No.	MIC (µg/mL)													PVL	Culture Type	Outcome	MLST	
	AMC	AMS	P	OX	CD	CRO	FOX	E	LEV	CIP	GEN	VA	LZD					SXT
MRSA01	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Blood	Survived	ST59
MRSA02	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Secretion	Survived	ST72
MRSA03	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST72
MRSA04	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Blood	Died	ST72
MRSA05	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST72
MRSA06	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST398
MRSA07	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Nasal swab	Survived	ST2690
MRSA08	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST6567
MRSA09	R	R	R	R	R	R	R	R	R	R	R	R	S	S	+	Pus	Survived	ST6568
MRSA10	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Secretion	Survived	ST6567
MRSA11	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Nasal swab	Survived	ST59
MRSA12	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Nasal swab	Died	ST6568
MRSA13	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Nasal swab	Survived	ST72
MRSA14	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST861
MRSA15	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST6570
MRSA16	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Nasal swab	Died	ST398
MRSA17	R	R	R	R	R	R	R	R	R	R	R	R	S	S	+	Puncture fluid	Survived	ST59
MRSA18	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Wound	Survived	ST72
MRSA19	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST59
MRSA20	R	R	R	R	R	R	R	R	R	R	R	R	S	S	+	Wound	Survived	ST59
MRSA21	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Nasal swab	Died	ST59
MRSA22	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Nasal swab	Died	ST7
MRSA23	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST72
MRSA24	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST72
MRSA25	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Puncture fluid	Survived	ST6567
MRSA26	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Puncture fluid	Survived	ST6569
MRSA27	R	R	R	R	R	R	R	R	R	R	R	R	S	S	+	Pus	Survived	ST6571
MRSA28	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST72
MRSA29	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST6572
MRSA30	R	R	R	R	R	R	R	R	R	R	R	R	S	S	-	Sputum	Survived	ST6573

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; AMC, amoxicillin-clavulanic acid; AMS, ampicillin-sulbactam; P, penicillin; OX, oxacillin; CD, clindamycin; CRO, ceftriaxone; FOX, cefoxitin; E, erythromycin; LEV, levofloxacin; CIP, ciprofloxacin; GEN, gentamicin; VA, vancomycin; LZD, linezolid; SXT, trimethoprim/sulfamethoxazole; PVL, Pantone Valentin leukocidin; MLST, multilocus sequence typing.

Table 2 The Characteristics and Typing of the X District of MRSA Spa Gene Repeats

Serial Number	Spa Repetitive Coding Feature	Repeat Number	Genotype	Strain	Proportion (%)
1	r07r23r12r12r17r20r17r12r12r17	10	t324	MRSA-2,3,4,5,8,10,23,24,29	30.00
2	r04r20r17r20r17r25r34	7	t437	MRSA-1,12,17,20,26	16.70
3	r08r16r02r25r02r25r34r24r25	9	t034	MRSA-6,16,27,30	13.33
4	r07r23r12r12r17r20r17r12r17	9	t664	MRSA-14,18	6.67
5	r04r20r17r20r17r45r16r34	8	t163	MRSA-19	3.33
6	r07r23r21r17r34r12r23r02r12r23	10	t091	MRSA-22	3.33
7	r07r23r12r17r20r17r12r12r17	9	t901	MRSA-28	3.33
8	r04r20r17r20r17r25r34r34r34	9	t8347	MRSA-11	3.33
9	r04r34r21r17r21r17r34r22r25	9	t4549	MRSA-15	3.33
10	r26r17r34r34r17r20r17r17r16	9	t9353	MRSA-7	3.33
11	r04r20r17r13	4	t2465	MRSA-9	3.33
12	r04r20r17r20r17r25	6	t3592	MRSA-21	3.33
13	r07r23r12r12r17r20r17r12r17r17	10	t13719	MRSA-25	3.33
14	r07r23r12r12r17r20r17r17r12r12r17	11	t19702	MRSA-13	3.33

Note: Proportion (%): Number of strains per genotype/Total number of strains (30 MRSA strains).

penicillin-binding protein, namely PBP2a, thereby leading to resistance. Simultaneously, *mecA* belongs to the SCCmec mobile genetic element. Therefore, the drug resistance gene *mecA* can be transferred horizontally with SCCmec, conferring antibiotic resistance to other *Staphylococcus aureus* strains. As a result, the MRSA

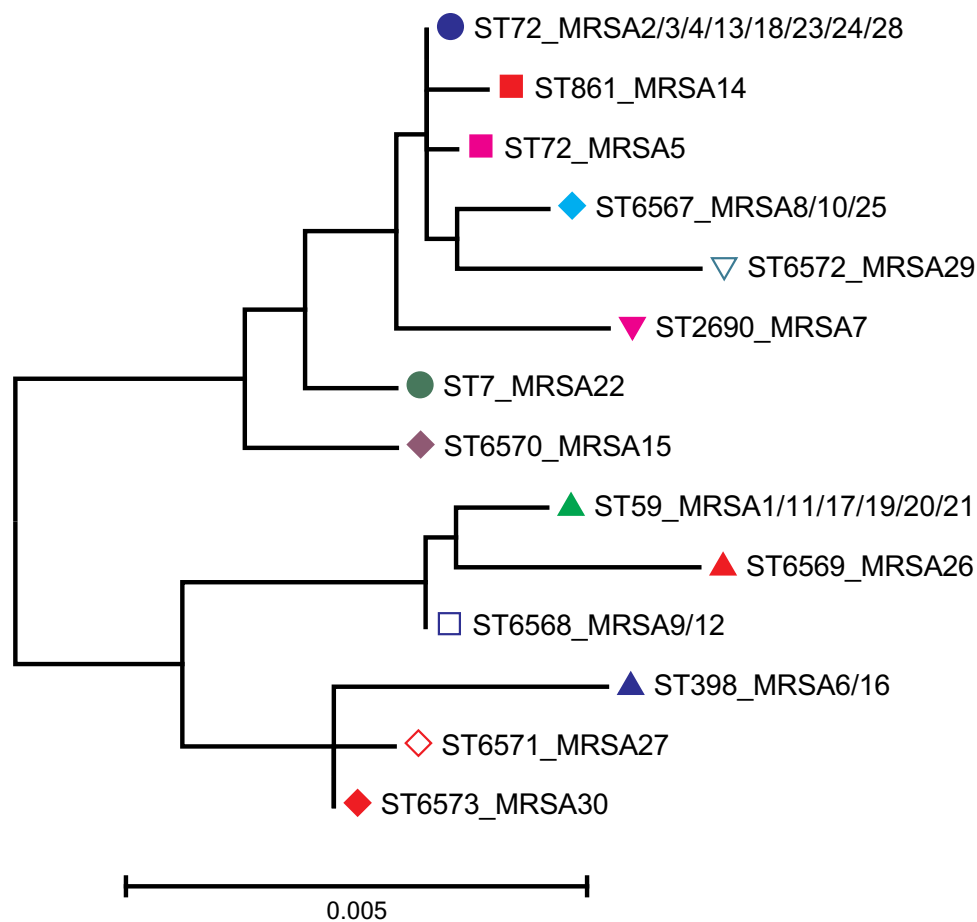
**Figure 1** Phylogenetic tree of 30 methicillin-resistant *Staphylococcus aureus* isolates

Table 3 Risk Factor Analysis of MRSA Colonization

Variables	P value	OR	95% CI (Lower Limit)	95% CI (Upper Limit)
Diabetes	0.724	2.025	0.040	101.548
Hemodialysis	0.756	1.890	0.034	105.534
ICU hospitalization history	0.007	0.054	0.007	0.447
Cerebral infarction and other embolism	0.036	44.900	1.281	1573.698
Lung disease	0.502	0.342	0.015	7.853
Nasal feeding tube	0.357	0.174	0.004	7.181
Hypertension	0.275	0.176	0.008	3.986
Tracheal intubation/Ventilator	0.003	91.916	4.674	1807.636
Heart failure	0.883	1.205	0.101	14.439

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

strains, in community and hospital environments, have become highly resistant to antibiotics.¹⁸ Unfortunately, there are reports that the resistance of *Staphylococcus aureus* to methicillin has increased, even in people not exposed to hospital risk factors.¹⁹

MRSA strains are mainly separated into hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA), based on its existence location and strain characteristics. HA-MRSA can usually be found in patients after a 48 h hospital stay, whereas, CA-MRSA can potentially be present in patients within 48 hours of outpatient or hospitalization visitation. HA-MRSA strains usually carry SCCmec type I, SCCmec type II, and SCCmec type III, whereas, CA-MRSA carried SCCmec type IV or SCCmec type V. However, multiple studies have demonstrated that the CA-MRSA and HA-MRSA strains can coexist within communities and hospital environments. For example, in Brazil, both SCCmec types IVa and V were detected in the HA-MRSA isolates.^{20,21}

This article is the first published report on the active screening and molecular epidemiological research of MRSA in hospitalized patients in Eastern Heilongjiang Province. The active MRSA screening was performed via nasal vestibular swabs from ICU patients. Using the active screening method, we isolated 7 MRSA strains. We further extracted 23 MRSA strains from clinical specimens. The *mecA* resistance gene was discovered in all examined strains, and only 4 strains (4/30; 13.33%) contained the PVL gene, which is similar to a report from a dental hospital in Egypt.²² Interestingly, studies show that SCCmec carries

the resistance gene, and the resistance gene can spread horizontally among different *staphylococcal* species.²³ Using multiplex PCR, we isolated three forms of SCCmec, namely, SCCmec type II, SCCmec type IVa, and SCCmec type V, of which SCCmec type IVa was the most abundant. This finding is in accordance with the SCCmec classification in Hainan and with a study by Alkharsah et al^{24,25}. We further used PCR to amplify the variable region of the *spa* gene in *Staphylococcus aureus*. Upon GenBank database comparison and analysis of the sequencing results, a total of 14 *spa* types were found, of which the main types were t324, t437, and t034. In addition, there were t9353, t2465, t8347, t664, and so on, among our samples. Notably, we also found a new classification, termed t19702. There is no general consensus on the ST typing of MRSA. The main types of MRSA strains in our hospital were ST72, ST59, ST398, ST861, and other ST types. Among them, we discovered 7 new ST types, namely, ST6567, ST6568, ST6569, ST6570, ST6571, ST6572, and ST6573. Our finding is consistent with a study conducted in Iran, who also reported MRSA ST typing, including ST1465, ST861, ST889 and ST772.²⁶ In Eastern Heilongjiang Province, the most important MRSA clones were ST59-SCCmecIV-t437 and ST72-SCCmecIV-t324. In Shenzhen, the main MRSA clone was ST59-SCCmecIV-t437.²⁷ In Northwest China, the most common clone was ST239-SCCmecIII-t030.²⁸ Based on published reports, the ST398 typing is a zoonotic clonotype.²⁹

MRSA infection can infect the skin and soft tissues, induce bacteremia, and endocarditis, and significantly increase the

risk of morbidity and mortality.³⁰ A combination of daptomycin and ceftaroline has been reported to lower fatality risk of MRSA bacteremia patients.³¹ In this study, a binary logistic regression analysis was performed to assess the risk factors of MRSA. Based on our analysis, cerebral infarction, embolism in other areas, ICU hospitalization, and invasive procedures like tracheal intubation and ventilator usage provided the main risk factors for MRSA colonization. Therefore, we recommend physicians to remain vigilant and provide timely intervention measures to high-risk patients, in order to prevent MRSA infection. The colonization rate of MRSA in ICU patients in mainland China is remarkably high, especially in patients whose nasal vestibular swabs test positive for MRSA.³² There are also reports of MRSA strains extracted from the tracheal intubation of ICU patients.³³ Other researchers have suggested that the ICU admission history may be a risk factor for MRSA infection.³⁴ Therefore, active monitoring of MRSA in ICU patients is crucial and must be considered. Additionally, there are emerging evidences of MRSA surges in non-hospital related environments. One study revealed that chronic skin diseases and invasive procedures, conducted in nursing homes, can elevate MRSA infection risk.³⁵ In addition, patients with weakened immunity and diabetes are prone to MRSA infection.^{36,37} Moreover, medical staff have been reported to carry MRSA, increasing the potential for transmission in both hospital and non-hospital environments.³⁸ There are also reports of livestock-related MRSA (LA-MRSA) colonization, suggesting a possible spread of MRSA strains between humans and animals.^{39,40} Special care needs to be given in all of these special cases to maintain MRSA infections to a minimum. In future research, we plan to screen ICU medical staff for MRSA, which has special significance for the prevention and control of MRSA in hospitals.

Conclusions

In summary, we report here, for the first time, the epidemiological characteristics of MRSA in Eastern Heilongjiang Province and recommend measures for active ICU screening of MRSA. Active screening proved to be an effective preventive measure for MRSA infection. The main MRSA clones in this area, according to our study, were ST72 and ST59. With the use of active ICU patient screening and risk factors analysis of MRSA colonization, clinical intervention can be performed on drug-resistant bacteria to prevent infection. This is of great significance in the improvement of patient survival and in preventing further spread of this drug-resistant bacteria.

Abbreviations

MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-sensitive *Staphylococcus aureus*; CoNS, Coagulase-negative *Staphylococci*; ICU, Intensive Care Unit; CLSI, Clinical and Laboratory Standard Institutes; PVL, Pantone Valentine leukocidin; Spa, *Staphylococcus aureus* protein A; SCCmec, *Staphylococcal* cassette chromosome mec; MICs, Minimum inhibitory concentrations; AST, Antimicrobial susceptibility test; PCR, Polymerase chain reaction; MLST, Multilocus sequence typing.

Data Sharing Statement

Any undisclosed data from this study is available upon request.

Ethics Approval and Consent to Participate

All protocols were agreed upon by the Ethics Committee of Jiamusi University Clinical Medical College (0326). Patient informed consent was waived as the samples were collected from existing specimens retrieved during routine procedures and did not require further involvement from patients. For patients who actively screened, we obtained oral consent from the patients.

Consent for Publication

Not applicable.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, drafting and revising the article, gave final approval of the version to be published, have agreed on the journal to which the article will be submitted and agreed to be accountable for all aspects of the work. The final manuscript was reviewed and approved by all authors.

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Disclosure

The authors have no competing interests.

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