Molecular characterization and susceptibility of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from hospitals and the community in Vladivostok, Russia

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Abstract

A prospective study was conducted during an 8-month period, from August 2006 to April 2007, to describe the epidemiology of *Staphylococcus aureus*-associated infections. In addition, the molecular characteristics, antimicrobial susceptibilities and antibiotic resistance determinants were identified in *S. aureus* isolates from hospitals and the community in Vladivostok, Russia. Among the 63 *S. aureus* isolates eligible for this study, methicillin resistance was observed in 48% (n = 30). Hospital-acquired strains accounted for 93% (28/30) of all methicillin-resistant *S. aureus* (MRSA) isolates. The major MRSA clone (sequence type (ST) 239, staphylococcal cassette chromosome *mec* (SCC*mec*) type III, Panton–Valentine leukocidin (PVL)-negative, with two related staphylococcal protein A gene (*spa*) types (types 3 and 351)) represented 90% of all of the MRSA isolates. This clone was multidrug-resistant, and 41% of isolates showed resistance to rifampicin. Community-acquired MRSA isolates (n = 2) were categorized as ST30, SCC*mec*IV, *spa* type 19, and PVL-positive, and as ST8, SCC*mec*IV, of a novel *spa* type 826, and PVL-negative. Eight different STs were detected among methicillin-susceptible *S. aureus* (MSSA) isolates, of which 55% were PVL-positive. One MSSA clone, which was categorized as ST121, *spa* type 273, and PVL-positive, caused fatal community-acquired pneumonia infections. The strains predominantly isolated in hospitals in Russia belonged to the multidrug-resistant Brazilian/Hungarian ST239 MRSA clone; however, this clone has new antibiotic susceptibilities. Additionally, the emergence of PVL-positive MSSA strains with enhanced virulence was observed, warranting continued surveillance.

Keywords: Antimicrobial susceptibility, community-acquired, hospital-acquired, molecular typing, Russia, *Staphylococcus aureus*
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Introduction

Staphylococcus aureus is of special concern because of its ability to cause a number of life-threatening conditions and its widening resistance to currently available antimicrobial drugs [1]. Methicillin-resistant *S. aureus* (MRSA), which harbours the staphylococcal cassette chromosome *mec* (SCC*mec*), has become a leading cause of hospital-acquired infections worldwide, accounting for >60% of *S. aureus* isolates in US hospitals [2]. Molecular epidemiological studies have shown the spread of several MRSA clones internationally, in the hospital setting. These epidemic hospital-acquired MRSA (HA-MRSA) clones have been identified as the Archaic/Iberian (sequence type (ST) 247, SCC*mec*I), Brazilian/Hungarian (ST239, SCC*mec*III), Berlin (ST45, SCC*mec*IV), New York/Japan (ST5, SCC*mec*II), paediatric (ST5, SCC*mec*IV), EMRSA-15 (ST22, SCC*mec*IV) and EMRSA-16 (ST36, SCC*mec*II) clones [3].

Since the mid-1990s, MRSA infection in healthy individuals who do not have any of the known risk factors for MRSA has increased. These community-acquired MRSA (CA-MRSA) strains have a different genetic background from the HA-MRSA strains, belong mainly to (ST1 (USA400; SCC*mec*IV), ST8 (USA300; SCC*mec*IV), ST30 (SCC*mec*IV), ST59 (USA1000; SCC*mec*IV), and ST80 (SCC*mec*IV), and are often associated with the production of Panton–Valentine leukocidin (PVL) [4]. PVL has been implicated in the pathogenesis of severe infections caused by CA-MRSA, especially pneumonia [5].

Methicillin-sensitive *S. aureus* (MSSA) isolates show greater genetic diversity than MRSA isolates, and they provide a pool of organisms for the emergence of new MRSA clones [6]. Hence, knowledge of the molecular characteristics of MSSA is essential for controlling the potential emergence of new epidemic MRSA clones.

Data on the antimicrobial resistance of *S. aureus* in Russia have been reported [7], but the data on clonality, virulence gene profiles and genetic determinants of antibiotic resistance remain incomplete. The aims of this study were to analyze the genetic characteristics of both community-acquired and hospital-acquired MRSA and MSSA strains isolated in Vladivostok, Russia, and to evaluate the antimicrobial susceptibilities of the isolates and the presence of antibiotic resistance genes.

Materials and Methods

Bacterial strains

S. aureus isolates were collected from paediatric and adult inpatients and outpatients using a systematic random sampling method at four hospital laboratories in Vladivostok (the largest city in the Primorsky region of Russia), from August 2006 to April 2007. The laboratories served the four Vladivostok city hospitals, which housed a combined total of 2124 beds and had more than 5000 outpatient visits per year. Each of the laboratories was asked to provide a maximum of two *S. aureus* isolates per week, excluding samples that were taken for 'screening' purposes. Patient data on demographics, reason for admission, history of prior hospitalization, outcome, site of *S. aureus* infection, and site of sample collection, and information on healthcare risk factors for MRSA infection, were collected using a standard case report form. HA-MRSA and CA-MRSA infections were defined as described previously [8]. This study was approved by the Ethics Committee of the Vladivostok City Hospital.

S. aureus isolates were identified in accordance with official Russian guidelines, using Gram staining, analysis of catalase production, a tube coagulase test in 5% rabbit plasma, and a lecithinase test performed on mannitol–salt agar. After confirmation of the identity of the strains at the Division of Bacteriology, Niigata University, Japan, using standard identification procedures [9], and exclusion of duplicate isolates collected from the same patient, 63 (of 170) *S. aureus* isolates were eligible for study. Data on the basic demographics of the patients and the clinical origin of *S. aureus* infection are shown in Table 1.

Positive controls for PCR assays were kindly provided by T. Yamamoto (Division of Bacteriology, Niigata University, Japan). *S. aureus* ATCC 29213 was used as a quality control strain in the MIC experiments.

Genotyping

Coagulase typing was performed using a coagulase typing kit (Denka Seiken Co. Ltd, Tokyo, Japan), according to the manufacturer's instructions. Pulsed-field gel electrophoresis (PFGE) was performed using a CHEF DR III apparatus (Nippon, Bio-Rad Laboratories) after *Sma*I digestion (Takara Bio Inc., Japan) to characterize all *S. aureus* isolates, as described previously [10]. Multiplex PCR-based protocols for allotyping the accessory gene regulator (agr) and SCC*mec*I–IV and for SCC*mec*IV subtyping (IVa, IVb, IVc, and IVd) were performed as previously described, using reference strains [11–13]. Staphylococcal protein A gene (*spa*) typing was performed using the eGenomics software package (http://tools.egenomics.com/) [14]. Multilocus sequence typing of all 30 MRSA isolates and 19 selected MSSA isolates was performed as described elsewhere [15].

Virulence gene analysis by PCR-based assays

PCR-based assays were performed as described elsewhere [16] for the following genes: four haemolysin genes (*hla*, *hld*, *hlg*, and *hlg-v*), two leukocidin genes (*lukM* and *lukE*), 18 staphylococcal enterotoxin (*se*) genes (*sea–see* and *seg–ser*), toxin shock syndrome toxin 1 (*tst*), three exfoliative toxin (*et*) genes (*eta*, *etb* and *etd*), and 11 adhesin genes (*icaA*, *icaD*, *cna*, *eno*, *fnbA*, *fnbB*, *ebpS*, *clfA*, *clfB*, *fib*, and *bbp*).

Susceptibility testing

Susceptibility testing of bacterial strains was performed using the agar dilution method according to the CLSI recommendations [17]. The tested antimicrobials included penicillin G, oxacillin, ampicillin, cefazolin, ceftazidime, cefotaxime, cefaclor, imipenem, meropenem, gentamicin, kanamycin, rifampicin, ciprofloxacin, levofloxacin, norfloxacin, trimethoprim, sulphamethoxazole, clindamycin, erythromycin, clarithromycin, azithromycin, linezolid, vancomycin, teicoplanin, chloramphenicol, doxycycline, minocycline, and tetracycline. The results of the susceptibility testing for streptomycin, fusidic acid and fosfomycin were interpreted in accordance with the recommendations of the Antibiotic Committee of the French Microbiological Society [18]. The susceptibility testing results for mupirocin were interpreted according to the manufacturer's recommendations [19]. The antimicrobial agents were gifts from their manufacturers. Inducible resistance to clindamycin was detected using the D-test with erythromycin (15 μg) and azithromycin (15 μg) disks [20].

TABLE 1. Basic demographics of patients and clinical origin and site of acquisition of 63 *Staphylococcus aureus* infections in Vladivostok, Russia, August 2006 to April 2007

Characteristics	Total no. (%) of patients/ infections, *n* = 63	Value for group (%)		p-Value
		MRSA, *n* = 30 (48)	MSSA, *n* = 33 (52)	
Patients				
Gender				
Female	21 (33)	8 (27)	13 (39)	0.2845
Male	42 (67)	22 (73)	20 (61)	
Age				
Mean years ± SD (range)	34.70 ± 20.03 (0–68)	41.00 ± 2057 (0–68)	28.97 ± 17.96 (0–68)	
Age group (years)				
0–34	30 (48)	10 (33)	20 (61)	0.0304[a]
35–68	33 (52)	20 (67)	13 (39)	
Clinical origin of infections				
Wound infection	33 (52)	21 (70)	12 (36)	0.0076[a]
Abscess (skin and soft tissue)	16 (26)	3 (10)	13 (39)	0.0094[a]
Pneumonia	9 (14)	3[b] (10)	6[b] (18)	0.4788
Bacteraemia	2 (3)	1[b] (3)	1[b] (3)	1
Joint infection	2 (3)	1 (3)	1 (3)	1
Peritonitis	1 (2)	1[b] (3)	0	0.4762

SD, standard deviation. Among three methicillin-resistant *S. aureus* (MRSA) pneumonia cases, only one had a fatal outcome; among six methicillin-susceptible *S. aureus* (MSSA) pneumonia cases, four were of community origin and all four had fatal outcomes.
[a]Significant differences.
[b]Cases with a fatal outcome.

Drug resistance gene analysis

Genes conferring resistance to β-lactams (*mecA*), tetracycline (*tetK* and *tetM*), aminoglycosides (*aac6′/aph2*) and macrolides and lincosamide (*ermA*, *ermB*, *ermC*, and *msrA/B*) were detected using PCR assays [20–22]. For fluoroquinolone resistance, mutations in *gyrA* (for DNA gyrase) and *grlA* (for topoisomerase IV) were detected with the primer set *gyrA*-F (5′-CAGTGAAATGCGTGAATC-3′) and *gyrA*-R (5′-CAATATCTTCCATTAACTCAGC-3′), and the primer set *grlA*-F (5′-GTGCATTGCCAGATGTTC-3′) and *grlA*-R (5′-TACCTTGAATAATACCACCAG-3′), respectively. These primer sets were designed on the basis on the gene sequences of MSSA strain 476 (GenBank accession number NC_002593). Screening for mutations in *rpoB*, which confer resistance to rifampicin, was performed as previously described [23].

Statistical analysis

Relationships between categorical variables were analyzed by the chi-square test or Fisher's exact test if 20% of the expected values were <5. A p-value of <0.05 was considered to be statistically significant.

Results

Characterization of *S. aureus* isolates

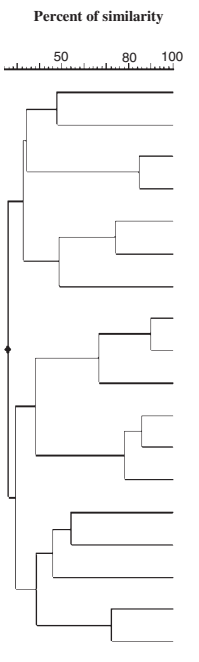
Six coagulase types were detected among the 63 *S. aureus* isolates: types IV, V, VII, VI, II, and III, representing 30 (48%), 16 (25%), seven (11%), five (8%), three (5%) and two (3%) isolates, respectively (Fig. 1). Fourteen different PFGE types (PFTs) were distinguished within the 63 *S. aureus* isolates (Fig. 1). The 30 MRSA isolates fell into four PFTs (B, D, E, and F). In contrast, the 33 MSSA isolates showed greater diversity than the MRSA isolates. Ten different PFTs (A, C, G, H, I, J, K, L, M, and N) were identified within the MSSA group, and included three PFTs that were split into subtypes (C, G, and I).

The *agr* typing allowed 42 (67%), 17 (27%) and four (6%) of the 63 *S. aureus* isolates to be classified into *agr* groups 1, 4, and 3, respectively (Fig. 1). Nearly all of the MRSA isolates (29/30) belonged to *agr* group 1. Two SCC*mec* types were identified among the 30 *mecA*-positive isolates. Twenty-seven (90%) of the *S. aureus* isolates were SCC*mec*III and three (10%) were SCC*mec*IVc (Table 2). Among the 63 isolates, 21 *spa* types were identified (Fig. 1). Twelve of the *spa* types were already recorded in the *spa* database (http://tools.egenomics.com/), and nine were novel types: 825, 826, 827, 828, 829, 830, 979, 980, and 981.

Finally, 11 ST types were identified, belonging to nine clonal complexes. One MSSA isolate had a novel ST, ST1211 (Fig. 1).

Clonal characterization of MRSA isolates

On the basis of epidemiological characteristics, 28 HA-MRSA and two CA-MRSA isolates were identified (Table 2). The major MRSA clone was exclusively HA-MRSA and comprised 90% of the isolates (27/30). Although PFGE identified two PFTs (D and E), both PFTs belonged to ST239, SCC*mec*III. PFTs D and E corresponded to *spa* type 3 (*n* = 16) and *spa* type 351 (*n* = 11), respectively. Data on the virulence gene profile and antimicrobial resistance are shown in Table 2.



PFGE patterns	Number of isolates	PFT	*spa* type	*agr* type	Coagulase	ST type	MRSA/ MSSA
	1	A	33	3	IV	30	MSSA
	1	B	19	3	IV	30	MRSA
	1	C1	184	1	II	25	MSSA
	1	C2	825*	1	II	25	MSSA
	16	D	3	1	IV	239	MRSA
	11	E	351	1	IV	239	MRSA
	2	F	826*	1	III	8	MRSA
	3	G1	466 (n=2), 827*	4	V	121	MSSA
	5	G2	830* (n=3), 828*, 829*	4	V	121	MSSA
	8	H	287	4	V	121	MSSA
	1	I1	979*	1	VII	1211**	MSSA
	1	I2	980*	1	VII	45	MSSA
	3	I3	73	1	VII	45	MSSA
	1	J	981*	3	VII	1	MSSA
	1	K	154	1	IV	6	MSSA
	1	L	23	4	II	5	MSSA
	5	M	105	1	VI	97	MSSA
	1	N	35	3	VII	1	MSSA

FIG. 1. *Sma*I macrorestriction patterns of 63 *Staphylococcus aureus* isolates from four hospital laboratories in Vladivostok, Russia and their genetic types. The pulsed-field gel electrophoresis (PFGE) types (PFTs) were defined by ≥80% similarity (UPMAG, Dice). Isolates with PFGE patterns with similarity greater than 95% were considered to belong to the same PFT. PFGE patterns of one representative isolate from each PFT are shown. The number of isolates in each PFT cluster is shown on the left side of the dendogram. The molecular characteristics of each bacterial strain are listed in Tables 2 and 3. *The novel *spa* type. **The novel sequence type (ST). MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

Strains belonging to *spa* type 351 were characteristically resistant to rifampicin and were isolated from all of the hospitals participating in this study. The molecular characterization and drug resistance profiles of the minor MRSA clones are shown in Table 2.

Clonal characterization of MSSA isolates

There were 33 MSSA isolates, separated into 17 (52%) HA-MSSA and 16 (48%) CA-MSSA strains (Table 3). The major MSSA clone comprised 48% of the isolates (16/33); they were primarily community-acquired (12 CA-MSSA and four HA-MSSA), and shared two PFTs (G and H). This clone was ST121, with six related *spa* types (828, 466, 287, 830, 829, and 827), which exhibited deletion and/or insertion of some repeats or point mutations in one of the repeats (Fig. 2). Notably, 57% (4/7) of isolates of the PFT H *spa* type 287 were community-acquired and caused pneumonia in patients, who had a mean age of 17.75 years. All of these community-acquired pneumonia cases were fatal. In spite of the difference in *spa* types, all isolates of this ST121 MSSA clone shared the same virulence gene pattern, and were PVL-positive. The molecular characterization of the minor MSSA clones is shown in Table 3.

In terms of drug resistance, 85% (28/33) of the MSSA isolates were resistant to penicillin and ampicillin. The MSSA isolates of hospital origin exhibited more antibiotic resistance than those of community origin. On the basis of the double-disk diffusion test results, all erythromycin-resistant and clindamycin-susceptible isolates showed an inducible macrolide–lincosamide–streptogramin B phenotype.

Drug resistance genes and sequence analysis

All phenotypically oxacillin-resistant isolates carried the *mecA* gene, and all of the gentamicin/kanamycin-resistant isolates possessed the *aacA*/*aphD* gene (Tables 2 and 3). The constitutive macrolide–lincosamide–streptogramin B phenotype and the *erm*(A) gene predominated among the erythromycin-resistant MRSA isolates (27/29), whereas the inducible phenotype and the *erm*(C) gene were found in all of the erythromycin-resistant MSSA isolates (7/7).

DNA sequencing of the rifampicin resistance-determining region of the rifampicin-resistant isolates revealed an H481N amino acid substitution.

Discussion

Reported here are the molecular characterization and antimicrobial susceptibilities of *S. aureus* isolates obtained from

TABLE 2. Molecular characteristics of methicillin-resistant *Staphylococcus aureus* strains isolated in Vladivostok, Russia within an 8-month period

Origin	No. of isolates, $n = 30$	Type				Presence of virulence genes		MIC for OXA, (mg/L)	Antimicrobial resistance pattern (% of strains[c]), non-β-lactam antibiotics[d]	Presence of drug resistance genes or gene mutation[e]
		PFGE	ST (clonal complex)	SCC*mec*	*spa*	Toxin genes[a]	Adhesin genes[b]			
Hospital-acquired	16	D	239 (8)	III	3	*lukE, hlg-v, sea, sek, seq*	*cna*	>128	GEN, ERY, CLI, TET, LVX, TMP, SMX, CHL (19%)	*mecA, aac6′/aph2, ermA, tetK, tetM, gyrA* (S84L), *grlA* (S80F)
	11	E	239 (8)	III	351	*lukE, hlg-v, sea, sek, seq*	*cna*	>128	GEN, ERY, CLI, TET, LVX (82%), SMX, CHL (18%), RIF	*mecA, aac6′/aph2, ermA, tetM, gyrA* (E88K), *grlA* (S80F), *rpoB* (H481N)
Community-acquired	1	F	8 (8)	IVc	826[f]	*lukE, hlg-v, sea*	–	64	GEN, ERY, CLI, CHL, RIF	*mecA, aac6′/aph2, ermC*
	1	F	8 (8)	IVc	826[f]	*lukE, hlg-v, sea*	–	32	ERY, CLI, CHL	*mecA, ermC*
	1	B	30 (30)	IVc	19	PVL, *egc*[g]	*cna, bbp*	32	–	*mecA*

OXA, oxacillin; GEN, gentamicin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; LVX, levofloxacin; TMP, trimethoprim; SMX, sulphamethoxazole; CHL, chloramphenicol; RIF, rifampicin; PFGE, pulsed-field gel electrophoresis; ST, sequence type.

[a]Other than three common genes: *hla, hlg,* and *hld.*

[b]Other than nine common genes: *icaA, icaD, eno, fnbA, fnbB, ebpS, clfA, clfB,* and *fib.*

[c]For <100%.

[d]No resistance was observed for vancomycin, teicoplanin, linezolid, minocycline, fusidic acid, or mupirocin. A representative drug of each tested antimicrobial group is shown.

[e]Genes *mecA, aac6′/aph2, ermA,C* and *tetK,M* code for resistance to methicillin, gentamicin, erythromycin–clindamycin, and tetracycline, respectively. Mutations in the *gyrA* and *rpoB* genes confer resistance to quinolones and rifampicin, respectively.

[f]The novel *spa* type.

[g]*egc*, enterotoxin gene cluster, (*seg, sei, sem, sen* and *seo.*)

outpatients and inpatients at four hospital laboratories in Vladivostok, Russia over an 8-month period in 2006–2007.

One major HA-MRSA ST239, SCC*mec*III clone, resembling the Brazilian/Hungarian clone, circulated in hospitals in Russia. The Brazilian/Hungarian clone accounts for 70–80% of the MRSA strains in the world, and its broad distribution may be due to its advantageous properties with respect to other clones, such as an enhanced ability to form biofilm and a tendency to acquire genes that confer resistance to different classes of antimicrobial agents [24].

In this study, the Brazilian/Hungarian clone was multidrug-resistant, which is in agreement with previous reports. Moreover, all of the HA-MRSA ST239-III, *spa* type 351 strains isolated in this study were also resistant to rifampicin. All rifampicin-resistant HA-MRSA ST239 isolates had the same H481N substitution in the rifampicin resistance-determining region. These observations suggest that it is the rifampicin-resistant Brazilian/Hungarian MRSA clone that is spreading through hospitals in Russia. Rifampicin is inexpensive, has a broad spectrum of antimicrobial activity, and is used in clinical practice in Russia when the aetiological agent of an infection is not yet confirmed and/or when *Mycobacterium tuberculosis* is suspected [25]. However, when rifampicin is used as monotherapy, *S. aureus* quickly develops resistance by selection for a point mutation that causes structural modifications in the cellular target of the drug. Therefore, rifampicin should be retained for the treatment of life-threatening *S. aureus* infections, e.g. necrotizing fasciitis, meningitis, or infections of bone and orthopaedic implants, but should not be used in monotherapy.

All HA-MRSA ST239 isolates belonging to the major MRSA clone were resistant to sulphamethoxazole, in contrast to the other MRSA clones (ST8 and ST30) and all of the MSSA clones isolated in this study. This result suggests that sulphamethoxazole might serve as a phenotypic marker with which to screen for the major HA-MRSA ST239 clone in Russia.

Also isolated from both community-acquired and hospital-acquired infections was the PVL-negative ST8 MRSA clone. The molecular characteristics of this clone were similar to those of the Lyon clone (ST8, SCC*mec*IV), which is present throughout hospitals in France, where it replaced the previously dominant gentamicin-resistant Iberian clone (ST247, SCC*mec*I) [26]. Although the prevalence of this clone is currently low in Russia, the tendency of this clone to replace other clones warrants its continuous monitoring in the coming years.

PVL is considered to be a marker of CA-MRSA infections in some countries. In this study, only one CA-MRSA isolate was PVL-positive. In contrast to PVL-positive MRSA, an exceptionally high prevalence (55%) of PVL-positive MSSA

TABLE 3. Molecular characteristics of methicillin-sensitive *Staphylococcus aureus* strains isolated in Vladivostok, Russia within an 8-month period

Origin	No. of isolates, *n* = 33	Type			Presence of virulence genes		MIC for OXA (mg/L)	Antimicrobial resistance pattern (% of strains[d])	Presence of drug resistance genes or gene mutation[e]
		PFGE	ST[a] (clonal complex)	*spa*	Toxin genes[b]	Adhesin genes[c]			
Hospital-acquired	3	G1, G2	121 (121)	828[f] (*n* = 1), 466 (*n* = 2)	PVL, *lukE*, *hlg-v*, *ecg*	*cna*, *bbp*	0.25	PEN (67), AMP (67), ERY, CLI (33), TET, CHL (33)	*ermC*, *tetK*
	1	H	121 (121)	287	PVL, *lukE*, *hlg-v*, *ecg*	*cna*, *bbp*	0.25	PEN, AMP, ERY, CLI, TET	*ermC*, *tetK*
	4	M	97 (97)	105	*lukE*, *hlg-v*	–	0.25	PEN (50), AMP (50), CHL (25)	–
	4	12, 13	45 (45)	73 (*n* = 3), 980[f] (*n* = 1)	*sec* (75%), *sel* (75%), *egc*[g]	*cna*	0.25	PEN (75), AMP (75), GEN (25), CHL (25)	*aac6′/aph2*
	1	J	1 (1)	981[f]	*lukE*, *hlg-v*, *sea*, *seh*	*cna*	0.25	PEN, AMP, ERY, TET, CHL	*ermC*, *tetK*
	1	N	1 (1)	35	*lukE*, *hlg-v*, *sea*, *seh*, *sek*, *seq*	*cna*	0.25	PEN, AMP, CHL	–
	1	C1	25 (25)	184	PVL, *lukE*, *hlg-v*, *egc*, *etd*	–	1	PEN, AMP, TET	*tetK*
	1	K	6 (6)	154	*lukE*, *hlg-v*, *sea*, *sec*, *sel*	*cna*	0.25	–	–
	1	L	5 (5)	23	*lukE*, *hlg-v*, *egc*, *seq*	–	0.25	PEN, AMP, ERY	*ermC*
Community-acquired	7[h]	H	121 (121)	287	PVL, *lukE*, *hlg-v*, *egc*	*cna*, *bbp*	0.125–0.5	PEN, AMP, ERY (14), TET (14), CHL (71)	*ermC* (14%), *tetK* (14%)
	5	G1, G2	121 (121)	830[f] (*n* = 3), 829[f] (*n* = 1), 827[f] (*n* = 1)	PVL, *lukE*, *hlg-v*, *egc*	*cna*, *bbp*	0.25	PEN, AMP, CHL	–
	1	C2	25 (25)	825[f]	PVL, *lukE*, *hlg-v*, *egc*, *etd*	–	1	PEN, AMP, CHL	–
	1	M	97 (97)	105	*lukE*, *hlg-v*	–	0.25	PEN, AMP, CHL	–
	1	A	30 (30)	33	*sea*, *egc*	*cna*, *bbp*	0.25	PEN, AMP	–
	1	11	1211[a] (45)	979[f]	*sec*, *sel*, *egc*	*cna*	0.25	PEN, AMP	–

OXA, oxacillin; PEN, penicillin G; AMP, ampicillin; GEN, gentamicin; ERY, erythromycin; CLI, clindamycin; TET, tetracycline; CHL, chloramphenicol; PFGE, pulsed-field gel electrophoresis; PVL, Panton–Valentine leukocidin ; ST, sequence type. A representative drug of each tested antimicrobial group is shown.

[a]ST was determined for one representative isolate of the same PFGE, *spa*, *agr* and coagulase type.

[b]Other than three common genes: *hla*, *hlg*, and *hld*.

[c]Other than nine common genes: *icaA*, *icaD*, *eno*, *fnbA*, *fnbB*, *ebpS*, *clfA*, *clfB*, and *fib*.

[d]For <100% resistance.

[e]Genes *aac6′/aph2*, *ermC* and *tetK* code for resistance to gentamicin, erythromycin–clindamycin, and tetracycline, respectively.

[f]The novel *spa* type.

[g]*egc*, enterotoxin gene cluster, (*seg*, *sei*, *sem*, *sen* and *seo* genes.)

[h]Four isolates caused community-acquired pneumonia with a fatal outcome.

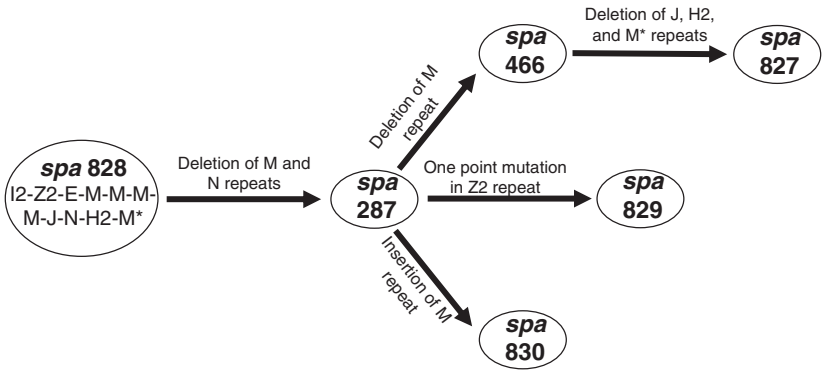


FIG. 2. Schematic figure of the proposed genetic relationship among *spa* types associated with Panton–Valentine leukocidin (PVL)-positive ST121 methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates. These *spa* types shared a common genetic background (similar order of the repeats and 100% homology among shared repeats), but differed in the deletion and/or insertion of some repeats or point mutations within the repeat. *spa* type 287 was unique for PVL-positive ST121 MSSA isolates (n = 4) that were associated with fatal community-acquired MSSA pneumonia in this study. Asterisks indicate the terminal M repeat.

isolates were found. These were represented by a dominant ST121 clone with high diversity in their *spa* types. These observations suggest that PVL-positive ST121 MSSA strains have the potential for epidemic spread in Russia.

Furthermore, in this study, the ST121, *spa* type 287 MSSA clone caused community-acquired pneumonia in young patients, resulting in 100% mortality. This clone contains several genes that mediate adhesion (e.g. *cna* and *bbp*) and toxin genes (PVL and *egc*, which encodes at least five superantigens, including staphylococcal enterotoxins G, I, M, N, and O). Sequence analysis of ST121, *spa* type 287 MSSA isolates revealed an R176H substitution in the PVL gene (the 'H variant' PVL) (data not shown). Recent reports suggested that the 'H variant' of PVL was associated with high virulence and mortality in a murine pneumonia model [27]. In contrast, Bubeck Wardenburg *et al.* [28] failed to observe a significant difference in virulence in a mouse pneumonia model that examined the 'R variant' PVL strains. Further studies are needed to clarify the pathogenesis of *S. aureus* pneumonia, but clinicians should take PVL production into account in the therapeutic management of community-acquired *S. aureus* pneumonia in Russia.

Some of the MSSA isolates had genetic backgrounds that were identical to those found in pandemic MRSA clones. Some examples of this include the following: ST45 MSSA (PFT I), which shared the same ST as the MRSA Berlin clone; ST1 MSSA (PFT J and PFT N), which had a genetic background identical to the Western Australian MRSA-1 clone; ST5 MSSA (PFT L), which corresponded to the New York/Japan clone; and clone ST30 MSSA (PFT A), which was a single-locus variant of the MRSA Southwest Pacific clone. These observations strongly suggest that MSSA strains are important both as causative agents of infections and as a potential reservoir of epidemic MRSA clones.

In conclusion, the Brazilian/Hungarian MRSA clone, which was resistant to nine groups of antimicrobials, including rifampicin, was found to be dominant in the hospital settings in Russia. Routine detection of this clone in clinical laboratories can be easily performed by detection of its resistance to sulphamethoxazole. Among the MSSA strains collected for this study, there was a high prevalence of the PVL gene in community isolates, which was a predictor of poor prognosis in patients with CA-MSSA pneumonia. These clones should be closely monitored, because of their apparently enhanced virulence, which makes them a substantial public health threat.

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Transparency Declaration

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