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].