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Fatal Pneumonia in HIV-Infected Patients from a Novel ST239 Methicillin-Resistant *Staphylococcus aureus* Carrying the Toxic Shock Syndrome Toxin-1 Gene in Krasnoyarsk, Siberian Russia

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Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated in the early 1960s and has continued to be a life-threatening multidrug-resistant bacterium in hospitals (1). MRSA includes diverse clones, and multilocus sequence type (ST) 239 MRSA is a hospital-acquired MRSA clone, found worldwide, in Asia, North America, South America, Europe, and Australia (2). ST239 MRSA (strain TW20) was also noted as an intensive care unit (ICU)-associated MRSA in London between 2002 and 2004 (3).

Toxic shock syndrome toxin-1 (TSST-1) is a superantigenic toxin of *S. aureus*, which is responsible for toxic shock syndrome (TSS) and also plays a role in immune evasion, allowing further invasion of *S. aureus* (4). TSST-1 is encoded by phage-related chromosomal islands, namely, *S. aureus* pathogenicity islands (SaPIs), which represent phage satellites and produce phage-like infectious particles (5). The ST239 MRSA lineage lacks the TSST-1 gene (*tst*).

In this study, we isolated MRSA strains from two HIV-infected patients who had fatal MRSA pneumonia. One was a 71-year-old woman who presented to the AIDS center in Krasnoyarsk (Siberian Russia) in January 2008, and the other was a 63-year-old man who presented to the center in August 2009. The MRSA strains isolated from the lung tissue of the woman was named OC76, and that isolated from the lung tissue of the man, OC3.

Next, we investigated the molecular characteristics of these MRSA strains OC3 and OC76. Molecular typing and virulence gene analysis were performed as described previously (6). The genetic characteristics of the strains are summarized in Fig. 1A. They shared the same genotype: ST239/*spa*3(t037)/*agr*1/SCC*mec*III.1.1.2 (IIIA)/CoaIV. SCC*mec* was a Brazilian type (SCC*mec*III.1.1.2 [IIIA]), representing the first of its type detected in Siberian Russia. The two strains were also characterized as carriers of *tst*.

TSST-1 production (the amount of TSST-1 in the cell-free culture supernatants from bacterial cultures [2.0×10^9 cfu/ml]) was measured by passive latex agglutination using a TST-RPLA kit (Denka Seiken, Tokyo, Japan), as per the manufacturer's instructions. The TSST-1 production levels of strains OC3 and OC76 were similar (200–400 ng/ml); other ST239 strains produced no detectable TSST-1, as expected.

Susceptibility testing of MRSA strains was performed using the agar-dilution method according to procedures previously described by the Clinical and Laboratory Standards Institute (CLSI) (7). The breakpoints for drug resistance were those described by the CLSI (7). Strains OC3 and OC76 were multidrug-resistant, similar to other ST239 MRSA strains (Fig. 1A). They exhibited uncommon resistance to rifampicin (MICs, ≥ 256 μg/ml) and chloramphenicol, similar to strain 16 K from Vladivostok, although the MRSA genotypes in these two geographic locations were different.

Pulsed-field gel electrophoresis (PFGE) analysis (with *Sma*I) of the MRSA strains was performed as described previously (6). The PFGE patterns of strains OC3 and OC76 were slightly different; they were also divergent from Vladivostok strains (Fig. 1B). The PFGE results along with the isolation of strains OC3 and OC76 from different patients in 2008 and 2009, strongly suggested that the infection with a novel *tst*-positive ST239 variant (designated as ST239 Krasnoyarsk variant in this study) persisted and spread among patients in Krasnoyarsk for at least 2 years since 2008, albeit with PFGE divergence. Such genetic divergence can be explained by the fact that the ST239 lineage possesses high potential of evolution (2). Further epidemiological studies are necessary to understand the spread of the ST239 Krasnoyarsk variants.

For the *tst* region analysis, the MRSA OC3 genome was analyzed by pyrosequencing using a genome sequencer FLX system (Roche Diagnostics, Branford, Conn., USA). The entire structure of SaPI (carrying *tst*)

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(A)

Type, gene, resistance	ST239 HA-MRSA Strain								
	OC3 Kras., Russia	OC76 Kras., Russia	10H Vlad., Russia	16K Vlad., Russia	PM31 Taiwan	ANS46 Australia	HU25 Brazil	TW20 UK	JKD6008 New Zealand
Type									
CC	8	8	8	8	8	8	8	8	8
ST	239	239	239	239	239	239	239	239	239
spa	3 (t037)	3 (t037)	3 (t037)	351 (t030)	3 (t037)	3 (t037)	390 (t138)	3 (t037)	3 (t037)
agr	1	1	1	1	1	1	1	1	1
SCC*mec*III (J1, J2, J3)	1.1.2 (IIIA)	1.1.2 (IIIA)	1.1.1	1.1.4 (III_6)	1.1.1	1.1.1	1.1.2 (IIIA)	1.1.1	1.1.new
Coa	IV	IV	IV	IV	IV	IV	IV	IV	IV
Virulence gene									
Enterotoxin									
tst	+	+	−	−	−	−	−	−	−
sea	−	−	+	+	+	+	−	+	−
sep	−	−	−	−	−	−	−	−	+
sek, seq	+	+	+	+	+	+	−	+	+
Drug resistance (Non-β-lactams)	G, K, T, E, C, L, Cp, R	G, K, T, E, C, L, Cp, R	G, K, T, E, C, L, Tr	G, K, T, E, C, L, Cp, R	G, K, T, E, C, L, Tr	K, T, E, C, Cp, Tr	G, K, T, E, C, L, Tr	G, K, T, E, C, L, Tr	G, K, T, E, C, L, Tr

(B)

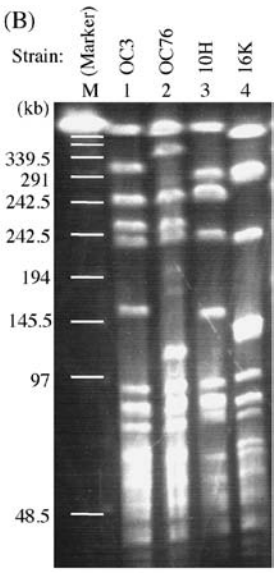
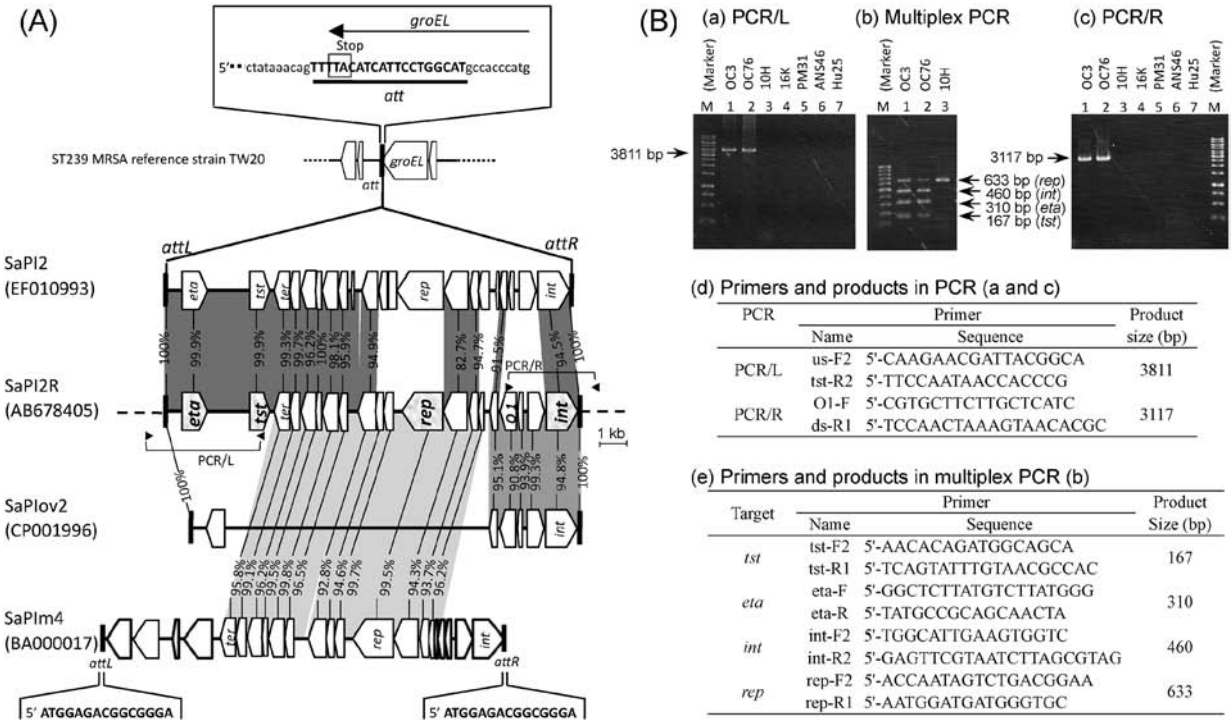


Fig. 1. Characteristics of ST239 MRSA strains OC3 and OC76 isolated from HIV patients in Krasnoyarsk (Siberian Russia), compared with other ST239 MRSA strains from Vladivostock (Far Eastern Russia) and other countries. Russian ST239 MRSA: strains OC3 and OC76, isolates in Krasnoyarsk; strains 10H and 16K, isolates in Vladivostok. In (A), the data of TW20 and JKD6008 are from GenBank accession numbers FN433596 and CP002120, respectively. G, gentamicin; K, kanamycin; T, tetracycline; E, erythromycin; C, clindamycin; L, levofloxacin; Cp, chloramphenicol; R, rifampicin; Tr, trimethoprim. In (B), PFGE patterns are shown. A lambda ladder (Bio-Rad Laboratories, Tokyo, Japan) was used as the molecular size standard (M).



(d) Primers and products in PCR (a and c)

PCR	Primer Name	Primer Sequence	Product size (bp)
PCR/L	us-F2	5′-CAAGAACGATTACGGCA	3811
	tst-R2	5′-TTCCAATAACCACCCG	
PCR/R	O1-F	5′-CGTGCTTCTTGCTCATC	3117
	ds-R1	5′-TCCAACTAAAGTAACACGC	

(e) Primers and products in multiplex PCR (b)

Target	Primer Name	Primer Sequence	Product Size (bp)
tst	tst-F2	5′-AACACAGATGGCAGCA	167
	tst-R1	5′-TCAGTATTTGTAACGCCAC	
eta	eta-F	5′-GGCTCTTATGTCTTATGGG	310
	eta-R	5′-TATGCCGCAGCAACTA	
int	int-F2	5′-TGGCATTGAAGTGGTC	460
	int-R2	5′-GAGTTCGTAATCTTAGCGTAG	
rep	rep-F2	5′-ACCAATAGTCTGACGGAA	633
	rep-R1	5′-AATGGATGATGGGTGC	

Fig. 2. The structure of SaPI2R (carrying *tst*) of strain OC3 (ST239 Krasnoyarsk variant isolated from an HIV patient) (A) and detection of SaPI2R in strain OC76 by PCR (B). In (A), homologous regions between four SaPI structures (SaPI2, SaPI2R, SaPIov2, and SaPIm4) are shaded; shaded numbers show percentage homology between the corresponding ORFs at the nucleotide level. For each SaPI, GenBank accession numbers are shown in parentheses. In (B), the PCR/L assay (a) with a primer set (us-F2 and tst-R2, shown in [d]) detects the left-side 3,811-bp boundary region (from a left-side chromosome site, 213-bp away from *attL*, to the *tst* gene in SaPI2R), as shown in Fig. 2A; ST239 Krasnoyarsk variant (strains OC3 and OC76) produced positive results, while other ST239 strains produced negative results. The multiplex PCR assay (b) with four primer sets (shown in [e]) detects four genes (*eta*, *tst*, *rep*, and *int*) in SaPI2R; only ST239 Krasnoyarsk variant gave all four products. And, the PCR/R assay (c) with a primer set (ORF-F4 and ds-R1, shown in [d]) detects the right-side 3,117-bp boundary region (from *orfO1* to a chromosome site [in the *groEL* gene], 125-bp away from *attR*), as shown in Fig. 2A; only ST239 Krasnoyarsk variant produced positive results. Bacterial strains, used in PCR or multiplex PCR assays, are those described in Fig. 1A.

of strain OC3 is shown in Fig. 2A. It was inserted into the 20-bp-attachment site sequence (*att*) of the *groEL* gene on the chromosome (Fig. 2A, upper part). The SaPI was 14,819 bp long, and flanked by 20-bp directly repeated *att* sequences (*attL* and *attR*), which were the same sequences as the *att* of *groEL*. The SaPI structure of strain OC3 contained *eta* (encoding *S. hyicus* exfoliatin A), *tst*, *ter* (encoding terminase, which cleaves multimeric DNA), *rep* (encoding replication initiator), and *int* (encoding integrase). The structure was a mosaic SaPI that most probably emerged through recombination between SaPI2, SaPIov2, and SaPIm4 (Fig. 2A, lower part); this novel mosaic SaPI was designated as SaPI2R. The GenBank accession number for the SaPI2R structure of strain OC3 is AB678405.

Multiplex PCR targeting four genes in SaPI2R (*eta*, *tst*, *rep*, and *int*) showed the presence of all four genes in strain OC76, as was observed for strain OC3 (but not in other ST239 MRSA strains [e.g., strain 10H] in Vladivostock) (Fig. 2B, b). Moreover, from the results of two PCR assays (PCR/L and PCR/R, as shown in Fig. 2A), we unambiguously concluded that OC76 also carried SaPI2R on the chromosome; other ST239 strains lacked SaPI2R (Fig. 2B, a and c).

HIV-infected patients have a higher risk of MRSA infections (8,9). In the USA, the incidence of MRSA infection in HIV patients in the community was 18-fold higher than that in the general population or >6-fold higher than that in HIV-negative populations, and the most notable clinical presentations in HIV patients are skin and soft tissue infections, but in some cases, the condition progressed to bacteremia or endocarditis (8). The most frequently isolated strain from HIV patients is USA300 (ST8, TSST-1-negative) (9).

In conclusion, a new geographic variant of the ST239 MRSA lineage emerged in Krasnoyarsk, Siberian Russia. This clone (designated as the ST239 Krasnoyarsk variant) was characterized by SCC*mec*IIIA (Brazilian SCC*mec*III), SaPI2R (carrying *tst*), and rifampicin resistance, and caused fatal pneumonia in HIV-infected patients in Russia, thus, necessitating attention.

Conflict of interest None to declare.

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