Global distribution of Panton-Valentine leukocidin–positive methicillin-resistant Staphylococcus aureus, 2006.

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Global distribution of Panton Valentine Leukocidin-positive methicillin-resistant *Staphylococcus aureus*: the situation in 2006.

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Abstract

We determined the *agr* type, multilocus sequence type (MLST), protein A gene type (*spa* typing), toxin gene profile and antibiotic resistance profile of 469 isolates of Panton Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* isolates (PVL-positive CA-MRSA) collected from around the world between 1999 and 2005 by the French National Reference Center for Staphylococci. We found that some continent-specific clones described in 2003, such as clone ST8, have now spread all over the world. Likewise, some PVL-positive CA-MRSA have spread to several countries on given continent. New clones have emerged (e.g. ST5) on new genetic backgrounds. PVL-positive CA-MRSA, that were usually susceptible to most antistaphylococcal antibiotics, have acquired new resistance determinants (e.g. to gentamicin) in certain countries. The major trait shared by all these clones is a short staphylococcal chromosomal cassette *mec* (SCC*mec*) element of type IV or V.

Introduction

By definition, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strains infect patients with no risk factors for acquiring an MRSA strain of hospital origin. CA-MRSA infections usually affect previously healthy young patients (1). They are mostly skin and soft-tissue infections, but deep-seated infections such as necrotizing pneumonia and disseminated invasive osteomyelitis have been described (2). Most CA-MRSA isolates produce the Panton-Valentine leukocidin (PVL) and harbor a type IV staphylococcal chromosomal cassette *mec* (SCC*mec*) element, but some isolates harboring the SCC*mec* V element have been reported (3). PVL-positive CA-MRSA clones have spread throughout the world (4).

In 2003, Vandenesch *et al.* described continent-specific PVL-positive CA-MRSA clones (mainly on an *agr3* background) and characterized them by their sequence types (ST) (4). The main European clone, ST80, was detected in France, Switzerland, the Netherlands, England, Belgium and Germany (5-10), but also in northern Europe (e.g. Denmark) where MRSA strains are rare in hospitals (11). One of the most prevalent PVL-positive CA-MRSA clones in the USA (USA300) belongs to ST8 (12); other US clones include USA400 (ST1), USA1000 (ST59) and USA1100 (ST30) (13,14). ST30 is also a major clone in Asia and Oceania (15,16) and is referred to as the South West Pacific clone (17). In Singapore, an international travel hub, several clones belonging to ST80, ST30 and ST59 have been reported (18). The prevalence of PVL-positive CA-MRSA varies considerably from one continent to another. In the USA, MRSA were isolated from 50% of patients presenting to emergency departments of 11 cities with skin and soft-tissue infections (97% of isolates belonged to clone USA300) (19). In Europe, the prevalence is lower, at approximately 1-3% (20,21).

Since 1999 the French National Center for Staphylococci has characterized 469 PVL-positive CA-MRSA isolates collected throughout the world. The isolates were typed by MLST, *spa* typing,

antibiotic resistance profiling and toxin and resistance gene analysis. Here we describe the evolution and spread of PVL-positive CA-MRSA clones since initial description.

Materials and methods

Bacterial isolates. Between 1999 and 2005, 469 PVL-positive CA-MRSA isolates were received from 17 countries by the French National Reference Center for Staphylococci. They were sent spontaneously to the Center for PVL gene detection and genomic characterization (clone designation).

DNA extraction. The strains were grown on brain-heart infusion agar or in brain-heart infusion broth at 37°C overnight. Genomic DNA was extracted with a standard procedure (22). Amplification of *gyr*A was used to confirm the quality of each DNA extract and the absence of PCR inhibitors. All PCR products were analyzed by electrophoresis on ethidium bromide-stained 1% agarose gels (Sigma, France).

Identification of *agr* alleles. The *agr* group (*agr* 1-4) was determined by PCR as previously described (23).

Detection of the *mecA* gene and SCC*mec* typing. The *mec*A gene (coding for methicillin resistance) was detected by PCR as described by Murakami *et al.* (24). The staphylococcal chromosomal cassette *mec* (SCC*mec* I-IV) was detected as described by Oliveira *et al.* (25) and SCC*mec* type V was detected as described by Ito *et al.* (26). The following reference strains, kindly provided by Herminia de Lencastre and Alexander Tomasz, were used as controls: COL (SCC*mec* I), BK2464 (SCC*mec* II), HU106 (SCC*mec* III), and BK2529 (SCC*mec* IV).

Detection of toxin genes. Sequences specific for staphylococcal enterotoxin genes (*sea-e, seh, sek, sem, seo*), as well as the toxic shock syndrome toxin gene (*tst*), exfoliative toxin genes

(*eta, etb, etd*), PVL genes (*luk*S-PV-*luk*F-PV), LukE-lukD leukocidin genes (*luk*E-*luk*D), the class F lukM leukocidin gene (*luk*M*)*, hemolysin genes (gamma (*hlg*), gamma variant (*hlgv*) and beta (*hlb)*) and epidermal cell differentiation inhibitor genes (*edinA/B/C*) were detected by PCR as described elsewhere (23,25,27,28).

Antimicrobial susceptibility testing. The MICs of penicillin, oxacillin, cefoxitin, kanamycin, tobramycin, gentamicin, erythromycin, clindamycin, tetracycline, pristinamycin, ofloxacin, fusidic acid, vancomycin, teicoplanin, fosfomycin, trimethoprim/sulfamethoxazole, rifampin, mupirocin, quinupristin/dalfopristin and linezolid were determined by using the standard agar dilution technique as recommended by the French Society for Microbiology.

Structural genes for resistance to tetracycline, aminoglycosides and macrolide-lincosamide-streptogramin (29) were identified by PCR. DNA was amplified in a model 2400 thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) with *Taq* (Qbiogene, Inc., Carlsbad, Calif.) or *Pfu* (Stratagene, La Jolla, Calif.) DNA polymerase, as recommended by the manufacturers. PCR elongation times and temperatures were adjusted to the expected size of the PCR product and to the nucleotide sequences of the primers, respectively.

***spa* typing**. *spa* typing was performed on PVL-positive MRSA isolates as previously described (30). The x region of the *spa* gene was amplified by PCR. *spa* types were determined with the Ridom Staph Type software (Ridom GmbH, Germany), which automatically detects *spa* repeats and assigns a *spa* type according to Harmsen *et al.* (31) and http://spaserver.ridom.de/. Applying the recently developed algorithm BURP (Based Upon Repeat Patterns) *spa* types were clustered into different groups with calculated cost between members of a group less or equal 6. *spa* types shorter than 3 repeats were excluded from analysis because no reliable deduction about ancestries can be made from these types. The new algorithm takes repeat-duplication/-deletion in addition to point mutation events into account when calculating the relatedness of different *spa*-

types. Due to speed constrains, a heuristic version of the EDSI-Alignment (Excisions, Duplications, Substitutions, Insertions), as described by Sammeth *et al*., was used (32). BURP *spa* clonal complexes (*spa*-CC) were automatically assigned by the Ridom Staph Type software.

Multilocus sequence typing (MLST). MLST was performed on representative strains of each clonal group, as described elsewhere (33,34). The allelic profile of each strain was obtained by sequencing internal fragments of seven housekeeping genes (*arcC, aroE, glpF, gmk, pta, tpi, yqiL*) and entering them on the MLST home page (http://saureus.mlst.net), where seven numbers depicting the allelic profile were assigned which defined a sequence type ST (33). Similar sequence types were grouped into clonal complexes (CC).

Results

agr and sequence types

The 469 PVL-positive CA-MRSA isolates were *agr* type 1 (n=46, 9.8%), *agr*2 (n=9, 1.9%) or *agr*3 (n=414, 88.3%); none was *agr*4 (Table 1). The 469 PVL-positive isolates belonged to 11 sequence types (ST): the *agr*1 isolates were ST8, ST59, ST22, ST766 or ST377; the *agr*2 isolates were all ST5; and the *agr*3 isolates were ST80, ST30, ST37, ST93 or ST1 (Table 1). None of the STs were shared by different *agr* types. The most frequent sequence type was ST80 (n=357, 76.1%), corresponding to the European clone.

spa types and *spa* clonal complexes

The *spa* types were specific for the *agr* type and the sequence type. Minor variations of *spa* types (deletions or duplications of SSR units) were observed in a number of isolates within the same ST. For instance, nine *spa* types were recognized among the 357 ST80 isolates, but t044 was the major *spa* type (n=333, 93.3%); eight of these *spa* types belonged to the same *spa* CC. A unique *spa* CC corresponded to each ST, except for ST1 isolates, which formed three different *spa* CC (Table 1).

Geographic origin and spread

A previous study (4) showed a limited number of clones and a limited geographic distribution. Schematically, ST80 was detected in Europe, ST8 and ST1 in the USA, and ST30 in Oceania. The results of the present study suggest intercontinental exchanges of several clones (Table 1): (i) the ST8 clone (USA300) from the USA towards Europe; (ii) the ST1 clone (USA400) from the USA towards Europe and Asia; (iii) the ST59 clone (USA1000) from the USA towards Asia; (iv) the ST80 clone from Europe towards Asia (18); and (v) the ST30 clone from Oceania

towards Europe. Countries with numerous international exchanges (e.g. Singapore) have the highest clonal diversity.

New clones have been detected since 2003. One, ST22, has been found in Europe only. Another new clone, ST766, that belongs to the same clonal group (CC22) as ST22, was found in Singapore (18). Clone ST377 (with a type V SCC*mec*) was reported simultaneously in Europe and Australia (3). Clone ST5 was detected in Europe only. Clone ST93 (the Queensland clone), described in Australia before 2003, has not yet been detected in other countries (17).

Toxin gene content

Comparison of the toxin gene distribution was used to determine the genetic background of the different clones with minor variations. For instance, ST80 isolates were all positive for *etd*, *luk*S-PV, *luk*F-PV and *edinA/B/C*; very few lacked *luk*DE or *hlgv* or harbored *hlB* (Table 2). Superantigenic toxin genes were detected in isolates belonging to the different STs, except for ST377, ST80 and ST93 (Table 2).

Antibiotic resistance

Isolates of each ST were grouped according to the number of antibiotic resistance determinants they harbored. Initial PVL-positive CA-MRSA isolates were susceptible to most antibiotics. For instance, 8 of the 25 ST8 isolates were resistant to penicillin and oxacillin alone, as were 17 of the 32 ST1 isolates and 18 of the 20 ST30 isolates (Table 3). ST80 isolates were initially resistant to penicillin, oxacillin, kanamycin and tetracycline, and intermediate to fusidic acid. Since 2003, new antibiotic resistance determinants have been acquired (e.g. gentamicin and ofloxacin). One ST8 isolate was resistant to penicillin, oxacillin, kanamycin, erythromycin, tetracycline and ofloxacin; one ST1 isolate was resistant to penicillin, oxacillin, kanamycin, tobramycin and gentamicin. A few ST80 isolates from Algeria were resistant to multiple antibiotics. Most PVL-

positive CA-MRSA strains with multiple antibiotic resistances were detected in Asia (Singapore, China) or Africa (Algeria).

Antibiotic resistance genes

Antibiotic resistance genes were sought in a subset of 153 ST80 isolates. The *aph3'-III* gene, encoding high-level resistance to kanamycin and neomycin, but also to amikacin and isepamycin, was detected in all 153 isolates (100%). The *tetK* efflux gene was detected in 125 (82%) of tetracycline-resistant isolates. The *ermC* gene, an erythomycin ribosome methylase, was detected in 61 (40%) of erythromycin-resistant isolates. The *far-1* gene was detected in 133 (87%) of fusidic acid-intermediate isolates.

SCC*mec* types

The SCC*mec* type was determined for 22 *agr*1 isolates (ten ST8, one ST59, one ST22, and ten ST377); five *agr*2 isolates (ST5); 190 *agr*3 isolates (179 ST80, nine ST30, two ST93, seven ST1). All the isolates were SCC*mec* type IV, except for the ten ST377 isolates, which were SCC*mec* type V.

Discussion

This study shows that (i) the continent-specific clones of PVL-positive CA-MRSA described in 2003 by Vandenesch *et al.* (4) have now spread to other continents. For instance, the ST1 clone USA400 is now detected in Europe and Asia. Some PVL-positive clones, such as ST1 and ST30, can now be considered pandemic, as they are detected in America, Europe and Asia; (ii) on a given continent, PVL-positive CA-MRSA have spread from country to country. For instance, in Europe, PVL-positive CA-MRSA were recently detected in Slovenia, Romania and Croatia; (iii) new PVL-positive CA-MRSA clones are emerging on different genetic backgrounds. While most of the clones described in 2003 by Vandenesch *et al.* (4) had an *agr*3 background, the newly described clones are *agr*1 or *agr*2; (iv) PVL-positive CA-MRSA, which were initially susceptible to most antistaphylococcal antibiotics, have acquired new antibiotic resistance determinants, to gentamicin and ofloxacin for instance.

The global ST distribution of PVL-positive CA-MRSA isolates in this study is of course dependent on the sources of the isolates received by the French National Reference Center for Staphylococci, and does not reflect the current epidemiology. Nevertheless, our results concord with other reports, confirming that ST80 is mainly detected in Europe (e.g. Denmark (11), Finland (35), Greece (36)), but also in Libya (6), while ST30 is pandemic (37).

PVL-negative hospital-acquired MRSA belong to five major clonal complexes (CC5, CC8, CC22, CC30, CC45). PVL-positive CA-MRSA of the same clonal classes were also detected in our study, with the exception of CC45, but the PVL+ MRSA strains showed a broader CC diversity. For instance, none of the ST80 isolates belonged to CCs harboring hospital strains. PVL-positive CA-MRSA are gradually causing an increasing number of hospital-acquired infections in countries, such as the USA, where their prevalence is high. Kourbatova EV *et al*. reported that, during the period 2003-2004, five prosthetic joint infections were caused by USA300 strains (38).

The worldwide spread of PVL-positive CA-MRSA is likely related to international travel. ST80 isolates recovered in France were mainly detected in patients originating from Algeria, a country that reported a high rate of community- and hospital-acquired infections due to ST80 isolates in 2006 (39). Maier *et al.* recovered ST22 strains from Turkish migrants in Germany (40). In some countries, such as Algeria, acquisition of new antibiotic resistance determinants could be related to antibiotic misuse, while the spread of multidrug-resistant strains could be facilitated by poor hygiene.

It is not known whether PVL-positive CA-MRSA clones arose through acquisition of the PVL phage by strains with a methicillin resistance background or, conversely, through acquisition of the SCC*mec* element by strains with a PVL-positive background. On analyzing the database of the French National Reference Center for Staphylococci, which contains over 5000 toxin gene profiles, we found isolates that were related to the PVL-positive MRSA clone ST80 but that lacked either the PVL genes (5 isolates) or the *mec*A gene (7 isolates) (data not shown). These isolates, like the ST80 clone, were *agr*3, *etd*+, *edin*A/B/C+; one isolate (PVL- *mec*A+) was ST80 and another (PVL+ *mec*A-) was ST635 (a single-locus variant of ST80). These "atypical" isolates were discovered in Algeria, Switzerland and France, and we are unable to state whether they are ancestors or descendants of the most prevalent strains.

Deep-seated infections due to PVL-positive *Staphylococcus aureus* can be extremely severe: for example, necrotizing pneumonia carries a mortality rate close to 75% (41). It is unclear whether the pathogenesis of these acute infections is related to the effect of PVL alone or in combination with other virulence factors such as superantigenic toxins. We found that some PVL-positive CA-MRSA clones (ST80) lacked any superantigenic toxin genes. Among the *S. aureus* virulence factors (not screened for here), ST30 strains are known to harbor the *bbp* gene encoding bone sialoprotein (37). The SCC*mec* elements detected in our collection were type IV or V, and corresponded to the smallest SCC*mec* element.

PVL-positive CA-MRSA are usually susceptible to most antistaphylococcal antibiotics. Clone ST80 is usually resistant to tetracycline (mediated by the *tetK* gene), intermediate to fusidic acid (*far1* gene) and resistant to kanamycin (*aph3'-III* gene). We observed the emergence of rare isolates with multiple resistances to antibiotics such as gentamicin and ofloxacin. From the therapeutic viewpoint, it is noteworthy that all the isolated were susceptible to trimethoprim-sulfamethoxazole, glycopeptides and linezolid.

In sum, since 2003 we have observed an impressive worldwide spread of PVL-positive CA-MRSA clones initially described at the beginning of this decade, and we have also detected PVL-positive CA-MRSA strains of other lineages. To counter this emerging global threat to public health, systematic surveillance of both hospital and community isolates is required, together with measures designed to limit their spread.

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agr type	ST	N (%)	CC	*spa* type	N (%)	Ridom motif	*spa* CC	Countries of detection before 2003 (4)	New countries of detection after 2003 (this work)	Other reports of the literature
***agr*1**		**46 (9.8)**								
	ST8	25 (54.3)	8					USA	The Netherlands, France, Spain, Switzerland,	Northern Norway (42), Greece (36)
				t008	25 (100.0)	r11-r19-r12-r21-r17-r34-r24-r34-r22-r25	singleton		French Polynesia	
	ST59	7 (15.2)						USA		
				t437	6 (75.0)	r04-r20-r17-r20-r17-r25-r34	8		The Netherlands, France, Singapore	Taïwan (43)
				t216	1 (12.5)	r04-r20-r17-r20-r17-r31-r16-r34	8			
	ST22	3 (6.5)	22							
				t005	2 (66.7)	r26-r23-r13-r23-r31-r05-r17-r25-r17-r25-r16-r28	4		The Netherlands, Germany	
				t310	1 (33.3)	r26-r23-r31-r05-r17-r25-r17-r25-r16-r28	4			
	ST766	1 (2.2)	22							
				t1276	1 (100.0)	r26-r23-r13-r23-r31-r05-r17-r25-r17-r24-r25-r16-r28	4		Singapore	
	ST377	10 (21.7)								
				t355	9 (90.0)		6		The Netherlands, France, Greece, Switzerland,	
				t595	1 (10.0)	r07-r56-r12-r17-r16-r16-r33-r31-r57-r12	6		Australia	
						r07-r56-r12-r17-r16-r16-r33-r31-r57-r31-r57-r12				
***agr*2**		**9 (1.9)**								
	ST5	9 (100.0)	5						France Switzerland, Algeria	
				t311	5 (55.5)	r26-r23-r17-r34-r20-r17-r12-r17-r16	5			
				t1277	3 (33.3)	r26-r23-r17-r34-r20-r17-r12-r17-r16-r16	5			
				t450	1 (11.1)	r26-r23-r17-r34-r16	5			
***agr*3**		**414**								
	ST80	**(88.3)**						France, Switzerland	Algeria, Singapore, Romania, Germany, Belgium,	Denmark (11), Northern Norway (42),
		357		t044	333 (93.3)	r07-r23-r12-r34-r34-r33-r34	1		Greece, Slovenia, The Netherlands	Finland (35), Sweden (11), Scotland (11),
		(83.2)		t131	9 (2.5)	r07-r23-r12-r34-r33-r34	1			Greece (36), England (8), Lybia (6), Croatia

			t376	8 (2.2)	r07-r23-r12-r34-r34-r34-r33-r34	1			(44)
			t639	2 (0.6)	r14-r12-r34-r34-r33-r34	1			
			t237	1 (0.3)	r07-r34-r34-r33-r34	1			
			t1199	1 (0.3)	r07-r23-r12-r02-r34	1			
			t1201	1 (0.3)	r07-r16-r34-r34-r33-r34	1			
			t1206	1 (0.3)	r07-r23-r12-r34-r34-r33-r34-r33-r34	1			
			t1200	1 (0.3)	r07-r23-r34	*			
ST30		30					New-Zealand, Western Samoa	The Netherlands, Australia, Japan, Switzerland,	
			t019	17 (75.0)	r08-r16-r02-r16-r02-r25-r17-r24	2		Singapore, China, French Polynesia	Sweden (45), Brazil (46), Uruguay (47),
	20 (4.8)		t021	1 (5.0)	r15-r12-r16-r02-r16-r02-r25-r17-r24	2			England (8), Hong-Kong (48)
			t318	1 (5.0)	r15-r12-r16-r16-r02-r16-r02-r25-r17-r24	2			
			t1273	1 (5.0)	r08-r16-r34-r02-r25-r17-r24	2			
ST37		30						The Netherlands	
			t914	1 (100.0)	r01-r12-r16-r02-r16-r02-r25-r24-r24-r24	2			
	1 (0.2)								
ST93							Australia		
			t202	4 (100.0)	r11-r17-r23-r17-r17-r16-r16-r25	singleton			
	4 (1)								
ST1						3	USA	France, Singapore	
			t128	18 (56.2)	r07-r23-r23-r21-r16-r34-r33-r13	3			Switzerland (6), Canada (49)
	32 (7.7)		t125	3 (9.4)	r07-r23-r23-r23-r23-r21-r13	3			
			t558	1 (3.1)	r07-r23-r23-r23-r21-r13	7			
			t175	8 (25.0)	r07-r23-r21-r16-r16-r33-r21-r16-r33-r13	7			
			t1274	1 (3.1)	r07-r23-r21-r16-r33-r21-r16-r33-r21-r16-r33-r13	singleton			
			t1272	1 (3.1)	r07-r23-r21-r17-r13-r34-r16-r33-r13				

agr: accessory gene regulator; ST: sequence type; CC: clonal complex; *spa* CC: *spa* clonal complex; *: excluded because ≤ 3 repeats

Table 1- Geographical distribution of PVL-positive CA-MRSA clones according their *agr*-type, ST and *spa*-type

agr type	ST	N (%)	Toxin genes constantly detected (100%)	Toxin genes unconstantly detected (%)
agr1		**46 (9.8)**		
	ST8	25 (54.3)	*luk*PV, *luk*DE	*hlgv* (95.8), *sek* (91.7), *sed* (16.7), *seb* (4.2), *hlB* (4.2)
	ST59	7 (15.2)	*luk*PV, *hlgv*	*hlB* (87.5), *sek* (87.5), *seb* (62.5), *lukDE* (12.5)
	ST22	3 (6.5)	*sem, seo, luk*PV, *hlg*	
	ST766	1 (2.2)	*sem, seo, luk*PV, *hlg*	
	ST 377	10 (21.7)	*luk*PV, *edinA/B/C, hlB, hlg*	
agr 2		**9 (1.9)**		
	ST5	9 (100)	*sem, seo, luk*PV, *luk*ED, *hlgv*	*edinA/B/C* (55.5)
agr 3		**414 (88.3)**		
	ST80	357 (83.2)	*etd, luk*PV, *edinA/B/C*	*luk*DE (99.7), *hlgv* (99.7), *hlB* (0.8)
	ST30	20 (4.8)	*sem, seo, lukPV, hlg*	*sek* (5.0), *tst* (5.0)
	ST37	1 (0.2)	*sec, sem, seo, tst, luk*PV, *hlg*	
	ST93	4 (1)	*luk*PV	
	ST1	32 (7.7)	*luk*PV, *seh, luk*DE, *hlgv*	*sea* (78.1), *sec* (68.7), *sek* (68.7), *seb* (25.0), *edinA/B/C* (3.1)

sea to *see, seh, sek, sem, seo*: staphylococcal enterotoxin type A to E, H, K, M, and O genes, respectively; *tst*: toxic shock toxin gene; *eta, etb, etd*: exfoliative toxin type A, B and D genes, respectively; *luk*PV: PVL genes; *luk*DE: LukE-lukD leukocidin genes; *luk*M: lukM leukocidin gene; gamma (*hlg*), gamma variant (*hlgv*) and beta (*hlb)*hemolysin genes; *edinA/B/C*: epidermal cell differentiation inhibitor; *agr*: accessory gene regulator; ST: sequence type

Table 2- Toxin gene content of PVL-positive CA-MRSA clones.

agr type	ST	N (%)	Antibiotic resistance profil[a]	N (%)	Countries of detection (N)
***agr*1**		**46 (9.8)**			
	ST8	25 (54.3)	P, OX	8 (32.0)	Spain (2), Switzerland (2), US (3), France (1)
			P, OX, K	1 (4.0)	Switzerland (1)
			P, OX, TE	3 (12.0)	Spain (1), The Netherlands (2)
			P, OX, K, E	6 (24.0)	France (1), The Netherlands (2), US (2)
			P, OX, E, OFL	1 (4.0)	France (1)
			P, OX, K, TE	1 (4.0)	French Polynesia (1)
			P, OX, K, E, OFL	1 (4.0)	US (1)
			P, OX, K, E, TE	1 (4.0)	Switzerland (1)
			P, OX, K, E, TE, OFL	1 (4.0)	Switzerland (1)
			P, OX, K, E, L, TE, MU	1 (4.0)	The Netherlands (1)
			P, OX, K, E, L, OFL, MU	1 (4.0)	US (1)
	ST59	7 (15.2)	P, OX, K, E, L, TE	5 (71.4)	France (2), The Netherlands (2), Singapore (1)

			P, OX	1 (14.3)	US (1)
			P, OX, K, T, G, E, L, TE	1 (14.3)	France (1)
	ST22	3 (6.5)	P, OX, K, T, G	2 (66.7)	The Netherlands (2)
			P, OX, FU	1 (33.3)	Germany (1)
	ST766	1 (2.2)	P, OX, K, T, G, E, TE, OFL	1 (100.0)	Singapore (1)
	ST377	10 (21.7)	P, OX, K, T, G	10 (100.0)	The Netherlands (1), France (1), Greece (5), Switzerland (2), Australia (1)
***agr* 2**		**9 (1.9)**			
	ST5	9 (100.0)	P, OX, TE, FU	8 (88.9)	France (3), Switzerland (5)
			P, OX, K, T, E, L, TE	1 (11.1)	Algeria (1)
***agr* 3**		**414 (88.3)**			
	ST80	357 (83.2)	P, OX, K	25 (7.0)	Algeria (9), France (13), Greece (1), Switzerland (2)
			P, OX, K,E	12 (3.4)	Algeria (5), France (6), Switzerland (1)
			P, OX, K, FU	19 (5.3)	Algeria (4), France (13), Switzerland (2)
			P, OX, K, TE	6 (1.7)	Algeria (1), France (5)
			P, OX, K, E, FU	8 (2.2)	Algeria (1), France (5), Switzerland (2)
			P, OX, K, E, L	1 (0.3)	France (1)
			P, OX, K, E, Rif	1 (0.3)	Algeria (1)
			P, OX, K, OFL, FU	1 (0.3)	Algeria (1)
			P, OX, K, TE, FU	205 (57.4)	Algeria (27), Belgium (1), France (147), Germany (1), Greece (3), The Netherlands (2), Slovenia (3), Switzerland (20), Singapore (1)
			P, OX, K, T, G	1 (0.3)	France (1)
			P, OX, K, E, L, FU	1 (0.3)	France (1)
			P, OX, K, E, TE, OFL	1 (0.3)	France (1)
			P, OX, K, E, TE, FU	59 (16.5)	Algeria (5), France (48), Romania (1), Switzerland (5)
			P, OX, K, E, L, TE, FU	2 (0.6)	France (2)
			P, OX, K, T, E, L, TE	1 (0.3)	Algeria (1)
			P, OX, K, T, G, OFL, FU	2 (0.6)	Algeria (2)
			P, OX, K, T, G, TE, FU	1 (0.3)	Algeria (1)
			P, OX, K, E, L, TE, OFL, FU	2 (0.6)	Algeria (2)
			P, OX, K, T, G, E, OFL, FU	1 (0.3)	Algeria (1)
			P, OX, K, T, E, L, OFL, FU	1 (0.3)	Algeria (1)
			P, OX, K, T, G, E, TE, FU	1 (0.3)	France (1)
			P, OX, K, T, G, OFL, FU, Rif	2 (0.6)	Algeria (2)
			P, OX, K, T, G, TE, FU, Rif	1 (0.3)	Algeria (1)

		P, OX, K, T, E, L, PRI, OFL, FU	2 (0.6)	Algeria (2)
		P, OX, K, T, G, E, L, PRI, OFL, FU	1 (0.3)	Algeria (1)
ST30	20 (4.8)	P, OX	18 (90.0)	The Netherlands (1), Australia (8), Japan (1), New-Zealand (4), Western Samoa (1), Switzerland (2), Singapore (1)
		P, OX, K, T	1 (5.0)	French Polynesia (1)
		P, OX, K, T, G, E, L	1 (5.0)	China (1)
ST37	1 (0.2)	P, OX, K, T, G, E, TE	1(100.0)	The Netherlands (1)
ST93	4 (100.0)	P, OX	3 (75.0)	Australia (3)
		P, OX, E	1 (25.0)	Australia (1)
ST1	32 (7.7)	P, OX	17 (53.1)	US (17)
		P, OX, E	10 (31.2)	US (9), France (1)
		P, OX, TE	4 (12.5)	US (4)
		P, OX, K, T, G	1 (3.1)	Singapore (1)

[a] penicillin (P), oxacillin, (OX), kanamycin (K), tobramycin (T), gentamicin (G), erythromycin (E), lincomycin (L), tetracycline (TE), pristinamycin (PRI), ofloxacin (OFL), fusidic acid (FU), rifampycin (Rif)

Table 3- Antibiotic resistance profil of PVL-positive CA-MRSA clone

Bibliography

1. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. Jama 2003,290:2976-84.
2. Bocchini CE, Hulten KG, Mason EO, Jr., Gonzalez BE, Hammerman WA, Kaplan SL. Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. Pediatrics 2006,117:433-40.
3. Garnier F, Tristan A, Francois B, Etienne J, Delage-Corre M, Martin C, et al. Pneumonia and new methicillin-resistant *Staphylococcus aureus* clone. Emerg Infect Dis 2006,12:498-500.
4. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 2003,9:978-84.
5. Dufour P, Gillet Y, Bes M, Lina G, Vandenesch F, Floret D, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: emergence of a single clone that produces Panton-Valentine leukocidin. Clin Infect Dis 2002,35:819-24.
6. Harbarth S, Francois P, Shrenzel J, Fankhauser-Rodriguez C, Hugonnet S, Koessler T, et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Switzerland. Emerg Infect Dis 2005,11:962-5.
7. Wannet WJ, Spalburg E, Heck ME, Pluister GN, Tiemersma E, Willems RJ, et al. Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in The Netherlands. J Clin Microbiol 2005,43:3341-5.

8. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J Clin Microbiol 2005,43:2384-90.
9. Denis O, Deplano A, De Beenhouwer H, Hallin M, Huysmans G, Garrino MG, et al. Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton-Valentine leucocidin genes in Belgium. J Antimicrob Chemother 2005,56:1103-6.
10. Wannet WJ, Heck ME, Pluister GN, Spalburg E, van Santen MG, Huijsdans XW, et al. Panton-Valentine leukocidin positive MRSA in 2003: the Dutch situation. Euro Surveill 2004,9:28-9.
11. Faria NA, Oliveira DC, Westh H, Monnet DL, Larsen AR, Skov R, de Lencastre H. Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. J Clin Microbiol 2005,43:1836-42.
12. Roberts JC, Krueger RL, Peak KK, Veguilla W, Cannons AC, Amuso PT, Cattani J. Community-associated methicillin-resistant *Staphylococcus aureus* epidemic clone USA300 in isolates from Florida and Washington. J Clin Microbiol 2006,44:225-26.
13. Mishaan AM, Mason EO, Jr., Martinez-Aguilar G, Hammerman W, Propst JJ, Lupski JR, et al. Emergence of a predominant clone of community-acquired *Staphylococcus aureus* among children in Houston, Texas. Pediatr Infect Dis J 2005,24:201-6.
14. Pan ES, Diep BA, Charlebois ED, Auerswald C, Carleton HA, Sensabaugh GF, Perdreau-Remington F. Population dynamics of nasal strains of methicillin-resistant *Staphylococcus aureus*--and their relation to community-associated disease activity. J Infect Dis 2005,192:811-18.
15. Takizawa Y, Taneike I, Nakagawa S, Oishi T, Nitahara Y, Iwakura N, et al. A Panton-Valentine leucocidin (PVL)-positive community-acquired methicillin-resistant *Staphylococcus aureus*

(MRSA) strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical PVL-negative MRSA strains found in Japan. J Clin Microbiol 2005,43:3356-63.

16. Smith JM, Cook GM. A decade of community MRSA in New Zealand. Epidemiol Infect 2005,133:899-904.
17. Vlack S, Cox L, Peleg AY, Canuto C, Stewart C, Conlon A, et al. Carriage of methicillin-resistant *Staphylococcus aureus* in a Queensland Indigenous community. Med J Aust 2006,184:556-9.
18. Hsu LY, Tristan A, Koh TH, Bes M, Etienne J, Kurup A, et al. Community associated methicillin-resistant *Staphylococcus aureus*, Singapore. Emerg Infect Dis 2005,11:341-2.
19. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med 2006,355:666-74.
20. Naas T, Fortineau N, Spicq C, Robert J, Jarlier V, Nordmann P. Three-year survey of community-acquired methicillin-resistant *Staphylococcus aureus* producing Panton-Valentine leukocidin in a French university hospital. J Hosp Infect 2005,61:321-9.
21. Del Giudice P, Blanc V, Durupt F, Bes M, Martinez JP, Counillon E, et al. Emergence of two populations of methicillin-resistant *Staphylococcus aureus* with distinct epidemiological, clinical and biological features, isolated from patients with community-acquired skin infections. Br J Dermatol 2006,154:118-24.
22. Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother 1999,43:1062-6.
23. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, et al. Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun 2002,70:631-41.

24. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Detection of methicillin-resistant *Staphylococcus aureus* by polymerase chain reaction. Rinsho Byori 1991,39:1325-30.

25. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2002,46:2155-61.

26. Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, ccrC. Antimicrob Agents Chemother 2004,48:2637-51.

27. Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, et al. Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. Infect Immun 2002,70:4987-96.

28. Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G. Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. J Clin Microbiol 2003,41:4465-7.

29. Strommenger B, Kettlitz C, Werner G, Witte W. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J Clin Microbiol 2003,41:4089-94.

30. Mellmann A, Friedrich AW, Rosenkotter N, Rothganger J, Karch H, Reintjes R, Harmsen D. Automated DNA Sequence-Based Early Warning System for the Detection of Methicillin-Resistant *Staphylococcus aureus* Outbreaks. PLoS Med 2006,3:e33.

31. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol 2003,41:5442-8.

32. Sammeth M, Weiniger T, Harmsen D, Stoye J. Alignment of Tandem Repeats with Excision, Duplication, Substitution and Indels (EDSI). WABI. LNBI 3692 2005.

33. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000,38:1008-15.

34. Urwin R, Maiden MC. Multi-locus sequence typing: a tool for global epidemiology. Trends Microbiol 2003,11:479-87.

35. Salmenlinna S, Lyytikainen O, Vuopio-Varkila J. Community-acquired methicillin-resistant *Staphylococcus aureus*, Finland. Emerg Infect Dis 2002,8:602-7.

36. Aires de Sousa M, Bartzavali C, Spiliopoulou I, Sanches IS, Crisostomo MI, de Lencastre H. Two international methicillin-resistant *Staphylococcus aureus* clones endemic in a university hospital in Patras, Greece. J Clin Microbiol 2003,41:2027-32.

37. Otsuka T, Saito K, Dohmae S, Takano T, Higuchi W, Takizawa Y, et al. Key adhesin gene in community-acquired methicillin-resistant *Staphylococcus aureus*. Biochem Biophys Res Commun 2006,346:1234-44.

38. Kourbatova EV, Halvosa JS, King MD, Ray SM, White N, Blumberg HM. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA 300 clone as a cause of health care-associated infections among patients with prosthetic joint infections. Am J Infect Control 2005,33:385-91.

39. Ramdani-Bouguessa N, Bes M, Meugnier H, Forey F, Reverdy ME, Lina G, et al. Detection of methicillin-resistant *Staphylococcus aureus* strains resistant to multiple antibiotics and carrying the Panton-Valentine leukocidin genes in an Algiers hospital. Antimicrob Agents Chemother 2006,50:1083-5.

40. Maier J, Melzl H, Reischl U, Drubel I, Witte W, Lehn N, Linde H. Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* in Germany associated with travel or foreign family origin. Eur J Clin Microbiol Infect Dis 2005,24:637-9.

41. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 2002,359:753-9.

42. Hanssen AM, Fossum A, Mikalsen J, Halvorsen DS, Bukholm G, Sollid JU. Dissemination of community-acquired methicillin-resistant *Staphylococcus aureus* clones in northern Norway: sequence types 8 and 80 predominate. J Clin Microbiol 2005,43:2118-24.

43. Wang CC, Lo WT, Chu ML, Siu LK. Epidemiological typing of community-acquired methicillin-resistant *Staphylococcus aureus* isolates from children in Taiwan. Clin Infect Dis 2004,39:481-7.

44. Krzyston-Russjan J, Tambic-Andrasevic A, Bukovski S, Sabat A, Hryniewicz W. First community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) strains in Croatia. Clin Microbiol Infect 2006,12:697-8.

45. Miklasevics E, Haeggman S, Balode A, Sanchez B, Martinsons A, Olsson-Liljequist B, Dumpis U. Report on the first PVL-positive community acquired MRSA strain in Latvia. Euro Surveill 2004,9:29-30.

46. Ribeiro A, Dias C, Silva-Carvalho MC, Berquo L, Ferreira FA, Santos RN, et al. First report of infection with community-acquired methicillin-resistant *Staphylococcus aureus* in South America. J Clin Microbiol 2005,43:1985-8.

47. Ma XX, Galiana A, Pedreira W, Mowszowicz M, Christophersen I, Machiavello S, et al. Community-acquired methicillin-resistant *Staphylococcus aureus*, Uruguay. Emerg Infect Dis 2005,11:973-6.

48. Ho PL, Tse CW, Mak GC, Chow KH, Ng TK. Community-acquired methicillin-resistant *Staphylococcus aureus* arrives in Hong Kong. J Antimicrob Chemother 2004,54:845-6.

49. Mulvey MR, MacDougall L, Cholin B, Horsman G, Fidyk M, Woods S. Community-associated methicillin-resistant *Staphylococcus aureus*, Canada. Emerg Infect Dis 2005,11:844-50.