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Community-acquired methicillin-resistant *Staphylococcus aureus* clones circulating in Belgium from 2005 to 2009: changing epidemiology

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Abstract The present study reports the evolution of the demographic characteristics and the molecular epidemiology of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Belgium from 2005 to 2009. Four hundred and ten CA-MRSA isolates were prospectively collected and screened for the presence of Panton–Valentin leucocidin (PVL) and toxic shock syndrome toxin 1 (TSST-1) encoding genes, while clinical information were recorded. PVL- and TSST-1-positive isolates were genotyped by pulsed-field gel electrophoresis (PFGE). Staphylococcal cassette chromosome *mec* (SCC*mec*) type, *spa* type and multilocus sequence type (MLST) were determined on representative isolates. One hundred and fifty-nine (39 %) isolates were PVL-positive. PVL-positive isolates were significantly more frequently isolated from skin or soft tissue than PVL-negative isolates, causing mainly subcutaneous abscesses and furuncles. Patients with PVL-positive CA-MRSA were significantly younger than patients with PVL-negative CA-MRSA. Eighty-seven percent of the PVL-positive isolates belonged to a limited number ($n=7$) of PFGE types belonging to sequence types (ST) ST80, ST8, ST30, ST5, ST152, ST338 and a new ST, a single-locus variant of ST1. A temporal evolution of the distribution of these PFGE types was observed, characterised by (1) the dissemination of the ST8-SCC*mec*IV *arc*A-positive (USA300) genotype and (2) a genetic diversification. Forty-seven (11 %) strains were TSST-1-positive, of which 65 % clustered into four PFGE types, all belonging to ST5. The epidemiology of CA-MRSA in Belgium is changing, as the rapid diffusion of the USA300 clone seems to occur, together with a clonal diversification.

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Introduction

The first methicillin-resistant *Staphylococcus aureus* (MRSA) emerged in the early 1960s, 2 years after methicillin was introduced in medical practice. Since then, MRSA strains have spread globally and caused countless nosocomial outbreaks, making MRSA one of the most common causes of nosocomial infections. Besides these healthcare-associated MRSA (HA-MRSA), MRSA infections now also occur in the community (community-acquired methicillin-resistant *S. aureus*, CA-MRSA). CA-MRSA infections usually occur in young and healthy patients without any healthcare-associated risks factors for MRSA acquisition. These CA-MRSA strains generally cause mild skin and soft tissue infections, but can also cause life-threatening infections, distinct from those caused by HA-MRSA strains, such as necrotising osteomyelitis and necrotising pneumonia [1]. Classically, CA-MRSA strains possess several distinctive characteristics as compared with HA-MRSA strains: (i) they are often producing the leucocidin of Panton–Valentine (PVL), (ii) they belong to genetic backgrounds unrelated to

those of pandemic HA-MRSA clones and (iii) they harbour a staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V [2].

The prevalence of CA-MRSA infections varies widely from one country to another [3]. For example, many differences exist between the epidemiology of CA-MRSA in the USA and in Europe. In the USA, CA-MRSA is one of the most common cause of skin and soft tissue infections in patients from emergency departments and one clone, the USA300 clone (ST8-SCC*mec* IV), is largely predominant [4]. This clone harbours the arginine catabolic mobile element (ACME), which is thought to enhance its colonising capacities [1]. In Europe, the prevalence of CA-MRSA is much lower but is increasing, especially in countries where the incidence of HA-MRSA is low, such as Denmark or the Netherlands [5]. Many different CA-MRSA clones are found, of which the ST80-SCC*mec* IV European clone is the most widely disseminated [5]. Recently, however, several European countries have been confronted with the rise of the prevalence of USA300 strains [3, 6].

In Belgium, the first PVL-positive MRSA were reported in 2002–2004 [7]. At that time, infections due to PVL-positive MRSA seemed to be sporadic cases and strains belonged to four major lineages (ST80, ST8, ST30 and ST88) [7]. The majority of strains had a genetic background that was distinct from those of Belgian HA-MRSA epidemic clones, and several cases were imported. The objectives of the present study were to update these data by following the demographic characteristics and molecular epidemiology of PVL-positive CA-MRSA colonisation and infection cases in Belgium from 2005 to 2009.

Materials and methods

Bacterial isolates, clinical and demographic information

Since 2002, all Belgian microbiology laboratories ($n\sim250$) are invited by the Reference Laboratory for *Staphylococcus aureus* (BRLS) to refer for detection of exotoxin genes all CA-MRSA strains defined as strains isolated from ambulatory patients or during the first 48 h of hospitalisation. Each isolate has to be sent with a case report form describing the demographics and clinical mandatory information: sex and age of the patient, clinical site and date of sampling. Additional optional information fields include history of hospitalisation or travel during the year preceding the sampling and if the case is sporadic or part of a cluster.

Identification and susceptibility testing

All isolates sent from January 2005 to December 2009 were confirmed to be MRSA both phenotypically (coagulase test

with human plasma and oxacillin susceptibility test using cefoxitin 30-μg discs) [8] and genotypically [detection of 16S rRNA, *mec*A and *nuc* genes by multiplex polymerase chain reaction (PCR) assay] [9].

Additionally, isolates collected between January 2005 and February 2008 were tested by disc diffusion for susceptibility to 12 antimicrobial agents (erythromycin, clindamycin, ciprofloxacin, gentamicin, kanamycin, tobramycin, fusidic acid, tetracycline, doxycycline, linezolid, trimethoprim/sulfamethoxazole, mupirocin). Isolates collected from March 2008 to December 2009 were tested by the Vitek 2 automated system (bioMérieux) using the AST-P549 card for the same antimicrobials, except for kanamycin and doxycycline, which are not present on the card, that were tested by disc diffusion, as described above. Clinical and Laboratory Standards Institute (CLSI) breakpoints were used for interpretation [10], except for fusidic acid and mupirocin, for which the Committee for Antimicrobial Testing of the French Society of Microbiology [11] and the British Society for Antimicrobial Chemotherapy (BSAC) [12] criteria were used, respectively.

Exotoxin genes detection

All MRSA isolates were screened for the presence of the PVL, toxic shock syndrome toxin 1 (TSST-1), and exfoliatin A and B encoding genes by PCR [13].

Molecular typing and characterisation

All exotoxin-positive isolates were genotyped by *Sma*I restriction of genomic DNA resolved by pulsed-field gel electrophoresis (PFGE) and classified as previously described [14]. The NARSA NRS384 strain was used for the USA300 reference profile.

For PVL- and TSST-1 positive isolates, SCC*mec* type and *spa* type were determined as previously described [15, 16] on randomly selected representative isolates (one per PFGE type per year). Multilocus sequence typing (MLST) was performed on one isolate per PFGE type randomly selected within the latter subset [17].

All PVL-positive isolates were further screened by PCR for the presence of the arginine deiminase gene (*arc*A, carried on the ACME) and the exfoliatin D encoding gene (*etd*), which are recognised as good markers for the USA300 and the European clones, respectively [18], and for the presence of enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*) [19]. Additionally, the presence of the antibiotic resistance genes *aph* (3′)-IIIa, *ant*(4′)-Ia, *aac*(6′)-Ie + *aph*(2″), *erm* (A, B and C) and *msr*A, *tet*K and *tet*M was searched by PCR on all kanamycin-, tobramycin-, gentamicin-, erythromycin- and tetracycline-resistant PVL-positive isolates, respectively [20–22].

Results

Participation and bacterial isolates

From January 2005 to December 2009, the BRLS received from 74 laboratories a total of 410 non-duplicated MRSA: nine laboratories of the Brussels-Capital region (that accounts for 10 % of the total Belgian population) sent 109 isolates (27 %), 44 laboratories located in the Flanders region (58 % of the Belgian population) sent 199 isolates (49 %) and 21 laboratories located in Wallonia (32 % of the population) sent 102 isolates (25 %). The relative proportion of isolates sent per region remained stable over the 5 years, except in 2009, where up to 45 % of the isolates received were from the Brussels-Capital region.

One hundred and fifty-nine isolates harboured the PVL-encoding genes, 47 the *tst* gene and two the *eta* gene. The number of PVL-positive isolates received per year increased progressively from 18 in 2005 to 48 in 2009, representing, every year, about 40 % (range 32 to 45 %) of the total number of isolates received (Fig. 1).
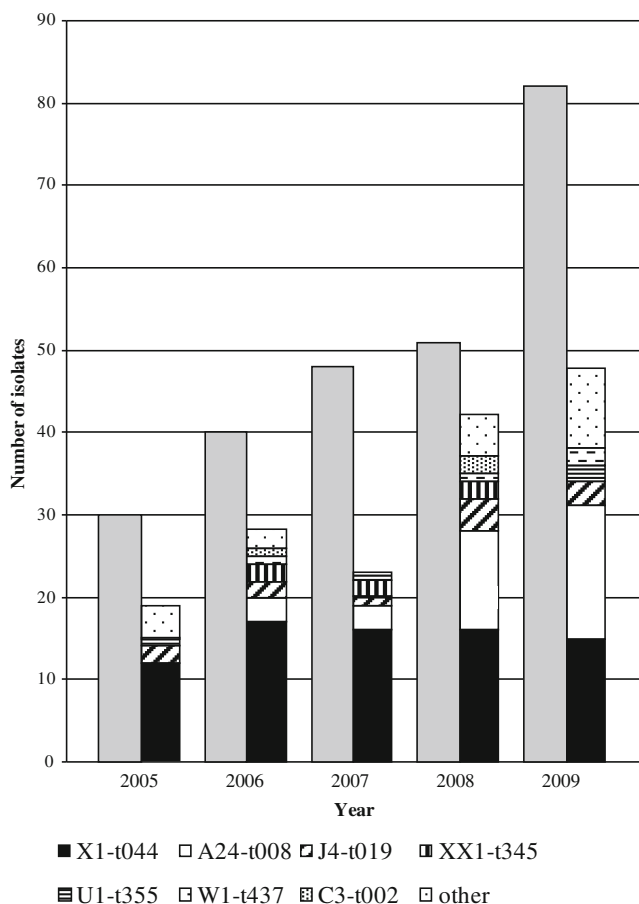
Fig. 1 Annual distribution of PVL-negative (*left grey bars*) and PVL-positive (*right black and white bars*) CA-MRSA, 2005–2009. For PVL-positive CA-MRSA, PFGE and *spa* types distribution are displayed

Demographic and clinical data

Three hundred and eighty case report forms (150 from PVL-positive and 230 from PVL-negative isolates) were available for analysis (Table 1). Patients with PVL-positive CA-MRSA had a Gaussian age distribution with a median of 23 years, which was significantly younger than the median observed among patients with PVL-negative CA-MRSA. The male/female ratio was 1.4. PVL-positive isolates were significantly more frequently isolated from skin or soft tissue (77 %) than PVL-negative isolates (37 %), causing significantly more subcutaneous abscesses and furuncles (Table 1). TSST-1-positive isolates were also observed more often in younger patients (median 20 years) compared to the other PVL-negative isolates and were significantly more frequently isolated from ear, nose and throat (ENT) specimens (8 out of the 40 evaluable forms reported an ear specimen).

Additional optional information was only available for about half of the isolates (see Table 1). When reported, hospitalisation or chronic pathology with frequent contacts with hospital were more frequently associated with PVL-negative isolates (85 cases), while travel within the preceding year was more frequently associated with PVL-positive isolates (22 cases). Few clusters were reported, three involving PVL-negative isolates ($n=11$) and five involving PVL-positive isolates ($n=14$). Four out of the five clusters involving PVL-positive isolates were related to travel abroad (Egypt, USA, Turkey and India).

Molecular typing results

Eighty-seven percent of the 159 PVL-positive isolates belonged to seven genotypes: PFGE X1-*spa* t044-ST80-SCC*mec* IV ($n=76$), A24-*spa* t008-ST8-SCC*mec* IV ($n=34$), J4-*spa* t019-ST30-SCC*mec* IV ($n=12$), XX1-*spa* t345-New ST (SLV of ST1)-SCC*mec* V ($n=6$), W1-*spa* t437–ST338-SCC*mec* V ($n=4$), U1–*spa* t355-ST152-SCC*mec* V ($n=4$) and C3-*spa* t002-ST5-SCC*mec* V ($n=3$) (Fig. 1, Table 2). All A24-*spa* t008-ST8-SCC*mec* IV strains carried the *arcA* gene.

A temporal evolution of the distribution of these different genotypes was observed (Fig. 1) and was mainly characterised by (1) the rising of the A24-*spa* t008-ST8-SCC*mec* IV genotype (from 0 % in 2005 to 33 % in 2009) and (2) a genetic diversification from six different PFGE types (belonging to three different MLST CCs) in 2005 to 14 PFGE types (belonging to nine CCs) in 2009. We found no significant association between clusters or travel abroad and a particular genotype, but the samples sizes were small. The four travel-related clusters were due to four distinct genotypes: X1-*spa* t044-ST80-SCC*mec* IV (Egypt), A24-t008-ST8-SCC*mec* IV (USA), J4-t019-ST30-SCC*mec* IV (Turkey) and XX1-t345-New ST (SLV of ST1)-SCC*mec* V (India).

Twenty-eight (65 %) of the 47 TSST-1-positive strains belonged to four PFGE types: C3 ($n=12$), C4 ($n=11$), C6

Table 1 Demographics, type of specimen and epidemiology of patients with CA-MRSA, 2005–2009

	Patients with:				p-value[a]
	PVL-positive MRSA (no. of evaluable reports)		PVL-negative MRSA (no. of evaluable reports)		
Demographics					
Sex ratio (M/F)	1.4	(153)	0.8	(230)	0.01
Median age (years)	23	(150)	42	(231)	<0.01[b]
Range	(0, 95)		(0, 96)		
Body site		(156)		(254)	<0.01
Blood	8		26		0.09
ENT	5		25		0.02
Respiratory tract	4		18		0.07
Screening at mucocutaneous site	16		77		<0.01
Skin and soft tissue	122		95		<0.01
Abscess	46		11		<0.01
Furuncle	38		9		<0.01
Cellulitis	5		12		0.04
Impetigo	6		6		0.88
Wound infection	23		55		<0.01
Not specified	4		2		ND
Other	1		13		ND
Epidemiology					
Recent travel abroad <1 year	22	(72)	9	(98)	<0.01
Hospitalisation <1 year or chronic pathology with frequent contacts with hospital	36	(98)	85	(143)	<0.01
Cluster-associated	14 (5 clusters)	(119)	11 (3 clusters)	(220)	ND

ENT ear, nose and throat; *ND* not done; *M* male; *F* female

[a] Chi-square test

[b] Mann–Whitney

(n=3) and G10 (n=5), all belonging to ST5 by MLST and harbouring *spa* t002 or related *spa* types, but distinct SCC*mec* types. C3 and C4 strains were SCC*mec* I, C6 strains were SCC*mec* I (n=1) or non-typable (NT, n=2), and G10 strains were SCC*mec* II. The two *eta*-positive isolates harboured unrelated PFGE types.

Susceptibility and resistance genes profiles

The majority of PVL-positive CA-MRSA isolates was kanamycin- and tetracycline-resistant (74 and 57 %, respectively), which was significantly more than among PVL-negative isolates (Table 3). Up to 41 % of strains were resistant to fusidic acid, which was not significantly more than that observed among PVL-negative isolates (Table 3). Thirty-seven percent of strains were erythromycin resistant. Resistance to gentamicin (4 %), trimethoprim/sulfamethoxazole (3 %) and doxycycline (7 %) was low, as well as resistance rates to tobramycin (6 %), clindamycin (2 %) and ciprofloxacin (15 %), which were significantly lower than for PVL-negative isolates (Table 3).

Resistance profiles, strongly linked to genotypes, followed the epidemiological changes over time (Table 3). This led to an increase in the proportion of erythromycin- and ciprofloxacin-resistant PVL-positive MRSA strains (due to the dissemination of the USA300 *arc*A-positive A24-ST8-SCC*mec* IV clone) and a decrease in the proportion of tetracycline- and fusidic acid-resistant PVL-positive MRSA strains (characteristic of the European X1-ST80-SCC*mec* IV clone). Of note, TSST-1-positive major clones also differed by their resistance profiles, and all C3, C4 and C6 ST5-SCC*mec* I isolates were fusidic acid-resistant as opposed to G10 ST5-SCC*mec* II isolates.

Among PVL-positive isolates, tetracycline resistance was mainly mediated by the *tet*K gene (96 %). The most prevalent aminoglycoside resistance gene was *aph*(3′)-IIIa, present in 94 % of the 126 kanamycin non-susceptible isolates (mostly belonging to X1-ST80-SCC*mec* IV and A24-ST8-SCC*mec* IV clones). *aac*(6′)-Ie + *aph*(2″) was found in the seven isolates combining kanamycin, tobramycin and gentamicin resistance (mainly U1-ST152-SCC*mec* V and C3-ST5-SCC*mec* V), while *ant*(4′)-Ia was rarely observed

Table 2 Distribution of 159 PVL-positive CA-MRSA by PFGE, *spa*, MLST, SCC*mec* types and toxin genes profile, 2005–2009

Type					Toxin genes detected (no. of positive isolates)
PFGE	*spa* (no. of isolates typed)	SCC*mec* (no. of isolates typed)	MLST (one isolate typed)	Total no. of isolates	
A23	t008 (1)	IV (1)	ST8	2	*sek* (2), *seq* (2)
A24	t008 (4)	IV (4)	ST8	34	*arc*A (34), *sek* (27), *seq* (32)
C3	t002 (2)	V (2)	ST5	3	*sed* (3), *seg* (3), *sei* (3), *sej* (3), *sem* (3), *sen* (3), *seo* (3), *sep* (3), *ser* (3)
J4	t019 (5)	IV (5[a])	ST30	12	*etd* (1), *seg* (11), *sei* (9), *sen* (9)
J9	t021 (1)	IV (1)	ST30	2	*seg* (2), *sei* (1), *sen* (1)
L1	t852 (2)	IV (2)	ST22	2	*seg* (2), *sei* (2), *semi* (2), *sen* (2), *seo* (2)
X1	t044 (5)	IV (5[a])	ST80	76	*etd* (73), *seg* (1), *sen* (1)
XX1	t345 (3)	V (3)	New ST	6	*sea* (6), *seg* (6), *sei* (6), *sem* (6), *sen* (6), *seo* (6)
W1	t437 (3)	V (3)	ST338	4	*seb* (4), *sek* (4), *seq* (4)
U1	t355 (3)	V (3)	ST152	4	None
ZA3	t311 (2)	IV (2)	ST5	2	*seg* (2), *sei* (2), *sem* (2), *sen* (2), *seo* (2)
Singletons				12	
Total				159	

Singleton: PFGE types represented by a single isolate

[a] One isolate was NT

(Table 3). Macrolides resistance was mediated by *msr*A in a majority of erythromycin-resistant isolates (61 %), which belonged mostly to the A24-ST8-SCC*mec* IV clone. By contrast, *erm*C, which was less frequent (34 %), was the gene harboured by the erythromycin-resistant X1-ST80-SCC*mec* IV isolates (Table 3).

Toxin genes profiles

The distribution of toxin genes was also strongly linked to the PFGE type. The X1-ST80-SCC*mec* IV type was associated with the carriage of *etD* and the lack of enterotoxin genes, while the A24 and A23 (ST8-SCC*mec* IV) isolates harboured the *sek* and *seq* enterotoxin genes. *sek* and *seq* were also present in W1-ST338-SCC*mec* V isolates, together with *seb*. The *egc* complex (*seg-sei-sem-sen-seo*) was present in L1-ST22-SCC*mec* IV and ZA3-ST5-SCC*mec* IV isolates, in XX1-New ST (single-locus variant of ST1)-SCC*mec* V isolates (in association with *sea*) and in C3-ST5-SCC*mec*V isolates (in association with *sed-sej-sel*). U1-ST152-SCC*mec* V isolates lacked any toxin gene targeted (Table 2).

Discussion

In Belgium, the first PVL-positive CA-MRSA isolate was reported in 2002, leading the BSRL to issue alert messages to microbiology laboratories about the need to refer CA-MRSA for the detection of exotoxin genes. At that time, infections due to PVL-positive CA-MRSA seemed to be sporadic cases and isolates belonged to four major lineages, the large majority belonging to the ST80-SCC*mec* IV European clone [7]. The present study updates these data by following the epidemiology of CA-MRSA cases from 2005 to 2009.

Our survey bears the obvious limitation of being voluntary based, which has the likely consequence of underestimating the true occurrence of CA-MRSA infections. Another drawback is the lack of answers in about half of the report forms concerning important epidemiological information such as healthcare-associated risk factors or travel abroad. However, it presents the advantage to be prospective, nationwide and to cover a 5-year period.

The number of CA-MRSA isolates received per year progressively increased from 48 in 2005 to 130 in 2009. As our survey is voluntary based, the reasons for this increase are difficult to ascertain. It could reflect a true increase in the CA-MRSA incidence, simply be due to an "artefactual" increase of sampling and/or sending due to a progressively growing awareness of laboratories and field practitioners regarding the CA-MRSA threat, or be the result of a mix of these two phenomenons. The majority of isolates received (about 60 % in each year) were PVL negative. PVL-negative isolates affected older patients than PVL-positive isolates, and about up to 30 % of them were recovered from admission screening samples. A recent study conducted in Belgian hospitals has demonstrated that previous healthcare exposure remains the main risk factor for MRSA carriage on admission [23]. However, when

Table 3 Resistance profile of PVL-positive and PVL-negative CA-MRSA, 2005–2009. For PVL-positive isolates, the resistance genes distribution per PFGE type is displayed

Antimicrobial Resistance genes	PVL-positive isolates								Total (159)	PVL-negative isolates	p-value[a]
	PFGE type and corresponding *spa* type									Total (251)	
	X1-t044 (76)	A24-t008 (34)	J4-t019 (12)	XX1-t345 (6)	W1-t437 (4)	U1-t355 (4)	C3-t002 (3)	Other (20)			
Kana	74	21	1	6	3	4	3	5	118	151	<0.01
Tobra	–	–	–	–	–	3	3	2	8	122	<0.01
Genta	–	–	–	–	–	3	3	1	7	18	0.35
*aph*3′	74	21	1	6	3	2	–	3	111		
*ant*4	1	–	–	–	–	–	–	3	4		
aac-aph	–	–	–	–	–	3	3	1	7		
Tetra	69	3	–	6	2	–	–	10	90	96	<0.01
*tet*M	–	–	–	–	–	–	–	4	4		
*tet*K	69	3	–	6	2	–	–	6	86		
Erythro	15	28	–	6	3	–	–	7	59	112	0.16
*msr*A	–	28	–	6	–	–	–	2	36		
*erm*A	–	1	–	–	–	–	–	1	2		
*erm*B	–	–	–	–	3	–	–	–	3		
*erm*C	15	2	–	–	–	–	–	4	21		
Clinda	–	–	–	–	3	–	–	1	4	65	<0.01
Doxy	3	–	1	5	1	–	–	2	12	8	0.08
Fusi	65	–	–	–	–	–	–	1	66	77	0.03
Cipro	1	14	–	6	–	–	–	4	24	138	<0.01
Sxt	–	–	–	1	–	–	–	4	5	12	0.58
Mupi	–	–	–	–	–	–	–	–	–	7	0.12
Line	–	–	–	–	–	–	–	–	–	–	ND

cipro ciprofloxacin; *clinda* clindamycin; *doxy* doxycycline; *erythro* erythromycin; *fusi* fusidic acid; *genta* gentamicin; *kana* kanamycin; *line* linezolid; *mupi* mupirocin; *sxt* trimethoprim/sulfamethoxazole; *tetra* tetracycline; *tobra* tobramycin

[a] Chi-square test

answered, the absence of previous hospitalisation or chronic pathology with frequent contacts with hospital was reported in 40 % of our PVL-negative CA-MRSA cases. In Belgium, the dissemination of HA-MRSA strains to long-term care facilities, serving as a reservoir of carriers, is a well-known phenomenon [24] that might account for part of these PVL-negative cases that could be considered as 'healthcare-associated CA-MRSA' (hCA-MRSA).

PVL-positive CA-MRSA received per year also tripled from 2005 to 2009, causing predominantly skin and soft tissue infections in young adults. Recent travel abroad was reported for a few cases. As compared to 2002–2004, the number of X1-ST80-SCC*mec* IV isolates remained stable (about 15/year), making the European clone the predominant PVL-positive CA-MRSA clone in Belgium. Similarly, the less frequent J4-ST30-SCC*mec* IV South West Pacific clone has remained stable since 2002–2004. On the other hand, the number of USA300 A24-ST8-SCC*mec* IV isolates has progressively increased and the USA300 clone became,

in 2009, as frequently reported as the European clone, with apparently no association with travel abroad. Interestingly, and despite the fact that—our survey being voluntary based —no firm conclusion can be drawn regarding the exact incidence of each genotype, this USA300 progressive increase, together with a growing number of several other recent but less diffused genotypes, seem to add themselves to the stable burden caused by the European clone instead of replacing it.

The large majority of PVL-positive CA-MRSA isolates belonged to genetic backgrounds that are distinct from those of nosocomial MRSA strains from Belgian hospitals, except for A23/A24-*spa* t008-ST8-SCC*mec* IV and C3-*spa* t002-ST5-SCC*mec* V, which appear related to the widely disseminated hospital A20/A21 t008-ST8-SCC*mec* IV and C3 t002-ST5-SCC*mec* IV MRSA clones, respectively. However, ST8 and ST5 CA-MRSA differ from ST8 and ST5 HA-MRSA by their mobile genes content: A21/A20-ST8-SCC*mec* IV HA-MRSA isolates harbour the *sea* gene, lack

PVL genes and carry *ant*(4′) and e*rm*A resistance genes [25, 26], while A24-*spa* t008-ST8-SCC*mec* IV CA-MRSA harbour PVL genes, *arc*A, *sek* and *seq*, *msr*A and *aph*3′. Similarly, C3-*spa* t002-ST5 CA-MRSA and HA-MRSA differ by the SCC*mec* type they harbour, the PVL and *aac-aph* genes. These multiple differences suggest, rather, a divergent evolution (in hospitals and in the community) of strains belonging to a common genetic background than the 'adaptation' of local HA-MRSA clones to the community setting as the source of these PVL-positive CA-MRSA clones.

Eighteen percent of the PVL-negative isolates were found to be TSST-1-positive, and the majority of them (65 %) belonged to four genotypes: C3, C4 and C6-ST5-SCC*mec* I, and G10-ST5-SCC*mec* II. The exact genetic relatedness of TSST-1-positive C3 ST5-SCC*mec* I and G10 ST5-SCC*mec* II CA-MRSA with the closely related C3 t002-ST5-SCC*mec* IV and G10 t002-ST5-SCC*mec* II HA-MRSA clones has to be further studied. However, the majority of G10 HA-MRSA strains do possess the *tst* gene [25], suggesting that G10 HA-MRSA could be a likely origin for the G10 CA-MRSA observed here. On the contrary, C3 TSST-1-positive CA-MRSA do differ from C3 HA-MRSA by the SCC*mec* type they harbour, their resistance to fusidic acid and the *tst* gene. These C3 TSST-1-positive CA-MRSA could have been imported from France, where a similar TSST-1-positive ST5 SCC*mec* I CA-MRSA clone has recently been reported [27].

In conclusion, the epidemiology of CA-MRSA in Belgium is changing, as the rapid diffusion of the USA300 clone seems to occur, together with a clonal diversification. These epidemiological changes underline the importance of surveillance and typing data to monitor the CA-MRSA evolving epidemiology and control the spread of these organisms in the general population.

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