Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton-Valentine leucocidin genes in Belgium

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**Objectives**: Worldwide spread of a limited number of Panton-Valentine leucocidin (PVL)-producing methicillin-resistant *Staphylococcus aureus* (MRSA) clones has been reported in various communities. The objective of this study was to describe the molecular characteristics of the first PVL-positive MRSA strains isolated in Belgium.

**Methods**: Clinical MRSA isolates (\(n = 41\)) collected from 2002 to 2004 from Belgian patients were investigated for the PVL gene by PCR. PVL-positive isolates were genotyped by PFGE, staphylococcal cassette chromosome mec (SCC\(\text{mec}\)) typing, spa sequence typing, accessory gene regulator (agr) polymorphism and multi-locus sequence typing (MLST). Susceptibility to 14 antimicrobials was determined by the disc diffusion method. Genes encoding resistance to tetracyclines, aminoglycosides and macrolide-lincosamide-streptogramin were determined by PCR.

**Results**: Sixteen isolates carried *lukS-lukF* genes that encode the PVL toxin. All but one isolate were community-acquired. Three patients reported recent travel to North Africa and South America. They were associated with skin or soft tissue infections, bacteraemia and peritonitis. By molecular typing, they belonged to five genotypes: ST80-SCC\(\text{mec}\) IV, ST8-SCC\(\text{mec}\) IV, ST30-SCC\(\text{mec}\) IV, ST153-SCC\(\text{mec}\) IV and ST88-SCC\(\text{mec}\) IV. They belonged to the agr type 3 except for ST8 strains, which showed agr type 1. All isolates were susceptible to fluoroquinolones. Approximately, half of them were resistant to tetracycline, fusidic acid and kanamycin. Tetracycline-resistant strains harboured the tet(K) gene and resistance to kanamycin was associated with the *aph(3\text{-Ia})* gene. The single erythromycin-resistant isolate harboured *msr(A/B)* genes conferring the M resistance phenotype.

**Conclusions**: These results indicate the recent emergence and sporadic importation into Belgium of PVL-positive community-associated MRSA strains belonging to five distinct clones.

Keywords: MRSA, PVL, molecular characteristics, MLST

**Introduction**

*Staphylococcus aureus* is a major pathogen responsible for various infections including bacteraemia, pneumonia, skin and soft tissue infections, and osteomyelitis. Over the last two decades, epidemic strains of methicillin-resistant *S. aureus* (MRSA) have disseminated widely in acute-care and long-term care facilities. Risk factors for MRSA acquisition in outpatients include history of past hospitalization or surgery, residence in chronic care facilities and injection drug use.\(^1\) More recently, MRSA infections have been reported from Australia, USA and Europe, in populations lacking previous contact with healthcare facilities.\(^2\)

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These community-acquired MRSA (CA-MRSA) strains have been associated with skin and soft tissue infections and necrotizing pneumonia. Children and young adults are the predominantly affected age groups. CA-MRSA strains frequently produce Panton-Valentine leucocidin (PVL). This bi-component pore-forming exotoxin is encoded by two co-transcribed genes, lukS-PV and lukF-PV, carried on a prophage integrated in the S. aureus chromosome. Moreover, PVL-positive MRSA strains belong to a restricted number of epidemic clones that are unrelated to hospital-acquired strains. In this report, we describe the clinical presentation of the first series of CA-MRSA infections caused by PVL-positive MRSA strains identified in Belgium and we define their phenotypic and genotypic features.

Materials and methods

Bacterial isolates

In 2002–2003, the Belgian Reference Laboratory for Staphylococci issued alert messages to microbiology laboratories about the need to refer MRSA isolates from community-acquired infection for detection of exotoxin genes. From 2002 to 2004, the Belgian Reference Laboratory for Staphylococci received 41 MRSA isolates referred for characterization of toxin production. S. aureus identification was confirmed by using the coagulase test with human plasma. Oxacillin susceptibility was tested by cefoxitin disc (60 μg) (Rosco Neo-Sensitabs, Taastraup, Denmark) according to the CLSI.

PCR for toxin detection

A multiplex PCR assay was performed for the detection of 16S rRNA and mecA and nuc genes as previously described. The presence of PVL, TSST-1, and exfoliatin A and exfoliatin B genes was tested by multiplex PCR assays as previously described. S. aureus ATCC 49775 control strain harbouring PVL genes and S. aureus FRI913 control strain harbouring TSST-1 genes were included in each run.

Antimicrobial susceptibility testing

PVL-positive isolates were tested by the disc diffusion method (Rosco Neo-Sensitabs, Taastraup, Denmark) for susceptibility to 14 antimicrobial agents, including erythromycin, clindamycin, pristinamycin, ciprofloxacin, gentamicin, kanamycin, tobramycin, minocycline, tetracycline, rifampicin, trimethoprim/sulfamethoxazole, fusidic acid, linezolid and mupirocin. A double disc diffusion method with erythromycin, rifampicin, trimethoprim/sulfamethoxazole, fusidic acid, linezolid and mupirocin was used to differentiate the MLS resistance phenotypes. CLSI susceptibility breakpoints were used for interpretation of inhibition zone diameters except for fusidic acid and mupirocin, which were interpreted according to the criteria of the Committee for Antimicrobial Testing of the French Society of Microbiology (CA-SFM). Resistance to vancomycin was tested by vancomycin screen with Brain Heart Infusion (BHI) agar (6 mg/L) (Becton-Dickinson, USA).

Molecular typing

All PVL-positive MRSA isolates were genotyped by the Smal macrorestriction analysis of genomic DNA resolved by PFGE, determination of staphylococcal cassette chromosome mec (SCCmec) type and multi-locus sequence typing (MLST) (http://www.mlst.net) as previously described. PFGE patterns were classified in groups according to the previously described nomenclature. The accessory gene regulator (agr) polymorphism was determined by PCR. DNA sequence analysis of the polymorphic repeat region of the protein A gene (spa typing) was performed as previously described.

PCR for tetracycline, aminoglycoside and MLS resistance gene detection

Tetracycline resistance genes encoding efflux pump system tet(K) or for ribosomal protection protein tet(M), the aminoglycoside modifying enzymes (AME) encoded by aac(6’)-Ie + aph(2’)-Ia and aph(3’)-IIa genes, the ribosomal methylase encoded by ermA(A) and ermA(C) and the macrolide efflux pumps encoded by msrA(A) and msrB(B) genes were tested by PCR as previously described.

Results and discussion

Exotoxin genes were detected in 23 (56%) out of 41 isolates, including PVL genes in 16 (40%), TSST-1 gene in 5 (12%), exfoliatin A gene in 1 (2%) and exfoliatin B gene in 1 (2%). Eighteen (44%) isolates did not harbour any toxin gene. The median age of patients from whom PVL-positive MRSA strains were isolated was 24 years (range: 1–70 years) and the proportion of female patients was 56%. All but one isolate were community-acquired. Fourteen patients had previous hospital exposure before illness and two had been hospitalized for surgery during the previous 12 months. Five patients had received previous β-lactam therapy. Three patients reported travel to North Africa (n = 2) and South America (n = 1) in the weeks preceding their illness. PVL-positive isolates were cultured from skin or soft tissue specimens from upper limbs (n = 6), trunk (n = 4), lower limbs (n = 3), throat swab (n = 1), blood (n = 1) and peritoneal fluid (n = 1). These isolates were associated with subcutaneous abscesses (n = 8), boils (n = 3), wound infection (n = 1), cellulitis with bacteremia (n = 1), peritonitis (n = 1) and colonization (n = 2). Patients were treated by surgical drainage (n = 8), topical antimicrobial therapy (n = 2) and/or by systemic antimicrobial therapy (n = 9). In six patients hospital admission was required for the management of their infection.

All PVL-positive isolates carried the SCCmec IV element (Table 1). They belonged to the agr type 3 except for PFGE group A strains, which showed agr type 1. They were clustered into four PFGE groups. Results of spa typing and MLST were in full agreement with the PFGE classification, except for two isolates which presented single repeat allele polymorphism by spa or MLST typing: one spa t131 isolate differed by a single deletion from t044 and one ST153 isolate was a single allele variant of ST80. All PVL-positive isolates were susceptible to ciprofloxacin, gentamicin and tobramycin. Another striking feature was that strains belonging to the PFGE X-ST80-SCCmec IV clone were resistant to fusidic acid, tetracycline and kanamycin (Table 1). A majority of PVL-positive isolates were resistant to tetracycline (56%), fusidic acid (56%) and kanamycin (56%) but showed only very occasionally resistance to erythromycin. Tetracycline-resistant isolates harboured the tet(K) gene in contrast with CA-MRSA strains from Germany that carry the tet(M) gene. Resistance to kanamycin was associated with the aph(3’)-IIa gene. The single erythromycin-resistant isolate harbouring msrA(B) genes conferring the M resistance phenotype. This mechanism of resistance is found in <1% of nosocomial MRSA isolates in Belgium.

In agreement with previous reports from other European countries, the majority of MRSA strains causing community infections in Belgium were of the ST80-SCCmec IV genotype.
These strains have a genetic background that is clearly distinct from those of nosocomial MRSA strains from Belgian hospitals. In addition to ST80, PVL-positive MRSA isolates belonging to at least three other genetic lineages were also found with lower frequency in this case series. Of these lineages, PVL-positive ST8-SCCmec IV strains have caused outbreaks of CA-MRSA infections in California and in the Netherlands. These strains appear closely related to the hospital MRSA clone PFGE A20-ST8-SCCmec IV, which is widely disseminated in Belgian hospitals. However, in contrast with community strains, PFGE A20-ST8-SCCmec IV nosocomial isolates are lacking PVL genes (O. Denis, unpublished results) and carry aut(r) and ermA resistance genes. These differences suggest a divergent evolution of strains belonging to a common genetic lineage in hospitals and the community. Another Belgian CA-MRSA lineage found in this study included ST30-SCCmec IV strains that were first isolated from community-acquired infections in native Australian populations. This lineage has also been described recently among CA-MRSA strains in Europe. It is closely related to the ‘UK epidemic MRSA 16’ (ST36-SCCmec IV), which is endemic in hospitals in Great Britain and other European countries.

Outbreaks of PVL-positive MRSA infections have been reported in many communities around the world. No such outbreak has been reported yet in Belgium. In this series, except for a cluster of two cases in the same family, cases occurred sporadically in different regions of the country. Three imported cases were associated with recent travel to North Africa (n = 2) (ST80-SCCmec IV) and Ecuador (n = 1) (ST8-SCCmec IV), suggesting the possibility of importing the infection from these countries into Belgium.

In conclusion, we report the emergence of PVL-positive CA-MRSA strains belonging to five different genetic lineages in Belgium, with a predominance of the ST80-SCCmec IV clone, which is widely disseminated in Europe. The presence of PVL genes in diverse genetic backgrounds suggests that their horizontal transfer into resident S. aureus has occurred repeatedly, sometimes affecting strains that are closely related to endemic nosocomial MRSA strains. In addition, travel-associated cases of these CA-MRSA infections illustrate the propensity of MRSA clones to spread over large geographical areas, underlying the potential for a pandemic of CA-MRSA disease.

Owing to the passive reporting design of this study, the data reported here are likely to underestimate the true occurrence of CA-MRSA infections in Belgium. As reported in previous surveys, CA-MRSA strains were clinically associated with severe skin and soft tissue infections. The emergence of hyper-virulent MRSA strains in the community highlights the importance of their rapid identification in infected patients to adapt antimicrobial therapy and implement appropriate infection control practices. Further study is required to monitor the incidence of CA-MRSA infections in the Belgian population.

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Transparency declarations

None to declare.

References


