

Prevalence of *Staphylococcus aureus* toxins and nasal carriage in furuncles and impetigo

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Summary

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Key words

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Conflicts of interest

None declared.

Background The precise role of *Staphylococcus aureus* toxins and nasal carriage in common skin infections remains unclear.

Objectives To seek correlations between toxin expression, *S. aureus* nasal carriage and clinical manifestations in patients with community-acquired furuncles and impetigo.

Methods From November 2004 to August 2005, we studied clinical data and bacteriological samples prospectively collected from 121 patients presenting with furuncles or impetigo.

Results Sixty-four patients (31 with furuncles and 33 with impetigo) had *S. aureus*-positive skin culture. Panton–Valentine leukocidin (PVL) genes were present in 13 of 31 (42%) isolates from furuncles and were associated with epidemic furunculosis. Exfoliative toxin genes were present in 10 of 10 (100%) and 12 of 21 (57%) bullous and nonbullous impetigo isolates, respectively. Nasal carriage of *S. aureus* was found in 58% of patients overall. It was strongly associated with chronic furunculosis but not with simple furuncles (88% vs. 29%, $P < 0.007$). Skin and nose isolates from a given patient always had identical characteristics. Methicillin-resistant *S. aureus* accounted for four of 64 (6%) positive skin cultures. **Conclusions** PVL is not involved in all types of furuncles but is associated with epidemic furunculosis. Both bullous and nonbullous forms of impetigo are associated with exfoliative toxins. *Staphylococcus aureus* nasal carriage is associated with the chronicity of furuncles.

Staphylococcus aureus is a frequent cause of skin and soft-tissue infections. It produces numerous virulence factors, including adhesins and toxins. Some toxins tend to be related with particular types of skin infection. Panton–Valentine leukocidin (PVL) genes tend to be expressed by *S. aureus* isolates from furuncles and skin abscesses, while exfoliative toxins (ETs) are associated with bullous impetigo and the scalded skin syndrome. Rare reports mention the detection of ETs in *S. aureus* strains from nonbullous impetigo.¹ Most *S. aureus* infections occurring in the community are due to methicillin-sensitive strains, but PVL-positive community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains are now emerging worldwide, especially in the U.S.A.^{2–4} ET-expressing CA-MRSA isolates have seldom been detected (mainly in Japan and Switzerland).^{5,6}

Staphylococcus aureus skin and soft-tissue infections are very frequent in the community, and most respond rapidly to simple local treatment. Some become extensive or chronic. It has been shown that *S. aureus* nasal carriage is more frequent in

chronic staphylococcal infections.^{7,8} Nasal decontamination with mupirocin can prevent relapses of chronic furunculosis.⁹

In this prospective study we analysed *S. aureus* strains isolated from patients with skin infections and nasal carriage, seen by a network of dermatologists working in private practices or hospital outpatient clinics. The aim was to identify links between clinical manifestations and toxin gene expression. We also compared the *S. aureus* isolates recovered from the skin lesion(s) and nose of each patient. Finally, we determined the prevalence of MRSA.

Patients and methods

Patients

Between 26 November 2004 and 26 August 2005 we prospectively collected standard data on patients presenting with furuncles or impetigo to a network of 100 dermatologists working in private practices or hospital outpatient clinics in

the Rhône-Alpes region of France. The dermatologists were asked to recruit all patients presenting with furuncles or impetigo. Patients with folliculitis or other infectious skin diseases or with an uncertain clinical diagnosis were excluded. The dermatologists classified the furuncles as *simple* when solitary, *acute and multiple* when several were disseminated in a patient with no previous history of furuncles, *carbuncles* when multiple furuncles occurred on the same part of the body, and *chronic* if several furuncles had occurred in the previous 6 months. Furuncles were considered to be epidemic if more than one case occurred within a family. Impetigo was classified as *bullous* if bullae were present, *nonbullous* if there were crusts but no bullae, and *secondary* if the impetigo occurred on a pre-existing skin lesion.

The following data were recorded in each case: clinical presentation, number of lesions, demographic characteristics, underlying conditions (atopic dermatitis, diabetes mellitus, immunosuppressive treatment, or a personal or familial history of furuncles or impetigo), risk factors for MRSA (recent hospitalization, contact with a hospitalized person or health-care worker in the previous 10 days), and antibiotic or anti-septic treatment before admission. The local ethics committee approved the protocol.

Specimen collection, bacterial identification and susceptibility testing

Two bacteriological samples were collected by physicians from each patient, using cotton wool swabs (Oxoid, Basingstoke, U.K.): one from the skin lesion, and the second on the anterior nares (to detect *S. aureus* nasal carriage). Patients with missing or unidentified swabs were excluded. The swabs were placed in Stuart medium (allowing survival of bacteria during transport) and mailed to the French Reference Center for Staphylococci. On reception the swabs were plated on CHROMagar Staph Aureus (CHROMagar, Paris, France), a chromogenic medium specific for *S. aureus*. Skin swabs from patients with impetigo were also plated on blood agar (bioMérieux, Marcy l'Étoile, France) to detect *Streptococcus pyogenes*. *Staphylococcus aureus* was identified on the basis of colony and microscopic morphology, coagulase testing with rabbit plasma (bioMérieux) and the Pastorex Staph-Plus kit (Bio-Rad, Marnes-la-Coquette, France). *Streptococcus pyogenes* was identified on the basis of colony and microscopic morphology, β -haemolysin evidence on blood agar (trypticase-soy agar with 5% horse blood) and the Streptococcal Grouping kit (Oxoid, Dardilly, France). Antimicrobial susceptibility was tested with the BD Phoenix device (Becton Dickinson Diagnostics, Franklin Lakes, NJ, U.S.A.).

Genotyping and macrorestriction analysis by pulsed field gel electrophoresis

Isolates were grown overnight on brain–heart infusion agar or in brain–heart infusion broth. Genomic DNA was used as the polymerase chain reaction (PCR) target, after extrac-

tion using a standard procedure.¹⁰ Sequences specific for PVL genes (*lukS-PV*, *lukF-PV*), ET genes A, B and D (*eta*, *etb*, *etd*), staphylococcal enterotoxin A, B, C, D, E, H, K, L, M and O genes (*sea-see*, *seh*, *sek-sem*, *seo*), the toxic shock syndrome toxin gene (*tsst*), epidermal-cell differentiation inhibitor (EDIN) genes, staphylococcal leukocidin Luk-DE and Luk-M genes (*lukE-lukD*, *lukM*), haemolysin genes [γ (*hlg*), γ variant (*hlgv*) and β (*hlb*)], and accessory gene regulator alleles (*agr-1* to *agr-4*) were detected by PCR, as previously described.^{11,12}

The *mecA* gene, which codes for methicillin resistance, was detected by PCR as described by Murakami *et al.*¹³ Amplification of *gyrA* was used to confirm the quality of each DNA extract and the absence of PCR inhibitors.¹⁴ All PCR products were analysed by electrophoresis through 1% agarose gels (Eurobio, Courtaboeuf, France). Isolates were genotyped by pulsed-field gel electrophoresis (PFGE) after *Sma*I restriction, as previously described.^{15,16} The PFGE patterns were digitized and analysed with the Taxotron typing system (Institut Pasteur, Paris, France). Strain relatedness was determined according to published guidelines.¹⁷ Isolates that differed by no more than three fragments were considered to be subtypes of a given clonal type.

Statistical analysis

Categorical data were compared by using the χ^2 test, Fisher's exact test and Fisher–Snedecor's test, implemented with Epi-Info software version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.). $P < 0.05$ was considered statistically significant.

Results

Patient characteristics and results of skin culture

Thirty-five dermatologists working in private practices and nine dermatologists working in seven hospital dermatology outpatient clinics collected data and samples from 123 patients. Two patients were excluded because bacteriological samples were missing (one patient) or unidentified (one patient). The final analysis therefore involved 121 patients. The median age was 30.2 years and the sex ratio was 1.09 (63 males/58 females). Seventy-two patients presented with furuncles, of whom 35 (49%) had chronic furunculosis. The median age of patients presenting with furuncles was 36.9 years (range 4–94). Forty-nine patients presented with impetigo, of whom 28 (57%) had nonbullous impetigo. The median age of patients presenting with impetigo was 20.8 years (range 7 days–67 years). Skin cultures grew *S. aureus* in 64 cases (52%), comprising 31 patients with furuncles and 33 patients with impetigo (Table 1 and Fig. 1). *Streptococcus pyogenes* was isolated in two patients with nonbullous impetigo, in association with *S. aureus* in both cases. Skin culture never yielded *S. pyogenes* alone in a patient with impetigo.

Table 1 Number of patients with *Staphylococcus aureus* isolated from a skin lesion and/or the nose

Clinical diagnosis	Number of patients	Isolation of <i>Staphylococcus aureus</i>		
		From skin lesion	From nose	From skin lesion and nose
Furuncles	72	31	27	19
Simple furuncle	25	7	7	2
Acute multiple furuncles	7	5	2	1
Chronic furunculosis	35	17	16	14
Carbuncle	5	2	2	2
Impetigos	49	33	20	18
Bullous impetigo	13	10	6	4
Nonbullous impetigo	28	21	13	13
Secondary impetigo	8	2	1	1
Total	121	64	47	37

Toxin detection in skin culture isolates

Panton–Valentine leukocidin and furuncles

PVL genes were detected in 13 of 31 (42%) *S. aureus* furuncle isolates and in 0% of impetigo isolates. Six of the 13 PVL-positive isolates (46%) were associated with epidemic furunculosis vs. two of the 18 PVL-negative isolates (11%). Epidemic furunculosis was then significantly associated with PVL-positive strains ($P < 0.04$) but there was no link between PVL-positive isolates and patient characteristics (age, sex, underlying conditions) or the clinical presentation (number of furuncles, chronicity).

Exfoliative toxins and impetigo

The *eta* and/or *etb* genes, coding for ETA and ETB, respectively, were found in 23 of 33 (70%) isolates from impetigo skin

cultures. All 10 isolates from bullous impetigo swabs harboured the *eta* and/or *etb* genes, compared with 12 of 21 (57%) nonbullous impetigo isolates. There was no association between the type of ET and patient characteristics or clinical presentation.

No significant association was found between impetigo or furuncles and the other toxins tested.

***Staphylococcus aureus* nasal carriage**

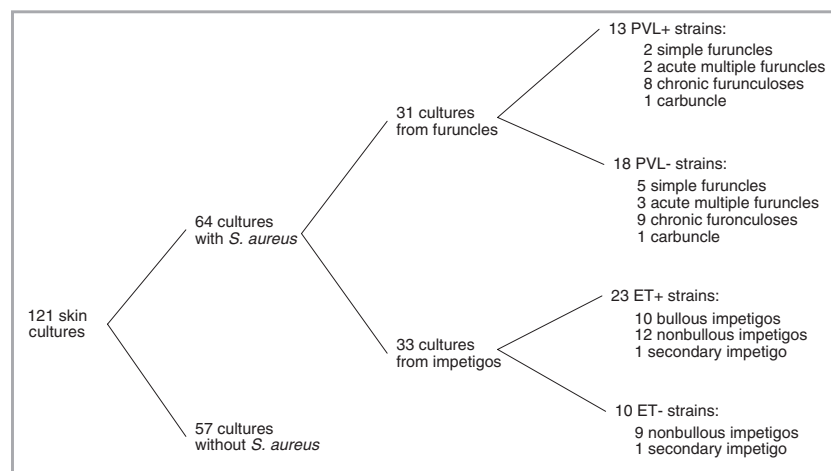
Nasal carriage of *S. aureus* was found in 37 of 64 (58%) patients with culture-confirmed *S. aureus* skin infection. The nasal carriage rate differed very significantly ($P < 0.007$) between patients with simple furuncles (two of seven, 29%) and patients with chronic furunculosis (14 of 16, 88%). The nasal carriage rates were 40% (4/10) and 62% (13/21) in patients with bullous and nonbullous impetigo, respectively (no significant difference). The nasal carriage rate was 46% (6/13) in patients with PVL-positive furuncles and 72% (13/18) in patients with PVL-negative furuncles (no significant difference).

Based on PFGE analysis and toxin expression, the same strain was present in the nares and in the skin lesion(s) of all 37 patients who were culture positive at both sites (Fig. 2).

Prevalence of methicillin-resistant *Staphylococcus aureus* in skin cultures

MRSA was isolated by skin culture in four patients (6%), of whom one had impetigo and three had furuncles (Table 2). Three of these patients had been in recent contact with health-care facilities, and their MRSA isolates belonged to the most prevalent MRSA clone (the Lyon clone) spreading in French hospitals, with an *agr* allele type 1, no PVL genes, and the enterotoxin A gene.¹⁸ PFGE typing of these strains confirmed that they belonged to the Lyon clone,¹⁸ which can be acquired in the community by patients with specific risk factors.¹⁹ One patient had no identified risk factor for MRSA acquisition; this patient’s strain was resistant to beta-lactams,

Fig 1. Distribution of patients according to the isolation of *Staphylococcus aureus* in skin cultures, the clinical presentation, and the presence of Panton–Valentine leukocidin (PVL) or exfoliative toxin (ET) genes.



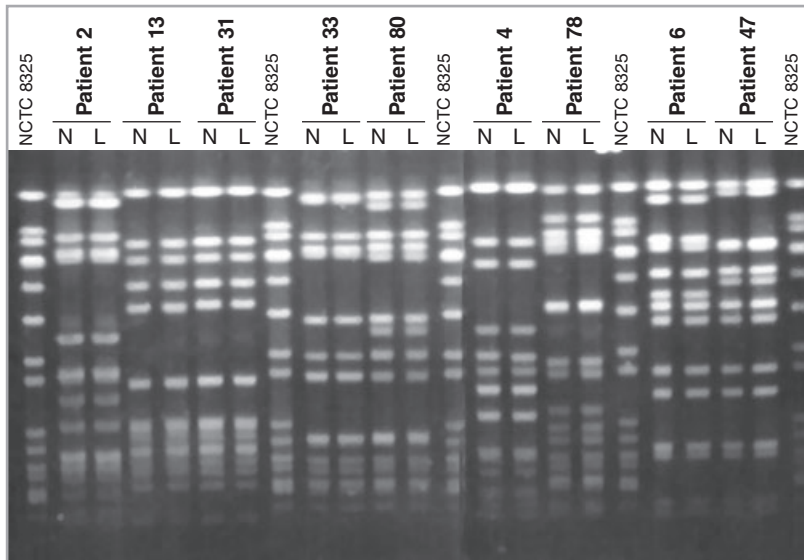


Fig 2. Comparison of pulsotypes of *Staphylococcus aureus* strains isolated from the nose (N) and skin lesions (L). NCTC 8325 is the molecular size reference strain.

Table 2 Epidemiological, phenotypic and genotypic data on methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from skin lesions

Patient	Skin infection	Age (years)/sex	Risk factors for MRSA	Antibiotic resistance ^a	<i>agr</i> ^b allele	<i>sea</i> ^c	PVL ^d	MRSA type ^f
22	Nonbullous impetigo	50/F	Recent contact with hospital	Peni, Oxa, Kana, Tobra, Oflo ^e	1	+	-	HCA-MRSA
27	Carbuncle	94/M	Age, diabetes mellitus, chronic dermatosis, healthcare worker in family	Peni, Oxa, Kana, Tobra, Tetra, Ery, Linco, Oflo ^e	1	+	-	HCA-MRSA
109	Chronic furunculosis	24/M	Severe atopic dermatitis, use of topical corticosteroids, recent hospitalization	Peni, Oxa, Tetra, Ery, Linco ^e	1	+	-	HCA-MRSA
42	Chronic furunculosis	21/F	None	Peni, Oxa, Kana, Tetra, Fusi ^e	3	-	+	CA-MRSA

^aAntibiotics to which the strain was resistant. ^bAccessory gene regulator. ^cStaphylococcal enterotoxin A. ^dPanton–Valentine leukocidin. ^ePeni, penicillin G; Oxa, oxacillin; Kana, kanamycin; Tobra, tobramycin; Tetra, tetracycline; Ery, erythromycin; Linco, lincomycin; Oflo, ofloxacin; Fusi, fusidic acid. ^fHCA-MRSA, healthcare-associated MRSA; CA-MRSA, community-acquired MRSA. + or -, presence or absence of the gene.

kanamycin, tetracycline and fusidic acid. The strain also harboured the PVL genes and an *agr* allele type 3. The characteristics of this strain, and its PFGE type, were similar to those of the predominant CA-MRSA clone spreading through Europe.³

Discussion

We studied 121 patients presenting with community-onset superficial skin infections and found that: (i) the PVL genes were detected in only 42% of *S. aureus* furuncle isolates (mainly in cases of epidemic furunculosis); (ii) bullous impetigo was always associated with ET genes, as well as 57% of strains causing nonbullous impetigo; (iii) *S. pyogenes* was isolated in only two cases of nonbullous impetigo, both of which also grew *S. aureus*; (iv) *S. aureus* nasal carriage was frequent (58% of patients with culture-confirmed *S. aureus* skin infection), but the rate was markedly different between patients with a simple furuncle (29%) and those with chronic furun-

culosis (88%); the nasal *S. aureus* isolate was always identical to the skin isolate; (v) MRSA was isolated in only four cases (6%), three corresponding to hospital strains and one being a true CA-MRSA.

Since the 1990s, PVL has been classically associated with furuncles.²⁰ In the first hospital-based series, Couppié *et al.* and Lina *et al.* found that, respectively, 86% and 93% of strains isolated from furuncles produced PVL.^{21,22} More recently, in a multicentre hospital study, Yamasaki *et al.* found that only 40% of *S. aureus* isolates from furuncles harboured PVL genes.²³ In our study, 42% of furuncle isolates were PVL positive. The striking differences between authors in the frequency of PVL genes detection could reflect differences in the studied populations. PVL detection rates would be higher in the most severe cases (hospital series), whereas most of our patients presented to community practices. PVL detection rates could also vary according to the area of the studied population (e.g. its prevalence is higher in countries with low economic

level^{24,25}). Finally, the differences observed between authors could also be related with the uncertainty of the clinical diagnosis (e.g. furuncle confounded with deep folliculitis), including in our own study as diagnoses were made by 44 different physicians. Unlike Yamasaki et al., who found that PVL-positive strains were associated with multiple inflammatory furuncles in young patients free of underlying systemic disorders,²³ we found no association between PVL genes and clinical manifestations. These results suggest that simple furuncles are not always associated with PVL-producing strains of *S. aureus*. In contrast, PVL was associated with epidemic furunculosis, which suggests that PVL-positive strains are not only more virulent but also more contagious, as witnessed by major outbreaks of furuncles due to PVL-positive *S. aureus* in jails, sports teams and semiclosed communities (native Americans, in rural Alaska, etc.).^{26–29} The epidemic potential of *S. aureus* PVL-positive isolates could be linked to the induction by leukocidin of the expression of cell wall-anchored proteins such as protein A, theoretically facilitating the adhesion of *S. aureus* on human epithelia.³⁰

Nonbullous impetigo, one of the most common skin diseases, can be caused by *S. pyogenes* alone, by *S. aureus* alone, or by the two bacteria in combination. *Streptococcus pyogenes* was long considered to be the main cause of nonbullous impetigo, a condition formerly called 'streptococcal impetigo'.³¹ In the 1990s, in American and Israeli studies, the importance of *S. aureus* rose dramatically but *S. pyogenes* was still isolated in about one-third of skin cultures, alone or together with *S. aureus*.^{32,33} In 2002, a large Dutch study of 160 children with impetigo showed that *S. pyogenes* was present in only 8.1% of cases, usually in association with *S. aureus*.³⁴ In our study, only two patients with nonbullous impetigo had both *S. pyogenes* and *S. aureus*, but no cases of pure streptococcal impetigo were found. These results confirm that the epidemiology of superficial skin infections is evolving rapidly and that treatment of impetigo should now target *S. aureus* more than *S. pyogenes*.

The association of bullous impetigo with staphylococcal ETA and ETB has been clearly demonstrated.³⁵ ETs act as serine proteases which cleave desmoglein 1 in the superficial epidermis, leading to blister formation. Our epidemiological results confirm these laboratory data, as all our cases of bullous impetigo were associated with ETA and/or ETB. Little is known about the pathophysiology of nonbullous impetigo. In our study, no particular toxin pattern was associated with this condition, but the high frequency of ETA and/or ETB genes in strains recovered from nonbullous impetigo (57%) suggests that bullous and nonbullous impetigo probably correspond to the same disease. Nonbullous impetigo possibly corresponds to a different quantitative expression of ETs in the epidermis and further studies should be performed to confirm this hypothesis.

Staphylococcus aureus is present as a commensal in the anterior nares of about 30% of healthy people.⁸ It has been shown that *S. aureus* nasal carriage is a risk factor for infections in patients with continuous peritoneal dialysis and for surgical wound infections.^{36,37} A higher rate of *S. aureus* nasal carriage has been described in patients with *S. aureus* skin infections.^{8,38} In our

study the *S. aureus* nasal carriage rate was about twice as high as that usually observed in the general population (58% vs. 30%). The nose and skin isolates always belonged to the same strain in a given patient. These results strongly suggest that the nasal isolate could play a role in the pathophysiology of skin infections. The nasal carriage rate was significantly higher in patients with chronic furunculosis (88%) than in those with a simple furuncle, whose nasal carriage rate (29%) was similar to that of the general population. This further supports the use of nasal decontamination in patients with chronic furunculosis.⁹ Mupirocin is probably the best drug for this purpose, although its widespread use could lead to the emergence of mupirocin-resistant *S. aureus* strains.^{39,40} In France, fusidic acid is also proposed in this indication although its use bears a high risk of selecting fusidic acid-resistant strains.⁴¹ Interestingly, nasal carriage of PVL-positive strains was infrequent in our patients, as in studies of outbreaks of PVL-positive *S. aureus* infections.^{25,27,42}

We genetically characterized our MRSA isolates and were thus able to distinguish between hospital MRSA that had spread to the community (healthcare-associated MRSA or HCA-MRSA) and true PVL-positive CA-MRSA. Del Giudice et al. prospectively studied community-acquired skin infections and found MRSA in 11% of cases, 8% of the strains corresponding to HCA-MRSA and 3% to PVL-positive CA-MRSA.⁴³ PVL-positive CA-MRSA strains are uncommon in Europe,⁴⁴ accounting for fewer than 1% of all MRSA isolates collected in France, for example. Their prevalence was also low in our study: only one CA-MRSA was isolated, from a patient with chronic furunculosis. PVL-positive CA-MRSA strains are an emerging threat, because of their global clonal spread. They have become hyperendemic in some parts of the world, and especially the U.S.A., where they are isolated from about 60% of patients with *S. aureus* skin infections.^{45,46} The other three MRSA isolates in our study were recovered from patients who had been in recent contact with healthcare facilities. Community-onset skin infections should be strictly monitored, as the epidemiology of *S. aureus* infections is constantly evolving.

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