EPIDEMIOLOGY

Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins

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Summary. DNA hybridisation of 309 consecutive *Staphylococcus aureus* clinical isolates with oligonucleotide probes specific for genes encoding Panton-Valentine leucocidin (*luk-PV*) and γ-haemolysin (*hlg*) revealed that 99% of randomly selected strains carried the *hlg* locus whereas only 2% harboured the *luk-PV* as well as the *hlg* loci. Only 1% of the strains did not possess either gene. In a clinical prospective study of independent *S. aureus* strains, 58 Panton-Valentine leucocidin (PVL)-producing isolates were shown to be responsible for primary skin infections, mainly furuncles (86%). Phage susceptibility patterns and pulsed field gel electrophoresis (PFGE) profiles of DNA were shown to be polymorphic epidemiological markers of PVL-producing strains. In eight patients with recurrent furuncles, the PVL-producing strains isolated either from furuncles or from the anterior nares were considered to be identical in each based upon phage sensitivity profiles or PFGE patterns.

Introduction

Among the toxins produced by *Staphylococcus aureus*, Panton-Valentine leucocidin (PVL) and γ-haemolysin are bi-component toxins which have been demonstrated recently to be members of a single family of toxins—the synergohymenotropic toxins.

The name Panton-Valentine leucocidin (PVL) was definitively given by Gladstone and Van Heyningen because of the exclusive biological activity of this toxin, formerly discovered by Panton and Valentine, upon human and rabbit polymorphonuclear cells. PVL was purified from the highly leucotoxic culture supernate of strain V8 (ATCC 49775). The biological activity of PVL resulted from the synergic action of two separate exoproteins, the S (32 kDa) and F (38 kDa) components. These two proteins are encoded by two co-transcribed genes, *luk*S-PV and *luk*F-PV, respectively. *Luk*S-PV and *Luk*F-PV are part of the first structural subgroup of the family of toxins.

The primary structure of these proteins has c. 72% identity to that of the components of each of the toxins belonging to a second two-component structural subgroup of toxins of *S. aureus*. The latter subgroup includes three closely related synergohymenotropic toxins (> 96% identity within this subgroup) referred to as "leucocidin R", "leucocidin from a methicillin-resistant *S. aureus*", and γ-haemolysin. The latter subgroup of toxins, called the γ-haemolysin subgroup, is encoded by a locus consisting of three open-reading frames: one of them (*hlg*A) is separately transcribed, and is located 570 bp upstream from the other two (*hlg*C and *hlg*B) which are co-transcribed. The genes *hlg*A and *hlg*C are structurally and functionally close to *luk*S-PV, whereas *hlg*B is structurally and functionally close to *luk*F-PV. The genes *hlg*C and *hlg*B are, like *luk*S-PV and *luk*F-PV, tandemly transcribed, but there is no *hlg*A-like open reading frame in the vicinity of the two PVL-encoding genes.

Although the PVL and γ-haemolysin group are closely related, it has to be emphasised that PVL is strongly cytolytic by the combined action of the S and F components upon human and rabbit polymorphonuclear cells (PMNs), monocytes and macrophages but not upon erythrocytes, whereas members of the γ-haemolysin subgroup are leucotoxic and also haemolytic by the association of HlgA or HlgC with HlgB.

Preliminary studies had shown that PVL-producing strains might be associated with cutaneous infec-
tions. The aim of this study was to determine the possible epidemiological association of γ-haemolysin- and PVL-producing strains with clinical syndromes.

**Materials and methods**

**Bacterial strains**

*S. aureus* isolates were identified as gram-positive cocci producing free coagulase, catalase and acetoin, but devoid of β-galactosidase activity. The reference *S. aureus* strains were: PVL-producing strain V8 (ATCC 49775) kindly provided by Dr S. Thornley (Wellcome Laboratories, London), and γ-haemolysin-producing strains Smith 5R and P83 generously given by N. El Solh (Institut Pasteur, Paris), and N. L. Norcross (Cornell University, Ithaca, NY, USA), respectively.

A preliminary study of the distribution of the genes encoding synergohymenotropic toxins included 309 consecutive isolates of *S. aureus* obtained from the bacteriology laboratory of the Strasbourg University General Hospital. The origin of the isolates is detailed in table I.

In a prospective study, the phenotypic expression of PVL was measured in 346 *S. aureus* strains (table II), 69 of which originated from blood cultures, 31 from asymptomatic nasal carriers and 246 from various forms of cutaneous infection.

**Phenotypic detection of PVL-producing strains**

The detection of PVL-producing strains was based on an immunoprecipitation assay as described earlier. Briefly, bacteria were grown in a CCY modified medium by the sac culture method. One colony of *S. aureus* was suspended in 20 ml of 0.02 M sodium phosphate buffer, 0.15 M NaCl, pH 7.5. This suspension was transferred to a 2-L Erlenmeyer flask containing a sterile dialysis bag filled with 120 ml of modified CCY medium. The cultures were incubated at 37°C for 18 h with vigorous shaking. The double immunoprecipitation test was performed in an agarose 0.6% w/v gel (Seakem-GTG, FMC products) prepared in phosphate-buffered saline (PBS, 20 mM Na-K-phosphate buffer, 150 mM NaCl, pH 7.5) with NaN3 0.5% w/v, pH 7.5. Wells (4 mm diam.) were cut in the gel, forming a crown of six wells with a central seventh well. F or S affinity-purified antibodies were added into the central well, whereas peripheral wells were charged with 35 μl of staphylcoccal culture supernate or 35 μl of purified S or F. After incubation for 18 h at 4°C, the presence of immunoprecipitation lines was recorded.

**DNA methods**

Restriction endonucleases and DNA modifying enzymes were used as recommended by the manufacturers (Gibco-Bethesda Research Laboratories, and New England Biolabs). To determine the presence of PVL- or γ-haemolysin-encoding genes, DNA was prepared by the following procedure. Bacteria were grown overnight in 10 ml of 2× TTY medium and then pelleted by centrifugation at 5000 g for 5 min at 0°C. After washing with 5 ml of distilled water, bacteria were resuspended in 2 ml of 0.05 M Tris-HCl buffer sucrose 25% w/v, pH 7.5, containing lyso- staphin (Biozyme, New York) 200 μg/ml, and lysozyme (Appligene Strasbourg, France) 250 μg/ml. After treatment for 1 h at 37°C, to the protoplast suspension was added 100 μl of SDS 10% w/v and 110 μl of 0.5 M EDTA, pH 8.0 and the mixture was heated for 10 min at 55°C. Nucleic acids were extracted twice with 2 ml of phenol:chloroform (1:1) and three times with diethylether before being precipitated with alcohol. After drying, nucleic acids were dissolved in 300 μl of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, containing RNAase A (Sigma) 20 μg/ml, and then stored at -20°C. The DNA fragments resulting from EcoRI restriction hydrolysates were electrophoresed on agarose gels and transferred on to Immobilon P membranes (Millipore, Molsheim, France). Southern blotting was performed as described previously with oligonucleotide probes specific for genes encoding each of the two components of the Panton-Valentine leucocidin and each of the three components of the γ-haemolysin group. The sequences for the probes were deduced from the sequence of the genes encoding the Panton-Valentine leucocidin and from the sequence of the genes encoding a member of the γ-haemolysins group, the leucocidin R. The nucleotide sequences of these two toxins were deposited at the EMBL/GenBank Data library with the numbers X 27200 and X 64389, respectively. Probes for genes encoding the S and the F components of PVL were 1500 5'-CCCCATTAGTACACAG-3' 1515 and 2386 5'-ATTTGATATTGTTAT-3' 2402, respectively. Probes for the three genes (hlgA, hlgC, hlgB) encoding leucocidin R (or γ-haemolysin) were 872 5'-AGCGAGTTTGAAATCACTTACGG-3' 894, 1076 5'-

Table I. Distribution of genes encoding synergohymenotropic toxins among 309 consecutive isolates of *S. aureus*

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number of isolates</th>
<th>Number of luk-PV-positive isolates*</th>
<th>Number of hlg-positive isolates†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood cultures</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Bronchoalveolar fluids and sputa</td>
<td>15</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Tracheal aspirations</td>
<td>106</td>
<td>2</td>
<td>106</td>
</tr>
<tr>
<td>Superficial lesions</td>
<td>89</td>
<td>3</td>
<td>89</td>
</tr>
<tr>
<td>Stools</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Catheters</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Urines</td>
<td>40</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>Others</td>
<td>9</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>309</td>
<td>5</td>
<td>307</td>
</tr>
</tbody>
</table>

*All these isolates hybridised with the two probes designed for the luk-PV locus.
†All these isolates hybridised with the three probes designed for the hlg locus.
To perform sequential hybridisations with various probes on the same membrane-immobilised DNA, Total exposure to a Fuji X-ray film at -70°C for 16 h.

Isolates these eight patients resulting in 24 PVL-producing kinase. Membranes were then washed twice for 20 min at 45°C in 1 x TE buffer at 90°C. These membranes were then dried dehybridisation was obtained by three 5-min washes in 6 x SSPE, 5 x Denhardt's solution, 10 SDS 0.05 % w/v. Hybridisation was done at the same temperature for 14 h in the same buffer containing 1 pmol of oligonucleotide, 5'-labelled with 32P by T4 polynucleotide kinase. Membranes were then washed twice for 20 min at 45°C in 1 x SSPE, SDS 0.05 % w/v and dried before exposure to a Fuji X-ray film at -70°C for 16 h. To perform sequential hybridisations with various probes on the same membrane-immobilised DNA, dehybridisation was obtained by three 5-min washes in TE buffer at 90°C. These membranes were then dried for 1 h at 80°C under vacuum. A pre-hybridisation step preceded each hybridisation with another oligonucleotide probe.

Epidemiological markers and PVL-producing strains

The polymorphism of PVL-producing staphylococci was studied in a cohort of 75 isolates. This investigation included the 58 PVL-producing independent isolates described in the prospective study (table II). Of the 58 patients from whom these strains were isolated, seven had several episodes of furuncles. An eighth epidemiologically related patient was also sampled: he belonged to the same family as one of the seven patients and was not included in the group of 58. Several nasal and furuncle swabs were taken from these eight patients resulting in 24 PVL-producing isolates; seven were considered to be distinct, and the remaining 17 were linked epidemiologically to these seven strains.

Phage typing

Phage susceptibility patterns were determined with the international set of typing phages according to Blair and Williams at routine test dilution (RTD) and at 100 x RTD. Two samples were considered as having different phage types when they differed by at least two phage susceptibilities.

Pulsed field gel electrophoresis

For typing by pulsed field gel electrophoresis (PFGE), the DNA preparations, Smal restriction endonuclease and electrophoretic conditions for PFGE on a transverse alternating field electrophoresis apparatus (GeneLine, Beckman) were as reported previously.

Results

Distribution of genes encoding synergohymenotropic toxins among hospital isolates of S. aureus

In our preliminary study, the distribution of PVL- and γ-haemolysin-encoding genes was examined among 309 consecutive hospital isolates of S. aureus. EcoRI-restricted DNA of each isolate was successively hybridised with lukS-PV-, lukF-PV-, hlgA-, hlgC- and hlgB-specific gene probes. The DNA from 307 isolates (99.3 %) hybridised with the three probes specific for hlgA, hlgC and hlgB. The hybridised EcoRI restriction fragments always had the same apparent size (> 8.3 kb) with hlgA and hlgC probes and all had a size of 4.0 kb when detected with the hlgB probe (fig. 1a and b). No apparent length variation of the detected DNA fragments was observed amongst the strains. No strain hybridised with only one or two of the three γ-haemolysin probes. Culture supernates of these 307 strains produced immunoprecipitation lines with affinity-purified PVL-antibodies. However, these lines failed to show complete identity with those obtained with purified LukS-PV or LukF-PV as antigens, because, as previously mentioned, PVL and γ-haemolysin share common sequences, and probably common epitopes.

DNA of only five strains (1.6%) hybridised with the two luk-PV probes. The expression of PVL was verified in these five strains by immunoprecipitation with affinity-purified PVL-antibodies. The precipitation lines had complete identity with those obtained with purified S or F antigens. Among these five isolates, three originated from skin infections (two abscesses and one whitlow); two other isolates were obtained from sputa. One of these came from a 78-year-old patient who developed pneumonia after surgery of the hip, with no cutaneous infection. The second isolate originated from a long-term immuno-suppressed 53-year-old woman, suffering from breast cancer and having bronchitis, but without any cutaneous lesion.

It has to be emphasised that, just like the reference S. aureus strain ATCC 49775, the DNA of all the PVL-producing strains hybridised not only with lukS-PV and lukF-PV probes, but also with hlgA, hlgC and hlgB.

Table II. Distribution of PVL-producing S. aureus strains among clinical samples taken from cutaneous infections, septicaemias and asymptomatic nasal carriers

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Number of strains tested</th>
<th>Number of PVL-positive strains</th>
<th>Number of PVL-negative strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>69</td>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>Nose</td>
<td>31</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Primary pyodermas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furunculosis</td>
<td>43</td>
<td>37</td>
<td>6</td>
</tr>
<tr>
<td>Abscess</td>
<td>19</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Whitlow</td>
<td>44</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td>Folliculitis</td>
<td>19</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Impetigo contagiosa</td>
<td>23</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Ecthyma</td>
<td>16</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Secondary pyodermas</td>
<td>82</td>
<td>1</td>
<td>81</td>
</tr>
<tr>
<td>Total</td>
<td>346</td>
<td>58</td>
<td>288</td>
</tr>
</tbody>
</table>

TGTGACCATCTAAATAAC-3' 1059, and 1592 5'-GGTTGGTGACATCAGTATCTCT-3' 1612, respectively.
Fig. 1. Toxin genotyping for *S. aureus* strains with *hlgA*-*, *hlgC*-*, *hlgB*-*, *lukS*-PV- and *lukF*-PV-specific oligonucleotide probes. Panel a: hybridisation with *hlgA*- and *hlgC*- specific probes; the DNA from 307 of 309 consecutive clinical isolates hybridised with an *EcoRI* fragment > 8.3 kb. Panel b: hybridisation with the *hlgB*-specific probe; the same DNA as in panel a hybridised with an *EcoRI* fragment of c. 4.0 kb. Panel c: hybridisation with *lukS*-PV- and *lukF*-PV- specific probes: the DNA from 11 PVL-producing strains identified from patients with furuncles hybridised with a unique *EcoRI* fragment whose size varied from 8.0 to c. 11 kb according to the strains. The size of the hybridised DNA fragments was deduced from the mobility of DNA from bacteriophage λ digested by *BsuEII* and 5'-labelled with 32P (lane 1 in each of the three panels).
### Table III. Distribution of phage types among 58 PVL-producing strains of *S. aureus*

<table>
<thead>
<tr>
<th>Phage groups</th>
<th>Phage types</th>
<th>Number (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>NT</td>
<td>22 (39)</td>
</tr>
<tr>
<td>II</td>
<td>3C/55</td>
<td>17 (29)</td>
</tr>
<tr>
<td></td>
<td>3A/3C/55/71</td>
<td>10 (18)</td>
</tr>
<tr>
<td>III</td>
<td>42E/81</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>47/54</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>54/75</td>
<td>3 (5.1)</td>
</tr>
<tr>
<td>V</td>
<td>94/96</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3A/3C/47/54</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>3A/3C/42E/47/54/81/95</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>3A/3C/55/71/6/47/75/81/94</td>
<td>1 (1.7)</td>
</tr>
</tbody>
</table>

NT, non-typable.

probes. These hybridisations with *hlg* genes occurred on *EcoRI* DNA fragments of the same size as those from isolates having only the locus for γ-haemolysin.

Finally, DNA of only two strains (0.7%) deemed subsequently to be contaminants did not hybridise with any of the five probes. Culture supernates did not contain synergohymenotropic toxin nor did they contain leucotoxic activity. A 1 in 10 dilution of the culture supernates was unable to lyse $10^5$ human PMNs. By comparison, dilutions of culture supernate ranging from 1 in 300 to 1 in 3000 of γ-haemolysin-producing strains and of PVL-producing strains were able to lyse $10^5$ adsorbed human PMNs.

### Clinical association of PVL-producing *S. aureus* strains

It appears that 99% of *S. aureus* isolates produce γ-haemolysin. Therefore, the clinical association between these strains and a clinical syndrome is not demonstrable. By contrast, the low frequency (2%) of PVL-producing strains makes it possible to seek an association with a clinical syndrome on the basis of reports of its role in septicaemia and in some cutaneous infections.

In a prospective study with 346 distinct strains of *S. aureus* (Table II), only one of 69 isolates from blood cultures produced PVL in the immunoprecipitation test. Similarly, no PVL-positive strain was isolated from the anterior nares of 31 asymptomatic nasal carriers.

Fifty-eight PVL-producing strains were isolated from 246 cutaneous infections. Most of them (56 of 58) were associated with primary cutaneous infections. This result was significant when compared with strains isolated from secondary cutaneous infections (*p* < 0.05). The 58 PVL-producing isolates were distributed between furuncles (64%), abscesses (13%) and whitlows (17%). Conversely, PVL-producing *S. aureus* strains were detected in 86% of the furuncles.

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*Fig. 2.* DNA fingerprint analysis from 58 PVL-producing *S. aureus* strains by transverse alternating PFGE showing 18 different profiles. Chromosomal DNA was digested with *SmaI* restriction endonuclease and the fragments were separated by PFGE. M, (lane T) are indicated in kb, and were obtained from bacteriophage *λ* concatemers (FMC products). The different fingerprints identified from the 58 strains are shown in lanes 1–18.
Fig. 3. Epidemiological investigation with PFGE of PVL-producing *S. aureus* strains from eight patients with recurrent furuncles. Chromosomal DNA was digested with *Sma*I restriction endonuclease before being electrophoresed. *M* (lane T) are indicated in kb, and were obtained from bacteriophage 4 concatemers (FMC products). PFGE profiles of reference strains ATCC 49775, P83 and Smith 5R are given in lanes 1-3, respectively. PFGE profiles of isolates from patients with recurrent furunculosis are shown in lanes 4-27. As seen at the bottom of the patterns, the isolates originated from eight patients (a-g). Two of the patients were from the same family (*a* and *a*₂). Isolates were sampled from furuncles (f) and anterior nares (n).
37% of the abscesses and 23% of the whitlows. Whitlows were considered as primary infections since no other micro-organism was identified. In eight patients with recurrent furuncles, a PVL-producing strain was isolated on at least two occasions, and at least once from the anterior nares. Only one PVL-positive isolate out of 58 was found as a superinfection of a cutaneous lesion.

All 58 cutaneous PVL-producing isolates were further characterised to eliminate the possibility of outbreaks of cutaneous lesions due to a limited number of strains. This strategy was designed to strengthen the epidemiological link between primary cutaneous lesions and PVL-producing strains. Three epidemiological markers were used: phage susceptibility patterns, genetic toxinotyping and PFGE electrophoretic DNA profiles.

**Phage types of PVL-producing strains**

As shown in table III, 10 phage types were obtained with the 58 PVL-producing strains. Most of the strains were non-typable (39%), or were in two types of phage group II (47%). From the strains isolated from the eight patients with recurrent furuncles, four different phage susceptibility patterns were identified. These isolates were identical whether or not they originated from the anterior nares or from furuncles of the same patient. The isolates from the two patients belonging to the same family had the same phage type (3C/55).

**Genetic toxinotyping of PVL-producing strains**

The lukS-PV and lukF-PV probes hybridised with a single EcoRI DNA fragment whose size varied from 8.0 kb to c. 11 kb, according to the strains. Among the 58 strains tested, there were at least five easily differentiated sizes of EcoRI DNA fragments that hybridised with the probes (fig. 1c), accounting for at least five genotypes. The hybridised DNA fragments corresponding to the isolates from the eight patients with recurrent furuncles were distributed into three sizes, 8.0, 8.5 and c. 9.3 kb (data not shown). These genotypes were always identical among all isolates obtained from each of the eight patients with recurrent furuncles, and among the two patients belonging to the same family.

**PFGE pattern**

DNA from the 58 PVL-producing strains was analysed after SmaI endonuclease digestion and 18 different PFGE patterns were identified (fig. 2). They showed wide variability, accounting for the polymorphism of PVL-producing strains. Since the most frequently encountered pattern was observed for only four clinically distinct strains, a predominant pattern for DNA from PVL-producing strains could not be defined. It should be noted that the PFGE pattern of the reference PVL-producing strain ATCC 49775 was not found amongst the 18 different PFGE profiles mentioned above.

As a second stage, the PFGE patterns of DNA from the 24 PVL-producing isolates obtained at various times and locations (anterior nares and furuncles) from the eight patients with recurrent furuncles were studied. Five different PFGE patterns were observed with the PFGE profiles being identical in nasal and furuncle isolates of a given patient. For the two persons from the same family (patients a1 and a2 of fig. 3) suffering from furuncles, the isolates had the same PFGE-profile (lanes 4–7 and 8–9).

**Discussion**

Although PVL and γ-haemolysin were known as bi-component toxins,5,8,11,15 they remained poorly differentiated until their primary structures were reported. It now appears that both toxins, despite different biological activities, belong to the same protein family, the synergohymenotropic toxins of *S. aureus*.4 However, the PVL and γ-haemolysin genes are not equally distributed, since γ-haemolysin coding genes are constitutive in most *S. aureus* isolates, whereas PVL genes are rarely encountered.

DNA of c. 99% of clinical isolates hybridised with all three γ-haemolysin-specific oligonucleotide probes. In all 307 strains producing γ-haemolysin, the probes specific for hlgA and hlgC recognised EcoRI DNA fragments of > 8.0 kb (c. 11 kb), while the hlgB probe recognised EcoRI DNA fragments of c. 4.0 kb. This result is in accordance with the EcoRI restriction maps of the hlg loci already sequenced in three different strains4,5,14,16 where there is always one EcoRI restriction site within the hlgC gene. This site is located between the hybridisation targets of hlgC and hlgB probes. In these three sequenced hlg loci, there is no EcoRI restriction site between the targets of hlgA and hlgC probes. Therefore, these two latter probes hybridise with an unique fragment with a size of c. 11 kb, as determined by Southern blotting with strains Smith 5R14 and P83.5 In all 307 strains with γ-haemolysin-encoding genes, the nucleotide sequences were not known, but hlgA and hlgC probes always hybridised with an EcoRI fragment of c. 11 kb. This suggests that in each of all these 307 strains too, hlgA and hlgC genes belong to the same 11-kb EcoRI fragment and that hlgA is always located in the vicinity of the co-transcribed hlgC and hlgB open reading frames, as was observed in the three previously sequenced hlg loci. Thus, it appears that the organisation of the γ-haemolysin locus is highly conserved in *S. aureus* strains.

The high frequency of hlg genes and γ-haemolysin expression in *S. aureus*, suggests that this toxin (and its variants) might be relevant to the pathogenicity of *S. aureus*. If such an hypothesis is true, the rare isolates that do not produce any synergohymenotropic toxin should be considered as less pathogenic, or even as...
commensals; indeed, this was observed with the only two non-toxigenic strains detected so far.

In contrast to the high frequency of \(\gamma\)-haemolysin coding genes, PVL coding genes are less frequently commensals; indeed, this was observed with the only preliminary survey produced PVL, three of which distributed since they are encountered in only 2% of randomly obtained isolates. Five isolates from the preliminary survey produced PVL, three of which originated from primary cutaneous necrotic lesions.

Another interesting result obtained in this study concerned the PVL-producing strains: five such isolates obtained in the preliminary survey as well as all the 75 other clinical isolates obtained from blood and skin infections, harboured not only the two genes coding for PVL, but also the three genes encoding \(\gamma\)-haemolysin. HlgB and F components are known to have structural homologies and can be classified as components of class F; HlgA, HlgC and S components also have structural homologies and, therefore, can be classified as components of class S. If all five proteins are produced in vivo, as they are in vitro, \(^4\) in PVL-producing strains, the association of a given protein of class S with a given protein of class F could generate six (F–S) molecular combinations produced by all PVL-producing strains. The biological activity of all these pairings remains to be investigated. In contrast, for the strains producing only \(\gamma\)-haemolysin, two toxic combinations only are possible: HlgA/HlgB and HlgC/HlgB.

PVL and \(\gamma\)-haemolysin are known to be structurally and functionally different and the prospective epidemiological study strengthened this difference. PVL-producing strains were not associated with bacteraemia in the preliminary study (table I) or in the prospective survey (table II). However, such strains were associated mostly with primary cutaneous infections, and especially with furuncles. These PVL-producing isolates were found in almost all furuncles and also in the anterior nares from all patients so far examined who had recurrent furuncles. A looser association was also observed with abscesses and whitlows. The ability to produce PVL could be at least considered as a good marker of the capability of a \(S.\) aureus strain to cause necrotising skin lesions. The strains were observed to be generally susceptible to most antibiotics having variable phenotypes for penicillin, doxycycline and erythromycin. PFGE profiles and lysotypes of PVL-positive \(S.\) aureus strains provided more polymorphic markers than toxin genotypes on DNA treated with EcoRI.

Recurrent furunculosis and small familial outbreaks of furuncles are sometimes observed and are difficult to cure. It is of particular interest to notice that amongst PVL-producing isolates from individuals belonging to the same family, the same epidemiological marker pattern was obtained. Moreover, in eight patients with recurrent furuncles, PVL-producing isolates were always obtained from the nose, and powerful epidemiological markers such as phage typing and PFGE analysis failed to distinguish between nose and furuncle strains. This finding may be important in the epidemiology of furuncles since nasal carriage was previously recognised as a constant feature in recurrent furunculosis.\(^{28}\) Therefore, epidemiological markers, including phenotypic toxinotyping (production of PVL) associated with PFGE typing, will be useful for identifying the reservoir of \(S.\) aureus between episodes of furunculosis. Therapeutic regimens specifically directed to this reservoir could be evaluated in an attempt to prevent these infections.

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