Epidemiological differences between the UK and Ireland versus France in Staphylococcus aureus isolates resistant to fusidic acid from community-acquired skin and soft tissue infections

A. R. Larsen1*, R. L. Skov1, V. Jarlier2 and A. S. Henriksen3

1Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen, Denmark; 2Service de Bactériologie-Hygienne, CHU Pitié-Salpêtrière, Paris, France; 3LEO Pharma, Industriparken 55, DK-2750 Ballerup, Denmark

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Objectives: To characterize the epidemiology of Staphylococcus aureus isolates resistant to fusidic acid isolated from patients with skin and soft tissue infections (SSTIs) in France, the UK and Ireland.

Methods: One hundred and thirty-six S. aureus isolates with an MIC of fusidic acid above 1 mg/L were isolated during the EPISA study from patients more than 2 years old attending their general practitioners for SSTIs. All isolates were related to clonal complex by a combination of PFGE, spa typing and multilocus sequence typing. The presence of toxin genes and of the fusB determinant was monitored to characterize each represented clonal complex.

Results: Eight different clonal complexes were identified. CC121 constituted the majority of the isolates from Ireland and the UK but was not represented in France. Among the other clonal complexes, CC8 and CC5 were the most common in the three countries, although the number of French isolates was limited. CC121 was the only clonal complex significantly associated with a skin infection, namely impetigo (P < 0.05). Toxin genes were present in CC121 and CC80. The fusB determinant was also detected in the same clonal complexes. Enterotoxins were found in four clonal complexes (CC1, CC5, CC8 and CC22).

Conclusions: The impetigo clone (CC121: ST123) was present in the majority of S. aureus isolates from the UK and Ireland but was not detected in France. This strain was associated with impetigo, exfoliative toxins and the fusB determinant. No other clonal complex appeared to be dominant in other types of skin infections.

Keywords: skin infections, clonal genotypes, impetigo clone

Introduction

Skin and soft tissue infections (SSTIs) are some of the most common infections both in hospitals and for general practitioners.1 While the majority of cases are superficial and may be treated with topical or oral drugs, severe cases of SSTI may require hospitalization and parenteral therapy. The majority of SSTIs are caused by aerobic Gram-positive cocci, specifically Staphylococcus aureus and streptococci.2 In a recent prospective epidemiological study (the EPISA study), 1387 patients with community-acquired SSTI were enrolled to investigate the susceptibility of S. aureus to 11 antibiotics in France, Ireland and the UK. In that study, the susceptibility pattern of S. aureus isolates from French patients was different from the pattern obtained with isolates from Irish patients or patients from the UK, especially with regards to susceptibility for fusidic acid.3 Fusidic acid has been widely used for the past 40 years for treating superficial skin infections such as impetigo.4 However, in recent years, the spread of a S. aureus epidemic clone with reduced susceptibility to fusidic acid especially in Scandinavia has reduced empirical use of fusidic acid.5–8 This clone has also been identified in the UK and in Ireland but not in France.7 The incidence of the clone among SSTI patients in the UK and Ireland has not yet been reported based on molecular characteristics of the strains but rather estimated by an ‘educated guess’ based on the typical MIC value (2–8 mg/L) reported for the clone. In the last couple of years, spread in the community of methicillin-resistant S. aureus (CA-MRSA) isolates belonging to clonal complex (CC) 80 has increased focus on fusidic acid as these isolates are susceptible to all antibiotics with the exception

*Corresponding author. Tel: +45-32688168; Fax: +45-32683231; E-mail: arl@ssi.dk

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of β-lactams, tetracycline, streptomycin, kanamycin and fusidic acid. Furthermore, those strains of CA-MRSA also express the Panton-Valentine leucocidin (PVL) toxin. Most reports on those strains are based on isolates selected for their toxin production and not for their susceptibility to fusidic acid. In this report, we characterized all S. aureus isolates resistant to fusidic acid collected from SSTI patients during a prospective epidemiological study in 2003–04 in Ireland, France and the UK.

Materials and methods

S. aureus isolates

The S. aureus isolates were obtained from the EPISA project, a surveillance programme conducted in 2003–04 in Ireland, France and the UK to determine the susceptibility of the community-acquired skin pathogens. Details of the study procedures have been published previously and are therefore only summarized here. Patients aged older than 2 years attending their general practitioner with SSTIs presumed to be due to S. aureus were enrolled. Patients hospitalized within the previous 4 weeks and those who had another SSTI or who had received any systemic or topical treatment with an antimicrobial agent in the previous 4 weeks were excluded.

Antimicrobial susceptibility

The in vitro antimicrobial susceptibility of the isolates was determined using broth microdilution according to CLSI (formerly NCCLS) methodology. CLSI breakpoints were used to define resistance to the following antibiotics: penicillin, rifampicin, gentamicin, erythromycin, clindamycin, tetracycline, ciprofloxacin, oxacillin and vancomycin. The BSAC breakpoints were used to define resistance to mupirocin (≥8 mg/L) and fusidic acid (≥2 mg/L) as there are no CLSI breakpoint criteria for these agents.

S. aureus isolates with an MIC of fusidic acid above 1 mg/L

All isolates with an MIC of fusidic acid above 1 mg/L were subjected to PFGE analysis according to the Harmony protocol. PFGE clusters were identified from a UPGMA dendrogram based on Dice coefficients. A similarity coefficient above 75% was selected to define the major clusters.

Representative isolates for each cluster, selected by showing the most diverse pattern within each major cluster, were typed by staphylococcal protein A (spa) typing and multi locus sequence typing (MLST) performed as previously described. The spa types (t) and MLST sequence types (ST) were assigned through the Ridom and MLST databases, respectively.

Each PFGE cluster was assigned to a CC based on spa typing and MLST.

Characterization of the clonal complexes

In total, 33 isolates representing each cluster were characterized by PCR with respect to toxin genes tsa, eta, etb and lukS-PV/lukF-PV encoding toxic shock syndrome toxin, exfoliative toxin A, exfoliative toxin B and PVL, respectively. Staphylococcal enterotoxin A–D (SEA–D) production was also determined using a SET-RPLA Toxin Detection Kit according to the manufacturer’s instructions (Oxoid, Basingstoke, UK).

The presence of the fusB gene was determined in the same isolates as described previously.

Statistical methods

Statistical analysis was done by the χ² test, P < 0.05 indicating significance.

Results

In the EPISA study, a total of 646 S. aureus were isolated from 631 patients (221 from 216 patients in the UK, 220 from 218 patients in Ireland and 205 from 197 patients in France). Of these, 136 isolates had a fusidic acid MIC above 1 mg/L. The MIC distribution of those isolates is shown in Figure 1.

The PFGE dendrogram determined eight clusters containing from 2 to 68 isolates. The number of representative isolates selected from the PFGE clusters for further characterization ranged from 2 to 9 and is shown in Table 1 together with their allocation to the eight clonal complexes.

The numbers of isolates from France, Ireland and the UK related to each clonal complex are also shown in Table 1. CC121 was isolated most frequently in Ireland and the UK, constituting 63% (34/54) and 49% (34/69) of the isolates, respectively. The CC121 isolates were characterized by the PFGE pattern shown in Figure 2, three different spa types (t171, t408 and t876) and sequence type ST123.

CC121 was isolated significantly more frequently from patients with impetigo (49 out of 68) than were the other clonal complexes (14 out 68) (P < 0.001) (Table 2).

Table 3 shows the antibiogram of the eight clonal complexes. Virtually all isolates were resistant to penicillin and most of the isolates resistant to erythromycin demonstrated inducible resistance to clindamycin with the exception of the isolates from CC8. Seven isolates, belonging to CC5 (n = 1), CC8 (n = 1), CC22 (n = 3) and CC80 (n = 2), were resistant to oxacillin. CC5, CC8 and CC22 included multiresistant isolates in contrast to CC121 where the isolates were only resistant to fusidic acid, penicillin and erythromycin in some cases.
Toxin genes were only found in isolates belonging to CC121 (etA and etB) and CC80 (lukS-PV/lukF-PV). SEA was found in CC1 and in one-third of CC5 and CC8 isolates, whereas SEB was only detected in one CC5 isolate in addition to SED and one CC8 isolate in addition to SEA. SEC was found in one CC5 isolate and in two CC22 isolates.

The resistant determinant to fusidic acid fusB was detected in all eight CC121 isolates and in half of the CC80 isolates characterized. Of the six CC5 isolates analysed, one demonstrated the presence of the fusB gene. One of the two CC8 isolates also demonstrated the presence of fusB.

Table 1. Clonal complex distribution of fusidic-acid-resistant S. aureus isolates by country

<table>
<thead>
<tr>
<th>Number of isolates characterized from PFGE clusters</th>
<th>spa type(s)</th>
<th>Clonal complex allocated</th>
<th>Toxin(s) detected (no. of isolates positive)</th>
<th>Number of isolates from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>France</td>
</tr>
<tr>
<td>3</td>
<td>t127</td>
<td>CC1</td>
<td>SEA (1)</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>t002, t045, t548, t878</td>
<td>CC5</td>
<td>SEA (2), SEB (1), SEC (1), SED (1)</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>t008, t068, t622</td>
<td>CC8</td>
<td>SEA (2), SEB (1)</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>t228</td>
<td>CC15</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>t022, t032</td>
<td>CC22</td>
<td>SEC (2)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>t031, t877</td>
<td>CC45</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>t044, t376</td>
<td>CC80</td>
<td>lukS-PV/lukF-PV (2)</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>t171, t408, t876</td>
<td>CC121</td>
<td>etA (6), etB (6)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2. Dendrogram of common PFGE patterns from each of the clonal complexes.
The isolates studied came from a large prospective multicentre study in three countries in Europe. The patients recruited were typical of those with skin infection in domiciliary practice and were recruited from geographically distinct regions of the countries concerned. Recent antimicrobial usage and hospitalization were exclusion criteria, thereby minimizing the selective influence of recent antimicrobial prescribing.

Determination of antimicrobial susceptibility was done by established methodology. Criteria for resistance were based on established CLSI recommendations. Although there are no CLSI breakpoints established for fusidic acid, those recommended by BSAC are widely accepted. Isolates were allocated to clonal complexes on the basis of established methodologies. PFGE was performed according to the Harmony protocol, and spa typing and MLST were used to allocate clonal complexes.\(^{19,22,23}\)

The isolates studied were likely to be representative of those present in the community in the countries studied and thus provide information on the epidemiological nature of \(S.\) aureus resistant to fusidic acid causing skin infections.

**Discussion**

The isolates studied came from a large prospective multicentre study in three countries in Europe. The patients recruited were typical of those with skin infection in domiciliary practice and were recruited from geographically distinct regions of the countries concerned.\(^{14,15}\) Recent antimicrobial usage and hospitalization were exclusion criteria, thereby minimizing the selective influence of recent antimicrobial prescribing.

Determination of antimicrobial susceptibility was done by established methodology. Criteria for resistance were based on established CLSI recommendations. Although there are no CLSI breakpoints established for fusidic acid, those recommended by BSAC are widely accepted. Isolates were allocated to clonal complexes on the basis of established methodologies. PFGE was performed according to the Harmony protocol, and spa typing and MLST were used to allocate clonal complexes.\(^{19,22,23}\)

The isolates studied were likely to be representative of those present in the community in the countries studied and thus provide information on the epidemiological nature of \(S.\) aureus resistant to fusidic acid causing skin infections.

**Table 2.** Clonal complex distribution of fusidic acid-resistant \(S.\) aureus isolates from the UK and Ireland by diagnosis

<table>
<thead>
<tr>
<th>Clonal complex (CC)</th>
<th>Number of isolates</th>
<th>Primary SSTI</th>
<th>Secondary SSTI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>impetigo</td>
<td>secondary-infected dermatoses</td>
</tr>
<tr>
<td>CC1</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>CC5</td>
<td>18</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>CC8</td>
<td>27</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>CC15</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CC22</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CC45</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CC80</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CC121</td>
<td>68</td>
<td>49</td>
<td>4</td>
</tr>
</tbody>
</table>

Of one hundred and thirty-six isolates, 21% of the isolates in the EPISA study were characterized and allocated to a clonal complex. There were eight clonal complexes identified, namely CC1, CC5, CC8, CC15, CC22, CC45, CC80 and CC121. Isolates belonging to CC121 were the most common, comprising 68 (50%) isolates. CC121 was found in Ireland and the UK only, and not in France. CC22, which is the genetic background for the epidemiological EMRSA-15 clone, prevalent in the UK and Ireland,\(^{26,27}\) was also found in this study in those two countries only. In contrast, the other clonal complexes dominated by CC8 (25 isolates) and CC5 (18 isolates) did not appear to be restricted to one particular country. However, we found three isolates belonging to CC80 in French patients. CC80 is known to constitute the genetic background of the European community-associated MRSA clone (ST80-IV), previously described to cause SSTIs in France.\(^{12}\)

In Ireland and the UK, CC121 was significantly associated with patients with impetigo. There was no obvious association of the other clonal complexes with any particular infection.

The genes encoding the exfoliative toxins \(etA\) and \(etB\) were detected in CC121 only. Similarly, the genes encoding PVL were detected in CC80 only. These results are consistent with previous reports on \(etA, etB\)\(^{28}\) and \(pvl.\)\(^{12}\) Staphylococcal enterotoxins were found in four clonal complexes but could not be only associated with clinically infected dermatitis as they were also detected in patients with furunculosis, impetigo or traumatic wound infections (data not shown).

There have been recent reports of the reduced susceptibility to fusidic acid in some countries.\(^{5,6,29–31}\) This was associated with the spread of a clonal epidemic strain,\(^7\) now identified as ST123.\(^{28}\) We have confirmed the presence of this clone in Ireland and the UK belonging to CC121. In our study, this clone accounted for 50% of the isolates with reduced susceptibility to fusidic acid. We confirmed that this clone has a typical fusidic acid MIC in the range of 2–8 mg/L.\(^{28}\) None of the CC121 isolates had acquired resistance to oxacillin.

We also confirmed that the decrease in susceptibility to fusidic acid in this clone is encoded by the \(fusB\) gene.\(^{7,28,32}\)

**Table 3.** Resistance profile of fusidic acid-resistant \(S.\) aureus related to clonal complexes

<table>
<thead>
<tr>
<th>Clonal complex (CC)</th>
<th>Total number of isolates</th>
<th>Number resistant to penicillin</th>
<th>oxacillin</th>
<th>erythromycin(^a)</th>
<th>tetracycline</th>
<th>ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC1</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CC5</td>
<td>18</td>
<td>16</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CC8</td>
<td>27</td>
<td>27</td>
<td>2</td>
<td>4</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>CC15</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CC22</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CC45</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CC80</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CC121</td>
<td>68</td>
<td>67</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>130</td>
<td>7</td>
<td>22</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\)Inducible resistance to clindamycin was observed using D-diffusion disc for all the isolates resistant to erythromycin with the exception of the isolates belonging to CC8.
The same determinant was identified in CC80 as previously described as well as in CC5 and CC8.33

In the UK and Ireland, this clone was predominantly associated with impetigo as reported previously from Sweden6 and Norway.5 The particular association of this clone with impetigo is not surprising in view of the toxin genes identified in CC121. Presence of \( etA \) and \( etB \) has previously shown to be associated with the ability of \( S. aureus \) to cause impetigo.34–36

The MIC of fusidic acid for the other clonal complexes ranged from 2 to more than 128 mg/L. This range is not unexpected in view of the absence of the \( fusB \) determinant in the majority of isolates tested from these complexes. The other known resistance mechanism involves various chromosomal single mutations in the \( fusA \) gene encoding the target site of fusidic acid, EF-G, and is known to give rise to a wide variation in MIC.7

In conclusion, we have confirmed in Ireland and the UK the presence of the clone (CC121) with reduced susceptibility to fusidic acid in association with impetigo. The clone contains the gene-encoding \( etA \) and \( etB \) toxin known to be associated with impetigo. This clone has a fusidic acid MIC of 2–8 mg/L. In this clone, the major mechanism of resistance involves the \( fusB \) determinant.

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Transparency declarations

None of the employees at Statens Serum Institut and Service de Bacteriologie-Hygiène received any direct or indirect payment from LEO Pharma. A. S. H. is an employee of LEO Pharma but holds no stock or options in the company.

References