A novel fusidic acid resistance determinant, fusF, in Staphylococcus cohnii

Hsiao-Jan Chen1, Wei-Chun Hung1, Yu-Tzu Lin1, Jui-Chang Tsai2,3, Hao-Chieh Chiu1,4, Po-Ren Hsueh4,5 and Lee-Jene Teng1,4*

1Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan; 2Center for Optoelectronic Medicine, National Taiwan University College of Medicine, Taipei, Taiwan; 3Division of Neurosurgery, Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan; 4Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan; 5Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

*Corresponding author. Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan. Tel: +886-2-23123456, ext. 66918; Fax: +886-2-23711574; E-mail: lj teng@ntu.edu.tw

Received 29 May 2014; returned 1 July 2014; revised 22 September 2014; accepted 22 September 2014

Objectives: To determine MICs of fusidic acid for and identify genetic determinants of resistance in Staphylococcus cohnii isolates.

Methods: Susceptibility to fusidic acid was determined by the standard agar dilution method in 24 S. cohnii subsp. urealyticus clinical isolates, 7 S. cohnii subsp. cohnii clinical isolates and 2 reference strains. Sequencing of a novel resistance determinant, fusF, and its flanking regions was performed by long and accurate PCR and inverse PCR. To evaluate the function of fusF, the MIC of fusidic acid was determined for recombinant Staphylococcus aureus carrying a plasmid expressing fusF.

Results: A total of 25 S. cohnii subsp. urealyticus (24 clinical isolates and 1 reference strain) and 2 S. cohnii subsp. cohnii displayed low-level resistance to fusidic acid (MICs 2–16 mg/L). Sequencing of a 4259 bp fragment from S. cohnii subsp. urealyticus ATCC 49330 revealed a novel resistance gene, designated fusF, which displayed 70.5% nucleotide and 67.3% amino acid identity to fusD. Expression of fusF in S. aureus confers resistance to fusidic acid.

Conclusions: A novel FusB-family gene, fusF, was identified as a major resistance determinant in S. cohnii clinical isolates resistant to fusidic acid.

Keywords: fusD, FusB family, staphylococci

Introduction

Staphylococcus cohnii subsp. urealyticus and S. cohnii subsp. cohnii, members of the CoNS, have been described as pathogens in human diseases.1,2 Antibiotic-resistant S. cohnii collected from ward environments has been reported.3 However, susceptibility testing with fusidic acid has not yet been reported in clinical isolates and any resistance determinants remain to be identified.

Fusidic acid acts by binding elongation factor G (EF-G) and blocks bacterial protein synthesis.4,5 Resistance to fusidic acid may result from alteration of the drug target site6–8 or may be due to the protection of the drug target site by genes encoding FusB-family proteins.9,10 Binding of FusB-family proteins to EF-G promotes the release of the EF-G/guanosine 5′-diphosphate complex in the presence of fusidic acid and thus rescues translation.11,12

The aim of this study was to describe susceptibility to fusidic acid in S. cohnii and identify any resistance determinants. A novel fusB-type gene was identified in the fusidic acid-resistant S. cohnii.

Materials and methods

Bacterial strains

A total of 24 S. cohnii subsp. urealyticus and 7 S. cohnii subsp. cohnii collected between 2000 and 2011 in the Bacteriology Laboratory of the National Taiwan University Hospital, a 2500 bed teaching hospital in northern Taiwan, as well as S. cohnii subsp. urealyticus ATCC 49330 and S. cohnii subsp. cohnii ATCC 29974, were used. The clinical isolates were initially identified by the Vitek 2 automated system (bioMérieux SA, Marcy-l’Étoile, France) and were then further divided into two subspecies based on the PCR–RFLP analysis of dnaJ13 and biochemical assays including lactose fermentation and the urease test.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the standard agar dilution method according to the guidelines of the CLSI.14 The breakpoint used to indicate fusidic acid resistance was 2 mg/L.15
Detection of fusidic acid resistance determinants

The DNA of the isolates was amplified with primers specific for fusB, fusC or fusD\textsuperscript{16} and a pair of degenerate primers for genes encoding FusB-family proteins (Uni-fusF and uni-fusR; Table S1), as Supplementary data at JAC Online. After DNA sequencing of fusF, a pair of specific primers (fusF 119-141F and fusF 546-572R; Table S1) was designed. To detect fusA mutations, the DNA was amplified with primers fusA-68 ~406\textsuperscript{16} and fusA-CR and then sequenced (Table S1) in four S. cohnii subsp. urealyticus (all resistant) and four S. cohnii subsp. cohnii (two resistant and two susceptible).

Sequencing of fusF and flanking regions

To determine the sequences of the entire fusF gene and its flanking regions, a long and accurate PCR corresponding ORFs. Susceptibility to fusidic acid: R, resistant; and S, susceptible.

Construction and expression of plasmids with fusF, fusB or fusD

To construct plasmids with fusF, fusB or fusD, specific PCR products were amplified from S. cohnii subsp. urealyticus ATCC 49330, Staphylococcus epidermidis NTUH-7778 (JF808726) and Staphylococcus saprophyticus ATCC 15305 (AP008934), respectively (Table S1). The constructions were generated by using pRM2 under the control of the xyl/tetO promoter,\textsuperscript{17} then transformed into Escherichia coli K12 ER2925 and confirmed by sequencing. The recombinant pRM2 derivatives were then transformed into Staphylococcus aureus RN4220 and S. aureus ATCC 29213 with a Gene pulser Xcell (Bio-Rad Laboratories, Hercules, CA, USA).\textsuperscript{18} Ampicillin (100 mg/L) and chloramphenicol (10 mg/L) were used for the selection of recombinant E. coli and S. aureus, respectively. Anhydrotetracycline hydrochloride (0.16 mg/L) was used to induce gene expression from the xyl/tetO promoter.\textsuperscript{17}

Nucleotide sequence accession numbers

The sequences of fusF elements from S. cohnii subsp. urealyticus ATCC 49330 and S. cohnii subsp. cohnii ATCC 29974 were deposited in GenBank under accession numbers AB934903 and AB934908, respectively.

Results

Susceptibility and fusidic acid resistance determinants

All isolates of 25 S. cohnii subsp. urealyticus (MICs ranging from 4 to 16 mg/L) and 2 of 8 of S. cohnii subsp. cohnii (MICs ranging from 0.125 to 4 mg/L) were resistant to fusidic acid. None of the fusidic acid-resistant S. cohnii possessed fusB, fusC or fusD. However, a 228 bp amplicon was generated by a pair of degenerate primers designed to amplify fusB-type genes. Each of the two subspecies of S. cohnii had the same fusA sequence. However, a total of seven amino acid differences between S. cohnii subsp. urealyticus and S. cohnii subsp. cohnii at positions 127, 213, 246, 288, 597, 691 and 695 in EF-G were found.

Sequence analysis of the fusF element

Sequencing of a 4259 bp fragment from S. cohnii subsp. urealyticus ATCC 49330 revealed a novel resistance gene, designated fusF, which displayed highest similarity to fusF in S. saprophyticus ATCC 15305, with 70.5% nucleotide and 67.3% amino acid identity (Figure 1 and Table S2). The sequences of the flanking genes were also most similar to those in S. saprophyticus ATCC 15305 (AP008934) (Figure 1). Sequence similarities between fusF and other fusB-type genes ranged from 50.4% to 70.5% in nucleotide sequence and 39.5% to 67.3% in amino acid sequence (Table S2).

Previous studies indicated that four conserved amino acids in the C-terminal domain of FusB-family proteins (Phe-156, Lys-184, Tyr-187 and Phe-208) are key regions for the interaction with EF-G.\textsuperscript{19} In FusF, three of the above residues were found; the missing residue was Phe-156, which was replaced by tyrosine (Tyr) (Figure S1).

Truncated fusF element in S. cohnii subsp. cohnii

Sequencing of a 4886 bp fragment revealed a truncated fusF in S. cohnii subsp. cohnii ATCC 29974. The truncated fusF (ORF3) was only 396 bp, showing 86.4% nucleotide identity to the 5\textsuperscript{\prime} end of fusF.

Susceptibility to fusidic acid\textsuperscript{a}

\begin{itemize}
  \item \textbf{S. saprophyticus} ATCC 15305 \hspace{1cm} R
  \item \textbf{S. cohnii subsp. urealyticus} ATCC 49330 \hspace{1cm} R
  \item \textbf{S. cohnii subsp. cohnii} ATCC 29974 \hspace{1cm} S
\end{itemize}

\textsuperscript{a}Susceptibility to fusidic acid: R, resistant; and S, susceptible.

Figure 1. Comparison of the genetic organization of the fusF gene and surrounding regions in S. saprophyticus ATCC 15305 (AP008934), S. cohnii subsp. urealyticus ATCC 49330 (AB934903) and S. cohnii subsp. cohnii ATCC 29974 (AB934908). Arrows represent putative ORFs. Homologous regions (>70% nucleotide identity) are shaded grey and the numbers in the shaded sections represent the percentage homologies of nucleotide sequences between the corresponding ORFs.
fusF (645 bp) in S. cohnii subsp. urealyticus (Figure 1). Downstream sequences of ORF3 also differed from those in the fusF element.

**fusF mediates resistance to fusidic acid**

To determine whether FusF is functionally similar to other FusB-family proteins, fusF, fusB and fusD were cloned into the plasmid pRM12 and introduced into S. aureus RN4200 (MIC=0.125 mg/mL) and ATCC 29213 (MIC=0.25 mg/mL). The MICs of fusidic acid for the recombinant S. aureus containing fusF (MIC=16 mg/mL), fusB (MIC=16 mg/mL) and fusD (MIC=1 mg/mL) were increased (Table 1), indicating that the function of FusF was similar to that of other FusB proteins that are associated with fusidic acid resistance.

**Discussion**

In the present study, a novel fusidic acid resistance determinant, fusF, was identified in fusidic acid-resistant S. cohnii. The fusD-like gene was designated fusF based on the cut-off value of ≤80% amino acid identity and represents a novel fusidic acid-resistance determinant that belongs to the FusB family of proteins. Flanking sequences of fusF suggested that the location of fusF may not be related to mobile genetic elements such as plasmids or pathogenicity islands, although more studies are required to confirm this. The fusF gene was not detected in other staphylococcal species, such as S. epidermidis, S. saprophyticus, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus caprae, Staphylococcus warneri and Staphylococcus capitis. Moreover, our results suggested that fusF was an intrinsic factor in S. cohnii subsp. urealyticus and may not be conserved in another subspecies, S. cohnii subsp. cohnii. A truncated fusF was found in susceptible strains of S. cohnii subsp. cohnii, lacking the C-terminal regions that were considered important for resistance to fusidic acid (Figure S1). The correlation of fusF structure with subspecies is of unknown significance, but may be due to genetic evolution.

The function of fusF was confirmed by the increase in MICs of fusidic acid from 0.125 or 0.25 to 16 mg/L for the recombinant S. aureus. Different from other FusB-family proteins, the amino acid at position 156 (Phe), the key residue related to interaction of FusB-family proteins and EF-G, was replaced by Tyr, a hydroxyl derivative of Phe, in FusF.

Although fusA mutations in S. aureus can confer high-level resistance to fusidic acid, it is unknown whether fusA in S. cohnii subsp. urealyticus behaves in the same way. Since all of the S. cohnii subsp. urealyticus isolates tested in this study were resistant to fusidic acid, there was no susceptible fusA-carrying S. cohnii subsp. urealyticus that could be used for comparison. Thus, it is unclear whether fusA is responsible for fusidic acid resistance in S. cohnii subsp. urealyticus.

In conclusion, a novel fusidic acid resistance determinant, fusF, was found in fusidic acid-resistant S. cohnii.

---

**Table 1.** MICs of fusidic acid for recombinant S. aureus strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus RN4200</td>
<td>0.125</td>
</tr>
<tr>
<td>ATCC 29213</td>
<td>0.25</td>
</tr>
<tr>
<td>Recombinant S. aureus RN4200 expressing FusB-family protein</td>
<td></td>
</tr>
<tr>
<td>RN4200/pRM12</td>
<td>0.0625</td>
</tr>
<tr>
<td>RN4200/pRM12:fusB</td>
<td>16</td>
</tr>
<tr>
<td>RN4200/pRM12:fusD</td>
<td>1</td>
</tr>
<tr>
<td>RN4200/pRM12:fusF</td>
<td>16</td>
</tr>
<tr>
<td>Recombinant S. aureus ATCC 29213 expressing FusB-family protein</td>
<td></td>
</tr>
<tr>
<td>ATCC 29213/pRM12</td>
<td>0.25</td>
</tr>
<tr>
<td>ATCC 29213/pRM12:fusB</td>
<td>16</td>
</tr>
<tr>
<td>ATCC 29213/pRM12:fusD</td>
<td>1</td>
</tr>
<tr>
<td>ATCC 29213/pRM12:fusF</td>
<td>16</td>
</tr>
</tbody>
</table>

---

**Funding**

This work was supported by a grant from the National Science Council of Taiwan (NSC 100-2320-B-002-014-MY3).

---

**Supplementary data**

Table S1, Table S2 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

**References**


