Prevalence of fusB in Staphylococcus aureus clinical isolates

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Fusidic acid (FA) resistance in Staphylococcus aureus markedly varied among different regions. Few data for FA resistance are available in China. In this study, FA susceptibility testing was performed, and the prevalence of fusB and fusC in 116 clinical isolates of S. aureus was investigated by PCR. Mutations in fusA were also determined by sequencing of PCR products. Molecular typing of fusB-positive strains was based on multilocus sequence typing (MLST), spa typing and pulsed-field gel electrophoresis (PFGE). A DNA fragment flanking fusB was sequenced. Transformation experiments were carried out in fusB-positive S. aureus. Of 116 S. aureus including 19 meticillin-resistant S. aureus (MRSA) and 97 meticillin-susceptible S. aureus (MSSA), four (3.5%) were resistant to FA with MICs of 6–12 μg ml⁻¹, including one MRSA from blood and three MSSA from wound exudates. All four FA-resistant isolates were found to be fusB gene positive. Three FA-resistant MSSA strains had the same MLST profile of ST630 and spa type of t377, whilst the MRSA strain belonged to ST630-t4549. Only one PFGE pattern was recognized for these four strains. No fusC and fusA mutations were detected in any of the isolates. FA resistance in fusB-positive clinical isolates could be transferred to S. aureus RN4220. The fusB gene was located in a transposon-like element, which had 99% identity with that found in pUB101. In conclusion, the FA resistance rate is low in S. aureus, and the fusB gene is responsible for the resistance.

INTRODUCTION

Fusidic acid (FA) is a bacteriostatic antibiotic that blocks bacterial protein synthesis by locking elongation factor G (EF-G) to the ribosome (O’Neill et al., 2007), and is considered a useful antibiotic for meticillin-resistant Staphylococcus aureus (MRSA) infections (Tsui et al., 2011). It is listed as the treatment of choice for skin and soft tissue infections (SSTIs), acute and chronic osteomyelitis, vertebral infection, septic arthritis and other device-related infections caused by S. aureus (Schöfer & Simonsen, 2010; Whitby, 1999).

FA-resistant S. aureus has been reported in many countries, and the prevalence of resistance was remarkably different among different countries (Wang et al., 2012), with resistance rates relatively low (<10%) in Asian countries except for Kuwait, Pakistan and South Korea. In Kuwait, resistance rates dramatically increased from 22% in 1994 to 92% in 2004. The overall FA resistance rates were 10.7% in 13 European countries; 1.4–3.1% in Italy, Poland, Spain and Sweden; 62.4% in Greece; and 19.9% in Ireland (Castanheira et al., 2010b). In the last decade, there has been an increase in the prevalence of FA-resistant staphylococci in a number of northern European countries. This has been particularly striking among strains of S. aureus causing impetigo due to clonal expansion of a strain called the epidemic European FA-resistant impetigo clone (EEFIC). This clone has been reported in the UK, France, Sweden, Norway and other European countries (O’Neill et al., 2007a). FA resistance rates in S. aureus have been found to be very low in the USA (0.3%) and higher in Canada and Australia (7.0% in both countries) (Pfaller et al., 2010).

Two major FA resistance mechanisms are involved in S. aureus: alteration of the drug target site, which is due to mutations in fusA (encoding EF-G), conferring high-level resistance, and protection of the drug target site by fusB family proteins, including fusB, fusC and fusD (Chen et al., 2005; Whitby, 1999).
et al., 2011; Cox et al., 2012; McLaws et al., 2011; Schöfer & Simonsen, 2010), which mediate low-level resistance. The fusB and fusC genes have been found in S. aureus and coagulase-negative staphylococci, and fusD is an intrinsic factor causing FA resistance in Staphylococcus saprophyticus (Castanheira et al., 2010a).

The fusB gene can be carried by plasmids such as by the 21 kb plasmid pUB101; however, it can also be chromosomal (O’Brien et al., 2002; O’Neill & Chopra, 2006). The FusB protein has been shown to bind EF-G and protect the staphylococcal translation apparatus from the inhibitory effects of FA (Cox et al., 2012).

Few data are available on the prevalence of FA resistance in China. A recent study showed that the resistance rate was 2.2% in S. aureus strains isolated from Chinese paediatric patients with SSTIs, and among four FA-resistant strains, two strains each carried fusB and fusC (Liu et al., 2012).

In this study, FA susceptibility was tested and the resistance mechanisms were determined by detection of the FA-resistant determinants fusB and fusC, and mutation of fusA among S. aureus clinical isolates. In order to understand the phylogenetic relationship of resistance determinant-containing isolates, homology genotypings were performed.

**METHODS**

**Bacterial isolates.** A total of 116 isolates of S. aureus were collected at Lishui Central Hospital, a 1200-bed teaching hospital in Zhejiang province of China, between February 2010 and November 2011. Only the first isolate from each patient was included, and each isolate was identified using a Vitek-2 Compact System with AST-GPI cards (bioMérieux). The pvl gene was detected by PCR as described previously (McClure et al., 2006). Of 116 S. aureus strains, 55 (47.41%) were isolated from abscess or wound exudates, 33 (28.45%) from sputum, 23 (19.83%) from blood and five (4.31%) from urine.

**Antimicrobial susceptibility of clinical isolates.** Of 116 S. aureus clinical isolates, 97 (83.6%) were meticillin-susceptible S. aureus (MSSA) and 19 (16.6%) were MRSA, and four (3.4%) were resistant to FA with MICs of 6–12 µg ml⁻¹ as determined by E-test. Among the four FA-resistant strains, one was MRSA isolated from a patient with bloodstream infection and three were MSSA from patients with wound infections. There was no significant difference in FA resistance rates between MRSA (3.1%, 3/97) and MSSA (5.3%, 1/19) ($\chi^2 = 0.6, P = 0.4$). The pvl gene was positive in 12 (10.3%) S. aureus strains, but none of these were MRSA-resistant strains. The three strains of fusB-positive MSSA had the same profile of antibiotic susceptibility, which was resistant to FA, penicillin and erythromycin, but susceptibility to cefazolin, chloramphenicol, fosfomycin, vancomycin, linezolid, sulfamethoxazole, tetracycline and rifampicin. The characteristics of the four FA-resistant clinical isolates of S. aureus are shown in Table 1.

**Detection of FA resistance determinants.** All isolates were tested for the presence of fusA mutation and the fusB and fusC genes using PCR as described previously (O’Neill & Chopra, 2006; O’Neill et al., 2004, 2007). Plasmid DNA was extracted by using a Plasmid Midi kit according to the manufacturer’s instructions (Qiagen). The bacterial cell wall was disrupted by lysostaphin (Sangon) before extraction.

**Molecular typing of fusB-positive strains.** To study the homology of the FA-resistant strains, S. aureus isolates were molecularly typed by multilocus sequence typing (MLST), staphylococcal protein A gene (spa) typing and pulse-field gel electrophoresis (PFGE) as described previously (Koreen et al., 2004; McDougal et al., 2003, Mendes et al., 2010; Saunders & Holmes, 2007). The homology of these strains was analysed with BioNumerics software version 5.0, and isolates were classified into the same PFGE group if their similarity coefficient was >80% (Carriço et al., 2005).

**Transformation experiments.** To determine whether FA resistance in clinical strains was transferable, plasmids were purified from FA-resistant S. aureus and transformed into S. aureus RN4220 using an electroporation system (Bio-Rad) (Nair et al., 2011), with selection on tryptic soy agar (Oxoid) (Chen et al., 2011) plates containing FA (6 µg ml⁻¹).

**Nucleotide sequencing of fusB and flanking regions.** To determine the sequence of fusB and its flanking regions, the plasmid of an FA-resistant transformant was extracted. A DNA fragment containing the fusB gene and its flanking regions was sequenced by primer walking.

**Statistical analysis.** Categorical variables were compared using a χ² or two-tailed Fisher’s exact test. The statistical analysis was carried out with spss version 13.0 software and the level of significance was set to α=5%.

**RESULTS**

**Antimicrobial susceptibility of S. aureus clinical isolates**

Of 116 S. aureus strains, 97 (83.6%) were meticillin-susceptible S. aureus (MSSA) and 19 (16.6%) were MRSA, and four (3.4%) were resistant to FA with MICs of 6–12 µg ml⁻¹ as determined by E-test. Among the four FA-resistant strains, one was MRSA isolated from a patient with bloodstream infection and three were MSSA from patients with wound infections. There was no significant difference in FA resistance rates between MRSA (3.1%, 3/97) and MSSA (5.3%, 1/19) ($\chi^2 = 0.6, P = 0.4$). The pvl gene was positive in 12 (10.3%) S. aureus strains, but none of these were MRSA-resistant strains. The three strains of fusB-positive MSSA had the same profile of antibiotic susceptibility, which was resistant to FA, penicillin and erythromycin, but susceptibility to cefazolin, chloramphenicol, fosfomycin, vancomycin, linezolid, sulfamethoxazole, tetracycline and rifampicin. The characteristics of the four FA-resistant clinical isolates of S. aureus are shown in Table 1.

**Screening of FA-resistant determinants and resistance transfer**

The FA resistance gene fusB was present in all four FA-resistant isolates, and there was no fusC or fusA mutation in the 116 S. aureus isolates (Table 1).

FA resistance in all four S. aureus isolates was successfully transformed into RN4220 by plasmid electroporation. The FA MICs of the transformants were 8–12 µg ml⁻¹. The fusB gene was detected in the plasmids from transformants by PCR.
Molecular typing of fusB-positive strains

The three FA-resistant MSSA clinical strains had the same MLST profile of ST630 and spa type t377, whilst the MRSA strain belonged to the type ST630-t4549, and only one PFGE pattern was recognized for these four strains (Fig. 1).

Analysis of DNA structure adjacent to fusB

A DNA fragment of 4797 bp containing the fusB gene was sequenced and showed 99 % nucleotide identity with that in plasmid pUB101 of S. aureus (GenBank accession no. AY373761.1), showing the tnp-1, hp-3, hp-4 and fusB leader peptide upstream of fusB and hp-1, hp-2 and tnp-2 downstream (Fig. 2).

DISCUSSION

Only one study could be found in the literature in PubMed prior to this study on the surveillance of FA resistance in S. aureus in mainland China, and it showed that FA resistance was low (2.2 %) in S. aureus isolates from paediatric patients with SSTIs (Liu et al., 2012). The present study showed that FA resistance in S. aureus was also low (3.5 %, 4/116) and there was no significant difference in FA resistance rates between MRSA and MSSA. The drawback of this study is that the S. aureus strains were collected from a single hospital, but the results may reflect the low FA resistance in China because of the limited use of FA in mainland China. FA is used intravenously for the treatment of MRSA infections in clinical practice in China, but the use of FA oral preparations is uncommon and FA topical treatment of superficial skin infection is rare.

This study showed that all four FA-resistant strains carried the acquired low-level resistance gene fusB, whilst no fusA mutation or fusC gene was found. Both fusB (2/186) and fusC (2/186) were responsible for the FA resistance in S. aureus isolates from Chinese paediatric patients studied previously (Liu et al., 2012). In central Taiwan region, the most common resistance determinant was fusC, which was found in 25 of 34 MRSA isolates (Chen et al., 2011). FA resistance could be transferred to S. aureus RN4220 by transformation, and the partial genetic structure was identical to that on plasmid pUB101 (Chen et al., 2010; O’Brien et al., 2002), indicating that the fusB gene was located on plasmids in the four FA-resistant S. aureus strains in this study. The fusB determinant was located on what initially appeared to be a genomic island of about 18 kb, which has been designated the ‘S. aureus resistance island’.

Table 1. Characteristics of four FA-resistant clinical isolates and their isolation source

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Isolation source</th>
<th>Meticillin resistance</th>
<th>Isolation date</th>
<th>FA MIC (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS64</td>
<td>65 Nephrology</td>
<td>Blood</td>
<td>MRSA</td>
<td>2 May 2011</td>
</tr>
<tr>
<td>LS18</td>
<td>42 Orthopaedics</td>
<td>Wound exudate</td>
<td>MSSA</td>
<td>5 March 2011</td>
</tr>
<tr>
<td>LS63</td>
<td>24 Orthopaedics</td>
<td>Wound exudate</td>
<td>MSSA</td>
<td>25 May 2011</td>
</tr>
<tr>
<td>LS88</td>
<td>42 Orthopaedics</td>
<td>Wound exudate</td>
<td>MSSA</td>
<td>20 July 2011</td>
</tr>
</tbody>
</table>

Fig. 1. Dendrogram of patterns generated by PFGE of the four FA-resistant S. aureus isolates using the BioNumerics software program. The four strains belonged to different subtypes of the same clonotype, with a genomic similarity of >80 % according to the dice coefficient. Strain number, MLST type and spa type are indicated to the right of the PFGE profiles. Strain LS-64 is MRSA and other three strains are MSSA.
island carrying $fusB$ (SaRI$fusB$) in the chromosome (O’Neill et al., 2004). These two phenomena may result in the flanking region containing transposon sequences.

On the basis of MLST and spa typing, previous studies have indicated that the majority of FA-resistant MRSA belong to two major spa types, t037 and t002, and the predominant sequence types were ST239, ST5, ST59 and ST254 (Chen et al., 2010). In this study, four $fusB$-positive $S$. aureus strains had the same MLST type of ST630 and were classified into the same PFGE group, although the spa types were different between the MRSA strain (t4549) and the three MSSA strains (t377). These results indicate that these strains might be clone related. ST630 is not a prevalent clone in China (Ho et al., 2012; Yan et al., 2009). To our knowledge, this is the first report of spa type t4549 in China.

In conclusion, the $fusB$ gene was found to be responsible for the low FA resistance in $S$. aureus in China, and the FA-resistant strains may be clone related.

Acknowledgements

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