Genetic analysis of high-level mupirocin resistance in the ST80 clone of community-associated meticillin-resistant Staphylococcus aureus

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Four community-associated meticillin-resistant Staphylococcus aureus (CA-MRSA) isolates expressing high-level mupirocin resistance (MIC > 1024 mg l⁻¹) were isolated from four sites of a diabetic patient and characterized for the genetic location of their resistance determinants and typed using PFGE, staphylococcal cassette chromosome mec (SCCmec), the coagulase gene and multilocus sequence typing to ascertain their relatedness. The presence of genes for resistance to high-level mupirocin (mupA), tetracycline (tetK) and fusidic acid (far1), Panton–Valentine leukocidin (PVL), accessory gene regulators (agr) and capsular polysaccharide (cap) were detected in PCR assays. The isolates were resistant to kanamycin, streptomycin, tetracycline, fusidic acid and cadmium acetate, and harboured mupA, tetK, far1, PVL, agr3 and cap8. They had identical PFGE patterns and coagulase gene type, possessed the type IV SCCmec element and belonged to sequence type 80 (ST80). However, they had three different plasmid profiles: (i) 28.0 and 26.0 kb; (ii) 28.0, 21.0 and 4.0 kb; and (iii) 41.0 and 4.0 kb. Genetic studies located the resistance to tetracycline, fusidic acid and cadmium acetate on the 28 kb plasmid and mupA on the related non-conjugative 26 and 21 kb plasmids. One of the 21 kb mupirocin-resistance plasmids was derived from the ~41 kb plasmid during transfer experiments. The emergence of high-level mupirocin resistance in the ST80-SCCmec IV MRSA clone demonstrates the increasing capacity of CA-MRSA clones to acquire resistance to multiple antibacterial agents. The presence of different plasmid profiles in genetically identical isolates creates difficulty in the interpretation of typing results and highlights the weakness of using plasmid analysis as the sole method for strain typing.

INTRODUCTION

Community-associated meticillin-resistant Staphylococcus aureus (CA-MRSA) was initially reported from patients in remote communities with no access to health-care facilities (Udo et al., 1994), but is now frequently reported in health-care facilities (Denis et al., 2005; Witte et al., 2005; Tenover et al., 2006). Besides carrying smaller-sized staphylococcal cassette chromosome mec (SCCmec) elements (types IV and V) than those of health-care-associated (HA)-MRSA (types I, II and III), CA-MRSA are generally characterized by their susceptibility to non-β-lactam antibiotics (Maltezou & Giamarellou, 2006). However, the trend appears to be changing, as some CA-MRSA clones have acquired multiresistance and the capacity to spread (Takizawa et al., 2005). In particular, the dominant CA-MRSA clone in North America (USA300) has acquired resistance to multiple antibacterial agents, including erythromycin, clindamycin, ciprofloxacin, tetracycline and high-level mupirocin, due to the acquisition of a multiresistance conjugative plasmid (Diep et al., 2006; Simor et al., 2007; Tenover et al., 2006). Similarly, a sequence type 30 (ST30) CA-MRSA clone isolated in Japan was reported to have acquired a multiresistance self-transmissible plasmid (Takizawa et al., 2005).

Mupirocin is a topical antibiotic with excellent anti-staphylococcal activity. It has been used successfully to eradicate the carriage of MRSA in health-care workers and patients, and has been invaluable in the control of MRSA outbreaks (Barrett, 1990). However, mupirocin resistance has occurred following its extensive and widespread use (Rahman et al., 1989; Udo et al., 1994, 2001; Pérez-Roth et al., 2006). Mupirocin resistance can be expressed as low-level (MIC 8–256 mg l⁻¹) or high-level (MIC ≥512 mg l⁻¹) resistance (Gilbart et al., 1993). High-level mupirocin resistance is encoded by a plasmid-mediated
mupA gene in most S. aureus isolates (Rahman et al., 1989; Udo et al., 1994; Udo & Jacob, 1998). Although high-level mupirocin resistance has been reported widely in HA-MRSA strains, it is rare in CA-MRSA clones outside the USA300 clone (Udo et al., 1994; Pérez-Roth et al., 2006). This paper describes the identification and determination of the genetic location of high-level mupirocin resistance in an ST80 clone of CA-MRSA isolated in a Kuwait hospital.

METHODS

MRSA isolates. Four MRSA isolates were obtained from a 52-year-old female diabetic patient admitted to a general hospital in Kuwait between 26 May and 14 June 2007. She presented for admission with a diabetic foot infection on 26 May 2007 and was admitted to an isolation room where she remained until discharged. Pre-admission screening of her nose, axilla and wound on 26 May 2007 yielded MRSA only from her axilla (isolate K6482). Subsequent screenings during hospitalization yielded MRSA from the diabetic wound (isolate K6531) on 3 June, ear (isolate K6533) on 5 June and forehead (isolate K6552) on 14 June 2007. She was treated with piperacillin/tazobactam, which was changed on 9 June 2007 to oral ciprofloxacin until she was discharged. No similar MRSA isolates were obtained from other patients in the hospital during or after her discharge.

Susceptibility testing. Susceptibility testing to antibacterial agents was performed by the disc diffusion method according to the Clinical and Laboratory Standards Institute’s guidelines (CLSI, 2006). Susceptibility to fusidic acid was interpreted following guidelines by the British Society for Antimicrobial Chemotherapy (BSAC, 2005). The MICs of metillin, mupirocin, fusidic acid and tetracycline were determined using Etest strips (AB Biodisk) according to the manufacturer’s instructions and using Etest strips (AB Biodisk) according to the Clinical Laboratory Standards Institute’s guidelines (CLSI, 2006). Susceptibility testing to antibacterial agents was performed by the disc diffusion method according to the Clinical Laboratory Standards Institute’s guidelines (CLSI, 2006). Susceptibility testing to antibacterial agents was performed by the disc diffusion method according to the Clinical Laboratory Standards Institute’s guidelines (CLSI, 2006). 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The 1.5 kb mupA PCR product was purified by elution from low-melting-point agarose following treatment with agarase (Roche Applied Science) according to the manufacturer’s instructions, and labelled by the random priming method with digoxigenin-11-UTP using the DIG High Prime labelling and detection kit (Roche Applied Science) to produce a mupA probe. EcoRI-digested, high-level mupirocin-resistance plasmids were transferred to a nylon membrane by Southern blotting. DNA hybridization, post-hybridization washes and detection by a colorimetric method were performed using the DIG High Prime labelling and detection kit according to the manufacturer’s instructions.

RESULTS AND DISCUSSION

The characteristics of the four MRSA isolates and their derivatives are summarized in Table 1. They were resistant to benzyl penicillin, metillin (MIC 16 mg l⁻¹), high-level mupirocin (MIC >1024 mg l⁻¹), tetracycline (MIC 64 mg l⁻¹), fusidic acid (MIC 24 mg l⁻¹), kanamycin, streptomycin and cadmium acetate, and susceptible to gentamicin, erythromycin, clindamycin, trimethoprim, ciprofloxacin, linezolid, vancomycin, teicoplanin, ethidium bromide and propamidine isethionate. They were positive for mupA, tetK and far1 encoding resistance to high-level mupirocin, tetracycline and fusidic acid, respectively. They were also positive for the PVL, agr3 and cap8 genes.

Plasmid analysis showed that the four isolates had three plasmid patterns (Fig. 1, lanes 1, 4 and 8). Isolate K6482 contained two plasmids (28 and 26 kb), K6531 and K6533 each contained three plasmids (28, 21 and 4 kb) and K6552 contained two plasmids (~41 and 4 kb). This suggested that the isolates were different. However, when the same four isolates were analysed by PFGE following SmaI digestion of their genomic DNA, the results showed that they had identical PFGE patterns (Fig. 2), suggesting that they were of the same clone. To resolve this apparent discrepancy between the results of plasmid analysis and PFGE typing, PFGE was repeated using EagI restriction enzyme to digest the genomic DNA. The result also yielded identical patterns, suggesting that they had a common origin (Fig. 2). All four isolates also had the same coagulase gene type (type 288), and contained type IV SCCmec genetic element (SCCmec IV). MLST of the four isolates...
also yielded identical results: they all belonged to ST80 (allelic profile 1-3-14-11-51-10), which confirmed them as CA-MRSA.

Genetic location of resistance determinants

In order to determine the genetic location of the high-level mupirocin resistance in these isolates, three of the isolates, K6482, K6531 and K6552, representing the three plasmid patterns, were selected and used in curing and transfer experiments.

Table 1. Properties of S. aureus isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Description</th>
<th>Resistant to:</th>
<th>Plasmid size (kb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>XU21</td>
<td>Recipient for conjugation</td>
<td>Nv, Rf</td>
<td>None</td>
<td>Udo &amp; Jacob (1998)</td>
</tr>
<tr>
<td>WBG1876</td>
<td>Recipient for MCT</td>
<td>Fa, Rf</td>
<td>None</td>
<td>Udo et al. (1994)</td>
</tr>
<tr>
<td>WBG4883</td>
<td>WBG541 containing pWBG636</td>
<td>Fa, Gm, Rf</td>
<td>39.2, 0</td>
<td>Udo &amp; Grubb (1990)</td>
</tr>
<tr>
<td>K6482</td>
<td>Clinical isolate obtained from axilla</td>
<td>Cd, Fa, Km, MupH, Sm, Tc</td>
<td>28.0, 26.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU796</td>
<td>K6482 cured of Cd, Tc and Fa resistance</td>
<td>Km, MupH, Sm</td>
<td>26.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU797</td>
<td>K6482 cured of MupH</td>
<td>Cd, Fa, Km, Tc, Sm</td>
<td>28.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU801</td>
<td>K6482 containing pWBG636</td>
<td>Cd, Fa, Gm, Km, MupH, Tc</td>
<td>39.2, 28.0, 26.0</td>
<td>This study</td>
</tr>
<tr>
<td>K6531</td>
<td>Clinical isolate obtained from wound</td>
<td>Cd, Fa, Km, MupH, Sm, Tc</td>
<td>28.0, 21.0, 4.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU798</td>
<td>K6531 cured of Cd, Tc and Fa resistance</td>
<td>Km, MupH, Sm</td>
<td>21.0, 4.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU799</td>
<td>K6531 cured of MupH</td>
<td>Cd, Fa, Km, Sm, Tc</td>
<td>28.0, 4.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU805</td>
<td>K6531 containing pWBG636</td>
<td>Cd, Fa, Gm, Km, MupH, Sm, Tc</td>
<td>39.2, 28.0, 21.0, 4.0</td>
<td>This study</td>
</tr>
<tr>
<td>K6533</td>
<td>Clinical isolate from ear</td>
<td>Cd, Fa, Km, MupH, Sm, Tc</td>
<td>28.0, 21.0, 4.0</td>
<td>This study</td>
</tr>
<tr>
<td>K6552</td>
<td>Clinical isolate from forehead</td>
<td>Cd, Fa, Km, MupH, Sm, Tc</td>
<td>44.0, 4.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU800</td>
<td>K6552 cured of MupH, Tc, Cd and Fa resistance</td>
<td>Km, Sm</td>
<td>4.0</td>
<td>This study</td>
</tr>
<tr>
<td>XU811</td>
<td>K6552 containing pWBG636</td>
<td>Cd, Fa, Gm, Km, MupH, Sm, Tc</td>
<td>39.2, 44.0, 4.0</td>
<td>This study</td>
</tr>
</tbody>
</table>

Cd, Cadmium acetate; Fa, fusidic acid; Gm, gentamicin; Km, kanamycin; MupH, high-level mupirocin resistance; Nv, novobiocin; Rf, rifampicin; Sm, streptomycin; Tc, tetracycline.

Fig. 1. Plasmids of CA-MRSA isolates and their derivatives. Two gels are shown. Lanes: M, molecular mass markers (band sizes are shown in kb – only the covalently closed circular forms are labelled); 1, K6482 clinical isolate containing 28 and 26 kb plasmids; 2, XU796 (K6482 cured of resistance to tetracycline, fusidic acid and cadmium acetate, and missing the 28 kb plasmid); 3, XU802 (mupirocin-resistant transconjugant containing the 26 kb mupirocin-resistance plasmid pXU20); 4, K6531 containing 28, 21 and 4.0 kb plasmids; 5, XU798 (K6531 cured of resistance to tetracycline, fusidic acid and cadmium acetate, and missing the 28 kb plasmid); 6, XU810 (mupirocin-resistant transconjugant following mobilization by pWBG636, carrying the 21 and 4.0 kb plasmids); 7, XU823 (mupirocin-resistant transconjugant following MCT, carrying the 21 kb mupirocin-resistance plasmid pXU21); 8, K6552 containing 44.0 and 4.0 kb plasmids; 9, XU812 (mupirocin-resistant transconjugant carrying the 21 and 4.0 kb plasmids following mobilization by pWBG636); 10, XU824 (mupirocin-resistant transconjugant following MCT, carrying the 21 kb plasmid pXU23).

Fig. 2. PFGE of SmaI- and Eagl-digested CA-MRSA isolates. Lanes: 1 and 6, molecular mass marker (lambda DNA concatemer); 2, K6482; 3, K6531; 4, K6552; 5, K6552.
Curing experiments resulted in the loss of high-level mupirocin resistance in all three isolates. Its loss was accompanied by loss of the 26 kb plasmid in K6482, and by loss of the 21 kb plasmid in K6531 (Table 1). High-level mupirocin resistance was lost together with resistance to cadmium acetate, tetracycline and fusidic acid with the loss of the ~41 kb plasmid in K6552 (Table 1). Resistance to tetracycline, fusidic acid and cadmium acetate were lost together with the loss of the 28 kb plasmid in K6482 and K6531 (Table 1; Fig. 1, lanes 2 and 5). These results suggested that resistance to cadmium acetate, tetracycline, fusidic acid and mupirocin were located on plasmids.

In order to isolate and characterize the resistance plasmids, the three isolates K6482, K6531 and K6552 were used as donors in MCT and conjugation experiments. None of the plasmids was transferred in these experiments. Further attempts to transfer and isolate the plasmids involved the use of the conjugative plasmid pWBG636 (Udo & Grubb, 1990) in mobilization experiments. For the mobilization experiments, derivatives of K6482, K6531 and K6552 carrying pWBG636 (XU801, XU805 and XU811, respectively) were conjugated with the recipient strain XU21, with selections made for the transfer of resistance to gentamicin (carried by pWBG636), mupirocin, tetracycline, fusidic acid and cadmium acetate. The results are summarized in Table 2. The mobilization experiments successfully transferred high-level mupirocin resistance together with the 26 kb plasmid (designated pXU20) from XU801 (Fig. 1, lane 3). The transfer of resistance to cadmium acetate, tetracycline and fusidic acid from strain XU801 was accompanied by the transfer of the 28 kb plasmid, designated pXU22 (designated strain XU803).

The transfer of high-level mupirocin resistance from XU805 (derived from K6531) was accompanied by the transfer of the 21 and 4.0 kb plasmids (Fig. 1, lane 6). One of these colonies was designated XU810. The transfer of resistance to cadmium acetate, tetracycline and fusidic acid from XU805 was accompanied by the transfer of a 28 kb plasmid. The transfer of high-level mupirocin resistance from XU811 resulted in the transfer of a 21 kb plasmid, which was not present in the parental isolate (K6552), and a 4.0 kb plasmid (Fig. 1, lane 9). One of these colonies was designated XU812.

Because strains XU810 and XU812 carried two plasmids (21 and 4.0 kb; Fig. 1, lanes 6 and 9), but expressed only high-level mupirocin resistance in addition to the chromosomal resistance of XU21, attempts were made to isolate their individual plasmids and determine their resistance phenotypes. Consequently, strains XU810 and XU812 were used as donors in MCT experiments with strain WBG1876 as the recipient. Mupirocin-resistant transipients carrying only a 21 kb plasmid were obtained in both experiments (Table 2; Fig. 1, lanes 7 and 10). This confirmed the location of high-level mupirocin resistance on the 21 kb plasmid. The 21 kb plasmid transferred from XU810 was designated pXU21, whilst that transferred from XU812 was designated pXU23. As no resistance phenotype was associated with the 4.0 kb plasmid, it was considered a cryptic plasmid.

### Characterization of the high-level mupirocin resistance plasmids

The presence of mupA on plasmids pXU20 (26.0 kb), pXU21 (21.0 kb) and pXU23 (21.0 kb) was confirmed by using them as templates in PCR assays for the detection of mupA. They all yielded the expected 1.5 kb amplified product for mupA. The three plasmids were then compared by EcoRI restriction analysis. The results presented in Fig. 3(a) showed that they are closely related. Plasmids pXU21 and pXU23 had identical EcoRI restriction patterns and differed from pXU20 in that pXU20 had a 12.0 kb fragment that was missing in pXU21 and pXU23. Also, plasmids pXU21 and pXU23 both contained a 6.6 kb fragment that was missing in pXU20. To confirm the location of mupA on the three plasmids, they were digested with EcoRI and used in a Southern blot hybridization experiment with a labelled mupA probe. The results presented in Fig. 3(b) showed that mupA was located on the 12.0 kb EcoRI fragment of pXU20 and on the 6.6 kb fragment of plasmids pXU21 and pXU23.

### Table 2. Transfer of high-level mupirocin-resistance in CA-MRSA

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Mode of transfer</th>
<th>Selection</th>
<th>Resistance transferred</th>
<th>Size of plasmid transferred (kb) [designation]</th>
</tr>
</thead>
<tbody>
<tr>
<td>XU801</td>
<td>XU21</td>
<td>Mobilization</td>
<td>MupH, Nv, Rf</td>
<td>MupH</td>
<td>26.0 [pXU20]</td>
</tr>
<tr>
<td>XU801</td>
<td>XU21</td>
<td>Mobilization</td>
<td>Nv, Rf, Tc</td>
<td>Cd, Tc, Fa</td>
<td>28.0 [pXU22]</td>
</tr>
<tr>
<td>XU801</td>
<td>XU21</td>
<td>Mobilization</td>
<td>Cd, Nv, Rf</td>
<td>Cd, Tc, Fa</td>
<td>28.0 [pXU22]</td>
</tr>
<tr>
<td>XU805</td>
<td>XU21</td>
<td>Mobilization</td>
<td>MupH, Nv, Rf</td>
<td>MupH</td>
<td>21.0, 4.0</td>
</tr>
<tr>
<td>XU805</td>
<td>XU21</td>
<td>Mobilization</td>
<td>Nv, Rf, Tc</td>
<td>Cd, Tc, Fa</td>
<td>28.0</td>
</tr>
<tr>
<td>XU805</td>
<td>XU21</td>
<td>Mobilization</td>
<td>Cd, Nv, Rf</td>
<td>Cd, Tc, Fa</td>
<td>28.0</td>
</tr>
<tr>
<td>XU811</td>
<td>XU21</td>
<td>Mobilization</td>
<td>MupH, Nv, Rf</td>
<td>MupH</td>
<td>21.0, 4.0</td>
</tr>
<tr>
<td>XU810</td>
<td>WBG1876</td>
<td>MCT</td>
<td>Fa, MupH</td>
<td>MupH</td>
<td>21.0 [pXU21]</td>
</tr>
<tr>
<td>XU812</td>
<td>WBG1876</td>
<td>MCT</td>
<td>Fa, MupH</td>
<td>MupH</td>
<td>21.0 [pXU23]</td>
</tr>
</tbody>
</table>

Cd, Cadmium acetate; Fa, fusidic acid; MupH, high-level mupirocin resistance; Nv, novobiocin; Rf, rifampicin; Tc, tetracycline.
High-level mupirocin resistance in ST80 CA-MRSA

To ascertain the relationship of pXU23 to the ~41 kb plasmid in K6552, pXU23 and a plasmid preparation of K6552 containing the ~41 kb and 4.0 kb plasmid were compared using EcoRI restriction analysis. Plasmid pXU23 shared common 9.8 and 4.6 kb fragments with the ~41 kb plasmid (not shown).

Our results demonstrated the carriage of plasmid-mediated, high-level mupirocin resistance in the ST80 clone of CA-MRSA isolated from different sites in a diabetic patient in a Kuwait hospital. Curing and transfer experiments demonstrated that our ST80 CA-MRSA isolates carried high-level mupirocin resistance located on closely related 26 and 21 kb non-conjugative plasmids, whilst resistance to cadmium acetate, tetracycline and fusidic acid were located on a 28 kb plasmid in two of the isolates. They all had chromosomal resistance to penicillin, meticillin, kanamycin and streptomycin. Although high-level mupirocin resistance has been reported in CA-MRSA obtained in Western Australia (Udo et al., 1994), ST146-IV MRSA clone (Pérez-Roth et al., 2006) and in the ST8 clone of USA300 (Diep et al., 2006; Tenover et al., 2006; Simor et al., 2007), this is believed to be its first report in the ST80 CA-MRSA clone. Such mupirocin resistance had been reported among HA-MRSA isolated in Kuwait hospitals (Udo & Jacob, 1998; Udo et al., 2001), but to the best of our knowledge this is the first report of high-level mupirocin resistance in CA-MRSA isolated in a Kuwait hospital. Its emergence in the ST80-SCCmec IV clone further demonstrates the ability of CA-MRSA to acquire multiple antibiotic-resistance properties.

The ST80-SCCmec IV isolates obtained in this study belonged to agr group 3 and had type 8 capsular polysaccharide. They had antibiotic-resistance profiles, including farI-mediated fusidic acid and tetK-mediated tetracycline resistance, that were similar to ST80-SCCmec IV CA-MRSA that have been isolated in some European countries (Denis et al., 2005; Witte et al., 2005) and recently in some Kuwait hospitals (Udo et al., 2008). As the ST80-SCCmec IV clone has been isolated in Kuwait hospitals (Udo et al., 2008), it is possible that one of these strains had acquired high-level mupirocin resistance. Alternatively, the patient could have acquired the mupirocin-resistant CA-MRSA clone from abroad. Apart from the fact that the patient was already colonized prior to presenting for admission shown by virtue of the isolation of the clone from a pre-admission screening sample, we could not establish how she acquired the organism. It is possible that she had been treated at another hospital in the country before presenting for the admission reported here. However, we could not obtain any history of overseas travel or treatment with mupirocin at any other hospital in the country.

Furthermore, the 21 and 26 kb mupirocin-resistance plasmids in the present ST80 CA-MRSA isolates were non-conjugative plasmids, and were smaller than the 32 and 38 kb conjugative mupirocin-resistance plasmids that were reported previously in HA-MRSA in Kuwait hospitals (Udo & Jacob, 1998; Udo et al., 2001). They also differed from the conjugative plasmid pSA03, which encodes high-level mupirocin resistance and resistance to macrolides, lincosamides and streptogramin in the ST8 CA-MRSA USA300 clone (Diep et al., 2006; Tenover et al., 2006). Therefore, they represent the acquisition of a new type of high-level mupirocin resistance plasmid in ST80 CA-MRSA isolates. As the ST80-SCCmec IV clone has already acquired resistance to kanamycin, tetracycline and fusidic acid (Witte et al., 2005), the acquisition of high-level mupirocin and cadmium resistance as shown in this report further increase its resistance repertoire.

Genetic studies with K6552 yielded interesting results. The loss of the ~41 kb plasmid together with resistance to mupirocin, cadmium acetate, tetracycline and fusidic acid in curing experiments suggested that it encoded resistance to these agents. However, mobilization and MCT experiments isolated a 21 kb mupirocin-resistance plasmid, pXU23, which was not detected in the parent but shared two EcoRI fragments (9.8 and 4.6 kb) with the ~41 kb plasmid in K6552. The similarity of the two EcoRI fragments between pXU23 and the ~41 kb plasmid suggests that pXU23 was derived from the ~41 kb plasmid during the transfer process, raising the possibility that the ~41 kb plasmid could have been formed by the union of a mupirocin-resistance plasmid and a plasmid encoding resistance to tetracycline, fusidic acid and cadmium acetate similar to the separate plasmids in K6531 and K6533 (Fig. 1, lane 4). Plasmid reassortment aided by transposable elements may explain the differences in the carriage of different-sized plasmids in genetically identical S. aureus isolates obtained from this patient.

Fig. 3. Southern blot analysis of mupirocin-resistance plasmids. (a) EcoRI restriction analysis of the mupirocin-resistance plasmids. Lanes: 1, lambda DNA digested with HindIII as a molecular mass marker; 2, pXU20 (12.0, 9.8 and 4.6 kb fragments); 3, pXU21 (9.8, 6.6 and 4.6 kb); 4, pXU23 (9.8, 6.6 and 4.6 kb fragments). (b) Localization of mupirocin resistance following the hybridization of the plasmids in (a) with a mupA-specific probe.

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The presence of different plasmid profiles in genetically identical isolates caused an interpretation dilemma from the molecular typing perspective. Whereas the results of typing using PFGE, the coagulase gene, SCCmec and MLST showed that the four isolates were identical, suggesting that the patient was colonized by the same strain, their different plasmid patterns suggested that they were different strains and that the different sites acquired them independently. It is also possible that one site was initially colonized followed by transmission to the other sites. The different plasmid patterns could then have evolved in vivo, similar to the events observed during the transfer of mupirocin resistance in Kf552. These questions are difficult to answer as it is not known how long the patient had been colonized before her presentation to the hospital. Nevertheless, this observation highlighted the weakness of using plasmid analysis as the sole method for strain typing and supports the use of a combination of typing methods to type MRSA strains.

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