ANTIMICROBIAL RESISTANCE

Genetic analysis of methicillin-resistant *Staphylococcus aureus* expressing high- and low-level mupirocin resistance

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Clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA) expressing high- and low-level mupirocin resistance were studied to determine the genetic location of mupirocin and other resistance determinants. Mupirocin resistance was confirmed by MIC determination with E-test strips. Curing and transfer experiments were used to establish the genetic location of the resistance determinants and the PCR with *mupA*-specific primers was used to detect the presence of *mupA* genes. High-level mupirocin-resistant isolates had MICs >1024 mg/L, whereas the low-level resistant isolates had MICs of 32–128 mg/L. The isolates carried plasmids ranging from 2.8 to 38 kb in size. All of them carried 26- and 3.0-kb plasmids, but only the high-level mupirocin-resistant isolates carried a 38-kb plasmid. Curing and transfer experiments revealed that the 26-kb plasmid encoded resistance to cadmium, mercuric chloride, propamidine isethionate and ethidium bromide and the 38-kb plasmid was a conjugative plasmid encoding high-level mupirocin resistance. One isolate, IBN287, carried both plasmid-borne high-level and chromosomal low-level mupirocin resistance. The *mupA* gene was detected on the 38-kb plasmid DNA but not in the genomic DNA of the low-level mupirocin-resistant isolates. The genomic DNA of strain IBN287 cured of the 38-kb mupirocin resistance plasmid did not contain *mupA*. The results suggest that different genes encoded low- and high-level mupirocin resistance in these isolates.

Introduction

Mupirocin resistance in *Staphylococcus aureus* is increasing with increasing use of the antibiotic to control the spread of methicillin-resistant *S. aureus* (MRSA) [1–5]. Two types of mupirocin resistance have been reported to date; a clinically significant high-level resistance (MIC >512 mg/L) and a low-level resistance (MIC 8–256 mg/L) with uncertain clinical significance [2]. High-level mupirocin resistance determinants have been reported to be carried on plasmids that vary in size, restriction patterns and their ability to be transferred in conjugation experiments [6–10]. On the other hand, the determinants for low-level mupirocin resistance are located on the bacterial chromosome [11]. High-level mupirocin resistance is mediated by a *mupA* gene [12–14] that was previously reported exclusively on mupirocin resistance plasmids [5–11, 15]. However, the *mupA* gene has also been

reported in the genomic DNA of a few *S. aureus* isolates expressing low-level mupirocin resistance, suggesting that the *mupA* gene may also be located in the chromosome [16, 17]. At present it is not clear whether the chromosomal *mupA* determinant is widespread and warrants further investigations in staphylococci from different geographical areas.

Mupirocin was introduced for clinical use at the Burns Unit, Ibn Sina Hospital, Kuwait in 1992 but resistance was not detected among *S. aureus* isolated in the unit until Nov. 1996. However, plasmid-borne high-level mupirocin resistance was detected in an isolate of *S. haemolyticus* isolated in the unit in 1995 [15]. From Nov. 1996, MRSA expressing low- and high-level mupirocin resistance were isolated from the Burns Unit. Pulsed-field gel electrophoresis (PFGE) of the mupirocin-resistant and mupirocin-susceptible MRSA from patients revealed that the mupirocin-resistant MRSA had identical or similar pulsed-field patterns to the mupirocin-susceptible MRSA isolated in the unit before the detection of mupirocin resistance. This suggested that a clone of MRSA already present in the Burns Unit had acquired the mupirocin resistance

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plasmid which later spread among MRSA isolates from different patients [5]. Following the initial detection of mupirocin resistance, all MRSA isolates from patients in the Burns Unit were screened for mupirocin resistance as part of a nationwide surveillance of MRSA for mupirocin resistance conducted from 1 May to 31 Oct. 1999. The results of susceptibility testing detected mupirocin resistance in 26% of these MRSA isolates and their pulsed-field patterns were different from those isolated in 1996–1997 [18], indicating that a new MRSA clone had been introduced into the Burns Unit. This study sought to determine the genetic location of antibiotic resistance determinants in the mupirocin-resistant MRSA and also to investigate whether the *mupA* gene conferred mupirocin resistance in low- and high-level mupirocin-resistant MRSA isolates from the Burns Unit in Kuwait.

Materials and methods

MRSA isolates

The properties of the isolates studied are presented in Table 1. A total of 72 MRSA isolates was obtained from patients in the Burns Unit of Ibn Sina Hospital Kuwait, as part of MRSA surveillance between May and Oct. 1999. They consisted of 36 isolates (50%) that expressed low-level mupirocin resistance and 19 isolates (26%) that expressed high-level mupirocin resistance. The isolates selected for this study were single patient isolates and were representatives of the common pulsed-field patterns.

Susceptibility to antimicrobial agents

Susceptibility to antimicrobial agents was tested by the disk diffusion method on Mueller Hinton agar with commercial antibiotic disks (Oxoid) as described previously [5]. Susceptibility to mupirocin was determined with disks containing 200 µg and 5 µg of mupirocin. Growth to the edge of the 200-µg mupirocin disk indicated high-level resistance while growth within a 14-mm zone of inhibition with the 5-µg mupirocin

disk indicated low-level resistance. The MIC of mupirocin was determined with E-test strips (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. Susceptibility to heavy metals and nucleic acid-binding compounds was tested with 6-mm disks impregnated with cadmium acetate (130 µg), propamidine isethionate (50 µg) and ethidium bromide (60 µg). *S. aureus* strain ATCC 25923 was used as a control strain. Mupirocin powder was a gift from SmithKline Beecham, Worthing.

β-lactamase production

β-Lactamase production was detected with a chromogenic cephalosporin (nitrocefin; Oxoid) according to the manufacturer's instructions.

Plasmid isolation and analysis

Plasmids were isolated by the cetyltrimethyl ammonium bromide method, separated by agarose gel electrophoresis and sized as described previously [5].

Curing of resistance and plasmids

Loss of resistance and plasmids was investigated by growing the organisms at 43°C overnight and screening single colonies for the loss of resistance by a replica plating method. Colonies that lost antimicrobial resistance were screened for plasmid loss by agarose gel electrophoresis.

Transfer of plasmid and resistance

Plasmids were transferred in mixed-culture transfer (MCT) and conjugation experiments as described previously [5, 10].

PCR

PCR was performed with previously published primers [16]: Mup1 (5'-CCC ATG GCT TAC CAG TTG A-3') and Mup2 (5'-CCA TGG AGC ACT ATC CGA A-3').

Table 1. Properties of *S. aureus* isolates

Isolate no.	Relevant properties	Plasmids	Source/reference
WBG248	RN450	None	10
WBG541	Fa ^R Rf ^R , mutant of WBG248	None	10
XU21	Nv ^R Rf ^R , mutant of RN4220	None	10
WBG1878	Fa ^R Rf ^R , ΦJ lysogen of WBG541	None	19
IBN238	mupL, Cm ^R , Cd ^R , Hg ^R , Pi ^R , Eb ^R	26, 4.4, 3.0, 2.8	This paper
IBN246	mupH, Cm ^R , Cd ^R , Hg ^R , Pi ^R Eb ^R	38, 26, 4.4, 3.0, 2.8	This paper
IBN252	mupL, Cm ^R , Cd ^R , Hg ^R , Pi ^R , Eb ^R	26, 4.4, 3.0, 2.8	This paper
IBN259	mupH, Cm ^S , Cd ^R , Hg ^R , Pi ^R , Eb ^R	38, 26, 3.0, 2.8	This paper
IBN260	mupH, Cm ^R , Cd ^R , Hg ^R , Pi ^R , Eb ^R	38, 26, 4.4, 3.0, 2.8	This paper
IBN266	mupH, Cm ^R , Cd ^R , Hg ^R , Pi ^R , Eb ^R	38, 26, 4.4, 3.0, 2.8	This paper
IBN267	mupL, Cm ^R Cd ^R , Hg ^R Pi ^R , Eb ^R	26, 4.4, 3.0, 2.8	This paper
IBN269	mupL, Cm ^S , Cd ^R , Hg ^R , Pi ^R , Eb ^R	26, 3.0, 2.8	This paper
IBN271	mupL, Cm ^S , Cd ^R , Hg ^R , Pi ^R , Eb ^R	26, 3.0, 2.8	This paper
IBN287	mupH, CmR, Cd ^R , Hg ^R , Pi ^R , Eb ^R	38, 26, 4.4, 3.0, 2.8	This paper
XU320	IBN246 cured of mupH	26, 4.4, 3.0, 2.8	This paper
XU322	mupL, IBN287 cured of mupH	26, 4.4, 3.0, 2.8	This paper
XU305	IBN252 cured of Cd ^R , Hg ^R , Pi ^R , Eb ^R	4.4, 2.8	This paper

The primers were purchased from Gibco BRL Custom primers (Life Technologies, UK). Amplification was performed with a kit from Gibco BRL. The amplification mixture consisted of 45 μ l of Supermix (22 mM Tris-HCl, pH 8.4; 55 mM KCl, 1.65 mM MgCl₂, 220 M dGTP, 220 M dATP, 220 M dCTP, recombinant Taq DNA polymerase 22 U/ml), bacterial DNA 3 μ l, primer solution 2 μ l in a total volume 50 μ l. A Perkin Elmer 9600 Thermocycler (Perkin Elmer, Cetus Norwalk, CT, USA) was programmed for 32 cycles with the following parameters: denaturation at 94°C for 3 min, annealing at 60°C for 45 s, extension at 72°C for 1 min and final extension at 72°C for 2 min. Amplified products were examined by agarose gel electrophoresis with agarose 1.5% w/v in 1 $\times$ TAE buffer for 2 h at 70 V.

Results

The 10 isolates investigated in this study were representatives of 55 mupirocin-resistant MRSA isolated between May and Oct. 1999 and consisted of five high-level and five low-level mupirocin-resistant isolates. The high-level resistant isolates had MIC values >1024 mg/L and belonged to two related pulsed-field patterns. The mupirocin MICs for the low-level resistant isolates ranged from 32 to 128 mg/L and these isolates belonged to three closely related pulsed-field patterns (not shown). All produced β -lactamase and were resistant to penicillin G, gentamicin, kanamycin, tobramycin, ciprofloxacin, tetracycline, cadmium, mercuric chloride, propamidine isethionate and ethidium bromide, but differed in their resistance to erythromycin, chloramphenicol and fusidic acid, as shown in Table 1. They harboured three-to-five plasmids per cell with sizes ranging from 2.8 kb to 38 kb (Table 1). All carried a 26-kb and a 3.0-kb plasmid but differed in the carriage of the 38-, 4.4- and 2.8-kb plasmids (Fig. 1). The 38-kb plasmid was present only in the high-level mupirocin-resistant isolates.

Location of resistance determinants

To isolate the plasmids and determine their resistance phenotypes, the high-level mupirocin-resistant isolates IBN246, IBN259, IBN260 and IBN287 and the low-level mupirocin-resistant isolates, IBN252, IBN267 and IBN271, were selected and used in curing and transfer experiments. In curing experiments, resistance to cadmium, mercuric chloride, propamidine isethionate and ethidium bromide were lost together from all the isolates whether they expressed low- or high-level mupirocin resistance and their loss was accompanied by the loss of a 26-kb plasmid in the colonies screened. Mupirocin resistance was lost from the high-level resistant isolates, IBN246 and IBN287; high-level mupirocin resistance was lost together with the 38-kb plasmid in both isolates. A colony of strain IBN246

Fig. 1. Plasmid contents of representative mupirocin-resistant MRSA. Lanes 1–3, MRSA isolates expressing low-level mupirocin resistance, they lack the 38-kb plasmid; 4–6, MRSA isolates expressing high-level mupirocin resistance, they carry the 38-kb plasmid.

cured of the 38-kb plasmid and mupirocin resistance had a mupirocin MIC of 2 mg/L and was designated XU320. The loss of the 38-kb plasmid in strain IBN287 resulted in colonies that still expressed low-level mupirocin resistance. One such colony, designated XU322, had a mupirocin MIC of 64 mg/L.

Transfer of resistance determinants

Each of the 10 isolates was used as a donor in separate conjugation and MCT experiments to transfer the plasmids to recipient strains and confirm the plasmid location of the cured resistance determinants. Results of the transfer experiments are summarised in Table 2. Chloramphenicol and high-level mupirocin resistance were transferred in both conjugation and MCT experiments. In the conjugation experiments, transconjugants were obtained on mupirocin and chloramphenicol selection plates. Those obtained on mupirocin selection plates were either resistant to mupirocin or to mupirocin and chloramphenicol. Colonies resistant to mupirocin carried a 38-kb plasmid or a 38-kb plasmid and a 3.0- or 2.8- kb plasmid. Colonies resistant to mupirocin and chloramphenicol carried a 38- and a 4.4-kb plasmid as shown in Table 2 and Fig. 2. Transconjugants from the chloramphenicol selection plates were also either resistant to chloramphenicol or to chloramphenicol and mupirocin, with plasmid profiles similar to those found in transconjugants from mupirocin selection plates.

Resistance to mupirocin, chloramphenicol, cadmium, mercuric chloride, propamidine isethionate and ethidium bromide was transferred in MCT experiments. Results of MCT for mupirocin and chloramphenicol

Table 2. Transfer of resistance determinants from high- and low-level mupirocin-resistant MRSA

Isolate no.	Mupirocin resistance	Mode of transfer	Resistance transferred	Plasmid transferred (kb)
IBN246	High-level	MCT	Mup, Cm	38, 4.4
		MCT	Cm	4.4
		MCT	Cd, Hg, Pi, Eb	26
IBN246	High-level	C	Mup, Cm	38, 4.4, 2.8
		C	Mup, Cm	38, 4.4
		C	Mup	38
		C	Cm	4.4
IBN252	Low-level	MCT	Cd, Hg, Pi, Eb	26
IBN259	High-level	MCT	Mup	38
		MCT	Cd, Hg, Pi, Eb	26
IBN259	High-level	C	Mup	38
IBN260	High-level	MCT	Mup, Cm	38, 4.4
		MCT	Mup	38
		MCT	Cm	4.4
		MCT	Cd, Hg, Pi, Eb	26
IBN260	High-level	C	Mup, Cm	38, 4.4
		C	Mup	38
		C	Cm	4.4
IBN267	Low-level	MCT	Cd, Hg, Pi, Eb	26
		MCT	Cm	4.4
IBN269	Low-level	MCT	Cd, Hg, Pi, Eb	26
IBN287	High-level	MCT	Mup, Cm	38, 4.4
		MCT	Mup	38
		MCT	Cm	4.4
		MCT	Cd, Hg, Pi, Eb	26
IBN287	High-level	C	Mup, Cm	38, 4.4
		C	Mup	38
		C	Cm	4.4

C, conjugation; Cm, chloramphenicol; Cd, cadmium; Eb, ethidium bromide; Hg, mercuric chloride; Mup, mupirocin; MCT, mixed-culture transfer; Pi, propamidine isethionate.

Fig. 2. Plasmids transferred in conjugation and MCT experiments. Lane **A**, isolate IBN246; **B**, a transconjugant of isolate IBN246 resistant to mupirocin and chloramphenicol carrying both 38-kb and 4.4-kb plasmids; **C** and **D**, mupirocin-resistant transconjugants of isolate IBN246 carrying only the 38-kb plasmid; **E**, a mupirocin- and chloramphenicol-resistant transconjugant carrying 38- and 4.4-kb plasmids in addition to a 2.8-kb cryptic plasmid; **G–K**, transciipients carrying the 26-kb plasmid encoding resistance to cadmium, mercuric chloride, propamidine isethionate and ethidium bromide; **L**, *S. aureus* strain WBG4483 containing plasmid molecular size markers.

resistance were similar to those obtained in conjugation experiments (Table 2). The transfer of resistance to cadmium, mercuric chloride, propamidine isethionate and ethidium bromide was accompanied by the transfer of the 26-kb plasmid present in the donors (Fig. 2).

PCR amplification of *mupA* DNA

The 38-kb plasmids encoding high-level mupirocin resistance and genomic DNA from the low-level mupirocin-resistant isolates were used in PCR experiments to amplify the *mupA* DNA. Genomic DNA from

isolates IBN287, XU320 and XU322 (IBN246 and IBN287 cured of high-level mupirocin resistance, respectively) were also tested. The results are summarised in Table 3, which also compares the mupirocin MICs and the genetic location of the mupirocin resistance determinant for each of the isolates. A PCR product of 1.6-kb corresponding to the *mupA* gene was detected in all the 38-kb plasmids and from genomic DNA of isolate IBN287, but not from the low-level mupirocin-resistant isolates and XU320 (the mupirocin-susceptible derivative of isolate IBN246). Similarly, no amplified product was obtained with the genomic DNA of XU322, the low-level resistant derivative of isolate IBN287. Fig. 3 shows the electrophoretic pattern of the PCR products.

Discussion

This study investigated the genetic basis of antimicrobial resistance determinants in low- and high-level mupirocin-resistant MRSA isolated in a Burns Unit. The isolates were more resistant to ciprofloxacin and mupirocin and the majority had PFGE patterns that were different from MRSA isolated in the same unit in 1996–1997 [18], suggesting the introduction of a new MRSA clone. However, they had resistance and plasmid profiles similar to the 1996–1997 isolates. This is not surprising, as unrelated strains can harbour plasmids with the same resistance phenotype. Curing and transfer experiments demonstrated that the 26-kb plasmid in these isolates coded for resistance to cadmium, mercuric chloride, propamidine isethionate and ethidium bromide, as shown earlier [5]. Similarly, the 4.4- and 38-kb plasmids in the present isolates coded for chloramphenicol and high-level mupirocin resistance, respectively. The present isolates also carried 3.0- and 2.8-kb cryptic plasmids. These plasmids were considered to be cryptic because they could not be associated with any resistance determinant. Although the 3.0- and 2.8-kb plasmids were co-transferred with the 38-kb plasmid, the strains carrying them were resistant only to mupirocin. Moreover, when the 38-kb plasmid was lost by curing, the resultant

Table 3. Location of *mupA* in mupirocin-resistant MRSA

Isolate no.	Mupirocin MIC (mg/L)	Location of mupirocin resistance	Presence of <i>mupA</i>
IBN238	32	chromosome	—
IBN246	>1024	plasmid (38 kb)	+
IBN252	64	chromosome	—
IBN259	>1024	plasmid (38 kb)	+
IBN260	>1024	plasmid (38 kb)	+
IBN266	>1024	plasmid (38 kb)	+
IBN267	64	chromosome	—
IBN269	128	chromosome	—
IBN271	32	chromosome	—
IBN287	>1024	plasmid (38 kb)	+
XU320	2	—	—
XU322	64	chromosome	—

+, present; —, not detected.

Fig. 3. PCR amplification of the *mupA* gene. Lane A, Φ lambda DNA digested with *Hind*III restriction endonuclease. The sizes in kb are from the top, 23.13, 9.4, 6.6, 4.3, 2.3, 2.0. Lanes B–E contain amplified products from the 38-kb plasmid DNA in: B, isolate IBN246; C, isolate IBN259; D, isolate IBN266; E, isolate IBN287. Lanes F–M, PCR products of genomic DNA from low-level mupirocin-resistant isolates: F, isolate IBN238; G, isolate IBN252; H, isolate IBN267; I, isolate IBN269; J, isolate IBN271; K, XU320; L, XU322; M, WBG248. None of the low-level resistant or susceptible isolates contained the *mupA* gene.

mupirocin-susceptible strains still carrying the small plasmids were resistant only to novobiocin and rifampicin, which are the resistance markers of the recipient strain, XU21. These results indicated that resistance to methicillin, gentamicin, kanamycin, streptomycin, tetracycline, trimethoprim, ciprofloxacin, fusidic acid and mupirocin (low-level only) were located on the bacterial chromosome.

As the 38-kb high-level mupirocin resistance plasmid mediated its own transfer in conjugation experiments it fits the description of a conjugative plasmid. In addition, it co-transferred with the 4.4-kb chloramphenicol resistance plasmid, as well as the 3.0- and 2.8-kb cryptic plasmids, demonstrating its ability to mobilise non-conjugative plasmids. This is a new property of mupirocin resistance conjugative plasmids. Although staphylococcal gentamicin resistance [19, 20] and the cryptic [21, 22] conjugative plasmids have been known to mobilise non-conjugative plasmids, the conjugative mupirocin resistance plasmids have only recently been reported to mobilise non-conjugative plasmids [10, 11, 15]. Therefore, the 38-kb plasmid belongs to a new class of mupirocin resistance conjugative plasmids that are capable of mobilising non-conjugative plasmids. In contrast, the 26-kb plasmids are not conjugative plasmids and were not mobilised by the 38-kb conjugative plasmid. In this regard, the 38-kb mupirocin resistance plasmid shares the feature of mobilising only certain non-conjugative plasmids with other staphylococcal conjugative plasmids [20–22].

The 38-kb conjugative mupirocin resistance plasmid was also transferred in MCT experiments (Table 2), confirming a previous observation that conjugative plasmids can transfer under conditions for both conjugation and MCT (or phage-mediated conjugation) [10]. It has also been observed that non-conjugative plasmids can be transferred by a phage-mediated mechanism in conjugation experiments if the donor contains prophages [10, 23]. Furthermore, the presence of prophages in the donor or recipient can increase the transfer frequencies of conjugative plasmids in conjugation experiments [10, 24]. These observations are in contrast to an earlier suggestion that conditions which favour conjugative transfer of resistance plasmids would inhibit transfer by MCT and *vice versa* [25].

Until recently the *mupA* genes encoding high-level mupirocin resistance was found only on plasmids. Its detection in genomic DNA of isolates expressing low-level mupirocin resistance [16, 17] suggested that high- and low-level mupirocin resistance may be mediated by the same genes, but their expression differed by virtue of their location and copy number. Alternatively, the *mupA* determinant may be carried on a transposable element like the gentamicin resistance transposon Tn4001/Tn3851 that has been found on physically unrelated plasmids and on the bacterial chromosome in staphylococcal isolates [26]. The presence of the determinants for high- and low-level mupirocin resistance in the same isolate (IBN287) further raised the possibility that the *mupA* determinant maybe a transposable element. Leski *et al.* [4] have reported a similar finding in *S. epidermidis*. However, the failure to detect *mupA* genes in the genomic DNA of XU322 (isolate IBN287 cured of high-level resistance plasmid) indicated that the determinants for low-level and high-level mupirocin resistance in this isolate were different. The *mupA* gene was not detected in the genomic DNA of any of the low-level mupirocin-resistant isolates. Similarly, Schmitz *et al.* [27] did not detect the *mupA* gene in low-level mupirocin-resistant isolates that they studied. Therefore more studies are needed to establish whether the observed chromosomal *mupA* [16, 17] is widespread in staphylococci from different geographical regions or whether it is due to the transposition of the *mupA* determinant or the integration of a mupirocin resistance plasmid into the bacterial chromosome in those isolates.

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References

1. Cookson BD. The emergence of mupirocin resistance: a challenge to infection control and antibiotic prescribing practice. *J Antimicrob Chemother* 1998; **41**: 11–18.
2. Vasquez JE, Walker ES, Franzus BW, Overbay BK, Reagan DR, Sarubbi FA. The epidemiology of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* at a Veterans' Affairs hospital. *Infect Control Hosp Epidemiol* 2000; **21**: 459–464.
3. Schmitz F-J, Lindenlauf E, Hoffmann B *et al.* The prevalence of low- and high-level mupirocin resistance in staphylococci from 19 European hospitals. *J Antimicrob Chemother* 1998; **42**: 489–495.
4. Leski TA, Gniadkowski M, Skoczyńska A, Stefaniuk E, Trzciński K, Hryniewicz W. Outbreak of mupirocin-resistant *Staphylococci* in a hospital in Warsaw, Poland, due to plasmid transmission and clonal spread of several strains. *J Clin Microbiol* 1999; **37**: 2781–2788.
5. Udo EE, Farook VS, Mokadas EM, Jacob LE, Sanyal SC. Molecular fingerprinting of mupirocin-resistant methicillin-resistant *Staphylococcus aureus* from a burn unit. *Int J Infect Dis* 1999; **3**: 82–87.
6. Rahman M, Connolly S, Noble WC, Cookson B, Phillips I. Diversity of staphylococci exhibiting high-level resistance to mupirocin. *J Med Microbiol* 1990; **33**: 97–100.
7. Morton TM, Johnston JL, Patterson J, Archer GL. Characterization of a conjugative staphylococcal mupirocin resistance plasmid. *Antimicrob Agents Chemother* 1995; **39**: 1272–1280.
8. Needham C, Rahman M, Dyke KGH, Noble WC. An investigation of plasmids from *Staphylococcus aureus* that mediate resistance to mupirocin and tetracycline. *Microbiology* 1994; **140**: 2577–2583.
9. Udo EE, Pearman JW, Grubb WB. Emergence of mupirocin resistance in methicillin-resistant *Staphylococcus aureus* in Western Australia. *J Hosp Infect* 1994; **26**: 157–165.
10. Udo EE, Jacob LE. Conjugative transfer of high-level mupirocin resistance and the mobilization of non-conjugative plasmids in *Staphylococcus aureus*. *Microb Drug Resist* 1998; **4**: 185–193.
11. Pawa A, Noble WC, Howell SA. Co-transfer of plasmids in association with conjugative transfer of mupirocin or mupirocin and penicillin resistance in methicillin-resistant *Staphylococcus aureus*. *J Med Microbiol* 2000; **49**: 1103–1107.
12. Gilbert J, Perry CR, Slocombe B. High-level mupirocin resistance in *Staphylococcus aureus*: evidence for two distinct isoleucyl-tRNA synthetases. *Antimicrob Agents Chemother* 1993; **37**: 32–38.
13. Dyke KGH, Curnock SP, Golding M, Noble WC. Cloning of the gene conferring resistance to mupirocin in *Staphylococcus aureus*. *FEMS Microbiol Lett* 1991; **77**: 195–198.
14. Hodgson JE, Curnock SP, Dyke KGH, Morris D, Sylvester DR, Gross MS. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. *Antimicrob Agents Chemother* 1994; **38**: 1205–1208.
15. Udo EE, Jacob LE, Mokadas EM. Conjugative transfer of high-level mupirocin resistance from *Staphylococcus haemolyticus* to other *Staphylococci*. *Antimicrob Agents Chemother* 1997; **41**: 693–695.
16. Ramsey MA, Bradley SF, Kauffman CA, Morton TM. Identification of chromosomal location of *mupA* gene encoding low-level mupirocin resistance in *Staphylococcal* isolates. *Antimicrob Agents Chemother* 1996; **40**: 2820–2823.
17. Ramsey MA, Bradley SF, Kauffman CA, Morton TM, Patterson JE, Reagan DR. Characterization of mupirocin-resistant *Staphylococcus aureus* from different geographic areas. *Antimicrob Agents Chemother* 1998; **42**: 1305.
18. Udo EE, Jacob LE, Mathew B. The spread of a mupirocin-resistant MRSA clone in Kuwait hospitals. Program of the First International Congress of Infectious and Tropical Diseases, Muscat, Oman, 2000.
19. Townsend DE, Bolton S, Ashdown N, Grubb WB. Transfer of plasmid-borne aminoglycoside-resistance determinants in staphylococci. *J Med Microbiol* 1985; **20**: 169–185.
20. Projan SJ, Archer GL. Mobilization of the relaxable *Staphylococcus aureus* plasmid pC221 by the conjugative plasmid pGO1 involves three pC221 loci. *J Bacteriol* 1989; **171**: 1841–1845.
21. Udo EE, Grubb WB. Transfer of resistance determinants from a multi-resistant *Staphylococcus aureus* isolate. *J Med Microbiol* 1991; **35**: 72–79.
22. Udo EE, Love H, Grubb WB. Intra- and inter-species mobilisation of non-conjugative plasmids in staphylococci. *J Med Microbiol* 1992; **37**: 180–186.
23. Pereira MSV, Barreto VP, Siqueira-Junior JP. Phage-mediated

- transfer of tetracycline resistance in *Staphylococcus aureus* isolated from cattle in Brazil. *Microbios* 1997; **92**: 147–155.
24. Zueva VS, Nesterenko LN, Dmitrenko OA, Akatov AK. Lysogeny of methicillin-resistant *Staphylococcus aureus* and the role of prophages in transfer of conjugative and non-conjugative plasmids. *J Chemother* 1991; **3**: 279–282.
 25. Townsend DE, Bolton S, Ashdown N, Grubb WB. Comparison of phage-mediated and conjugative transfer of staphylococcal plasmids *in vitro* and *in vivo*. *J Med Microbiol* 1986; **22**: 107–114.
 26. Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol Rev* 1987; **51**: 88–134.
 27. Schmitz FJ, Fluit AC, Lindenlauf E, Scheuring S, Kohrer K. Molecular analyses of possible mechanisms coding for low-level mupirocin resistance in clinical *Staphylococcus aureus* isolates. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 649–650.