Intra- and inter-species mobilisation of non-conjugative plasmids in staphylococci

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Summary. The ability of *Staphylococcus aureus* conjugative plasmids to mobilise non-conjugative resistance plasmids from clinical isolates of *S. aureus* and *S. epidermidis* was studied. Plasmids which could not be transferred by transduction or mixed-culture transfer were transferred from phage-typable and non-typable *S. aureus* and from *S. epidermidis*. Plasmids encoding single resistance determinants were transferred by mobilisation whereas multiple-resistance plasmids were transferred as co-integrates between the conjugative and non-conjugative plasmids. This study demonstrates that mobilisation is a useful tool for the transfer and study of staphylococcal plasmids and illustrates how antibiotic resistance could be transferred between staphylococci *in vivo*.

Introduction

Plasmids are important carriers of resistance genes and have been reported in nearly all species of medically important bacteria. Plasmid-determined antimicrobial resistance in *Staphylococcus aureus* can be transferred between strains *in vitro* by transduction, phage-mediated conjugation, or mixed-culture transfer (MCT) and conjugation.¹ Increasing numbers of recent isolates of *S. aureus* are untypable with the international set of typing phages.^{2–7} Consequently, phage-dependent methods of plasmid transfer cannot be used to transfer plasmids from the non-typable isolates because these methods require that the donor and recipient be lysed or lysogenised by appropriate bacteriophages.⁸

Coagulase-negative staphylococci (CNS) are involved increasingly in infections, especially in the elderly and in immunocompromised patients.^{9–15} They are often multi-resistant to antimicrobial agents and frequently harbour multiple plasmids.^{10, 14, 15} However, plasmids in CNS have not been studied extensively because of the lack of a suitable method for plasmid transfer.

Conjugation, unlike transduction and MCT, appears to be independent of bacteriophages¹ and, therefore, does not depend on phage susceptibility. The majority of conjugative plasmids reported in *S. aureus* and *S. epidermidis* can mobilise and transfer non-conjugative plasmids.^{16–24} However, the ability of conjugative plasmids to mobilise non-conjugative plasmids has not been exploited as a tool for isolating and studying plasmids from staphylococcal isolates.

In this study, the ability of the conjugative plasmid

pWBG637²⁰ to transfer non-conjugative resistance plasmids from *S. aureus* and *S. epidermidis* was investigated. The study was motivated by previous findings that the conjugative plasmids pWBG636 and pWBG642²⁵ could transfer from *S. aureus* to *S. epidermidis* and *Streptococcus faecalis* and back to *S. aureus*.²⁶ The results presented here indicate that conjugative plasmids are a useful tool for transferring plasmids in staphylococci *in vitro* and add further support to the premise that they are important in the transmission of antibiotic resistance *in vivo*.²⁶

Materials and methods

Bacterial strains

The bacterial isolates used in this study are listed in table I. Because plasmid pWBG637 has no selective markers, derivatives of it labelled with transposons were used. These were: pWBG636, a 39.2-kb plasmid labelled with the gentamicin-resistance transposon Tn3851 and pWBG642, a 39.9-kb plasmid labelled with the erythromycin-resistance transposon Tn551.²⁵ Another conjugative plasmid pWBG653 of 44 kb and encoding resistance to gentamicin, kanamycin and neomycin²⁵ was also used. The recipients were plasmid-free strains; strain WBG541 was resistant to fusidic acid and rifampicin²⁷ and strain WBG4515 was resistant to streptomycin and novobiocin.¹⁹ Isolate WBG7407 was provided by Dr B. Cookson, Central Public Health Laboratory, Colindale Avenue, London.

Media

Brain Heart Infusion Agar (BHIA), Brain Heart Infusion Broth, Trypticase Soy Broth and Mueller

Hinton Agar were purchased from Gibco Diagnostics (Madison, WI, USA).

Bacteriophage typing

This was performed at the State Health Laboratory Service of Western Australia with phages of the International Phage Typing Set.

Susceptibility testing

Susceptibility to antimicrobial agents was tested by the disk diffusion and replica plating methods described previously.^{27, 28}

Loss of resistance at 43.5°C

Tests for this were performed as described previously.²²

Isolation and analysis of plasmid DNA

Plasmids were isolated by the cetyltrimethyl-ammonium bromide method.²⁹ Agarose gel electrophoresis (AGE) and mol.-wt determination of plasmid DNA were performed on horizontal gels incorporating agarose (Sigma) 0.5% w/v as described previously.¹⁹ Restriction endonuclease digestion of plasmids was performed according to the manufacturer's instructions and the fragments were separated on agarose 0.8% w/v gels. Phage λ DNA (Pharmacia, Uppsala, Sweden) digested with *Hind*III or *Hind*III and *Eco*RI (Toyobo, Co. Ltd, Osaka, Japan) were used as size standards.

Conjugation

Conjugation experiments were performed in TSB in the presence of polyethylene glycol (PEG) 40% w/v as described previously.^{1, 22} Mobilisation experiments were performed by first transferring one of the conjugative plasmids to the strain to be tested. The resulting transconjugants were then used as donors in a further conjugation experiment with strain WBG541. Transconjugants were selected on BHIA containing (mg/L) fusidic acid (Fa), 10 and rifampicin (Rf), 2.5, and one of the following (see table II): streptomycin (Sm), 100; kanamycin (Km), 75; gentamicin (Gm), 8; erythromycin (Em), 5; tetracycline (Tc), 5; chloramphenicol (Cm), 10; trimethoprim (Tp), 2.5; propamidine isethionate (Pi), 10; ethidium bromide (Eb), 120; or cadmium (Cd), 2×10^{-2} M. Transconjugants were screened for the presence of plasmids by AGE.

Results

Mobilisation of non-conjugative plasmids from S. aureus isolates

A total of 20 *S. aureus* isolates was studied for transfer of resistance plasmids by mobilisation with

the conjugative plasmids pWBG636 and pWBG642. The isolates were resistant to a range of antimicrobial agents and contained at least two plasmids/cell. The resistance and plasmid profiles of representative isolates are shown in table I. They were mostly untypable at 100RTD with phages of the International Typing Set. Only isolates WBG4761, WBG4762, WBG4918, WBG4920, WBG4856 and WBG7407 were susceptible to the International Set of Typing phages.

None of the isolates, except WBG7407, transferred resistance determinants by conjugation before receiving either of the conjugative plasmids, indicating that they did not contain conjugative plasmids. However, resistance was transferred from the isolates after either of the conjugative plasmids pWBG636 or pWBG642 was introduced and the resultant strains were tested for conjugation with the recipient strain, WBG541.

The results of the mobilisation experiments (table II) demonstrated transfer of plasmids ranging from small 3.2–4.4-kb plasmids, encoding single resistances to Tc, Sm or Cm, and 5.2-kb plasmids, encoding resistance to Cm and Sm, to plasmids of 15.0–38.0 kb encoding resistance to multiple antimicrobials.

Plasmid analysis performed on the transconjugants revealed that single resistance plasmids encoding Tc, Sm or Cm resistance were mobilised either with or without co-transfer of the conjugative plasmid. In approximately one third of the transconjugants, only the single resistance plasmid was transferred while the remainder carried both the single resistance plasmid and the conjugative plasmid.

Both plasmids were maintained separately and no evidence of recombination between the single resistance and the conjugative plasmid was apparent. In contrast, the multi-resistance plasmids were either transferred alone or as recombinants with the conjugative plasmids. This is illustrated with the results obtained when plasmids pWBG659 and pWBG700, both encoding resistance to Pc, Cd and arsenate (Asa), were transferred from WBG1004 and WBG4761 respectively with pWBG636 (table II). Two types of transconjugants were obtained with both isolates. One type had plasmids encoding resistance to Pc, Cd, and Asa which gave *Eco*RI restriction fragments identical with those of plasmids pWBG659 and pWBG700 from isolates WBG1004 and WBG4761 respectively (data not shown). The other type of transconjugants also contained a single plasmid (e.g. pWBG635 and pWBG686 from isolates WBG1004 and WBG4761 respectively, table II) and were resistant to Pc, Cd, Asa, Gm and Km. In curing experiments on this type of transconjugant, resistance to all five antimicrobials was lost concomitantly with the plasmids. *Eco*RI restriction enzyme analysis (fig. 1) indicated that plasmids pWBG635 and pWBG686 were recombinants between pWBG659 and pWBG636, and pWBG700 and pWBG636, respectively, and were not formed as a result of transposition of the Tn3851 of pWBG636 to either pWBG659 or pWBG700.

Two 4.4-kb plasmids, one encoding resistance to Sm

Table I. Resistance and plasmid profiles of representative staphylococcal isolates

Isolates nos	Resistance profile	Plasmid sizes (kb)
<i>S. aureus</i>		
WBG1004	Pc, Cd, Asa, Sm	38.8, 4.4
WBG1006	Pc, Cd, Asa, Sm, Tc	36.8, 4.4
WBG4761	Pc, Cd, Asa, Sm, Cm, Tc, Mn	36.3, 5.2
WBG4762	Pc, Cd, Sm, Cm, Tc, Mn	20.8, 5.2, 4.4
WBG4856	Pc, Cd, Tc, Tp, Su, Eb	34.5, 4.4, 2.8, 1.9
WBG4918	Pc, Cd, Tc, Sm, Hg, Pma	23.8, 4.4, 3.8
WBG4920	Pc, Cd, Sm, Cm, Tc, Eb, Su	20.8, 5.2, 4.4
WBG4922	Pc, Cd, Asa, Eb, Sm, Tc, Su	28.4, 18.2, 8.8, 4.4, 3.0
WBG4928	Pc, Cd, Hg, Pma, Tc	22.6, 4.4
WBG4940	Pc, Cd, Sm, Tc, Cm, Su	30.4, 10.7, 5.3, 4.4, 3.5
WBG4945	Pc, Cd, Hg, Pma, Cm	24.0, 4.4, 3.5
WBG4948	Pc, Cd, Tc, Cm	28.5, 4.4, 3.5
WBG7407	Mu, Pc, Cd, Tc	20.2, 4.4
<i>S. epidermidis</i>		
WBG7193	Mc, Asa, Em, Tc, Tp, Su, Eb, Pi	NS
WBG7195	Mc, Pc, Gm, Km, Nm, Sm, Tc, Pi	NS
WBG7196	Pc, Cd, Hg, Asa, Pma, Pi, Su	NS
WBG7200	Pc, Cd, Asa, Eb, Pi, Cm, Gm, Km, Nm, Tp	NS
WBG7216	Mc, Pc, Asa, Km, Em, Tc, Tp, Su, Hg, Pma, Eb, Pi	NS

Asa, arsenate; Cd, cadmium; Cm, chloramphenicol; Eb, ethidium bromide; Em, erythromycin; Gm, gentamicin; Hg, mercury; Km, kanamycin; Mc, methicillin; Mn, minocycline; Mu, mupirocin; Nm, neomycin; Pc, benzyl penicillin; Pi, propamidine isethionate; Pma, phenyl mercuric acetate; Sm, streptomycin; Su, sulphamethoxazole; Tc, tetracycline; Tp, trimethoprim; NS, not sized.

and the other to Tc, were transferred from isolate WBG1006. Plasmid analysis of WBG1006 revealed that it contained only one 4.4-kb band as demonstrated by AGE (fig. 2). When both Tc and Sm resistance was lost from WBG1006 after growth at 43.5°C, the 4.4-kb band was also lost, whereas the 4.4-kb band was retained when either Tc resistance or Sm resistance was lost separately (fig. 2). Both plasmids were mobilised by plasmid pWBG636. Of 222 Sm-resistant transconjugants tested, all were resistant to Sm only and contained a 4.4-kb plasmid band. However, of 222 Tc-resistant transconjugants screened, 221 were resistant to Tc and only one was resistant to Tc and Sm. Both types of Tc-resistant transconjugants contained a 4.4-kb plasmid. These results indicate that WBG1006 contained two 4.4-kb plasmids, one encoding Tc resistance and the other Sm resistance. Two 4.4-kb plasmids, one encoding Tc resistance and the other Sm resistance were also transferred separately from isolate WBG4918 (table II).

Analysis of isolate WBG7407

Isolate WBG7407 was resistant to mupirocin (Mu), Pc, Tc and Cd and contained a 4.4-kb Tc-resistance plasmid, a plasmid of about 20 kb encoding resistance to Pc and Cd, and a conjugative Mu-resistance determinant.³⁰ When WBG7407 was examined in conjugation experiments with WBG541, only Mu resistance was transferred and none of 222 Mu-resistant transconjugants screened co-transferred resistance to Tc, Pc or Cd, indicating that the Tc-

resistance and the Pc- and Cd- resistance plasmids were not mobilised by the conjugative Mu-resistance determinant. However, when pWBG636 was transferred to WBG7407 it mobilised both these plasmids (table II). The Tc-resistance plasmid was mobilised as a separate plasmid whereas the Pc- and Cd-resistance plasmid was transferred recombined with pWBG636.

Mobilisation of plasmid pWBG632 by pWBG653

It has been reported previously that the Tc-resistance plasmid pWBG632 present in isolate WBG1024 was not mobilised by the conjugative plasmid pWBG637³¹ although pWBG637 mobilises pWBG3, a Tc-resistance plasmid²⁰ and has mobilised other Tc-resistance plasmids in this study (table II). Plasmid pWBG632 was studied further to determine whether it could be mobilised by another conjugative plasmid, pWBG653. Plasmid pWBG653 was transferred to strain WBG4856 (carrying pWBG632) and a resultant transconjugant was examined for conjugation with WBG541. Tc resistance was transferred to WBG541 and the transconjugants contained pWBG632 alone or both pWBG632 and pWBG653 (table II).

Mobilisation of non-conjugative plasmids from *S. epidermidis*

The *S. epidermidis* isolates were resistant to a wide range of antimicrobial agents (table I) and harboured many plasmid bands (not shown). The sizes of these plasmids could not be determined in the parental

Table II. Mobilisation of plasmids in staphylococci

Mobilising plasmid	Donor strain*	Selective markers	Transferred determinants	Plasmid and sizes (kb)
<i>S. aureus</i>				
pWBG636	WBG1004	FaRfSm	Sm	4.4
	WBG1004	FaRfSm	Sm, Gm	4.4, 39.2 (pWBG636)
	WBG1004	FaRfCd	Pc, Asa, Cd	38.8 (pWBG659)
	WBG1004	FaRfCd	Pc, Asa, Cd, Gm	78.4 (pWBG635)
	WBG1004	FaRfGm	Gm	39.2 (pWBG636)
	WBG1004	FaRfGm	Gm, Sm	4.4, 39.2 (pWBG636)
	WBG1004	FaRfGm	Gm, Pc, Asa, Cd	78.4 (pWBG635)
	WBG1006	FaRfSm	Sm	4.4 (pWBG623)
	WBG1006	FaRfSm	Sm, Gm	4.4, 39.2 (pWBG636)
	WBG1006	FaRfTc	Tc	4.4 (pWBG622)
	WBG1006	FaRfTc	Tc, Sm	4.4, 4.4
	WBG1006	FaRfTc	Tc, Gm	4.4, 39.2 (pWBG636)
	WBG1006	FaRfCd	Nil	Nil
	WBG1006	FaRfGm	Gm	39.2 (pWBG636)
	WBG1006	FaRfGm	Gm, Tc	4.4, 39.2 (pWBG636)
	WBG1006	FaRfGm	Gm, Sm	4.4, 39.2 (pWBG636)
	WBG4761	FaRfSm	Sm, Cm	5.2
	WBG4761	FaRfSm	Sm, Cm, Gm	5.2, 39.2 (pWBG636)
	WBG4761	FaRfCm	Sm, Cm	5.2
	WBG4761	FaRfCd	Pc, Asa, Cd	39.8 (pWBG700)
	WBG4761	FaRfCd	Gm, Pc, Asa, Cd	79.1 (pWBG686)
	WBG4761	FaRfTc	Nil	Nil
	WBG4762	FaRfSm	Sm, Cm	5.2
	WBG4762	FaRfSm	Gm, Sm, Cm	5.2, 39.2 (pWBG636)
	WBG4762	FaRfCm	Sm, Cm	5.2
	WBG4762	FaRCm	Gm, Sm, Cm	5.2, 39.2 (pWBG636)
	WBG4918	FaRfTc	Tc	4.4
	WBG4918	FaRfSm	Sm	4.4
	WBG4918	FaRfGm	Gm	39.2 (pWBG636)
	WBG4918	FaRfGm	Gm, Sm	4.4, 39.2 (pWBG636)
	WBG4918	FaRfGm	Gm, Tc	4.4, 39.2 (pWBG636)
	WBG4920	FaRfSm	Sm, Cm	5.2
	WBG4920	FaRfSm	Gm, Sm, Cm	5.2, 39.2 (pWBG636)
	WBG4920	FaRfCm	Sm, Cm	5.2
	WBG4920	FaRfCm	Gm, Sm, Cm	5.2, 39.2 (pWBG636)
	WBG4920	FaRfTc	Tc	4.4
	WBG4920	FaRfTc	Gm, Tc	4.4, 39.2 (pWBG636)
	WBG4920	FaRfCd	Cd	20.8
	WBG4920	FaRfCd	Gm, Cd	NS
pWBG642	WBG4940	FaRfCm	Cm	3.5
	WBG4940	FaRfCm	Cm, Em	3.5, 39.9 (pWBG642)
	WBG4940	FaRfSm	Nil	Nil
	WBG4940	FaRfEm	Em	39.9 (pWBG642)
	WBG4940	FaRfEm	Em, Cm	3.5, 39.9 (pWBG642)
	WBG4948	FaRfCm	Cm	3.5
	WBG4948	FaRfCm	Em, Cm	3.5, 39.9 (pWBG642)
	WBG4948	FaRfCd	Nil	Nil
	WBG4948	FaRfEm	Em	39.9 (pWBG642)
	WBG4948	FaRfEm	Em, Cm	3.5, 39.9 (pWBG642)
pWBG636	WBG7407	FaRfTc	Tc	4.4
	WBG7407	FaRfTc	Tc	4.4, 39.2 (pWBG636)
pWBG653	WBG7407	FaRfCd	Gm, Pc, Cd	NS
	WBG4856	FaRfTc	Tc	4.4
pWBG636	WBG4856	FaRfTc	Tc	4.4, 44.0 (pWBG653)
<i>S. epidermidis</i>				
pWBG636	WBG7193	FaRfEm	Em, Eb, Pi	39.0
pWBG642	WBG7195	FaRfGm	Gm, Km	39.9 (pWBG684)
	WBG7195	FaRfKm	Gm, Km	39.9 (pWBG684)
	WBG7195	FaRfTc	Tc	4.4
	WBG7195	FaRfTc	Em, Tc	4.4, 39.9 (pWBG642)
	WBG7196	FaRfEb	Pc, Pi, Eb	17.0
	WBG7196	FaRfEb	Em, Pc, Pi, Eb	17.0, 39.9 (pWBG642)
	WBG7196	FaRfEm	Em	39.9 (pWBG642)
	WBG7196	FaRfEm	Em, Pc, Pi, Eb	17.0, 39.9 (pWBG642)
pWBG636	WBG7200	FaRfCm	Cm	4.5
	WBG7200	FaRfCm	Gm, Cm	4.5, 39.2 (pWBG636)
	WBG7200	FaRfEm	Em, Tp	42.0
	WBG7216	FaRfTc	Tc, Pc, Km	28.0
	WBG7216	FaRfKm	Tc, Pc, Km	28.0
	WBG7216	FaRfPi	Tp, Su, Pi	17.1

See footnote to table I; Fa, fusidic acid; Rf, rifampicin.

* Clinical isolate which has received a conjugative plasmid.

Fig. 1. *EcoRI* digests of plasmids pWBG636, pWBG635, pWBG659, pWBG700 and pWBG686: lane 1, pWBG636 Gm^r, tra⁺, the 1.6- and 1.4-kb fragments are not visible in this gel; 2, pWBG635 Gm^rPc^rCd^rAsa^r; 3, pWBG659 Pc^rCd^rAsa^r; 4, pWBG700 Pc^rCd^rAsa^r; 5, pWBG686 Gm^rPc^rCd^rAsa^r.

Fig. 2. Plasmids of isolate WBG1006 and its derivatives: lane 1, WBG4483 carrying mol.-wt markers; 2, WBG1006; 3, WBG1006 cured of Tc^r; 4, WBG1006 cured of Sm^r; 5, WBG1006 cured of Sm^r and Tc^r; 6, a Tc^r transconjugant carrying a 4.4-kb plasmid, pWBG622; 7, a Sm^r transconjugant carrying a 4.4-kb plasmid, pWBG623 (the OC form is prominent in this gel); 8, a transconjugant carrying the Tc^r plasmid pWBG622 and the conjugative plasmid pWBG636.

isolates because it was difficult to ascertain which of the bands represented covalently-closed circular or open-circular forms. However, the plasmids were sized

Fig. 3. *EcoRI* digest of plasmids pWBG642 and pWBG684. Lane 1, *HindIII* digest of phage λ DNA (the faint bands are partial digests); 2, pWBG642 Em^r, tra⁺; 5-8, pWBG684 Gm^rKm^r, tra⁺.

and their resistance phenotypes determined once they were transferred to strain WBG541.

Small 4.4-4.5-kb plasmids encoding resistance to Tc or Cm and 17-42-kb plasmids encoding multiresistance were transferred from *S. epidermidis* (table II). Linked resistance to Tc, Pc and Km were transferred from WBG7216 and were borne on a single 28.0-kb plasmid. Resistance to the nucleic-acid-binding compounds³² Pi and ethidium bromide (Eb) were transferred from WBG7196 and WBG7193 and were found to be encoded on 17.0-kb and 39.0-kb plasmids. The latter plasmid also carried a Pc-resistance gene.

Transposon displacement

The *S. epidermidis* isolate WBG7195 was resistant to methicillin, Gm, Km, neomycin, Sm, Tc and Pi. When the conjugative plasmid pWBG642 (Em resistance) was used to transfer plasmids from WBG7195, resistance to Gm and Km was transferred and the transconjugants examined contained plasmids of the same size as pWBG642 but encoded resistance to Gm and Km. One of these plasmids was designated pWBG684. Proof that plasmid pWBG684 encodes resistance to Gm and Km was obtained when one of the transconjugants carrying pWBG684 was examined by conjugation with strain WBG4515. Gm and Km resistance was transferred to strain WBG4515 and the transconjugants were shown to contain pWBG684. This result also indicates that pWBG684 is a conjugative plasmid. *EcoRI* restriction enzyme analysis

indicated that pWBG684 had *EcoRI* restriction patterns similar to those of pWBG642 (fig. 3) although it encoded resistance to Gm and Km instead of Em. It would appear that the Gm and Km resistance determinants had displaced the Em-resistance transposon Tn551 in plasmid pWBG642 and occupied its site. This phenomenon is described as 'transposon displacement'.

Discussion

The increasing incidence of untypable *S. aureus* isolates has not only limited the use of phage typing for studying the epidemiology of *S. aureus* but it has meant that neither transduction nor MCT, which require both donor and recipient cells to be susceptible to the transferring phage,⁸ can be used for transferring plasmids from non-typable *S. aureus* isolates.

We investigated the use of plasmid mobilisation by conjugative plasmids as an alternative to phage-mediated methods for transferring plasmids from staphylococci. This has been made possible by the discovery of conjugative plasmids that have no resistance phenotypes.^{20, 22} One of these plasmids has been labelled with different resistance transposons²⁵ and this has enabled them to be used with isolates with a range of resistance profiles. The results presented have demonstrated that mobilisation by conjugative plasmids can be an effective tool for transferring non-conjugative plasmids from typable and non-typable *S. aureus* and *S. epidermidis*, an organism for which phage-mediated transfer systems are not well characterised.

Two separate 4.4-kb plasmids, one encoding Tc resistance and the other Sm resistance, were transferred from isolates WBG1006 and WBG4918. On one occasion both Tc- and Sm-resistance plasmids were co-transferred on Tc selection but this did not appear to result from recombination between the two replicons as the transconjugant had the same 4.4-kb band as WBG1006. A recombinant plasmid would have been expected to be larger than that in WBG1006. The transfer of two separate 4.4-kb plasmids, one encoding Tc resistance and the other Sm resistance, confirms earlier reports that determinants for Tc and Sm resistance co-transduced from other *S. aureus* isolates existed as separate replicons.^{33, 34}

The results indicate that, whereas the small 3.2–5.2-kb plasmids were mobilised and were transferred with or without the conjugative plasmid, the large plasmids appeared to be transferred via co-integrate formation. Plasmids pWBG635 and pWBG686 appear to be co-integrates of pWBG659 and pWBG636, and pWBG700 and pWBG636, respectively (fig. 1), which have not resolved in the transconjugants.

Mobilisation was more successful with *S. aureus* than with *S. epidermidis* plasmids. It is possible that some of the plasmids in the *S. epidermidis* isolates that appeared not to be mobilised were cryptic and,

therefore, no positive selection pressure was available to obtain transconjugants, even if they were mobilisable.

The transfer of Gm and Km resistance from *S. epidermidis* isolate WBG7195 yielded plasmid pWBG684 which was conjugative and similar in size (39.9 kb, table II) to, and had the same *EcoRI* restriction endonuclease pattern as, plasmid pWBG642 (fig. 3). However, plasmid pWBG684 differed from pWBG642 in encoding resistance to Gm and Km and not to Em. It appears that pWBG684 was pWBG642 in which the Gm-Km-resistance determinants had displaced Tn551 and occupied its site. Consequently, this phenomenon has been referred to as 'transposon displacement'. The Gm-Km-resistance determinant of WBG7195 is probably a transposon similar to Tn4031, a Gm-resistance transposon reported in *S. epidermidis*.³⁵ The fate of Tn551 after the displacement is uncertain. It was not transposed to the chromosome of WBG541 as none of the Gm-Km-resistant transconjugants was resistant to Em. It would appear that little, if any, of Tn551 remained on the plasmid as the acquisition of the determinants for Gm and Km resistance did not result in any detectable increase in size of the plasmid. Further analysis is required to understand this new phenomenon. However, it represents another method whereby conjugative plasmids are able to acquire and disseminate resistance genes.

As not all the plasmids detected in the isolates were mobilised by pWBG636 and pWBG642, it is possible that some of these plasmids could be mobilised by other conjugative plasmids. Support for this view is three-fold: (i) the Tc-resistance plasmid pWBG632 was not mobilised by pWBG637 but was mobilised by pWBG653; (ii) pWBG636 was able to mobilise a Tc-resistance plasmid from isolate WBG7407 which was not mobilised by the conjugative Mu-resistance determinant of WBG7407; and (iii) the report that the conjugative plasmid pG01 could not mobilise plasmids pT181 and pE194,²⁴ whereas plasmids pE194 and pWBG3, a plasmid similar to pT181, were mobilised by pWBG637.²⁰ Therefore, the use of different sets of conjugative plasmids should increase the number of plasmids transferred in staphylococci.

Mobilisation of non-conjugative plasmids in staphylococci may be similar to the situation in *Escherichia coli*, in which it has been observed that some plasmids can be efficiently mobilised by one conjugative plasmid and not by another. An example is plasmid RSF100 which is mobilised efficiently by *IncP* plasmids, but poorly by *IncI* and *IncW* plasmids and not at all by *IncF* plasmids.³⁶ Although the conjugative plasmids in *S. aureus* belong to at least three incompatibility groups,^{25, 37} it is not yet known whether incompatibility plays any role in the mobilisation of plasmids in *S. aureus*.

Although these studies have all been performed *in vitro*, the demonstration that conjugation can occur *in vivo*^{38–40} suggests that the mobilisation described here

could also occur *in vivo*. If this is true, these results should increase our understanding of how antimicrobial resistance may be disseminated in clinical staphylococcal isolates.

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