Transfer of resistance determinants from a multi-resistant *Staphylococcus aureus* isolate

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Summary. The clinical isolate *Staphylococcus aureus* WBG1024 was resistant to cadmium, benzyl penicillin, kanamycin, neomycin, streptomycin, tetracycline and trimethoprim and harboured a conjugative plasmid pWBG637 (34.5 kb) and non-conjugative plasmids of 23.8, 4.4, 2.8 and 1.9 kb. Transduction and mixed-culture transfer experiments demonstrated that the 4.4-kb plasmid (pWBG632) encoded resistance to tetracycline and the 23.8-kb plasmid (pWBG628) encoded resistance to cadmium, benzyl penicillin, kanamycin, neomycin and streptomycin. The conjugative plasmid pWBG637 was able to mobilise a further 4.4-kb plasmid (pWBG633) encoding streptomycin resistance and recombined with the multi-resistance plasmid pWBG628 to produce transconjugants of various resistance phenotypes.

Introduction

Plasmid-mediated resistance to antimicrobial agents among pathogenic bacteria constitutes a major clinical and economic problem world-wide. Consequently, antibiotic resistance plasmids have been the subject of extensive genetic and biochemical studies. Plasmid transfer *in vitro* is used to characterise plasmids and establish the genetics and spread of plasmid-linked resistance genes. In *Staphylococcus aureus*, resistance can be transferred *in vitro* by transduction,¹ mixed-culture transfer (MCT; phage-mediated conjugation)² and conjugation.^{3–6} Multi-resistant *S. aureus* isolates commonly harbour two or more plasmids that often vary in size and resistance phenotypes. In such circumstances, an effective study of plasmid-linked resistance determinants depends upon the successful transfer of plasmids from resistant isolates to suitably marked sensitive, and plasmid-free, recipients. This paper describes the transfer of resistance determinants from a multi-resistant *S. aureus* isolate WBG1024 by a combination of transduction, MCT and conjugation.

Materials and methods

Bacterial strains

The bacterial strains and plasmid are listed in table I.

Media

Brain Heart Infusion Broth (BHIB), Brain Heart Infusion Agar (BHIA), Trypticase Soy Broth (TSB)

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

Mutation of *S. aureus* strain WBG6522

An overnight culture (10 ml) of strain WBG6522 was pelleted by centrifugation and the deposit was spread on to BHIA plates containing rifampicin (Rf) 25 mg/L and incubated overnight at 37°C. Colonies were subcultured on to another Rf plate and incubated at 37°C. Rf resistance was confirmed with a 25-mg Rf disk. The Rf-resistant mutant was then induced to mutate to fusidic acid (Fa) resistance by adding 0.1 ml of ethyl methane sulphonate to 10 ml of a late log-phase culture and incubating at 37°C for 30 min with gentle shaking. The culture was washed twice in BHIB, resuspended in 1.0 ml of BHIB, incubated at 37°C for 2 h, and 0.1 ml was spread on to a BHIA plate containing Fa 5 mg/L. After incubation overnight at 37°C, resistant colonies were purified on Fa plates and resistance to both Fa and Rf was confirmed by disk sensitivity tests. This strain was designated WBG6522RF.

Antibiotic susceptibility testing

This was performed on MHA plates by multipoint replication or with impregnated disks.^{12,13}

Loss of resistance at 43.5°C

This was performed as described previously.¹²

Resistance transfer

Transduction and MCT were performed as described previously.⁶ Phage 85 of the International Phage Typing Set was used for the transduction

experiments. Recipient colonies which had received transferred determinants by MCT are referred to as transciipients. Conjugation was performed in the presence of polyethylene glycol 40% w/v as previously described.⁶ Selections were made on BHIA containing the following agents (mg/L): kanamycin (Km) 75; streptomycin (Sm) 100; tetracycline (Tc) 5; trimethoprim (Tp) 2.5; Fa 5; Rf 25; novobiocin (Nb) 5; acridine yellow (Ay, Gurr Ltd, London) 8.4; and ethidium bromide (Eb) 120. For benzyl penicillin (Pc), 2U was used and for cadmium (Cd), 2×10^{-2} M.

Plasmid isolation, restriction endonuclease analysis, Southern blotting and DNA hybridisation

Plasmid DNA was isolated by the cetyltrimethylammonium bromide (CTAB) method,¹¹ digested with restriction endonucleases according to the manufacturers' instructions and analysed by agarose- (0.8% w/v) gel electrophoresis in TAE buffer (1.6 M Tris base, 40 mM edetic acid, 0.8 M sodium acetate, pH 7.2). Phage λ DNA digested with the restriction endonuclease *Hind*III was used as the mol. wt standard. Undigested plasmids were electrophoresed in TAE buffer in agarose 0.5% w/v and the plasmids of strain WBG4483⁶ were used as mol. wt standards. Plasmids to be probed were isolated

by the CTAB method, purified by caesium chloride-ethidium bromide gradient centrifugation¹² and digested with the restriction endonuclease *Hpa*II, then electrophoresed and transferred to nitrocellulose filters (BioRad Laboratories) by the method of Southern.¹⁴

Purified plasmids pWBG637 and pWBG628 were labelled with ³²P (dCTP) by nick translation.¹⁵ The ³²P-labelled probe was hybridised to blotted DNA at 65°C. After hybridisation, the filters were subjected to the following washes: twice in $2 \times$ SSC (NaCl 17.5 gm; sodium citrate 8.82 gm; pH 7.0)–sodium dodecyl sulphate (SDS) 0.3% for 5 min each; once in $2 \times$ SSC–SDS 0.1% for 10 min and once in $0.2 \times$ SSC–SDS 0.1% for 10 min; all washes were at room temperature. This was followed by washing in $0.1 \times$ SSC–SDS 0.1% at 50°C for 60 min and then for 30 min. The filters were then exposed to Kodak X-Omat AR film at –70°C for autoradiography.

Results

The clinical isolate WBG1024 was resistant to Cd, Pc, Km, neomycin (Nm), Sm, Tp, Tc, Eb, Ay and susceptible to Rf, Fa, Nb, methicillin, gentamicin, erythromycin, spectinomycin, mercury, phenylmercuric acetate, arsenate, and propamidine isethionate (table I). It contained plasmids of 34.5, 23.8, 4.4, 2.8

Table I. *S. aureus* strains and plasmids

Strain no.	Properties	Plasmids, kb	Reference no.
WBG541	RN450 mutated to chromosomal Fa ^R , Rf ^R	None	8
WBG879	WBG541 lysogenised with phage 85	None	8,9
WBG742	RN450 mutated to chromosomal Nb ^R	None	10
WBG1876	WBG541 lysogenised with phage J	None	9
WBG4483	RN450 transductants and transconjugant	pWBG615, 40.3 pWBG115, 22.5 pWBG3, 4.4 pE194 3.5	6
WBG1024	Clinical isolate Pc ^R , Km ^R , Nm ^R , Sm ^R , Tp ^R , Tc ^R , Cd ^R	pWBG637, 34.5 pWBG628, 23.8 pWBG632, 4.4 pWBG633, 4.4 pWBG634, 2.8 pWBG651, 1.9	This study, 7
WBG4527	WBG541 transconjugant, Sm ^R	pWBG637, 34.5 pWBG633, 4.4	7
WBG4856	WBG1024 cured of Km ^R , Nm ^R , Sm ^R	pWBG637, 34.5 pWBG632, 4.4 pWBG634, 2.8 pWBG651, 1.9	This study
WBG4530	WBG879 transciipient Pc ^R , Cd ^R , Km ^R , Nm ^R , Sm ^R	pWBG628, 23.8	This study
WBG4844	WBG879 transciipient Tc ^R	pWBG632, 4.4	This study
WBG6178	WBG879 transciipient Ay ^R	pWBG634, 2.8	This study
WBG4526	WBG541 transconjugant Km ^R , Nm ^R , Sm ^R	pWBG629, 39.1	This study
WBG4528	WBG541 transconjugant Pc ^R , Km ^R , Nm ^R , Sm ^R	pWBG630, 40.2	This study
WBG4529	WBG 541 transconjugant Pc ^R , Cd ^R , Km ^R , Nm ^R , Sm ^R	pWBG631, 53.2	This study
WBG4529A	WBG742 carrying pWBG631	pWBG631, 53.2	This study
WBG6522	RN981, <i>rec</i> [–]	None	10
WBG6522RF	WBG522 mutated to chromosomal Fa ^R , Rf ^R	None	This study
WBG6522A	WBG6522 transductants and transconjugants; Pc ^R , Cd ^R , Km ^R , Nm ^R , Sm ^R , Tc ^R	pWBG637, 34.5 pWBG628, 23.8 pWBG3, 4.4	This study

Ay, acridine yellow; Cd, cadmium; Pc, benzyl penicillin; Km, kanamycin; Nm, neomycin; Sm, streptomycin; Fa, fusidic acid; Rf, rifampicin; Nb, novobiocin; Tc, tetracycline; Tp, trimethoprim; *rec*[–], recombination deficient; superscript ^R, resistance.

and 1.9 kb (fig. 1) and was lysed by phages 6/47/54/84/85 of the International Phage Typing Set.

To determine the resistance phenotypes of the plasmids in isolate, WBG1024, it was used as donor in transduction, MCT and conjugation experiments. Resistance to Cd, Ay, Tc, Pc, Km, Nm and Sm was transferred by transduction and MCT. Screening of 404 transductants and transciipients revealed that Tc resistance (Tc^R) was not co-transferred with any other phenotype whereas resistance to Cd, Pc, Km, Nm and Sm were always co-transferred irrespective of whether the selection was with Km, Sm or Cd. The transfer of Tc^R and Ay^R corresponded to the transfer of a 4.4-kb and a 2.8-kb plasmids respectively and the transfer of resistance to Cd, Pc, Km, Nm and Sm to the transfer of the 23.8-kb plasmid pWBG628 (fig. 1). This relationship was further confirmed by curing experiments on isolate WBG1024 and its transductants and transciipients. However, when plasmid pWBG628 was cured from isolate WBG1024, only resistance to Km and Nm was lost, indicating that isolate WBG1024 contained additional resistance determinants for Cd, Pc and Sm.

Tp^R and Pc^R were transduced independently and in neither case were plasmids detected in the transductants. No transfer of Eb^R was obtained by transduction or MCT.

When isolate WBG1024 was examined in conjugation experiments with the standard recipient strain WBG541, transconjugants containing plasmids with

four different resistance phenotypes were obtained (table II). Plasmids of the pWBG631 type encoded resistance to Cd, Pc, Km, Nm, and Sm and were obtained by selection with Km, Cd or Sm. The pWBG630 plasmid type encoded resistance to Pc, Km, Nm and Sm and transconjugants were obtained on Km and Sm, whereas plasmids of the pWBG629 type encoding resistance to Km, Nm and Sm were obtained only by selection with Km. Transconjugants of the remaining type were obtained only by Sm selection and these corresponded to the transfer of the 4.4-kb Sm^R plasmid pWBG633 which was mobilised by the conjugative plasmid pWBG637 as previously reported.⁷

Table II. Transconjugants from strains WBG1024/WBG541

Selective agent	Resistance transferred	Plasmid (kb) transferred
Km	Km, Nm, Sm	39.1, pWBG629
	Pc, Km, Nm, Sm	40.2, pWBG630
	Pc, Cd, Km, Nm, Sm	53.2, pWBG631
Cd	Pc, Cd, Km, Nm, Sm	53.2, ND
	Pc, Km, Nm, Sm	40.2, ND
Sm	Pc, Cd, Km, Nm, Sm	53.2, ND
	Sm	4.4, pWBG633
	Sm	4.4, pWBG633
	Sm	34.5, pWBG637

For abbreviations, see footnote to table I; ND, not designated.

Fig. 1. Plasmids of isolate WBG1024 and its derivatives. Lane 1, WBG4483, plasmid mol. wt markers; 2, WBG1024, pWBG637 (34.5 kb), pWBG628 (23.8 kb), pWBG632/pWBG633 (4.4 kb), pWBG634 (2.8 kb), pWBG659 (1.9 kb); 3, pWBG628 Pc^R Cd^R Km^R Nm^R Sm^R tra^- ; 4, pWBG637 tra^+ (34.5 kb); 5, pWBG629 Km^R Nm^R Sm^R tra^+ (39.1 kb); 6, pWBG630 Pc^R Km^R Nm^R Sm^R tra^+ (40.2 kb); 7, pWBG631 Pc^R Cd^R Km^R Nm^R Sm^R tra^+ (53.2 kb); 8, pWBG634 Ay^R (2.8 kb); 9, pWBG632 Tc^R tra^- (4.4 kb); 10, WBG4856, WBG1024 cured of pWBG628.

Plasmids pWBG629 (Km^R Nm^R Sm^R), pWBG630 (Pc^R Km^R Nm^R Sm^R) and pWBG631 (Cd^R Pc^R Km^R Nm^R Sm^R) were all found to be conjugative when their transconjugant hosts WBG4526, WBG4528 and WBG4529, respectively, were examined in conjugation experiments as donors with WBG742 as recipient and selection made with Km and Sm. The plasmids transferred to strain WBG742 had the same phenotype and gave the same *Eco*RI restriction enzyme fragments as the donor plasmids. Plasmid pWBG629 transferred at highest frequency (3.0×10^{-4} /donor), then plasmid pWBG630 (2.0×10^{-6} /donor) and plasmid pWBG631 at the lowest frequency (1.6×10^{-7} /donor). In a parallel experiment, plasmid pWBG628 was found not to be conjugative when the host strain WBG4530 was mated with strain WBG742.

Restriction endonuclease analysis

As plasmids pWBG629, pWBG630 and pWBG631 were not detected in the parent isolate WBG1024 (fig. 1) but had the resistance phenotypes of plasmid pWBG628 and were conjugative, they appear to have arisen by recombinations between plasmids pWBG628 and pWBG637 during conjugation. Consequently the plasmids were compared by restriction

endonuclease analysis. Plasmids pWBG637, pWBG629, pWBG630 and pWBG631 were found to be related when digested with *EcoRI* (fig. 2). Plasmid pWBG628 was cut only once with *EcoRI* and, consequently, no relatedness with the other plasmids was evident. However digestion with *HpaII* (fig. 3A) and *ClaI* (not shown) demonstrated that plasmids pWBG637, pWBG629, pWBG630, pWBG631 and pWBG628 shared some restriction fragments.

The relatedness was confirmed by hybridisation experiments. ^{32}P -labelled plasmid pWBG637 hybridised with fragments of plasmids pWBG629, pWBG630 and pWBG631 but not against fragments of plasmid pWBG628 (fig. 3B), whereas ^{32}P -labelled plasmid pWBG628 hybridised with some fragments of plasmids pWBG629, pWBG630 and pWBG631 but not with plasmid pWBG637 (fig. 3C). These results indicated that although plasmids pWBG637 and pWBG628 had no detectable homology, plasmids pWBG629, pWBG630 and pWBG631 resulted from recombination between them.

Fig. 2. *EcoRI* restriction endonuclease analysis of plasmids pWBG637, pWBG628, pWBG629, pWBG630 and pWBG631. Lane 1, phage λ DNA digested with *HindIII*; 2, pWBG637; 3, pWBG629; 4, pWBG630; 5, pWBG631; 6, pWBG628; 7, λ DNA digested with *HindIII* and *EcoRI*.

Effect of *Rec* background

To study how recombination was occurring between plasmids pWBG637 and pWBG628, experiments were conducted in a *rec⁻* background. Isolate WBG1024 was examined in conjugation experiments with the *rec⁻* recipient WBG6522RF. All the transconjugants were resistant to Cd, Pc, Km, Nm, Sm and harboured single plasmids which varied in size. Analysis of representative plasmids from the transconjugants revealed that they were conjugative and, although closely related, they varied in their *EcoRI* restriction endonuclease digestion patterns (fig. 4).

In the other experiment, a *rec⁻* donor WBG6522A was used. This was constructed by first transducing plasmid pWBG628 to strain WBG6522 and then employing this strain as a recipient in a mating with strain WBG4872 (carrying plasmids pWBG637 and pWBG63) and selecting for Km and Tc resistance. Since plasmid pWBG637 mobilises plasmid pWBG63, Tc^{R} transconjugants were screened by agarose-gel electrophoresis for carriage of plasmids pWBG637, pWBG63 and pWBG628. One such strain (WBG6522A) was then tested for conjugation with strain WBG541, a *rec⁺* recipient (table III). Transconjugants were obtained only with Tc selection. None of the Tc^{R} transconjugants were resistant to Pc, Km, Nm, Sm or Cd.

Behaviour of plasmid pWBG631 in MCT

Since plasmid pWBG631 ($\text{Pc}^{\text{R}}\text{Km}^{\text{R}}\text{Nm}^{\text{R}}\text{Sm}^{\text{R}}\text{Cd}^{\text{R}}$) is conjugative and has the full resistance determinants of plasmid pWBG628, it could be a co-integrate of plasmids pWBG637 and pWBG628. Therefore, it was investigated for its ability to resolve into the component plasmids. Strain WBG4529 (carrying plasmid pWBG631) was grown on antibiotic-free BHIA at 37°C and at 43.5°C, and single colonies were screened for loss of resistance to Cd, Pc, Km, Nm and Sm. No resistances were lost at 37°C but all five resistance determinants were lost together at 43.5°C and their loss was accompanied by the loss of plasmid pWBG631, indicating that the resistances were maintained stably on the plasmid. Plasmid pWBG631 was transferred to strain WBG742 by conjugation and one of the transconjugants (WBG4529A) was used as donor in a MCT experiment with strain WBG1876 (table IV). Transcipients obtained by Km and Sm selection were all resistant to Cd, Pc, Km, Nm and Sm. Two types of resistance phenotype were obtained by Cd selection. One type was resistant to Cd, Pc, Km, Nm and Sm and the other to Pc and Cd. Transcipients of the $\text{Cd}^{\text{R}}\text{Pc}^{\text{R}}\text{Km}^{\text{R}}\text{Nm}^{\text{R}}\text{Sm}^{\text{R}}$ phenotype carried plasmids of the same size irrespective of their selection. *EcoRI* digestion of representative plasmids such as pWBG680 produced the same *EcoRI* restriction endonuclease digestion fragments and were different from *EcoRI* fragments of the $\text{Pc}^{\text{R}}\text{Cd}^{\text{R}}$ plasmids such as pWBG681 (fig. 5).

Fig. 3. DNA-DNA hybridisation analysis. (A), *Hpa*II restriction digest of plasmids. Lane 1, pWBG637; 2, pWBG629; 3, pWBG630; 4, pWBG631; 5, pWBG628. (B) Autoradiograph of gel in panel A after Southern transfer and hybridisation with 32 P-labelled plasmid pWBG637 as probe. No hybridisation was detected with plasmid pWBG628 (lane 5). (C) Autoradiograph of gel in panel A after Southern transfer and hybridisation with 32 P-labelled plasmid pWBG638 as probe. No hybridisation was detected with plasmid pWBG637 (lane 1).

Fig. 4. *Eco*RI restriction endonuclease analysis of WBG1024/WBG6522RF transconjugants. Lanes 1 and 8, phage λ DNA digested with *Hind*III and *Eco*RI respectively; 2, pWBG637; 3-7, different $Pc^R Cd^R Km^R Nm^R Sm^R$ plasmids.

Discussion

The clinical isolate, *S. aureus* WBG1024 has been analysed for susceptibility to antimicrobial agents, plasmid content and transfer of resistance determinants. Results of curing and transfer experiments indicate that only Tp^R and Eb^R did not have a plasmid location.

Isolate WBG1024 harbours six plasmids; two of these, pWBG632 (Tc^R) and pWBG633 (Sm^R) have the same molecular size (4.4 kb) and could not be distinguished on agarose gel-electrophoresis of plasmid DNA from isolate WBG1024. However, transfer experiments clearly demonstrated their separate existence in isolate WBG1024. Whereas plasmid pWBG632 was transferred by transduction and MCT, it was not mobilised by the conjugative plasmid pWBG637, although plasmid pWBG637 does mobilise another Tc^R plasmid, pWBG3.⁷ On the other hand, plasmid pWBG633 was mobilised by plasmid pWBG637 during conjugation but was not transferred by transduction or MCT. The results of curing, transduction and MCT experiments demonstrated that a separate Sm^R determinant was borne, together with Cd, Pc, Km and Nm resistances, on plasmid pWBG628. The resistance phenotype of plasmid pWBG628 is similar to that of a plasmid reported by Lacey.¹⁶ However, unlike this plasmid,¹⁶ the

Table III. Effect of Rec background on recombination

Donor strain no.	Recipient strain no.	Selective agent	Resistance transferred	Plasmid (kb)
WBG1024	WBG6522RF	Km	Pc, Cd, Km, Nm, Sm	c. 53.0*
	(<i>rec</i> ⁻)	Sm	Pc, Cd, Km, Nm, Sm	c. 53.0*
WBG6522A	WBG541	Km	Nil	Nil
(<i>rec</i> ⁻)		Sm	Nil	Nil
		Tc	Tc	4.4, pWBG3

*Transconjugants harboured plasmids of slightly different molecular sizes (see also fig. 4).

Table IV. Analysis of WBG4529A/WBG1876 transci-
pients

Selective agent	Resistance transferred	Plasmid (kb)
Km	Pc, Cd, Km, Nm, Sm	pWBG680, 40.2
Sm	Pc, Cd, Km, Nm, Sm	pWBG680, 40.2
Cd	Pc, Cd, Km, Nm, Sm	pWBG680, 40.2
	Pc, Cd	pWBG681, 36.1

Fig. 5. *EcoRI* restriction endonuclease analysis of WBG4529A/WBG1876 transci-
pients. Lane 1, phage λ DNA digested with *Hind*III; 2, pWBG637; 3 and 4, pWBG680 (Pc^RCd^RKm^RNm^RSm^R);
5 and 6, pWBG681 (Pc^RCd^R); 7, pWBG628; 8, λ DNA digested with *Hind*III and *Eco*RI.

Km^RNm^RSm^R determinants of plasmid pWBG628 were not observed to have a chromosomal location.

It appears that isolate WBG1024 carries two sets of genes encoding resistance to Pc and Cd. Derivatives of isolate WBG1024 that had lost plasmid pWBG628 after growth at 43.5°C were still resistant to Pc and Cd. These determinants could be different from those of plasmid pWBG628 or they could be the same, and their duplication could be a result of transposition. Pc^R transposons are well documented in *S. aureus*¹⁷⁻²⁰ although there has been no report of Cd transposition. Pc^R and Cd^R appear not to be closely linked because Pc^R could be transduced without Cd^R and transconjugants were obtained (e.g., with plasmid pWBG630) which did not carry Cd^R but encoded the other resistance determinants of plasmid pWBG628. Isolate WBG1024 could, therefore, carry separate transposons for Pc^R and Cd^R.

The conjugative plasmid pWBG637 mediated transfer of non-conjugative plasmids in two ways: (i) mobilisation of the Sm^R plasmid, pWBG633, and (ii) recombination with plasmid pWBG628 to form new conjugative resistance plasmids such as pWBG629, pWBG630 and pWBG631. Although mobilisation is well documented^{3-7, 21, 22} there has been no previous report of recombination similar to that observed between plasmids pWBG637 and pWBG628 in *S. aureus*.

No homology was detected between plasmids pWBG637 and pWBG628. However, this does not exclude the possibility that recombination may occur between short regions (RS) of complementary DNA²³ not detected in Southern hybridisation experiments. The failure to obtain recombinants when a *rec*⁻ strain was used as donor in conjugation experiments suggests that some Rec-mediated homologous recombination may be occurring. Non-homologous recombination is usually mediated by transposable elements.^{24, 25} A feature of transposon-mediated recombination and that involving RS sequences is the formation of co-integrates²³⁻²⁵ which may resolve into component parts. Plasmid pWBG631 appeared to be a co-integrate of plasmids pWBG637 and pWBG628 but no resolution of the plasmid was detected when the transconjugants were grown without selective pressure and plasmid pWBG631 and all its resistance determi-

nants were lost together in curing experiments. Although two different plasmids resulted from MCT of plasmid pWBG631, these plasmids differed from plasmids pWBG628 and pWBG637 in their *EcoRI* restriction endonuclease digest fragments and it appears that they resulted from the constraints of packaging a c. 53-kb plasmid by phages rather than co-integrate resolution.

Iordanescu^{26, 27} and Novick *et al.*²⁸ have observed recombination between compatible staphylococcal plasmids during transduction. However, recombinations between plasmids pWBG628 and pWBG637 were detected only in conjugation and not in transduction or MCT experiments. Of 404 transcipts and transductants tested, in none had any of the resistance determinants of plasmid pWBG628 been deleted. Furthermore, all the transductants and transcipts analysed for plasmids carried single plasmids of the same size as seen in isolate WBG1024. Recombination can occur between incompatible plasmids when a cell is forced to maintain them.²⁹ However, plasmids pWBG628 and pWBG637 are compatible and are stably maintained in isolate WBG1024, which eliminates incompatibility as the mitigating factor in their recombination.

Although recombination was observed only from a *rec*⁺ donor, conjugation occurred from both *rec*⁺ and

rec⁻ donors indicating that conjugation occurs independently of the host's *rec* status. A similar observation has been reported by El Sohl *et al.*³⁰

Although plasmids pWBG637 and pWBG628 have not been demonstrated to share homologous sequences, it is clear that the *rec* system is necessary for the observed recombination. This does not exclude any transposition events but so far there is no evidence for the presence of transposons and no recombination occurred in a *rec*⁻ background. The role of this kind of recombination in the evolution of resistance plasmids deserves further study.

The clinical significance of the conjugative plasmid pWBG637 is its ability to enhance the spread of resistance plasmids by mobilisation⁷ and, as demonstrated in this study, by recombining with plasmids such as pWBG628 to generate new resistance plasmids. This has provided an insight into the evolution and spread of resistance plasmids. So far, no other role has been determined for plasmid pWBG637. However it may have other yet undetermined functions to warrant its carriage in the cell.

This work was supported by a NH&MRC grant to WBG and a Curtin University of Technology research scholarship to EEU. We are grateful to the State Health Laboratory Service for phage typing.

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