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NOTES

Conjugative Transfer of High-Level Mupirocin Resistance from *Staphylococcus haemolyticus* to Other Staphylococci

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A conjugative plasmid, pXU10, encoding high-level mupirocin resistance was transferred from a *Staphylococcus haemolyticus* isolate, CN216, to other coagulase-negative staphylococci and a restriction deficient *Staphylococcus aureus* strain, XU21, but not to clinical isolates or a restriction-proficient laboratory strain (strain WBG541) of *S. aureus*. However, from XU21 it was cotransferred with a 3.5-kb chloramphenicol resistance plasmid to WBG541. The results demonstrated the ability of pXU10 to mobilize nonconjugative plasmids.

High-level resistance to mupirocin (MICs, > 1,000 mg/liter) was first reported in the United Kingdom in 1987 (10) and subsequently in Australia (13, 16), the United States (4, 7), and New Zealand (5). High-level mupirocin resistance is usually mediated by a plasmid-encoded *mupA* gene (9, 11, 12). These plasmids, some of which are conjugative, vary considerably in size and in their resistance phenotypes (4, 9–12, 16). However, the conjugative mupirocin resistance plasmids differ from gentamicin resistance (1, 3, 6, 15), cryptic (18, 20), and trimethoprim resistance (19) conjugative plasmids because they usually carry only the mupirocin resistance determinant and do not appear to mobilize nonconjugative plasmids (18). The majority of reports of high-level mupirocin resistance have been for *Staphylococcus aureus*, with few reports of high-level mupirocin resistance among coagulase-negative staphylococci (2, 11, 12). Here we report on the isolation of a conjugative plasmid, pXU10, encoding high-level mupirocin resistance from *Staphylococcus haemolyticus* and present evidence that it can mobilize nonconjugative plasmids.

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Mupirocin was introduced for clinical use at the Burn Unit, Ibn Sina Hospital, Kuwait, in 1992 and was used continuously over the next 2 years. Between April 1994 and May 1995, of 395 staphylococci tested, only 1 isolate, cultured from an intravascular catheter tip of a patient treated previously with mupirocin, expressed high-level resistance. The isolate, designated CN216, was identified as *S. haemolyticus* by biochemical tests with the API 20 Staph identification kit (bioMerieux Sa, Marcy l'Etoile, France).

Strain CN216 was studied for its resistance to antimicrobial agents and its ability to transfer them to other staphylococci in conjugation experiments. Susceptibility to antimicrobial agents was tested by the disk diffusion method as described previously (8) by using commercially available disks (UniPath, Basingstoke, England) on Mueller-Hinton agar (BBL, Cockeysville, Md.). The MIC of mupirocin was determined by the agar dilution method with an inoculum of 10^5 CFU/ml and incubation at 37°C for 18 h. CN216 grew to the edge of a 200-μg mupirocin disk in the disk diffusion test, and the mupirocin MIC for the isolate was >1,024 μg/ml. It was also resistant to the antimicrobial agents listed in Table 1. Plasmid analysis (14) revealed that it contained plasmids of approximately 40, 24, 3.5, 1.8, and 1.6 kb.

Strain CN216 was used as the donor in conjugation experiments in which the staphylococcal strains listed in Table 1 were used as recipients. Conjugation was performed by filter mating and by the polyethylene glycol (PEG) methods described previously (15, 18). Strain WBG541 was derived from strain RN450 by sequentially selecting for resistance to rifampin and fusidic acid (15), and strain XU21 was derived from strain RN4220 by selecting for resistance to novobiocin and rifampin. Transconjugants were selected on brain heart infusion agar containing fusidic acid (5 μg/ml), tetracycline (5 μg/ml), novobiocin (5 μg/ml), rifampin (2.5 μg/ml), mupirocin (10 μg/ml), chloramphenicol (10 μg/ml), and erythromycin (5 μg/ml). Transfer frequencies were expressed as the number of transconjugants per number of donor cells.

The results of the conjugation experiments are summarized in Table 2. No resistance was transferred from strain CN216 to strain WBG541, a standard *S. aureus* recipient, on any of the selection plates by either the filter mating or the PEG method. Failure to obtain transfer of resistance from CN216 to WBG541 after three attempts necessitated its conjugation with XU21, a restriction-deficient *S. aureus* recipient. Surprisingly, transconjugants were obtained on mupirocin and chloramphenicol selection plates (Table 2). These were screened for plasmids and the cotransfer of unselected resistance by replica plating. Nineteen percent of the transconjugants from the mupirocin selection plates were resistant to mupirocin and chloramphenicol. The rest were resistant to mupirocin only. Those resistant to both mupirocin and chloramphenicol harbored a large plasmid designated pXU10 and a 3.5-kb plasmid. One of these strains was designated XU118. Those resistant to mupirocin alone harbored pXU10 alone (e.g., XU120) or pXU10 with a 1.8- and/or a 1.6-kb plasmid. Thirty-two percent of the transconjugants from the chloramphenicol selection plates were resistant to chloramphenicol and mupirocin, similar to those obtained on mupirocin selection plates. The rest were resistant only to chloramphenicol and contained either a

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TABLE 1. Properties of staphylococcal strains tested

Isolate	Species	Resistance[a]	Reference or source
WBG541	*S. aureus*	Rf, Fa	15
XU21	*S. aureus*	Nv, Rf	This study
Sa35	*S. aureus*	Pc, Cd, Tc, Fa	This study
Sa40	*S. aureus*	Pc, Cd, Fa	This study
Sa358	*S. aureus*	Mc, Pc, Gm, Sm, Em, Tp, Tc, Cm, Cf, Fa, Cd	This study
Sa434	*S. aureus*	Mc, Pc, Gm, Sm, Em, Tp, Tc, Cd, Fa	This study
CN13	*S. epidermidis*	Pc, Cd, Fa	This study
CN55	*S. epidermidis*	Mc, Pc, Gm, Sm, Em, Tc, Fa	This study
CN230	*S. saprophyticus*	Pc, Cd, Tc, Fa	This study
CN80	*S. haemolyticus*	Mc, Pc, Km, Sm, Tc, Cd, Fa	This study
CN40	*S. haemolyticus*	Pc, Cd, Fa	This study
CN216	*S. haemolyticus*	Mc, Pc, Gm, Km, Sm, Em, Tc, Cm, Cf, Tp, Rf, Cd, Mup	This study
XU118	*S. aureus*	Mup, Cm, Nv, Rf	This study
XU120	*S. aureus*	Mup, Nv, Rf	This study

[a] Abbreviations: Mc, methicillin; Pc, benzylpenicillin; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Em, erythromycin; Tc, tetracycline; Cm, chloramphenicol; Cf, ciprofloxacin; Tp, trimethoprim; Cd, cadmium; Mup, mupirocin; Nv, novobiocin; Rf, rifampin; Fa, fusidic acid.

3.5-kb plasmid or a 3.5-kb plasmid together with a 1.8-kb plasmid or 1.8- and 1.6-kb plasmids. These results indicated that pXU10 is a conjugative plasmid and encodes mupirocin resistance and that the 3.5-kb plasmid encodes chloramphenicol resistance.

Strains XU118, resistant to mupirocin and chloramphenicol, and XU120, resistant to mupirocin only, were then conjugated with strain WBG541. The results (Table 2) demonstrated that the resistance determinants were transferred from XU118 and XU120 to WBG541. However, a strain of XU21 carrying only the 3.5-kb plasmid did not transfer chloramphenicol resistance to WBG541 (data not shown). These results suggested that the failure to obtain transfer of resistance from CN216 to WBG541 in the initial experiment may be due to a restriction-modification barrier between the two species. This notion was tested by conjugating CN216 with clinical isolates of *S. aureus*, *Staphylococcus epidermidis*, and *S. haemolyticus* as recipients and selecting for mupirocin-resistant transconjugants. Mupirocin resistance was transferred to the coagulase-negative staphylococci (Table 2) but not to *S. aureus* (data not shown).

TABLE 2. Conjugative transfer of resistance determinants[a,b]

Donor	Recipient	Selection	Resistance transferred	Transfer frequency
CN216	WBG541	Mup, Rf, Fa	Not detected	Not detected
	WBG541	Cm, Rf, Fa	Not detected	Not detected
	WBG541	Em, Rf, Fa	Not detected	Not detected
	WBG541	Tc Rf, Fa	Not detected	Not detected
CN216	XU21	Mup, Nv	Mup,	1.7×10^{-4}
			Cm, Mup	2.2×10^{-8}
CN216	XU21	Cm, Nv	Cm,	1.2×10^{-6}
			Cm, Mup	1.4×10^{-6}
CN216	CN40	Mup, Fa	Mup,	1.5×10^{-4}
CN216	CN80	Mup, Fa	Mup,	1.1×10^{-4}
CN216	CN230	Mup, Fa	Mup,	2.1×10^{-6}
CN216	CN55	Mup, Fa	Mup,	1.5×10^{-6}
CN216	CN13	Mup, Fa	Mup,	1.4×10^{-6}
XU118	WBG541	Mup, Fa	Mup,	1.5×10^{-4}
	WBG541	Cm, Fa	Cm,	1.2×10^{-6}
XU120	WBG541	Mup, Fa	Mup,	1.2×10^{-4}

[a] No transfer was obtained from CN216 to any of the *S. aureus* isolates.
[b] Abbreviations: Mup, mupirocin; Cm, chloramphenicol; Fa, fusidic acid; Rf, rifampin; Nv, novobiocin.

When pXU10 was digested with the *Eco*RI restriction enzyme, it was found to have a size of 31.5 kb and fragments of 7.8, 6.6, 6.1, 4.9, 2.7, 1.8, and 1.6 kb; this was less than the estimated size of 40 kb calculated from studies with the undigested DNA of CN216. The digested plasmid DNA was transferred to a nylon membrane in a Southern blot experiment and was probed with a digoxigenin-11-dUTP-labeled *mupA* gene probe (12). The *mupA* gene probe hybridized to a 4.9-kb fragment of pXU10 (data not shown), indicating that the mupirocin resistance of pXU10 is mediated by the *mupA* gene.

This study has demonstrated the conjugative transfer of pXU10, which encodes high-level mupirocin resistance from an *S. haemolyticus* isolate to coagulase-negative staphylococci and to a restriction-deficient *S. aureus* strain but not to clinical isolates or a restriction-proficient laboratory strain of *S. aureus*. This was an unexpected observation because transfer of the conjugative gentamicin resistance (1, 3, 6, 15) and the cryptic conjugative plasmid pWBG637 (17, 18) between *S. aureus* and *S. epidermidis* is well documented, although their transfer between *S. aureus* and *S. haemolyticus* has not been reported. Attempts to transfer mupirocin resistance from another *S. haemolyticus* isolate designated D2 to *S. aureus* by Connolly et al. (2) was also unsuccessful. This may be due to the restriction-modification system between the two species.

Because the mupirocin resistance of pXU10 is mediated by the *mupA* gene, it is related to other conjugative mupirocin resistance plasmids (4, 10, 11, 12) except pJ3358 (9), which also encodes tetracycline resistance. However, pXU10 is smaller and can mobilize nonconjugative plasmids. The failure of the 3.5-kb chloramphenicol resistance plasmid to transfer from a XU21 background to WBG541 in the absence of pXU10 demonstrated clearly that its transfer from CN216 to XU21 and from XU118 to WBG541 was mediated by pXU10. Besides the 3.5-kb chloramphenicol resistance plasmids, pXU10 also mobilized the 1.8- and 1.6-kb plasmids in CN216. This ability to mobilize nonconjugative plasmids distinguishes pXU10 from other conjugative mupirocin resistance plasmids. Of the five

types of staphylococcal conjugative plasmids (20), only the gentamicin (1, 3, 6, 15) and trimethoprim (19) resistance plasmids and those without a resistance phenotype (17, 18, 20) mobilized nonconjugative plasmids. It was reported that during the conjugative transfer of pJ3358, which encodes resistance to mupirocin and tetracycline, a small tetracycline resistance plasmid was sometimes obtained on tetracycline selection (9). The tetracycline resistance plasmid probably excised from pJ3358 during conjugation. A similar event was observed with pWBG715, a nonconjugative plasmid encoding resistance to mupirocin, tetracycline, trimethoprim, and cadmium (16). In a mixed-culture transfer experiment involving pWBG715, two transferrants on a tetracycline selection plate were resistant only to tetracycline but contained no detectable plasmid. The tetracycline resistance plasmid in this case was excised from pWBG715 and was inserted into the chromosome. On the basis of being able to mobilize nonconjugative plasmids, pXU10 appears to represent a new type of mupirocin resistance plasmid.

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