In Vivo Conjugal Transfer of R Plasmids in *Neisseria gonorrhoeae*

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In vivo R plasmid transfer between two *Neisseria gonorrhoeae* strains was detected in the absence of antibiotic pressure.

In 1976, penicillin-resistant β-lactamase-producing strains of *Neisseria gonorrhoeae* were reported throughout the world (2, 10, 11). β-Lactamase production by these strains was shown to be plasmid mediated (3, 6, 7). Two distinct R plasmids, one 3.2×10^6 daltons (3.2 Mdal) and one 4.4×10^6 daltons (4.4 Mdal) in size, were described and characterized (12). The strains carrying the 3.2-Mdal plasmid appear to result from the spread of a single clone, whereas the 4.4-Mdal R plasmid is found in a number of different gonococcal strains (12). More recently, we (13) as well as other (3, 6, 8) have shown that a 24.5-Mdal indigenous gonococcal plasmid has sex factor activity and can promote in vitro transfer of itself and the 4.4-Mdal R plasmid, which itself is non-conjugative. Therefore, we have speculated that the presence of the 24.5-Mdal plasmid may have contributed to the successful dissemination of the 4.4-Mdal R plasmid among a variety of gonococcal auxotypes in nature (13). To further support this hypothesis, we wanted to determine whether in vivo R plasmid transfer could be detected and if the 24.5-Mdal plasmid was required for transfer. Man is the natural host for *N. gonorrhoeae*, and at the present time most animal models do not mimic the human infection (1). However, small laboratory animals with subcutaneously implanted chambers have been used to study immunity and various aspects of pathogenesis in a gonococcal infection (1). Therefore, we used subcutaneous chambers implanted on the backs of guinea pigs as our in vivo model. The independently isolated clinical strains CDC67 and CDC66 were used as donors. They were β-lactamase producers which harbored the 4.4-Mdal R plasmid, required no amino acids for growth on the defined medium of Catlin (4, 5), and were sensitive to 10 μg of nalidixic acid and 5 μg of rifampin per ml. Donor strain CDC67 carries the 24.5-Mdal plasmid, whereas CDC66 does not carry the 24.5-Mdal plasmid. The recipient strain F62 requires proline for growth on the

defined medium of Catlin and is resistant to 10 μg of nalidixic acid and 5 μg of rifampin per ml. All three strains carry the cryptic indigenous 2.6-Mdal plasmid (7). Chambers were made from stainless-steel wire coiled to a 5-mm diameter and 20 mm in length and implanted on the backs of Hartley guinea pigs (500 to 700 g) as described by Arko (1). One week after surgery, a small amount of fluid was removed with a syringe from each chamber and plated on Kellogg's medium (9) to check for sterility. If the chambers were sterile they were used the following day.

Each parental strain was passed separately through guinea pig chambers in at least three different animals to adapt them to in vivo growth. The innoculum for the chamber was prepared by removing colonies from six plates of *N. gonorrhoeae* grown overnight on Kellogg's medium (9). The cells were suspended in a sterile proteose peptone broth (9% proteose peptone, 1% NaCl). Matings were performed by inoculating into each chamber 10^8 donor gonococci followed by 10^8 recipient gonococci. Total volume per chamber was between 0.6 and 0.8 ml. The gonococcal population was sampled at 24, 48, and 72 h. The fluid was removed with a syringe and 18-gauge needle (1-mm outside diameter) and placed directly on a Kellogg plate supplemented with 1 μg of penicillin per ml and 10 μg of nalidixic acid per ml or immediately diluted in broth and blended in a Vortex mixer, to reduce the possibility of mating occurring in the broth, and then plated. Other samples were diluted, blended in a Vortex mixer, and plated on Kellogg's medium supplemented either with 1 μg of penicillin per ml or 10 μg of nalidixic acid per ml for colony counts of parental strains. Single colonies appearing on the selective medium were restreaked four times on Kellogg's medium supplemented with 1 μg of penicillin per ml and 10 μg of nalidixic acid per ml and then tested for resistance to rifampin and requirement for proline.

The results of several 24-h mating experi-

TABLE 1. *Twenty-four-hour in vivo R plasmid transfer*

Donor strain	Plasmid complement ($\times 10^6$ daltons)	Recipient strain	Plasmid complement ($\times 10^6$ daltons)	No. of donor cells	No. of recipient cells	No. of penicillin-resistant transconjugants per 10^5 recipients[a]
N. gonorrhoeae CDC67	24.5 4.4 2.6	*N. gonorrhoeae* F62	2.6	1×10^6	2×10^5	1,000
N. gonorrhoeae CDC66	4.4 2.6	*N. gonorrhoeae* F62	2.6	4×10^5	2×10^6	0

[a] The data presented are from two sets of experiments. In later CDC67-F62 matings, a range of 10^1 to 10^3 penicillin-resistant cells per 10^5 recipients was found.
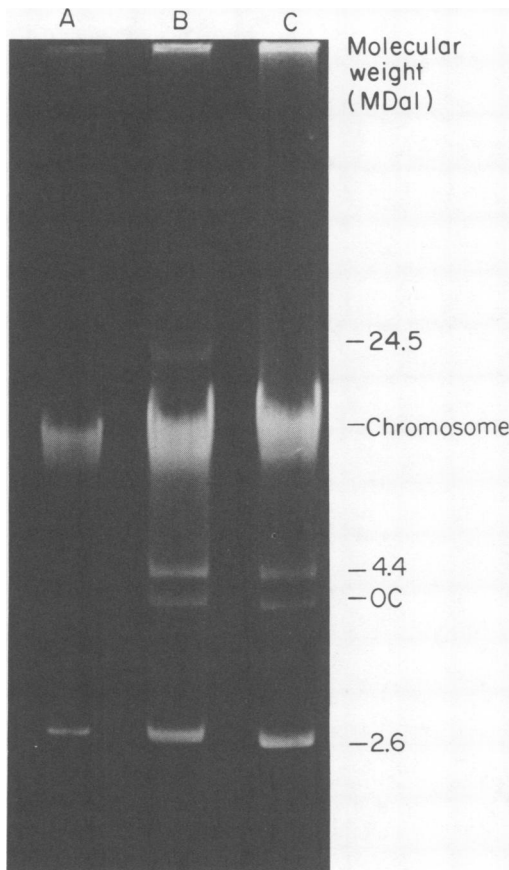

FIG. 1. *Agarose gel electrophoresis of ethanol-precipitated deoxyribonucleic acid from cleared lysates of recipient and transconjugants of N. gonorrhoeae F62. (A) Penicillin-sensitive recipient strain of N. gonorrhoeae F62 containing the 2.6-Mdal plasmid. (B) Penicillin-resistant transconjugant containing the 24.5-Mdal plasmid, the 4.4-Mdal R plasmid and the indigenous 2.6-Mdal plasmid. (C) Penicillin-resistant transconjugant containing the 4.4-Mdal R plasmid and the indigenous 2.6-Mdal plasmid. OC refers to the open circular form of the 2.6-Mdal plasmid.*

ments are summarized in Table 1. No β-lactamase-producing recipients could be isolated from matings in which the donor CDC66, which lacks the 24.5-Mdal plasmid, was used. However, colonies always appeared when the donor strain CDC67 was used. All the colonies examined from these matings were found to be resistant to rifampin and to require proline for growth on Catlin's medium, suggesting that the colonies did represent F62 cells which had acquired penicillin resistance. The frequency of in vivo transfer ranged between 10^{-2} and 10^{-4} per plated recipient cell and is similar to the previously reported in vitro frequencies (13). The plasmid component of representative colonies was determined by agarose gel electrophoresis, and 9 of 10 isolates examined had acquired both the 4.4-Mdal R plasmid and the 24.5-Mdal conjugative plasmid; a representative isolate is illustrated in Fig. 1, slot B. The tenth isolate carried the 4.4-Mdal R plasmid but not the 24.5-Mdal plasmid and is illustrated in Fig. 1, slot C. All transconjugants also carried the indigenous 2.6-Mdal plasmid.

Therefore, we have been able to detect R plasmid transfer in an animal model only when the donor strain carries the 24.5-Mdal plasmid along with the R factor. Transfer can be detected in the absence of any antibiotic pressure. These data strengthen the hypothesis that the 24.5-Mdal plasmid played a role in the spread of the 4.4-Mdal plasmid in nature from gonococci to gonococci.

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