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Capsule Homology Does Not Increase the Frequency of Transformation of Linked Penicillin Binding Proteins PBP 1a and PBP 2x in *Streptococcus pneumoniae*

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Penicillin resistance is mainly confined to a limited number of *Streptococcus pneumoniae* serotypes. Given linkage between the capsular biosynthesis locus and two penicillin binding proteins, we tested whether capsule homology increases transformation rates of penicillin resistance. Transformation rates in homologous donor-recipient pairs were no higher than expected, falsifying this hypothesis.

Resistance to β -lactam antibiotics in *Streptococcus pneumoniae* has spread globally and is increasing in many countries (3, 11). Worldwide, pneumococcal resistance to β -lactams is associated with a subset of serotypes, with very high levels in, for example, 9V and 19A and zero or low prevalence in types 1 to 4 and many others (6).

The reason for this association between serotype and drug resistance is unknown. One possibility is that resistance arises rarely and randomly within serotypes and that the growing use of antimicrobial agents has led to an increase in the frequency of those serotypes in which resistance happened to arise (2). We recently showed that, because the genes encoding two penicillin binding proteins, PBP 2x and PBP 1a, are located within 10 to 15 kbp of the capsular biosynthesis (*cps*) operon, it is possible to transfer DNA containing both a novel capsular type (*cps* operon) and novel PBP alleles that confer resistance to penicillin and cephalosporins in a single transformation event (10). If such transfers were sufficiently frequent, acquisition of penicillin resistance by a new strain might often be accompanied by a shift of serotype to the donor type, preserving the association between penicillin nonsusceptibility and serotype. However, although the frequency of cotransfer of the *cps* operon and one or both *pbp* genes was elevated above the rate of random cotransformation in our prior study, it was still low in absolute terms, raising doubts about the generality of this explanation. We therefore considered an additional hypothesis, tests of which are reported here. The *cps* locus of different serotypes is heterogeneous in gene content and sequence (12). Given that the *pbp* genes are linked in the genome to the *cps* locus, we hypothesized that transfer of *pbp* genes by transformation might be more efficient when donor and recipient have the same serotype than when they have different serotypes, because of the large region of homology that would be present in the former case.

To test this hypothesis, we performed experiments in vitro to estimate the efficiency of transformation of penicillin resistance genes from a nonsusceptible to a susceptible strain, comparing transformation rates observed when the donor and recipient had the same capsular type with rates observed when the capsular type differed between strains. All strains used were variants of *S. pneumoniae* TIGR4 (8), differing in capsular serotype and susceptibility to selected antibiotics.

We used a previously described serotype 14, otherwise isogenic variant of TIGR4 (9), and we created additional, otherwise isogenic TIGR4 variants bearing serotypes 8, 9V, and 11A by the same procedure (9). These strains served as recipients in our experiments. In addition, we transformed the previously described TIGR4J strain (9), which contains the Janus cassette in the *cps* locus, successively with PCR products of resistant alleles of *dhfr*, *pbp2x*, and *pbp1a* and isolated transformants by successive selection on blood agar (BA) containing 25 mg of trimethoprim/liter, then 0.3 mg of cefotaxime/liter, and then 0.07 mg of penicillin/liter. The resulting penicillin-nonsusceptible, trimethoprim-resistant strain was transformed separately with DNA from the TIGR4 isogenic capsule variants to create four strains bearing the *pbp* and *dhfr* markers and themselves isogenic except for the *cps* locus. These strains served as donors in the experiments.

To test our main hypothesis, DNA extracted from each donor was used at a final concentration of 1 μ g/ml to transform 20-fold-diluted recipient cultures harvested at an optical density at 600 nm of 0.5 (7). Total recipients were enumerated by plating on BA, while nonsusceptible transformants were enumerated on BA containing 0.04 or 0.05 mg of penicillin/liter or 25 mg of trimethoprim/liter. Three experiments were performed for each donor-recipient pair. We calculated the normalized log-frequency of transformation to penicillin nonsusceptibility as the logarithm (base 10) of the ratio of penicillin-nonsusceptible transformants to trimethoprim-resistant transformants in each experiment, using the ratio to control for possible interexperiment variation in conditions. If capsule homology promotes *pbp* gene exchange, then this frequency should be elevated in donor-recipient pairs of the same serotype.

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TABLE 1. Normalized log-frequency (standard deviation) of transformation to penicillin nonsusceptibility^a

Recipient serotype	Donor serotype ^b			
	8	9V	11A	14
8	-0.52 (0.06)	-0.69 (0.10)	-0.50 (0.07)	-0.68 (0.09)
9V	-2.28 (0.10)	-2.49 (0.27)	-1.96 (0.06)	-2.46 (0.48)
11A	-1.18 (0.07)	-1.21 (0.07)	-1.51 (0.08)	-1.39 (0.12)
14	-1.76 (0.28)	-1.84 (0.02)	-1.63 (0.15)	-1.64 (0.20)

^a See text for explanation.

^b Boldface indicates frequencies for homologous pairs.

The data (shown for 0.04 mg of penicillin/liter in Table 1 and not shown, but similar except for a ~3-fold-lower frequency, for 0.05 mg of penicillin/liter) suggested that homologous transformation frequencies were almost exactly those expected based on heterologous transformation frequencies of the same types. Formally, we tested for such an effect by a linear regression of log-frequency against dummy variables for each donor and each recipient and a dummy variable for homologous transformation. The effect of homology was estimated as -0.1 (95% confidence interval, -0.3, 0.1), indicating that the effect of homology was nearly zero and at most quite small, associated with between a 24% reduction and a 14% increase in transformation frequency.

In our experimental system, the presence of a homologous capsule in donor and recipient does not increase the transformation frequency of penicillin binding proteins conferring nonsusceptibility to β -lactams. A limitation of this study is the artificial, in vitro setting, in which transformation was performed in the presence of competence-stimulating peptide (7). With the caveat that in vivo conditions may differ, we reject our initial hypothesis that such a mechanism could account for the serotype association of penicillin nonsusceptibility in pneumococci. The mechanism(s) underlying this association should be general enough to account for the association of resistance to other, unrelated drug classes with the same serogroups (4, 6).

Interestingly, we found that transformation to penicillin resistance was as efficient for serotypes rarely resistant to penicillin, 8 and 11A, as for types commonly found to be resistant, 9V and 14 (raw transformation frequency data not shown). Pediatric strains of serotypes-serogroups 6, 9, 14, 19, and 23 are carried for long periods of time, perhaps resulting in more exposure to selecting antimicrobial agents and/or to donor DNA from resistant strains (1, 5). Our findings suggest that the serotype association with drug resistance in general, and penicillin resistance in particular, is probably due not to an increased ability to generate resistant variants but to an in-

creased frequency of exposure to transforming DNA (due to longer carriage) and/or to greater selective pressure from antimicrobials (because susceptible strains are more likely to be cleared by antibiotic treatment when they are carried for a long period).

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