Efficient Dissemination of Integrative and Conjugative Elements Conferring Multidrug Resistance in *Streptococcus pneumoniae* in an *Ex Vivo* Human Nasopharyngeal Biofilm

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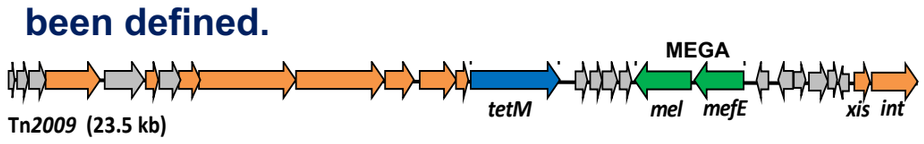
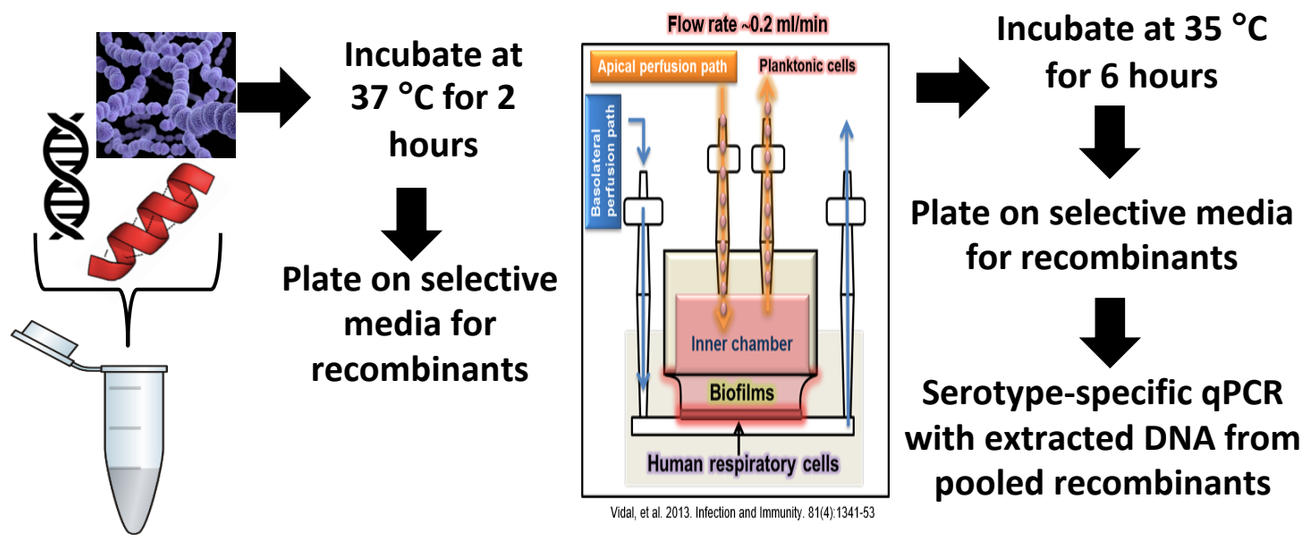
Background and Aims

- Naturally competent *Streptococcus pneumoniae* (*Spn*) horizontally exchanges >20 kb multidrug resistance elements known as ICEs.
- ICE element Tn*2009* (23.5 kb) carries *tetM* and the Macrolide Efflux Genetic Assembly (Mega), conferring tetracycline and macrolide resistance, respectively.
- Although *Spn* can transform 2-6 kb of DNA *in vitro* and >25 kb *in vivo*, the molecular mechanism for large ICE dissemination has not been defined.

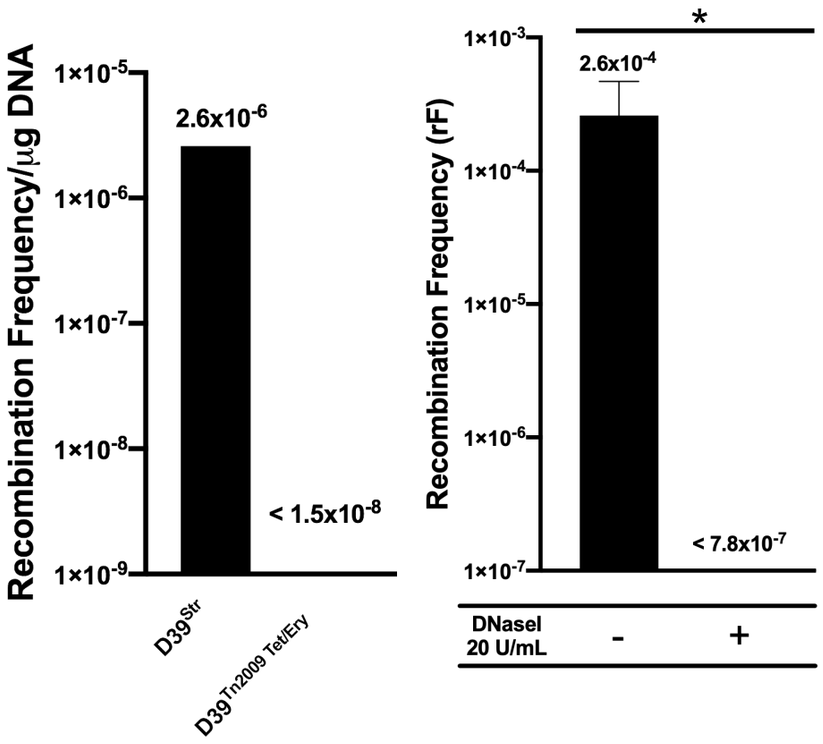


Methods



Results

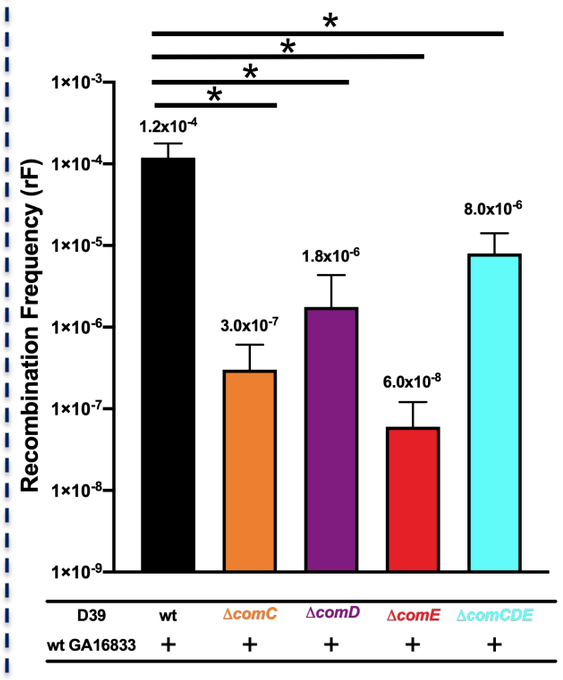
Tn*2009* was not taken up by recipient D39 via *in vitro* transformation (left). However, Tn*2009* from *Spn* donor GA16833$^{Tet/Ery}$ was transferred to recipient D39^{Str} in the *ex vivo* nasopharyngeal biofilm bioreactor, which was prevented with DNaseI addition (right).



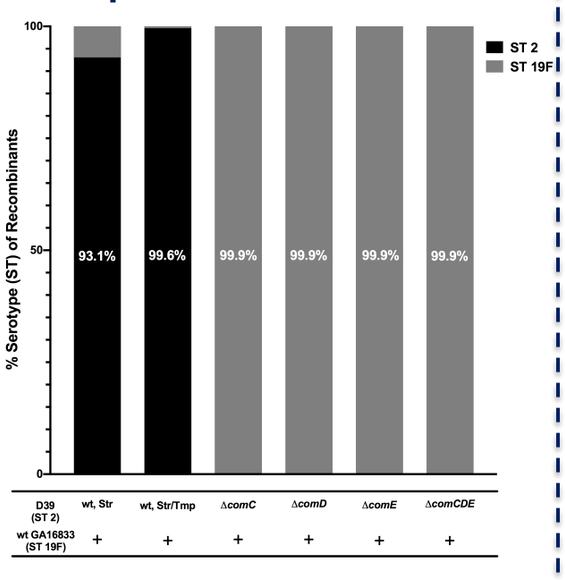
Whole genome sequencing of bioreactor D39 recombinants revealed that intact Tn*2009* is transferred within variably-sized, flanking donor DNA fragments, suggesting ICE integration via homologous recombination.

Recombinant Antibiotic Selection	Upstream of Tn*2009*	Downstream of Tn*2009*
Tet+Str	9 kb	7 kb
Ery+Str	12 kb	30 kb
Tet+Ery+Str	13 kb	3 kb
Ery+Str+Tmp	5 kb	7 kb

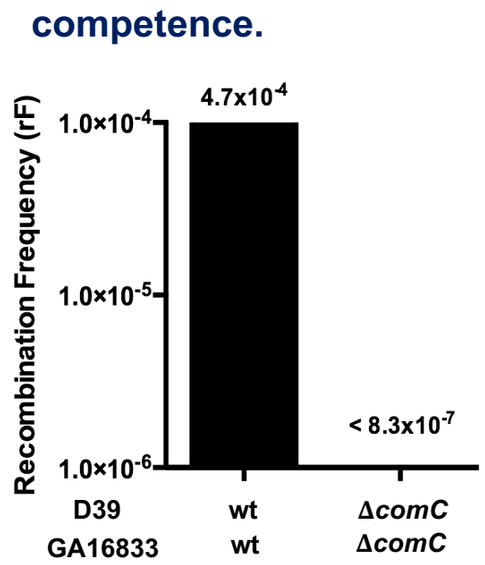
Competence development by recipient D39^{Str} was required for uptake of Tn*2009* from donor GA16833.



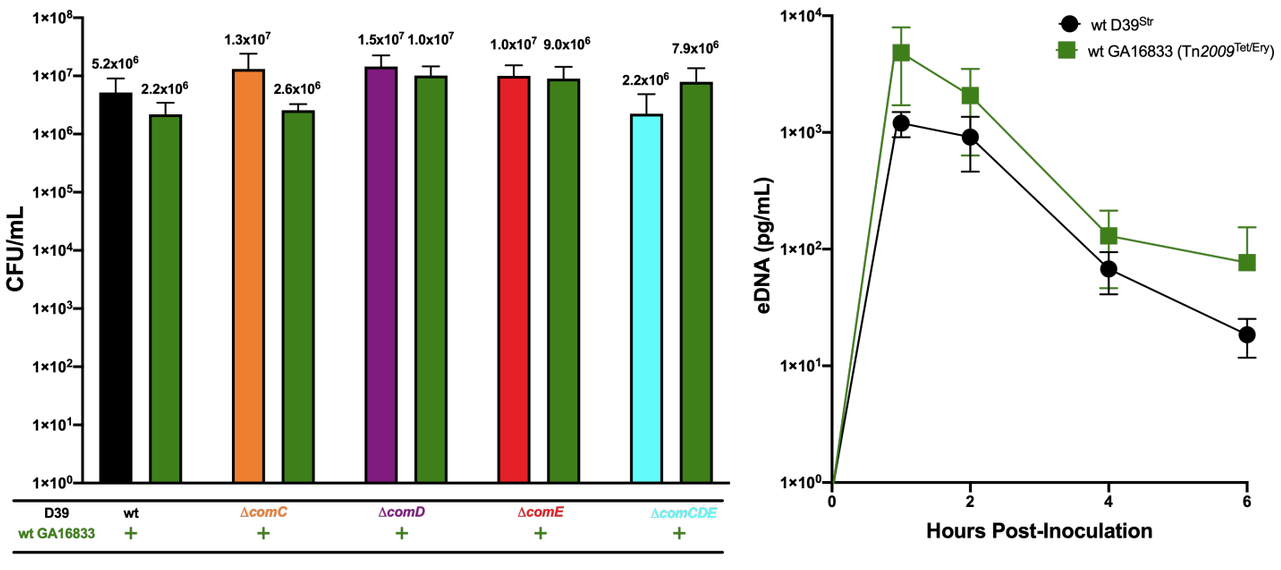
Competence inactivation of recipient D39^{Str} allowed for GA16833 to acquire Str resistance.



No recombinants are recovered when D39 and GA16833 are unable to develop competence.



Similar bacterial densities and extracellular DNA concentrations from both strains in the bioreactor co-inoculations ensured equal opportunity for recombination.



Conclusion

- Tn*2009* dissemination among *Spn* strains requires competence development (*comCDE*) by the recipient strain.
- ICE transference is highly efficient in the *ex vivo* bioreactor system and yields a 10,000-fold higher recombination frequency as compared to *in vitro* conditions.
- A *com*-mediated dominance influences the spread of antibiotic resistance determinants carried by ICEs.

Acknowledgements

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