

Figure S1: Schematic illustration of the study design and experimental procedures performed on the clinical strains (Hi-122, Hi-142, Hi-143, Hi-147, and Hi-228), recipient strain (Rd-Rif), and transconjugants.

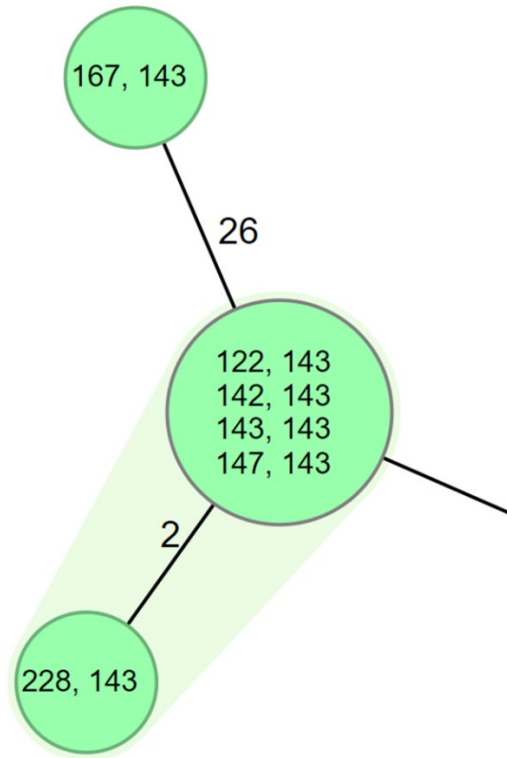


Figure S2: Section of Minimum Spanning Tree (MST) showing results from conventional and core-genome multi-locus sequence typing (MLST and cgMLST) of the five strains investigated in this study together with a collection of 213 clinical isolates of *H. influenzae* from Norway and Sweden (BioProject PRJEB49398<sup>1</sup>). MST cluster distance threshold = 25. Strains Hi-122, Hi-142, Hi-143, Hi-147, Hi-228 belonged to the same MST cluster and were epidemiologically related (Oslo, Norway, June 2016 – January 2017). The closest related strain (Hi-167) was collected in a different geographical area (Trøndelag county, Norway, July 2017) and lacked transferable resistance genes. The closest related ST was ST183 (561 allelic differences). Numbers within circles indicate ID and ST; numbers between circles indicate allelic differences.

<sup>1</sup> [Cefotaxime-resistant Haemophilus influenzae \(ID 815576\) - BioProject - NCBI \(nih.gov\)](https://www.ncbi.nlm.nih.gov/bioproject/49398)

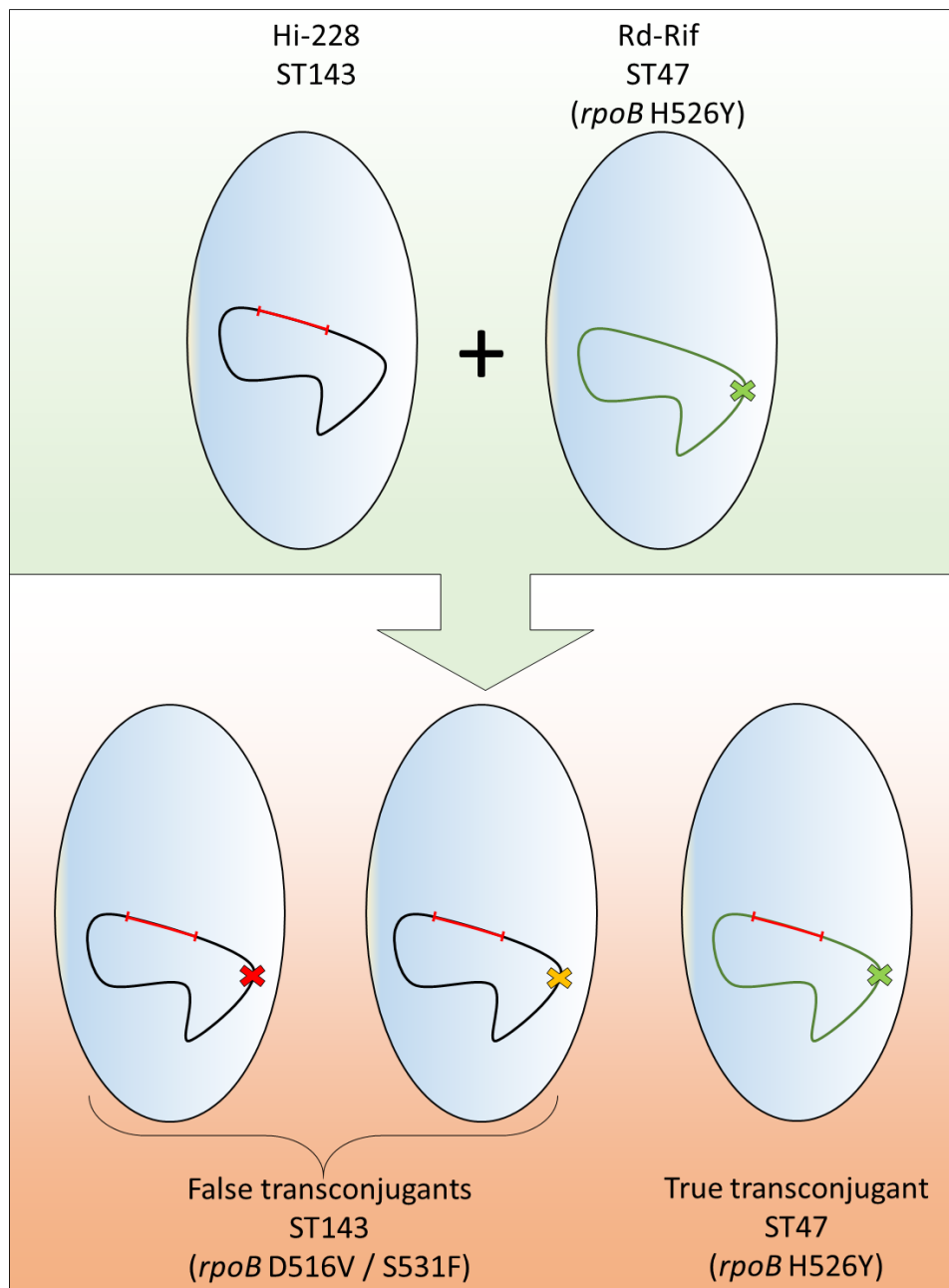


Figure S3: Illustration of the conjugation experiments performed in this study, resulting in both false and true transconjugants. Top: donor (Hi-228) and recipient (Rd-Rif), bottom: false and true transconjugants. When plated on selective agar plates (rif<sup>+</sup>azm plates), a relatively high proportion of Hi-228 colonies acquired rifampicin resistance-associated substitutions in the *rpoB* gene, resulting in false transconjugants. *Tn7100* indicated in red, donor genome indicated in black, recipient genome indicated in green, substitutions in the *rpoB* gene indicated by colored crosses and described in the figure.