Supplemental Methods

Genetic complementation. To genetically complement gene disruptions, a system for chromosomal complementation was developed, as no plasmids have been identified to date for use in *K. kingae*. A site within a ~1500bp region in the *K. kingae* strain 269-492 genome that is devoid of ORFs, tRNA genes, and other obvious genetic elements was chosen for the complementation locus. The 5' homologous targeting region was amplified from strain 269-492 with primers 5'comp F and 5'comp R, digested with MunI and EcoRI, and ligated into EcoRI-digested pUC19, generating pC5'. Digestion and sequencing were used to confirm proper orientation of the insert resulting in destruction of the correct EcoRI site. The 3' homologous targeting region was amplified from strain 269-492 with primers 3'comp F and 3'comp R, digested with HindIII, and ligated into HindIII-digested pC5', generating pC5'3'. Sequencing was used to confirm proper orientation of the insert. The *ermC* erythromycin-resistance cassette was amplified from pIDN4 with primers compErm F and compErm R, digested with EcoRI and KpnI, and ligated into EcoRI/KpnI-digested pC5'3', generating pCErm. The resulting construct retains a portion of the pUC19 multiple cloning site (MCS) containing the restriction sites KpnI, BamHI, XbaI, SalI, PstI, and SphI for use in cloning the complementation inserts.

To complement the *knh* disruption, the *knh* gene and ~300bps of sequence upstream of the start codon were amplified with primers comp*knh* F and comp*knh* R, digested with KpnI and BamHI, and ligated into KpnI/BamHI-digested pCErm, generating pCE/*knh.* The construct was then linearized with NdeI and transformed into KK03*knh*. To complement the *pilT* disruption, the *pilTU* operon and ~300bps of sequence upstream from the start codon were amplified with primers comp*pilTU* F and comp*pilTU* R, digested with KpnI and BamHI, and ligated into KpnI/BamHI-digested pCErm, generating pCE/*pilTU*. Due to the fact that the *K. kingae pilT* mutant is not competent for transformation, pCE/*pilTU* was linearized with NdeI and transformed into strain KK03 first, followed by targeted disruption of the WT *pilT* gene.

Transcript levels. RNA was extracted using TRIreagent (Sigma) and the RNeasy minikit by following the lipid-rich tissue protocol (Qiagen). Residual DNA was digested with RQ1 DNase (Fisher Scientific), which was inactivated prior to generating cDNA with random hexamers and Superscript II (Invitrogen). Primer sets used in these experiments are listed in Table S1. To normalize RNA/cDNA levels, *ftsZ* was used as the internal control. Error bars represent standard deviation of three independent experiments.