Identification of genes important for survival of *Yersinia pestis* in a flea vector
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*Yersinia pestis*, the bacterial causative agent of plague, is transmitted to mammalian hosts via flea bites and the number of plague cases has been increasing in recent decades. *Y. pestis* is also a potential candidate to be used as a biological weapon and there is currently no vaccine licensed in the U.S., making this a concern for public health. Therefore, understanding the genetic factors that play a role in allowing *Y. pestis* to maintain successful flea infections and undergo transmission to humans is very important. Unfortunately, few of these “transmission factors” are currently known.

To better understand mechanisms involved in survival in the flea, a library of *Y. pestis* transposon mutants, created through signature-tagged mutagenesis (STM), was introduced into a natural flea vector to discover mutants that were unable to maintain an infection. Three mutants that displayed decreased survival in fleas also showed increased sensitivity to antibiotics similar to factors that fleas produce in order to deal with infection. The antibiotic-resistant phenotype was not regained when the STM mutants were complemented with wild-type copies of the transposon-disrupted genes. This indicates that the antibiotic-sensitive phenotype in these mutants was not due to a single gene inactivation that can be easily restored simply by reintroduction of the wild type gene.

Other possible causes of the observed antibiotic sensitivity in these transposon mutants that were investigated include: altered genomic content in the mutants, effects due to gene copy number in complemented strains, and polar effects of transposon insertions on neighboring genes. PCR screening for the presence of native *Y. pestis* plasmids in the STM mutants indicated that the mutants all retained their expected plasmid content. Complementation of the mutant strains with larger genomic regions encompassing the target gene, as well as neighboring genes on either side, did not restore antibiotic resistance, suggesting that a polar effect on genes in the immediate vicinity of the transposon insertion is unlikely to explain the antibiotic-sensitive phenotype.

The inability to restore wild-type phenotypes may be due to improper gene expression levels in the mutants or complementation constructs. To further investigate these mutants, new sets of low copy number plasmids with inducible promoters are under construction for additional complementation studies. By controlling gene expression levels, these new vectors may enable the identification of the genetic factors responsible for antibiotic sensitivity in these mutants and shed light on novel mechanisms involved in the survival of *Y. pestis* in fleas.