

Mobile Genetic Elements and Resistance in Enterococci

KEYWORDS

ENTEROCOCCUS; CONJUGATION, GENETIC; ANTIBIOTICS

In the past decade there have been dramatic increases in the prevalence of multi-resistant gram-positive pathogens in United States hospitals, notably ampicillin- and vancomycin-resistant enterococci (VRE). Unknown until the late 1980's, VRE now represent roughly 25% of enterococci isolated from infections throughout the hospital. Recent data convincingly associates infection with these strains and increased mortality, amplifying the importance of understanding their spread and preserving the activity of the few antibiotics available for treatment. Our laboratory has focused for years on the mechanisms by which enterococci stockpile and disseminate multiple resistance determinants. We propose to continue this work by completing our structural analysis of Tnvamp, a large chromosomal element that transfers both vancomycin and ampicillin resistance between strains of *Enterococcus faecium*. Data accumulated in experiments funded by the present Merit Review have allowed us to refine our hypotheses regarding the structure and movement of this important resistance element. We now have compelling evidence that transfer of Tnvamp begins by a circularization of the element and ends (most commonly) by recombination with the recipient chromosome across homologous sequences.

The first specific aim of the present proposal is to fully characterize the structure of Tnvamp as it exists in transconjugants CVI 33 and T2. Once we have identified the limits of the element in these two transconjugants, we will compare these limits with other transconjugants, to determine whether transfer occurs consistently with the same discreet package of DNA. These structural studies are important to understanding Tnvamp, but also have the potential to suggest important related projects. One such related project, that has recently been funded by a NIH RO-1 grant, is an analysis of the factors responsible for high-level ampicillin resistance in enterococci.

The second specific aim will test our hypothesis that transfer of Tnvamp is encoded within the embedded VanB-type Tn916-like transposon Tn5382. There are several open reading frames within Tn5382 that exhibit significant homology with genes associated with DNA transfer in other species. Using a shuttle vector that is temperature-sensitive for replication, we will interrupt candidate open reading frames within Tn5382. Once the gene interruptions are confirmed, we will test the impact of the interruptions on transfer of Tnvamp. If we find that transfer is reduced or eliminated with the disruption of the gene, we will reintroduce the interrupted gene on a plasmid and determine whether we can complement these functions in trans.

The third specific aim represents a somewhat new direction for our laboratory, although it employs techniques that have been used for years. We will investigate the mechanisms by which enterococci become resistant to linezolid, the most promising of the recently introduced antibiotics with activity against VRE. We will first test the hypotheses that linezolid resistance is due to a point mutation within the 23S ribosomal RNA and that the level of resistance correlates with the absolute or relative number of 23S rRNA genes that have the mutation. Our initial strategy will be to place the 23S rRNA gene downstream of the inducible vanHB/vanB/vanXB promoter and introduce this into a VanB enterococcal strain on shuttle plasmid pTCV-lac. In this construct, we should be able to increase expression of a mutated 23S rRNA gene in the presence of vancomycin and examine the correlation between exposure to vancomycin and linezolid resistance. If we are able to make this inducible system work, we will proceed to mutate several sites within the 23S gene, that have been implicated in prior studies of resistance, in an effort to obtain detailed structure function information on the interaction of linezolid, and by extension other antibiotics of this class. These investigations will yield important information about the mechanisms by which enterococci acquire and disseminate resistance genes, knowledge that will inform future strategies for minimizing resistance spread. They will also yield important information about structural details of the mechanism of action of an important new class of antimicrobial agents, information that will be useful in the design and development of newer members of this class.