

Microbial Strategies for Acquiring Resistance

T.A. Cebula and J.E. LeClerc

Microbes are simple critters. They eat to survive. They survive to divide and multiply. And division and multiplication give the vast reservoir of organisms from which genetic variants arise. As William Hayes pointed out years ago, it is particular to the realm of microorganisms that only such numbers allow the needed mutant to be culled from its environment, now to survive and multiply in a changed and hostile one (ref. 1). Viewing the antibiotic resistance problem from this simple perspective, it is not peculiar that resistant mutants, preexisting even at barely detectable numbers, rise to prominence in a population of pathogens confronted by exposure to antibiotic in an otherwise sumptuous environment.

Much of what we know about the processes that give rise to resistant mutants (DNA replication, repair and mutagenesis) and the mechanics of selection that drive these resistant mutants to prominence was learned from the laboratory-attenuated strains of *Escherichia coli* K12 and *Salmonella typhimurium* LT2. The current clinical situation, however, in which antibiotic resistance is increasing dramatically among human pathogens, compels us to inspect "natural" strains, rather than lab-attenuated ones, and there we have much to learn about the mechanisms and mechanics that bacteria use in their feral settings. Do these bacteria possess novel ways to control mutation in response to changing environments? Does recent experience with natural strains cause us to rethink notions about optimal rates of mutation? And does exchange of DNA between organisms, now recognized as the important determinant of virulence factors, bear as well on the origination of chromosomal resistance factors?

We found one means that natural strains may use to spawn genetic variants is the hypermutability phenotype, a surprising result since hypermutable strains (mutators) are thought to be rare among bacterial populations due to the deleterious effects of mutations (ref. 2). Examination of isolates of pathogenic *E. coli*, including serotype O157:H7, and *Salmonella* outbreak strains showed the unexpectedly high incidence of mutators, accounting for 1-5% of isolates compared with an expected frequency of <0.001%. Genetic analysis revealed that the defects responsible for the mutator phenotype in these strains were in the methyl-directed mismatch repair pathway; they are therefore more prone to both chromosomal mutation and promiscuous exchange of DNA between species. More particularly, eight out of ten mutators carried defects in the *mutS* region of the chromosome, a region that shows extensive genetic variability among the *E. coli*, *Shigella*, and

Salmonella strains that we have analyzed and among the set of genomes for which the entire sequence is known. It is in this region, for instance, that the *Salmonella* 40-kb pathogenicity island (SPI 1) is located (ref. 3), thought to have been acquired by a horizontal transfer event. The increased promiscuity associated with the mutator phenotype may be a mechanism for pathogens, or potential pathogens, to gain genetic traits such as these virulence genes or the development of new antibiotic resistances. In turn, strong selection for a phenotype as beneficial as antibiotic resistance causes the bacterial pathogen--an asexual organism--to carry along the mutator locus that likely spawned it.

Molecular characterization of the genomes of emerging pathogens has revealed the presence of genetic elements suggesting that these genomes are indeed mosaics of old, familiar pathogens. The *mutS* region in *E. coli* O157:H7 contains unique DNA sequence (3 kb) not found in the lab-attenuated *E. coli* K12 strain. It is likely to have been derived from *Shigella dysenteriae* by gene exchange, as evidenced by identical sequence abutting a mobile insertion element (IS1) found in *Shigella*. Related sequence (85% similarity) in inverted orientation is found in *Salmonella typhimurium*. Another novel element (0.5 kb) located upstream of the *mutS* gene in *Shigella dysenteriae* has been identified at the same nucleotide site in the uropathogenic strain ECOR48, a mutator, and in an enteropathogenic *E. coli* of the O55:H7 serotype, the closest known siblings of O157:H7 strains (4). Such results make possible the construction of molecular pathways to describe the evolution of these emerging pathogens. These data could also help to explain how resistance determinants become linked and are now so readily inherited.

References

- (1) Hayes, W *The Genetics of Bacteria and their Viruses* (Wiley, New York, 1968) pp. 179-223.
- (2) LeClerc, JE, Li, B, Payne, WL and Cebula, TA (1996) *Science* **274**, 1208-1211.
- (3) Mills, DM, Bajaj, V and Lee, CA (1995) *Mol. Microbiol.* **15**, 749-759.
- (4) Whittam, TS, Wolfe, ML, Wachsmuth, IK, Orskov, F, Orskov, I and Wilson, RA (1993) *Infect. Immun.* **61**, 1619-1629.