

Triple threat: One bacterium, three plasmids

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Researchers from Australia found something completely new while conducting a genetic study of the pathogenesis of an enteric disease in birds. They report what is believed to be the first bacterial strain to carry three closely related but independently conjugative plasmids.

The authors were researching the pathogenesis of avian necrotic enteritis, a disease caused by the bacterium *Clostridium perfringens* which causes necrotic lesions in the [small intestines](#) of infected birds. This disease is economically important to the [poultry industry](#) as acute clinical disease leads to increased mortality of birds and subclinical disease leads to decreased weight and loss of productivity.

In order to cause disease, the bacterium must be capable of producing the toxin netB. In this study the researchers examined the location of netB gene within the Australian necrotic enteritis isolate EHE-NE18, a strain that was also resistant to tetracycline. They found the gene was encoded on a plasmid, a piece of DNA which exists inside the bacterium but is separate from the chromosomal DNA. Plasmids can also replicate independently and can be transferred to other bacteria.

Additional research identified two other plasmids that shared a similar DNA sequence but coded for different processes. One encoded a different toxin, called beta2, and the other coded for the tetracycline resistance.

"To our knowledge, this is the first report of a bacterial strain that carries three closely related by different independently conjugative plasmids.

These results have significant implications for our understanding of the transmission of virulence and [antibiotic resistance genes](#) in [pathogenic bacteria](#)," write the authors.

More information: Necrotic Enteritis-Derived *Clostridium perfringens* Strain with Three Closely Related Independently Conjugative Toxin and Antibiotic Resistance Plasmids, 27 September 2011 *mBio* vol. 2 no. 5 e00190-11. doi: 10.1128/mBio.00190-11

ABSTRACT

The pathogenesis of avian necrotic enteritis involves NetB, a pore-forming toxin produced by virulent avian isolates of *Clostridium perfringens* type A. To determine the location and mobility of the netB structural gene, we examined a derivative of the tetracycline-resistant necrotic enteritis strain EHE-NE18, in which netB was insertionally inactivated by the chloramphenicol and thiamphenicol resistance gene catP. Both tetracycline and thiamphenicol resistance could be transferred either together or separately to a recipient strain in plate matings. The separate transconjugants could act as donors in subsequent matings, which demonstrated that the tetracycline resistance determinant and the netB gene were present on different conjugative elements. Large plasmids were isolated from the transconjugants and analyzed by high-throughput sequencing. Analysis of the resultant data indicated that there were actually three large conjugative plasmids present in the original strain, each with its own toxin or antibiotic resistance locus. Each plasmid contained a highly conserved 40-kb region that included plasmid replication and transfer regions that were closely related to the 47-kb conjugative tetracycline resistance plasmid pCW3 from *C. perfringens*. The plasmids were as follows: (i) a conjugative 49-kb tetracycline resistance plasmid that was very similar to pCW3, (ii) a conjugative 82-kb plasmid that contained the netB gene and other potential virulence genes, and (iii) a 70-kb plasmid that carried the cpb2 gene, which encodes a different pore-forming toxin, beta2 toxin.

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