

D001

Clarithromycin Resistance in *Helicobacter pylori*: Resistance Mechanism and Effect of Clarithromycin Resistance on Bacterial Eradication in Infected Adults

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Outcome of *Helicobacter pylori* infection was analyzed in 114 dyspeptic patients treated with a triple therapy including clarithromycin. The clarithromycin resistance (20.2%) was mainly caused by an A2142G mutation in the 23S rRNA gene of *H. pylori*. *H. pylori* eradication was obtained in all patients with clarithromycin-susceptible isolates but not in patients with clarithromycin-resistant isolates ($P = 0.0001$). We conclude that there is a significant difference in bacterial eradication rate between patients infected with clarithromycin-resistant *H. pylori* isolates and clarithromycin-sensitive ones. These results do not appear to be biased by any differences in pathogenicity because of the similar CagA expression statuses of the isolates in both groups. We also suggest that *H. pylori* antimicrobial susceptibility testing of first biopsy culture is useful before choosing the first triple therapy in infected patients and that clarithromycin should not be used in case of primary resistance.

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D002

***Caenorhabditis elegans* as a Model Host for *Escherichia coli* O157:H7**

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Caenorhabditis elegans has been a model host for many bacterial pathogens. In this study, we determine whether the animal can be a model host for *Escherichia coli* O157:H7 causing hemolytic uremic syndrome, which is fatal to human host. The cell number of *C. elegans* was scored over 14 days while cells in L4 stage were continuously fed with several bacterial strains including *E. coli* O157:H7(O157) on NGM plate over the period. The time required for 50% of *C. elegans* to die (time to death 50, TD50) when feeding on *E. coli* O157 was determined to be 4 days, whereas TD50 when fed with *Salmonella typhimurium* was 5 days. The intoxication with shiga toxins, potent toxins, may be the main cause of the marked reduction of the life span. Currently, we are studying the cause of the worm death by less-virulent O157 mutants (*stx1*, *stx2*, *eae*, etc). Based on these results, it is suggested that *C. elegans* is a good host model to study the pathogenesis of *E. coli* O157:H7.

D003

Expression and Purification of a Chimeric Protein BoNT/A(Hc)-PA4 for Dual Protection Against Anthrax and Botulism

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Botulism and anthrax are regarded as major bioterror target diseases. Carboxy terminus of the botulinum neurotoxin type A heavy chain [BoNT/A(Hc)] and carboxy-terminal domain of protection antigen (PA4) are known as binding sites to each receptor. To develop a vaccine candidate which contains dual protection against anthrax and botulism, a chimeric protein consisting of a BoNT/A(Hc) linked to the PA4 was constructed and expressed in *Bacillus brevis* by using pNU212, and purified by gel filtration on a Hiloal 16/60 Superdex 200 column followed by anion exchange on a fast performance liquid chromatography with a Mono-Q column. The purified recombinant protein was obtained with yield of 2 mg/liter from this procedure, and was confirmed by SDS-PAGE and immunoblot analysis. FPLC-purified BoNT/A(Hc)-PA4 chimeric protein was used to vaccinate BALB/c mice, and could induce IgG specific for both *C. botulinum* toxin A and *B. anthracis* PA. These results suggest a possibility of the BoNT/A(Hc)-PA4 chimeric protein as a potential vaccine candidate to protect against both anthrax and botulism. [Supported by grants from KNIH]

D004

Detection of *Melissococcus pluton*, *Paenibacillus alvei* and *Bacillus subtilis* by Multiplex PCR Analysis

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Melissococcus pluton is the causative agent of European foulbrood (EFB), a disease of honeybee larvae. EFB was related to *Streptococcus apis*, *Paenibacillus alvei* and *Bacterium eurydice*. The other bacteria considered to cause EFB are generally associated secondary invader bacteria which dramatically accelerated the death of the infected larvae. Also, *Bacillus subtilis* may be existent at the infected larvae because of soil bacteria. We had a grasp of the problem that was confirmed by Multiplex PCR Analysis. The method was used to confirm each DNA sequences in the specific 16S rRNA gene for *Melissococcus pluton*, *Paenibacillus alvei* and *Bacillus subtilis*. It improved sensitive diagnosis and rapid species identification. This detection-system would be useful for the control of EFB in apiculture.