Susceptibility to these drugs increased as much as 32-fold for the ΔcptAΔpmrC and ΔpmrAB mutants. At the same time, susceptibility to the hydrophilic antibiotics cephalothin and norfloxacin, which pass through outer membrane porins, remained unchanged. Susceptibility to vancomycin, a hydrophilic antibiotic that does not pass through porins, and thus can only reach its target if the outer membrane is disrupted, increased 4-fold for the ΔcptAΔpmrC mutant. In support of a mechanism by which the addition of pEtN to LPS affects permeability and/or integrity of the outer membrane, we observed that outer membrane permeability to Nile Red, a hydrophobic and fluorescent compound, was increased for the mutants compared to wild-type. In addition, substantial outer membrane disruption was detected for the ΔcptAΔpmrC and ΔpmrAB mutants by monitoring the leakage of ß-lactamase from the periplasm. In conclusion, this work has revealed the importance of pEtN modifications of LPS in maintaining outer membrane integrity and restricting access to hydrophobic compounds through the bacterial outer membrane.

II43

Identification And Characterization Of A Helicobacter Sialyltransferase From Gt-42

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Helicobacter bizzozeronii, a canine gastric species belonging to the "Helicobacter heilmannii" type 2, is the only non-pylori Helicobacter spp. isolated in vitro from human patients with gastritis. "H. heilmannii" comprises at least five different Helicobacter species observed occasionally (0.17-2.3%) in gastric biopsies of human with upper gastrointestinal symptoms. The genome sequence of a canine (CCUG 35545T) and a human (CIII-1) H. bizzozeroni isolates, identified a cluster of 5 and 4 genes, respectively, involved in the biosynthesis and transfer of N-acetylneuraminic acid. We are characterizing this locus in different canine and human strains and we found that it is likely phase-variable. That locus shows high homology with a similar cluster of genes involved in the biosynthesis of sialylated LOS in Campylobacter jejuni. Moreover, H. bizzozeronii and H. acinonychis (a gastric Helicobacter linked to gastritis in large felines) seem to be the only Helicobacter species having these genes. The LPS profile analysis of multiple canine and human H. bizzozeronii strains performed with and without neuraminidase treatment verified the presence of sialic acid in the LPS. These results were confirmed by HPEAC-PAD analysis for both canine and human isolates. Moreover, preliminary dot blot analysis showed that H. bizzozeronii cells bind Cholera Toxin, suggesting mimicry with human gangliosides. We are presenting data on the cloning and characterization of the Cst-II like protein HBS-01 and HBS-02 which appear to encode GT-42 family sialyltransferases. We were able to show that HBS-02 is indeed a functional sialyltransferase with very different acceptor specificity from the Campylobacter Cst-I/II enzymes.

II47

Aurintricarboxylic Acid is a Potent Inhibitor of Influenza Ā and B Virus Neuraminidases

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Influenza viruses cause serious infections that can be prevented or treated using vaccines or antiviral agents, respectively. While vaccines are effective, they have a number of limitations, and influenza strains resistant to currently available anti-influenza drugs are increasingly isolated. This necessitates the exploration of novel anti-influenza therapies. We investigated the potential of aurintricarboxylic acid (ATA), a potent inhibitor of nucleic acid processing enzymes, to protect Madin-Darby canine kidney cells from influenza infection. We found, by neutral red assay, that ATA was protective, and by RT-PCR and ELISA, respectively, confirmed that ATA reduced viral replication and release. Furthermore, while pre-treating cells with ATA failed to inhibit viral replication, pre-incubation of virus with ATA effectively reduced viral titers, suggesting that ATA may elicit its inhibitory effects by directly interacting with the virus. Electron microscopy revealed that ATA induced viral aggregation at the cell surface, prompting us to determine if ATA could inhibit neuraminidase. ATA was found to compromise the activities of virus-derived and recombinant neuraminidase. Moreover, an oseltamivir-resistant H1N1 strain with H274Y was also found to be sensitive to ATA. Finally, we observed additive protective value when infected cells were simultaneously treated with ATA and amantadine hydrochloride, an anti-influenza drug that inhibits M2-ion channels of influenza A virus. Collectively, these data suggest that ATA is a potent anti-influenza agent by directly inhibiting the neuraminidase and could be a more effective antiviral compound when used in combination with amantadine hydrochloride.