Characterization and persistence of potential human pathogenic vibrios in aquatic environments

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i föreläsningssalen, våning 3, Guldhedsgatan 10A, Göteborg, torsdagen den 7 juni 2012 kl. 13.00

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Avhandlingen baseras på följande papper:

I B. Collin, A.-S. Rehnstam-Holm, S.-M. Ehn Börjesson, A. Mussagy and B. Hernroth
Characteristics of potentially pathogenic vibrios in sub tropic Mozambique compared to isolates from tropic India and boreal Sweden
Submitted

II B. Collin & A.-S. Rehnstam-Holm
Occurrence and potential pathogenesis of *Vibrio cholerae, Vibrio parahaemolyticus* and *Vibrio vulnificus* on the South Coast of Sweden.
FEMS Microbiology Ecology. 2011; 78: 306-313.

III B. Collin, A.-S. Rehnstam-Holm, B. Lindmark, A. Pal, S. N. Wai and B. Hernroth
The origin of *Vibrio cholerae* influences uptake and persistence in the blue mussels *Mytlius edulis*
Journal of Shellfish Research, 2012, 31: 87-92

IV B. Collin, B. Hernroth, and A.-S. Rehnstam-Holm
The importance of marine sediments as a reservoir for human pathogenic *Vibrio cholerae* in cold water conditions
Submitted

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Characterization and persistence of potential human pathogenic vibrios in aquatic environments

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Vibrio spp.*,* natural inhabitants of aquatic environments, are one of the most common causes of bacterial gastroenteritis in the world, being spread to humans via the ingestion of seafood, contaminated drinking water or exposure to seawater. The majority of *Vibrio* spp. are avirulent, but certain strains may sporadically be human pathogenic. *Vibrio cholerae* may cause cholera and fatal wound infections, *Vibrio parahaemolyticus* may cause gastroenteritis and *Vibrio vulnificus* may cause wound infections and sepsis. To expand current knowledge of the occurrence, ecological niche and persistence of potential human pathogenic *Vibrio* spp. in aquatic environments, occurrence and laboratory studies were performed.

The seasonal variation of *Vibrio* spp. in clams and mussels from Mozambique and Sweden were studied, with isolated strains characterized and compared with those isolated from water samples collected in India. Results showed that the numbers of *Vibrio* spp. in Mozambican clams peaked during the warmer rainy season and that the dominating species was *V. parahaemolyticus.* Biochemical fingerprinting and virulence screened by PCR revealed a high similarity among strains from the different aquatic environments. However, isolate functional hemolytic analyses and antibiotic resistance patterns differed between strains; Swedish and Indian strains were less sensitive to the tested antibiotics and had a lower hemolytic capacity than those from Mozambique. Molecular analysis of bacterial DNA from Swedish mussels showed the presence of the three *Vibrio* spp. most commonly linked with human illness, as well as their associated virulence genes. The strains isolated from marine and clinical environments were equally and highly harmful to the tested eukaryotic cells.

The persistence of clinical *V. cholerae* in aquatic environments was investigated *in vivo.* Strains were exposed to mussels, with bacterial uptake and elimination then examined. The mussels were able to avoid the most potent strain by complete closure of shells. The less potent strain was accumulated in mussel tissue in low levels and one marine control strain to a higher degree. Mussels eliminated the pathogenic strain less efficiently than they did the marine strain. One clinical and one marine strain were then exposed to 4°C for 21 days, with the temperature then increased to 20°C. The clinical strain was more prone to lose culturability than the marine strain at 4°C, the former performed significantly better in regaining culturability after the temperature up-shift. Subsequently, the persistence of the clinical strain in natural bottom sediment, incubating as above, was studied and results showed a similar decrease in culturable numbers in the sediment as in the water. As the clinical *V. cholerae* strains did not carry any of the standard set of virulence genes, the ability to change from non-culturable to culturable may be of great importance to strain pathogenicity. The results also show that natural bottom sediment may be a potential reservoir of human pathogenic *Vibrio* spp.

Key words: *Vibrio cholerae, Vibrio parahaemolyticus,* Mozambique, Sweden, molluscs, occurrence, persistence, sediment, TCBS, PCR, PhP, antibiotic resistance

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