Pathogenic *Vibrio* spp. – a health hazard in the German North Sea?

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Introduction

Vibrio spp. are ubiquitous bacteria naturally found in marine and estuarine waters. Pathogenic representatives of this genus can cause severe human diseases such as sea food poisoning and wound or ear infections. As temperatures above 20°C promote *Vibrio* spp. proliferation, vibrioses are primarily important in tropical and subtropical regions. However, since 1994, outbreaks of *Vibrio*-related wound infections among bathers have frequently been reported for the Baltic Sea coast following heat waves (e.g. Andersson & Ekdahl 2006, Hoyer et al. 1995, Lukinmaa et al. 2006, Statens Serum Institut 2006). For the more saline waters of the North Sea, only single cases of *Vibrio*-related wound infections have been reported so far (e.g. Schets et al. 2006). Due to a deadly case of *V. vulnificus* wound infection at the German Baltic Sea coast in 2003, the Governmental Institute of Public Health of Mecklenburg-Vorpommern and the Governmental Institute of Public Health of Lower Saxony (NLGA) have started to routinely investigate selected bathing sites along the German Baltic Sea and North Sea coast for *V. vulnificus* occurrence during summer. The organism was frequently found along the entire German Baltic Sea coast given that water temperatures exceeded 20°C (Hauk et al. 2010); at the North Sea coast, it so far occurred only sporadically (NLGA, pers. communication).
The number of *Vibrio*-related infections caused by pathogenic *Vibrio* spp. worldwide has steadily increased in recent years and there is growing concern that non-cholera-vibrioses may represent an emerging disease in a warming climate, even in the temperate seas of Northern Europe. Within the framework of the research program KLIWAS (Impacts of Climate Change on Waterways and Navigation), the Federal Institute of Hydrology thus initiated a yearlong monitoring program in cooperation with the NLGA in order to gain a better understanding of the ecology of potentially pathogenic *Vibrio* spp. in the German North Sea. Preliminary results from 11 bathing sites are shown here for the period September ´09 – May ´10; further findings will be presented at the conference.

Methods

Sampling site

The monitoring started in September 2009. Water and sediment samples for *Vibrio* analyses were taken monthly at 11 bathing sites along the North Sea coast and within the estuaries of the rivers Ems and Weser in the federal state of Lower Saxony (Germany). Water samples were retrieved as described in the EU bathing water directive 76/160/EEC. Sediment samples were taken in aseptic 100ml-polythene beakers. Sensor measurements were used to determine temperature and conductivities (as a proxy for salinity) at each site during sampling.

Vibrio analyses

Water and sediment samples were tested for *V. vulnificus*, *V. alginolyticus*, *V. parahaemolyticus* and *V. cholerae* using the most probable number series (MPN) approach. For sediment MPNs, declining volumes of sediment were directly weighed into tubes containing peptone water and incubated overnight at 36°C. MPN tube contents were inoculated onto TCBS and ChromeAgar plates and incubated again. For investigation of water samples, 10 ml of a series of water dilutions were filtered and filters incubated on TCBS and ChromeAgar overnight at 36°C. Suspicious colonies were confirmed via API tests.

Results

A total of 70 sediment and 72 water samples were retrieved between September 2009 and May 2010. Sampling in Bremerhaven, Dedesdorf and Burhave was started in December 2009. Due to ice cover, several bathing sites could not be sampled in winter (marked with* in Fig.). *V. vulnificus* was not detected at any of the sampling sites during the investigation period (-2°C to 18°C water temperature). *V. cholerae* (non-O1, non-O139-serovars) was detected thrice (Borkum, Duhnen, Dyksterhusen). *V. alginolyticus* was the predominant *Vibrio* species and detected at all sampling sites with 71% of water and 91% of sediment samples being positively tested. *V. parahaemolyticus* occurred at all sampling sites, however was only the second most dominant species with 39% of water and 64% of sediment samples being positively tested for this organism. Sediments were more often positively tested for these two species than water samples and germ concentrations were approximately 1 order of magnitude higher in sediments than in the water, a phenomenon already observed before (Vezzulli et al. 2009). Both species were detected at a wide range of salinities (1-33 ‰), however both detection rates and concentrations were lower at sampling sites that were influenced strongly either by the open sea (Norderney, Borkum) or fresh water (Dedesdorf, Bremerhaven, Fig. 1A & 1B). Concentrations as high as >1000 germs/100 mg (sediments) or >100 germs/100 ml (water), respectively, were observed at salinities between 11 and 28 ‰ only.

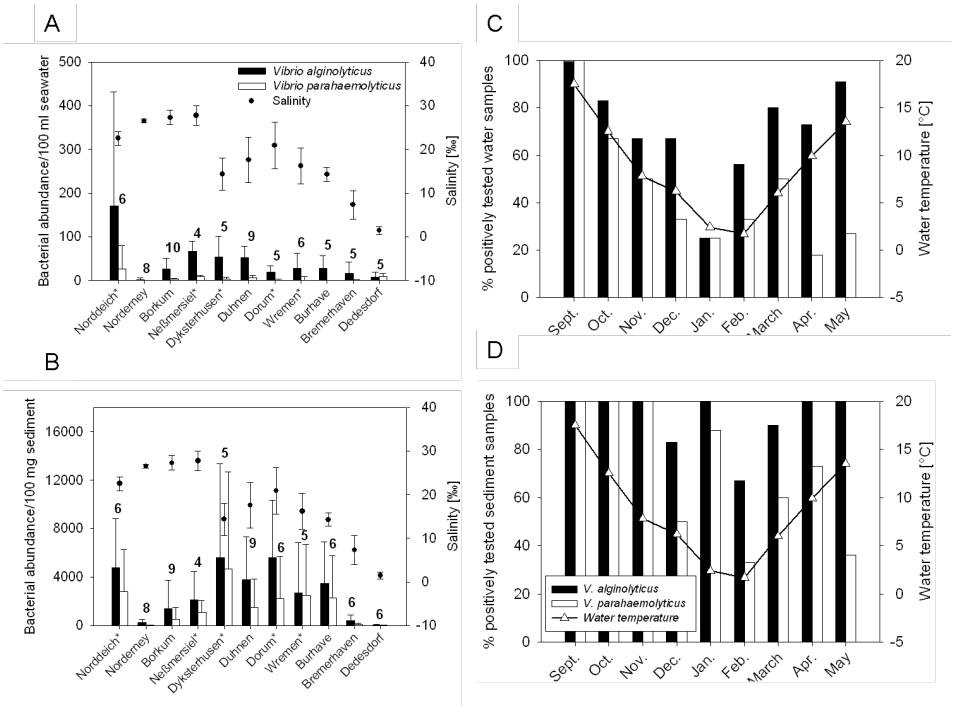

Fig. 1: Left: Mean *Vibrio* spp. concentrations for the period September 2009 to may 2010 separated by sampling sites in A: water samples, B: sediment samples. Numbers above columns indicate number of analyzed samples. Right: Temperature effect on detection rates at all 11 sampling sites in A: water samples, B: sediment samples.

Detection of *V. parahaemolyticus* and *V. alginolyticus* was strongly related to water temperature. While 100 % of water samples were positively tested in September (~18°C), only 25% of water samples were positively tested in January (~0°C; Fig. 2A). The temperature effect was less pronounced in the sediments, particularly for *V. alginolyticus* (Fig. 2B). Concerning absolute *Vibrio* numbers, the effect of temperature was not significant, however the data might be insufficient to draw definite conclusions.

Discussion

The preliminary results of this study show that potential pathogens such as *V. alginolyticus* and *V. parahaemolyticus* can be frequently found even in temperate coastal regions such as the German North Sea. Both organisms were detected at all 11 investigated bathing sites in Lower Saxony (Germany). Although *V. vulnificus* was so far the main causative of wound infections among bathers in Germany, reports from adjacent European countries, such as the Netherlands, Denmark and Poland, show that *V. alginolyticus* and *V. parahaemolyticus* may pose a severe human health hazard also in Northern Europe. Although detectable at a wide salinity range of 1 to 33 ‰, both organisms occurred more frequently and also more abundant at salinities of 11-28 ‰, which confirms previous findings. These salinities are predominantly found along the mainland coast of Lower Saxony and at the southern beaches of the East Frisian Islands, thus these areas might potentially be more at risk for *Vibrio* incidences than sites on the northern part of the islands or sites strongly influenced by fresh water. Presence and abundancies of *V. alginolyticus* and *V. parahaemolyticus* in the water were strongly related to temperature. Elevated abundancies at high temperatures suggest that climate change effects – rising sea water temperatures and higher frequencies and duration of heat waves - may potentially influence *Vibrio* spp. distribution in Northern Europe. Sediment Vibrios were consistently more abundant than those in the water and more resistant towards cold temperatures. Thus, sediments seem to serve as a potential hideaway and reservoir for these organisms in winter, especially for *V. alginolyticus*. The monitoring program will be continued (at least) until winter 2010 which will allow us to pinpoint more clearly possible trends in terms of *Vibrio* spp. ecology in the North Sea already during the conference. Further investigations will need to clarify the actual pathogenicity and growth preferences of isolated strains in order to allow an adequate risk assessment. Lab experiments on that effect are planned in the near future.

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