Publishable summary

This research investigated 1) the occurrence and distribution of antibiotic resistance (AR) in marine sediment bacteria (MSB) in relation to environmental antibiotic exposure, 2) the physiological costs for sustaining AR, 3) the effect of antibiotics on MSB community structure and function and 4) the potential transfer of AR bacteria along marine food chains. Sampling was performed in coastal waters off the town of Sisimiut, Greenland during the summers of 2009, 2010 and 2012. Sisimiut is Greenland´s second largest town (5000 inhabitants) with a regional hospital that has been operative since the 1950´s. Sisimiut was chosen as study site since it is located along a vast unpopulated pristine coastline, while simultaneously producing hospital and municipal sewage, which is discharged untreated into the bay "Ulkebugten" right off the town shore (Fig. 1). Pristine and highly polluted areas are thus found in close proximity.

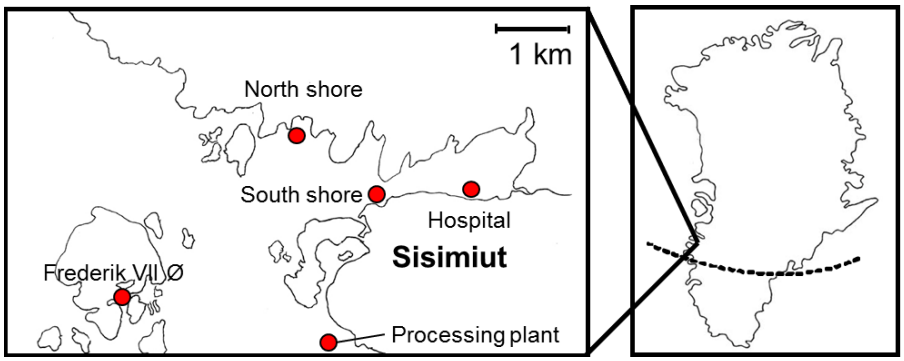

Figure 1. Greenland, Sisimiut and sampling sites (indicated with red circles).

Results show that AR was more prevalent in sediment bacteria communities closer to the sewage outlet as compared to the pristine site. Resistance towards natural (like Penicillin) and semi-synthetic β-lactams was present at all sites while resistance towards the synthetic fluoroquinolone Ciprofloxacin only occurred at the contaminated sites. Bacterial gut flora of mussels and stationary fish sampled at the same sites replicated the MSB-AR responses (Fig. 2). These results indicate that the occurrence of AR towards synthetic fluoroquinolones is anthropogenic while β-lactam resistance is part of the natural bacterial background AR. Results also show that AR patterns are recurrent at different trophic levels along the food chain within each site and that these AR patterns differ among sites depending on levels of sewage exposure. Results from analyses of sediment antibiotics concentrations and molecular analyses of resistance genes are yet to be retrieved. A sum of antibiotics tested and candidate genes to be analyzed is presented in Table 1.

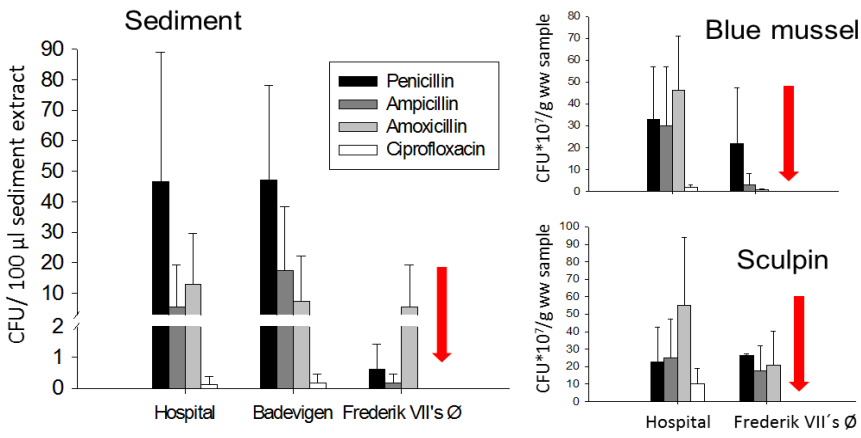

Figure 2. Number of bacterial colonies (CFU: Colony Forming Units) appearing on agar plates containing either of the four antibiotics. Bacteria originate from samples of gut tissue or marine sediments collected at sites located at increasing distance from the Sisimiut hospital sewage outlet, *i.e.* Hospital, North shore (only sediments), Frederik VII Ø. Numbers are means of six replicates ± SD. Arrows indicate lack of resistance towards Ciprofloxacin at the clean site, Frederik VII´s island.

Table 1. Antibiotics used for testing antibiotic resistance in marine sediment bacteria communities. "Candidate resistance genes" indicates genes mediating resistance to the corresponding antibiotic, and will be considered for qPCR analysis of DNA extracted from marine sediments.

Antibiotic	Group	Year tested for MSB-AR*	Candidate resistance genes
Penicillin G	β-laktam	2009, 2010, 2012	*cfx(A)*
Penicillin V	β-laktam	2010	
Ampicillin	β-laktam	2009, 2010, 2012	*blaTEM,bla-Q-1F,bla-Q-2F,amp(C)*
Amoxicillin	β-laktam	2009, 2010, 2012	
Meropenem	β-laktam	2012	
Erythromycin	Macrolide	2012	*erm(F),elm(A),erm(B),mef(a)*
Roxithromycin	Macrolide	2010	
Tetracyclin	Tetracyclin	2012	*tet (A),tet(C),tet(G),tet(M),tet (Q),tet(R)*
Ciprofloxacin	Fluoroquinolone	2009, 2010, 2012	*qnr(A),qnr(B),qnr(S)*
Gentamicin	Aminoglycoside	2012	*aacA-aphD*

*MSB-AR: marine sediment bacteria community associated antibiotic resistance determined using plate culturing techniques.

All aims and objectives have been assessed except number 2; the physiological cost for sustaining AR. Instead resources were used to investigate the impact of increasing levels of CO_2 in the world oceans, leading to ocean acidification, and antibiotics exposure on marine bacterial communities in sediments and the free water mass. Antibiotics are polar organic contaminants, which change forms, bioavailability and toxicity in relation to pH. Ocean acidification will lead to a change in the future marine environmental "toxicity map" of polar contaminants, yet this topic is vastly unexplored. Our findings show a clear predictable combined effect on both bacterial community structure and function in pelagic microbial communities (including both bacteria and phytoplankton) but not on soft sediment bacterial or meiofaunal communities.

The Recruited researcher (MG) has been running the research group independently, mainly involving BSc and MSc students (total of 8 persons over 3 years) and with shorter engagements of qualified laboratory technicians. Two other larger grants on the same topic were financed during the grant period. The host institute provided suitable laboratory facilities, administrative support and extensive teaching opportunities. At this point MG has left her Assistant Professorship at the host institute for an employment as researcher at the Norwegian Polar Institute, Norway. MG is still running this project at the host institute at distance through the employment of a postdoctoral fellow.