Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries

Antimicrobial resistance (AMR) in bacteria and associated human morbidity and mortality is increasing. The use of antimicrobials in livestock selects for AMR that can subsequently be transferred to humans. This flow of AMR between reservoirs demands surveillance in livestock and in humans. We quantified and characterized the acquired resistance gene pools (resistomes) of 181 pig and 178 poultry farms from nine European countries, sequencing more than 5,000 Gb of DNA using shotgun metagenomics. We quantified acquired AMR using the ResFinder database and a second database constructed for this study, consisting of AMR genes identified through screening environmental DNA. The pig and poultry resistomes were very different in abundance and composition. There was a significant country effect on the resistomes, more so in pigs than in poultry. We found higher AMR loads in pigs, whereas poultry resistomes were more diverse. We detected several recently described, critical AMR genes, including mcr-1 and optrA, the abundance of which differed both between host species and between countries. We found that the total acquired AMR level was associated with the overall country-specific antimicrobial usage in livestock and that countries with comparable usage patterns had similar resistomes. However, functionally determined AMR genes were not associated with total drug use.
A sampling and metagenomic sequencing-based methodology for monitoring antimicrobial resistance in swine herds

Objectives
Reliable methods for monitoring antimicrobial resistance (AMR) in livestock and other reservoirs are essential to understand the trends, transmission and importance of agricultural resistance. Quantification of AMR is mostly done using culture-based techniques, but metagenomic read mapping shows promise for quantitative resistance monitoring.

Methods
We evaluated the ability of: (i) MIC determination for Escherichia coli; (ii) cfu counting of E. coli; (iii) cfu counting of aerobic bacteria; and (iv) metagenomic shotgun sequencing to predict expected tetracycline resistance based on known antimicrobial consumption in 10 Danish integrated slaughter pig herds. In addition, we evaluated whether fresh or manure floor samples constitute suitable proxies for intestinal sampling, using cfu counting, qPCR and metagenomic shotgun sequencing.

Results
Metagenomic read-mapping outperformed cultivation-based techniques in terms of predicting expected tetracycline resistance based on antimicrobial consumption. Our metagenomic approach had sufficient resolution to detect antimicrobial-induced changes to individual resistance gene abundances. Pen floor manure samples were found to represent rectal samples well when analysed using metagenomics, as they contain the same DNA with the exception of a few contaminating taxa that proliferate in the extraintestinal environment.

Conclusions
We present a workflow, from sampling to interpretation, showing how resistance monitoring can be carried out in swine herds using a metagenomic approach. We propose metagenomic sequencing should be part of routine livestock resistance monitoring programmes and potentially of integrated One Health monitoring in all reservoirs.

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Impact of Sample Type and DNA Isolation Procedure on Genomic Inference of Microbiome Composition

Explorations of complex microbiomes using genomics greatly enhance our understanding about their diversity, biogeography, and function. The isolation of DNA from microbiome specimens is a key prerequisite for such examinations,
but challenges remain in obtaining sufficient DNA quantities required for certain sequencing approaches, achieving accurate genomic inference of microbiome composition, and facilitating comparability of findings across specimen types and sequencing projects. These aspects are particularly relevant for the genomics-based global surveillance of infectious agents and antimicrobial resistance from different reservoirs. Here, we compare in a stepwise approach a total of eight commercially available DNA extraction kits and 16 procedures based on these for three specimen types (human feces, pig feces, and hospital sewage). We assess DNA extraction using spike-in controls and different types of beads for bead beating, facilitating cell lysis. We evaluate DNA concentration, purity, and stability and microbial community composition using 16S rRNA gene sequencing and for selected samples using shotgun metagenomic sequencing. Our results suggest that inferred community composition was dependent on inherent specimen properties as well as DNA extraction method. We further show that bead beating or enzymatic treatment can increase the extraction of DNA from Gram-positive bacteria. Final DNA quantities could be increased by isolating DNA from a larger volume of cell lysate than that in standard protocols. Based on this insight, we designed an improved DNA isolation procedure optimized for microbiome genomics that can be used for the three examined specimen types and potentially also for other biological specimens. A standard operating procedure is available from https://dx.doi.org/10.6084/m9.figshare.3475406.

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Organisations: National Food Institute, Research Group for Genomic Epidemiology, University of Copenhagen
Authors: Knudsen, B. E. (Intern), Bergmark, L. (Intern), Munk, P. (Intern), Lukjancenko, O. (Intern), Priemé, A. (Ekstern), Aarestrup, F. M. (Intern), Pamp, S. J. (Intern)
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Aminobacter MSH1-Mineralisation of BAM in Sand-Filter Depends on Biological Diversity
BAM (2,6-dichlorobenzamide) is a metabolite of the pesticide dichlobenil. Naturally occurring bacteria that can utilize BAM are rare. Often the compound cannot be degraded before it reaches the groundwater and therefore it poses a serious threat to drinking water supplies. The bacterial strain Aminobacter MSH1 is a BAM degrader and therefore a potential candidate to be amended to sand filters in waterworks to remediate BAM polluted drinking water. A common problem in bioremediation is that bacteria artificially introduced into new diverse environments often thrive poorly, which is even more unfortunate because biologically diverse environments may ensure a more complete decomposition. To test the bioaugmentative potential of MSH1, we used a serial dilution approach to construct microcosms with different biological diversities. Subsequently, we amended Aminobacter MSH1 to the microcosms in two final concentrations; i.e. 105 cells mL−1 and 107 cells mL−1. We anticipated that BAM degradation would be most efficient at “intermediate diversities” as low diversity would counteract decomposition because of incomplete decomposition of metabolites and high diversity would be detrimental because of eradication of Aminobacter MSH1. This hypothesis was only confirmed when Aminobacter MSH1 was amended in concentrations of 105 cells mL-1. Our findings suggest that Aminobacter MSH1 is a very promising bioremediator at several diversity levels.

General information
Groundwater chemistry determines the prokaryotic community structure of waterworks sand filters

Rapid sand filtration is essential at most waterworks that treat anaerobic groundwater. Often the filtration depends on microbiological processes, but the microbial communities of the filters are largely unknown. We determined the prokaryotic community structures of 11 waterworks receiving groundwater from different geological settings by 16S rRNA gene-based 454 pyrosequencing and explored their relationships to filtration technology and raw water chemistry. Most of the variation in microbial diversity observed between different waterworks sand filters could be explained by the geochemistry of the inlet water. In addition, our findings suggested four features of particular interest: (1) Nitrospira dominated over Nitrobacter at all waterworks, suggesting that Nitrospira is a key nitrifying bacterium in groundwater-treating sand filters. (2) Hyphomicrobiaceae species were abundant at all waterworks, where they may be involved in manganese oxidation. (3) Six of 11 waterworks had significant concentrations of methane in their raw water and very high abundance of the methanotrophic Methylococcaceae. (4) The iron-oxidizing bacteria Gallionella was present at all waterworks suggesting that biological iron oxidation is occurring in addition to abiotic iron oxidation. Elucidation of key members of the microbial community in groundwater-treating sand filters has practical potential, for example, when methods are needed to improve filter function.
Fungal-bacterial consortia increase diuron degradation in water-unsaturated systems

Bioremediation of pesticide-polluted soil may be more efficient using mixed fungal-bacterial cultures rather than the individual strains alone. This may be due to cooperative catabolism, where the first organism transforms the pollutant to products which are then used by the second organism. In addition, fungal hyphae may function as transport vectors for bacteria, thereby facilitating a more effective spreading of degrader organisms in the soil. A more rapid mineralization of the phenylurea herbicide diuron was found in sand with added microbial consortia consisting of both degrading bacteria and fungi. Facilitated transport of bacteria by fungal hyphae was demonstrated using a system where herbicide-spiked sand was separated from the consortium by a layer of sterile glass beads. Several fungal-bacterial consortia were investigated by combining different diuron-degrading bacteria (Sphingomonas sp. SRS2, Variovorax sp. SRS16, and Arthrobacter globiformis D47) and fungi (Mortierella sp. LEJ702 and LEJ703). The fastest mineralization of 14C-labeled diuron was seen in the consortium consisting of Mortierella LEJ702, Variovorax SRS16, and A. globiformis D47, as measured by evolved 14CO2. In addition, the production of diuron metabolites by this consortium was minimal. Analyses of 16S rDNA suggested that bacteria were transported more efficiently by LEJ702 than by LEJ703. Finally, it was determined that the fungal growth differed for LEJ702 and LEJ703 in the three-member consortia. This study demonstrates new possibilities for applying efficient fungal-bacterial consortia for bioremediation of polluted soil. © 2013 Elsevier B.V.

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Organisations: University of Copenhagen, Aarhus University, Geological Survey of Denmark and Greenland
Authors: Ellegaard-Jensen, L. (Ekstern), Knudsen, B. E. (Intern), Johansen, A. (Ekstern), Albers, C. N. (Ekstern), Aamand, J. (Ekstern), Rosendahl, S. (Ekstern)
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Large-scale bioreactor production of the herbicide-degrading Aminobacter sp. strain MSH1

The Aminobacter sp. strain MSH1 has potential for pesticide bioremediation because it degrades the herbicide metabolite 2,6-dichlorobenzamide (BAM). Production of the BAM-degrading bacterium using aerobic bioreactor fermentation was investigated. A mineral salt medium limited for carbon and with an element composition similar to the strain was generated. The optimal pH and temperature for strain growth were determined using shaker flasks and verified in bioreactors. Glucose, fructose, and glycerol were suitable carbon sources for MSH1 ($\mu = 0.1 \text{ h}^{-1}$); slower growth was observed on succinate and acetic acid ($\mu = 0.01 \text{ h}^{-1}$). Standard conditions for growth of the MSH1 strain were defined at pH 7 and 25 °C, with glucose as the carbon source. In bioreactors (1 and 5 L), the specific growth rate of MSH1 increased from $\mu = 0.1 \text{h}^{-1}$ on traditional mineral salt medium to $\mu = 0.18 \text{ h}^{-1}$ on the optimized mineral salt medium. The biomass yield under standard conditions was 0.47 g dry weight biomass/g glucose consumed. An investigation of the catabolic capacity of MSH1 cells harvested in exponential and stationary growth phases showed a degradation activity per cell of about 3×10−9 μg BAM h−1. Thus, fast, efficient, large-scale production of herbicide-degrading Aminobacter was possible, bringing the use of this bacterium in bioaugmentation field remediation closer to reality.

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Web of Science (2017): Indexed yes
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Fungal hyphae stimulate bacterial degradation of 2,6-dichlorobenzamide (BAM)

Introduction of specific degrading microorganisms into polluted soil or aquifers is a promising remediation technology provided that the organisms survive and spread in the environment. We suggest that consortia, rather than single strains, may be better suited to overcome these challenges. Here we introduced a fungal bacterial consortium consisting of Mortierella sp. LEJ702 and the 2,6-dichlorobenzamide (BAM)-degrading Aminobacter sp. MSH1 into small sand columns. A more rapid mineralisation of BAM was obtained by the consortium compared to MSH1 alone especially at lower moisture contents. Results from quantitative real-time polymerase chain reaction (qPCR) demonstrated better spreading of Aminobacter when Mortierella was present suggesting that fungal hyphae may stimulate bacterial dispersal. Extraction
and analysis of BAM indicated that translocation of the compound was also affected by the fungal hyphae in the sand. This suggests that fungal bacterial consortia are promising for successful bioremediation of pesticide contamination. (C) 2013 Elsevier Ltd. All rights reserved.
2,6-dichlorobenzamide (BAM), Consortium, Bacterial dispersal, Pesticide biodegradation, Fungal–bacterial interactions, ENVIRONMENTAL, DEGRADING BACTERIA, SOIL, BIOAUGMENTATION, MINERALIZATION, BIOREMEDIATION, REMEDIATION, TRANSPORT, SELECTION, SURVIVAL, FIELD, Fungal-bacterial interactions, aquifer, bioremediation, fungal bacterial consortium, mineralisation, moisture content, polluted soil, Fungi Plantae (Fungi, Microorganisms, Nonvascular Plants, Plants) - Phycomycetes [15900] Mortierella genus, Gram-Negative Aerobic Rods and Cocci Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Pseudomonadaceae [06508] Aminobacter genus, Microorganisms (Bacteria, Eubacteria, Microorganisms) - Bacteria [05000] bacteria common, Plantae (Fungi, Microorganisms, Nonvascular Plants, Plants) - Fungi [15000] fungi common, 2,6-dichlorobenzamide pesticide, pollutant degradation, 31000, Physiology and biochemistry of bacteria, 37015, Public health - Air, water and soil pollution, 39008, Food microbiology - General and miscellaneous, 54512, Phytopathology - Nonparasitic diseases, 54600, Pest control: general, pesticides and herbicides, hyphal, quantitative real-time polymerase chain reaction qPCR laboratory techniques, genetic techniques, Bioprocess Engineering, Pesticides, Pollution Assessment Control and Management, Bacteria, Benzamides, Biodegradation, Environmental, Environmental Pollutants, Environmental Pollution, Groundwater, Hyphae, Soil Microbiology, Water Microbiology, 2008-58-4 2,6-dichlorobenzamide, Aquifers, Biodegradation, Bioremediation, Fungi, Hydrogeology, Microbiology, Polymerase chain reaction, Soil pollution, Bacterial degradation, Pesticide contaminations, Quantitative real-time polymerase chain reaction, Remediation technologies

**Projects:**

**EFFORT: Ecology from farm to fork of microbial drug resistance and transmission**

EFFORT will study the complex epidemiology and ecology of antimicrobial resistance and the interactions between bacterial communities, commensals and pathogens in animals, the food chain and the environment. This will be conducted by a combination of epidemiological and ecological studies using newly developed molecular and bio-informatics technologies. EFFORT will include an exposure assessment of humans from animal and environmental sources. The ecological studies on isolates will be verified by in vitro and in vivo studies. Moreover, real-life intervention studies will be conducted with the aim to reduce the use of antimicrobials in veterinary practice. Focus will be on understanding the eco-epidemiology of antimicrobial resistance from animal origin and based on this, predicting and limiting the future evolution and exposure to humans of the most clinically important resistance by synthesising different sources of information in our prediction models.

Through its results, the EFFORT research will provide scientific evidence and high quality data that will inform decision makers, the scientific community and other stakeholders about the consequences of AMR in the food chain, in relation to animal health and welfare, food safety and economic aspects. These results can be used to support political decisions and to prioritise risk management options along the food chain.
Knudsen, Berith Elkær (Intern)
Other:
Carlsson, Susanne (Intern)
Project Manager, academic:
Aarestrup, Frank Møller (Intern)

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Name of research programme: EU FP7
Amount: 1,450,568.00 Euro
Project