The role of outer membrane proteins and lipopolysaccharides for the sensitivity of Escherichia coli to antimicrobial peptides

Bacterial resistance to classical antibiotics is emerging worldwide. The number of infections caused by multidrug resistant bacteria is increasing and becoming a serious threat for human health globally. In particular, Gram-negative pathogens including multidrug resistant Escherichia coli are of serious concern being resistant to the currently available antibiotics. All Gram-negative bacteria are enclosed by an outer membrane which acts as an additional protection barrier preventing the entry of toxic compounds including antibiotics and antimicrobial peptides (AMPs). In this study we report that the outer membrane component lipopolysaccharide (LPS) plays a crucial role for the antimicrobial susceptibility of E. coli BW25113 against the cationic AMPs Cap18, Cap11, Cap11-1-18m², melittin, indolicidin, cecropin P1, cecropin B, and the polypeptide antibiotic colistin, whereas the outer membrane protease OmpT and the lipoprotein Lpp only play a minor role for the susceptibility against cationic AMPs. Increased susceptibility toward cationic AMPs was found for LPS deficient mutants of E. coli BW25113 harboring deletions in any of the genes required for the inner part of core-oligosaccharide of the LPS, waaC, waaE, waaF, yaaG, and gmhA. In addition, our study demonstrates that the antimicrobial activity of Cap18, Cap11, Cap11-1-18m², cecropin B, and cecropin P1 is not only dependent on the inner part of the core oligosaccharide, but also on the outer part and its sugar composition. Finally, we demonstrated that the antimicrobial activity of selected Cap18 derivatives harboring amino acid substitutions in the hydrophobic interface, are non-active against wild-type E. coli ATCC29522. By deleting waaC, waaE, waaF, or waaG the antimicrobial activity of the non-active derivatives can be partially or fully restored, suggesting a very close interplay between the LPS core oligosaccharide and the specific Cap18 derivative. Summarizing, this study implicates that the nature of the outer membrane component LPS has a big impact on the antimicrobial activity of cationic AMPs against E. coli. In particular, the inner as well as the outer part of the core oligosaccharide are important elements determining the antimicrobial susceptibility of E. coli against cationic AMPs.
Dissection of the antimicrobial and hemolytic activity of Cap18: Generation of Cap18 derivatives with enhanced specificity

Due to the rapid emergence of resistance to classical antibiotics, novel antimicrobial compounds are needed. It is desirable to selectively kill pathogenic bacteria without targeting other beneficial bacteria in order to prevent the negative clinical consequences caused by many broad-spectrum antibiotics as well as reducing the development of antibiotic resistance. Antimicrobial peptides (AMPs) represent an alternative to classical antibiotics and it has been previously demonstrated that Cap18 has high antimicrobial activity against a broad range of bacterial species. In this study we report the design of a positional scanning library consisting of 696 Cap18 derivatives and the subsequent screening for antimicrobial activity against Y. ruckeri, A. salmonicida, S. Typhimurium and L. lactis as well as for hemolytic activity measuring the hemoglobin release of horse erythrocytes. We show that the hydrophobic face of Cap18, in particular I13, L17 and I24, is essential for its antimicrobial activity against S. Typhimurium, Y. ruckeri, A. salmonicida, E. coli, P. aeruginosa, L. lactis, L. monocytogenes and E. faecalis. In particular, Cap18 derivatives harboring a I13D, L17D, L17P, I24D or I24N substitution lost their antimicrobial activity against any of the tested bacterial strains. In addition, we were able to generate species-specific Cap18 derivatives by particular amino acid substitutions either in the hydrophobic face at positions L6, L17, I20, and I27, or in the hydrophilic face at positions K16 and K18. Finally, our data showed the proline residue at position 29 to be essential for the inherent low hemolytic activity of Cap18 and that substitution of the residues K16, K23, or G21 by any hydrophobic residues enhances the hemolytic activity. This study demonstrates the potential of generating species-specific AMPs for the selective elimination of bacterial pathogens.

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Draft Genome Sequence of Acinetobacter johnsonii C6, an Environmental Isolate Engaging in Interspecific Metabolic Interactions

Acinetobacter johnsonii C6 originates from creosote-polluted groundwater and performs ecological and evolutionary interactions with Pseudomonas putida in biofilms. The draft genome of A. johnsonii C6 is 3.7 Mbp and was shaped by mobile genetic elements. It reveals genes facilitating the biodegradation of aromatic hydrocarbons and resistance to antimicrobials and metals.

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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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Comparative Evaluation of the Antimicrobial Activity of Different Antimicrobial Peptides against a Range of Pathogenic Bacteria

The rapid emergence of resistance to classical antibiotics has increased the interest in novel antimicrobial compounds. Antimicrobial peptides (AMPs) represent an attractive alternative to classical antibiotics and a number of different studies have reported antimicrobial activity data of various AMPs, but there is only limited comparative data available. The mode of action for many AMPs is largely unknown even though several models have suggested that the lipopolysaccharides (LPS) play a crucial role in the attraction and attachment of the AMP to the bacterial membrane in Gram-negative bacteria. We compared the potency of Cap18, Cap11, Cap11-1-18m2, Cecropin P1, Cecropin B, Bac2A, Bac2A-NH2, Sub5-NH2, Pyrrhocoricin, Apidaecin and Metalnikowin I towards Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli, Aeromonas salmonicida, Listeria monocytogenes, Campylobacter jejuni, Flavobacterium psychrophilum, Salmonella typhimurium and Yersinia ruckeri by minimal inhibitory concentration (MIC) determinations. Additional characteristics such as cytotoxicity, thermo and protease stability were measured and compared among the different peptides. Further, the antimicrobial activity of a selection of cationic AMPs was investigated in various E. coli LPS mutants.

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Characterization of Extended spectrum beta-lactamases (ESBL)-producing Escherichia coli obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of 3rd or 4th generation cephalosporins

Objectives: To compare and characterize extended-spectrum b-lactamase (ESBL)-producing Escherichia coli from pigsties, pig farmers and their families on farms with previous high or no use of third- or fourth-generation cephalosporins.

Methods: Twenty farms with no third- or fourth-generation cephalosporin use and 19 herds with previous frequent use were included. The ESBL-producing isolates detected in humans and pigs were characterized by ESBL genotype, PFGE, susceptibility to non-b-lactam antibiotics and phylotype, and selected isolates were characterized by multilocus sequence typing (MLST). Furthermore, transferability of blaCTX-M-1 from both human and pig isolates was studied and plasmid incompatibility groups were defined. The volunteers answered a questionnaire including epidemiological risk factors for carriage of ESBL-producing E. coli. Results: ESBL-producing E. coli was detected in pigs on 79% of the farms with high consumption of cephalosporins compared with 20% of the pigs on farms with no consumption. ESBL-producing E. coli was detected in 19 of the 195 human participants and all but one had contact with pigs. The genes found in both humans and pigs at the same farms were blaCTX-M-1 (eight farms), blaCTX-M-14 (one farm) and blaSHV-12 (one farm). At four farms ESBL-producing E. coli isolates with the same CTX-M enzyme, phylotype, PFGE type and MLST type were detected in both pigs and farmers. The majority of the plasmids with blaCTX-M-1 were transferable by conjugation and belonged to incompatibility group IncI1, IncF, or IncN. Conclusions: The present study shows an increased frequency of ESBL-producing E. coli on farms with high consumption of third- or fourth-generation cephalosporins and indicates transfer of either ESBL-producing E. coli or plasmids between pigs and farmers.

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Survival and Growth of Epidemically Successful and Nonsuccessful Salmonella enterica Clones after Freezing and Dehydration.

The spread of epidemically successful nontyphoidal Salmonella clones has been suggested as the most important cause of salmonellosis in industrialized countries. Factors leading to the emergence of success clones are largely unknown, but their ability to survive and grow after physical stress may contribute. During epidemiological studies, a mathematical model was developed that allowed estimation of a factor (q) accounting for the relative ability of Salmonella serovars with different antimicrobial resistances to survive in the food chain and cause human disease. Based on this q-factor, 26 Salmonella isolates were characterized as successful or nonsuccessful. We studied the survival and growth of stationary- and exponential-phase cells of these isolates after freezing for up to 336 days in minced meat. We also investigated survival and growth after dehydration at 10°C and 82% relative humidity (RH) and 25°C and 49% RH for 112 days. Stationary-phase cells were reduced by less than 1 log unit during 1 year of freezing, and growth was initiated with an average lag phase of 1.7 h. Survival was lower in exponential-phase cells, but lag phases tended to be shorter. High humidity and low temperature were less harmful to Salmonella than were low humidity and high temperature. Tolerance to adverse conditions was highest for Salmonella Infantis and one Salmonella Typhimurium isolate and lowest for Salmonella Derby and one Salmonella Typhimurium DT170 isolate. Dehydration, in contrast to freezing, was differently
tolerated by the Salmonella strains in this study, but tolerance to freezing and dehydration does not appear to contribute to the emergence of successful Salmonella clones.
Adjustment of pH of enrichment media might improve selective isolation of MRSA from pig samples

Methicillin resistant Staphylococcus aureus (MRSA) have emerged in livestock in several countries worldwide in recent years. MRSA may colonise in low numbers which makes both epidemiological studies and the implementation of control programmes difficult. Methods for selective isolation of MRSA from animal samples have been developed. However, obtaining sufficient sensitivity has been a challenge. Staphylococcus aureus is normally found on the skin, surviving and growing under extreme conditions: dry environment with high salt and low pH. In the selective isolation so far used high salt concentrations has been the main selection. We hypothesized that also pH adjustment could be used for selection of this species. In this study we compared the growth of MRSA and back ground flora in enrichment media at several different combinations of salt and pH. Background flora isolates were obtained from pig swabs. Initially a total of seven strains, including: two MRSA, two enterococci, two CNS one Aerococcus viridans and one Proteus spp. strains, were tested for growth in Mueller Hinton II broth with pH ranging from 4 to 5.5 and salt addition of 4% to 7%. In the next step, these strains were tested for growth in 12 different combinations of salt (5%- 6.5%) and pH (4.5-5.5). For assessment of the growth of S. aureus in pH adjusted media, further 14 MRSA and 13 MSSA were tested in a similar way. In a preliminary study using reference strains (data not shown) it was observed that pH ≤5.5 as well as salt concentrations ≥4% did allow growth of S. aureus but was inhibiting the growth of enterococci. Subsequent growth experiments with isolates from background flora showed an inhibitory effect of pH below 5 for Aerococcus spp and enterococci, whereas less effect was observed on CNS and Proteus spp. In the assays using different combinations of pH and salt, the pH showed, in general, a larger effect than the salt concentration on growth. MRSA and MSSA strains were partially inhibited by pH below 4.5 and grew with a moderate growth rate at pH 5.5 with lower salt concentration (5%). The growth of enterococci strains was completely inhibited by pH ≤5.5 at any salt concentrations tested (5- 6.5%), whereas the growth of Proteus spp. was only inhibited totally at pH of 4.5, but the growth rate could be reduced combining pH at 5 or 5.5 with high salt concentrations. In conclusion, pH adjustment of enrichment media might improve sensitivity of methods for detection of S. aureus by reducing background flora growth. Moreover, the combination of pH adjustment with reduction of the currently used salt enrichment concentration might increase the sensitivity of the detection of MRSA. For screening purposes it will still be necessary to have further steps for the selective enrichment and isolation of MRSA. Further studies are underway to evaluate the value of this under field conditions.

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The survival of Listeria monocytogenes during long term desiccation is facilitated by sodium chloride and organic material

One specific DNA-subtype, as determined by RAPD, of Listeria monocytogenes persisted in a fish slaughterhouse for years, even during months with no production where the plant was cleaned and kept dry. We hypothesised that tolerance to desiccation could be a factor in explaining the persistence of L monocytogenes in food processing environments and the purpose of the present study was to determine ability of L monocytogenes to survive desiccation on stainless steel under simulated food processing conditions. Viable counts of eight different L. monocytogenes strains exposed to different soils and relative humidities (RHs) during desiccation decreased significantly.
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