Magnitude and determinants of plasmid transfer from exogenous donor strains to complex microbial communities

Klümper, Uli; Dechesne, Arnaud; Riber, Leise; Droumpali, Ariadni; Brandt, K.K.; Sørensen, S.; Smets, Barth F.

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<td>Mobilizing antibiotic resistance genes through anthropogenic use of antibiotics</td>
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ORGANIZATION AND IMPRINT

Venue
HKK Hotel Wernigerode
Harzer Kultur- & Kongresshotel
Pfarrstrasse 41 • 38855 Wernigerode (Germany)

Date
17–21 May 2015

Conference Homepage
www.antibiotic-resistance.de

Conference Chair
Kornelia Smalla
Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI)
Institute for Epidemiology and Pathogen Diagnostics
Braunschweig, Germany

International Scientific Committee
Kristian Brandt, Denmark
Will Gaze, United Kingdom
Joakim Larsson, Sweden
Amy Pruden, United States
Kornelia Smalla, Germany
Pascal Simonet, France
James Tiedje, United States
Ed Topp, Canada
Elizabeth M. H. Wellington, United Kingdom
Yong-Guan Zhu, China

Conference Organization
Conventus Congressmanagement & Marketing GmbH
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edar@conventus.de • www.conventus.de

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Editorial Deadline 5 May 2015
Dear colleagues,

The increasing problems with multiple resistant pathogens led to a critical discussion of the anthropogenic use of antibiotics worldwide.

The widespread and continued global growth in antibiotics consumption is the driver of antibiotic resistance, and identification of the factors fostering the dissemination of transferable antibiotic resistances is needed to determine effective interventions. The 3rd international symposium on the Environmental Dimension of Antibiotic Resistance (EDAR-3) will focus on effects of anthropogenic use of antibiotics on the microbiomes in various ecosystems and the implications for human health.

Recent discoveries using omics technologies have provided not only new insights into the natural reservoirs of antibiotic resistances but also into their broader roles in an organism and for community function. The fate of antibiotics in environmental compartments such as aquatic or agro-ecosystems will be discussed regarding short- and long-term effects, in particular on resistance development and dissemination.

The aim is an improved qualitative and quantitative understanding of the processes involved, such as antibiotic selection, co-selection by metals or other agents, and the role of the bacterial mobilome to provide a basis for the discussion of regulatory considerations and management options and to identify possible limitations and research needs. The development of technologies and management options to reduce environmental pollution by antibiotics and resistance determinants from, e.g. sewage or production sites might contribute to avoid negative impacts on the environment.

We are delighted that you are joining EDAR-3 to discuss the most recent research results and their implications for human health. Welcome to Wernigerode!

Kornelia Smalla
Conference chair
Julius Kühn-Institut, Braunschweig
GENERAL INFORMATION

Opening Hours

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<tr>
<td>Poster Exhibition</td>
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Internet

Wireless internet (WLAN) is provided by the conference hotel and is available for all congress attendees. Vouchers will be available at the Check-In desk.

City Map

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Key

HKK Hotel                  Conference Venue
Fürstlicher Marstall      Venue Conference Dinner
Castle                    Venue Welcome Reception
ProkaGENOMICS 2015
6th European Conference on Prokaryotic and Fungal Genomics

29 September–2 October 2015
GÖTTINGEN • GERMANY

www.prokagenomics.org
The Media Check-In is located in the lecture hall.
GENERAL TIPS FOR AUTHORS AND PRESENTERS

Submitting Your Presentation/Technical Information
Please prepare your presentation in 4:3 aspect ratio.

A presentation notebook with a PDF reader and MS Office PowerPoint 2010 will be provided. The use of personal notebooks is possible upon agreement. However, it may interrupt the flow of the program in the lecture hall. Please provide an adapter for VGA if necessary.

A notebook, presenter and laser pointer are available at the speaker’s podium in the lecture hall. If necessary a technical supervisor can help you.

Please note that certain encodings for video and audio files could lead to problems. Please visit our Media Check-In for further information in advance.

Media Check-In
The Media Check-In is located in our plenary hall.

Please submit your presentation at the Media Check-In desk in the plenary hall ideally the day before your presentation, but no later than 2 hours before the presentation should begin. You may view and/or edit your presentation.

For submission, please use a USB flash drive, CD or DVD disc, which should not be protected with software.

Speaking Time
Please prepare your presentation for the allotted amount of time. Chairs and moderators may interrupt should you overrun your time limit. Speaking time is assigned as follows (speaking + discussion time):

1. Invited Speakers  30 + 5 minutes
2. Abstract Authors  15 + 5 minutes

Poster Sessions
Posters should be no larger than DIN A0 (84.1 cm x 118.9 cm). Poster pinboards are 120 cm x 150 cm. They are only to be used with the designated pins. Pinboards will be numbered. You will find your poster number in the program book on pages 11–18. Poster presenters are asked to be present during their poster sessions.

Posters should be erected on 18 May by 16:00 hrs and removed on 20 May by 16:00 hrs. All posters that have not been removed by then will be considered as waste.
SCIENTIFIC PROGRAM • SUNDAY, 17 MAY 2015

17:15-17:30 Welcome
Kornelia Smalla, Germany

17:30-18:05 Opening Lecture
Antibiotics at sub-inhibitory concentrations
Fernando Baquero, Spain

18:30-20:00 Welcome Reception at Wernigerode Castle
see page 28

SCIENTIFIC PROGRAM • MONDAY, 18 MAY 2015

08:30–10:25 SESSION I EVOLUTION OF ANTIBIOTIC RESISTANCE
Chair Jan Dirk van Elsas, The Netherlands

08:30 Phylogenomic transduction networks reveal genetic barriers for phage-mediated lateral gene transfer
Tal Dagan, Germany

09:05 Magnitude and determinants of plasmid transfer from exogenous donor strains to complex microbial communities
Barth F. Smets, Denmark

09:25 The origin of Beta-lactamase encoding genes – Are we misannotating resistance genes using sequencing data in microbial ecology?
Lars Hansen, Denmark

09:45 Insights into the resistome of bacterial communities from environments shaped by exceptional antibiotic selection pressures
Nachiket Marathe, Sweden

10:05 Bacterial antibiotic resistance can be induced by disinfection byproducts via mutagenesis
Lu Lv, China

10:25–10:55 Coffee Break
10:55–14:20  MOBILIZING ANTIBIOTIC RESISTANCE GENES THROUGH ANTHROPOGENIC USE OF ANTIBIOTICS

Chairs
Steven Djordjevic, Australia
Amy Pruden, United States

10:55  Xenobiotics, xenogenetics and evolution – Tracking antibiotic resistance from environment to clinic, and back again
Michael Gillings, Australia

11:30  Class 1 integrons as markers of antibiotic resistance
Will Gaze, United Kingdom

11:50–13:00  Lunch Break

13:00  Antibiotic use selects for strains with increased permissiveness for broad-host-range resistance plasmids within a local population
Holger Heuer, Germany

13:20  Turn up the signal – wipe out the noise: Gaining insights into antibiotic resistance of bacterial communities using metagenomic data
Johan Bengtsson-Palme, Sweden

13:40  Dissemination of tetracycline resistance genes from a conventional dairy farm via manure into field soil
Dana Elhottova, Czech Republic

14:00  What’s the road to resistance? – 15 years of antibiotic molecules application to farm soil do not significantly increase resistance genes abundance
Joseph Nesme, Germany
MOLECULAR STUDIES REVEAL LINKS BETWEEN THE HOSPITAL AND THE ENVIRONMENT

Chairs
Lisa Durso, United States
Will Gaze, United Kingdom

14:20
The rifamycin antibiotic resistome
Gerry Wright, Canada

14:55
Co-occurrence of antibacterial biocide, metal and antibiotic resistance genes in bacterial genomes and plasmids reveals novel insights in their co-selection potential
Chandan Pal, Sweden

15:15
Phylogeny and comparative genomics unveil independent diversification trajectories of qnrB and genetic surroundings within Citrobacter sp.
Luísa Peixe, Portugal

15:35
Exploiting unique molecular signatures created during the evolution of complex resistance regions to track imminent threats by multiple antibiotic resistant pathogens
Steven Djordjevic, Australia

15:55–16:25
Coffee Break

16:25–19:00
POSTER SESSION I

Topics
Evolution of antibiotic resistance see pages 18–19

Fate and effects of antibiotics in the agro-ecosystem see pages 21–22

Mobilizing antibiotic resistance genes through anthropogenic use of antibiotics see page 19

Molecular studies reveal links between the hospital and the environment see pages 20
**FATE AND EFFECTS OF ANTIBIOTICS IN THE AGRO-ECOSYSTEM**

Chair
Kristian Koefoed Brandt, Denmark

08:30
**Antibiotic resistance gene discovery in the swine intestinal microbiome**
Heather Allen, United States

09:05
**Manure fertilization increases resident antibiotic-resistant bacteria in soil**
Nikolina Udikovic-Kolic, Croatia

09:25
**Antibiotic resistance gene concentrations in agroecosystems following beef manure or poultry litter deposition**
Kimberly Cook, United States

09:45
**Fate and effects of veterinary medicines in soil**
Jan Siemens, Germany

10:20–10:50
Coffee break

**SESSION IV continued**
Chair
Kimberly Cook, United States

10:50
**Antibiotics in plant agriculture**
Fiona Walsh, Ireland

11:25
**Antibiotic resistant bacteria from livestock husbandry are released with biogas plant digestate into the environment**
Stefanie P. Glaeser, Germany

11:45
**Metals as co-selective agents in soil bacterial communities**
Kristian Koefoed Brandt, Denmark

12:05
**Transport of antibiotics in wastewater irrigated soil columns and their relation with the increase of resistance genes**
Kathia Lueneberg, Mexico

12:25–13:25
Lunch Break
SESSION IV continued
Chair Pascal Simonet, France

13:25 Resistance and mobilome of fresh produce – the missing link
I 6 Ed Topp, Canada

14:00 Effects of advanced treatment on the removal of antibiotic resistance genes in wastewater treatment plant
0 FAT 6 Hong Chen, China

14:20 The role of wildlife in disseminating antibiotic-resistant bacteria to and from livestock facilities
0 FAT 7 Alan B. Franklin, United States

14:40 Antibiotic resistance genes in manure-amended soil and vegetables at harvest
0 FAT 8 Min Qiao, China

15:00–15:30 Coffee Break

15:30–18:00 POSTER SESSION II
Topics Dissemination of antibiotics and antibiotic resistance genes through aquatic ecosystems/sewage treatment plants see pages 23–26

Mitigation strategies and how to evaluate them see page 26
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<tr>
<td>08:30</td>
<td>The contribution of the mobilome in the dissemination of antibiotic resistance in rivers, sediments and farm slurries</td>
<td>Elizabeth M. H. Wellington, United Kingdom</td>
<td>United Kingdom</td>
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<tr>
<td>09:05</td>
<td>Exploring plasmid-based dissemination of antibiotic resistance genes in environmental matrices</td>
<td>Xavier Bellanger, France</td>
<td>France</td>
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<td>09:25</td>
<td>Distribution and fate of antibiotic resistance bacteria and genes in enhanced anaerobic digestion of sewage sludge with microwave pretreatment</td>
<td>Juan Tong, China</td>
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<td>09:45</td>
<td>Transferable antibiotic resistance in rivers and estuaries</td>
<td>Isabel Henriques, Portugal</td>
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<td>Antibiotic resistance genes in urbanizing watershed</td>
<td>Yong-Guan Zhu, China</td>
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<td>11:25</td>
<td>Multidrug-resistant human-associated bacteria from wastewater treatment plants and their receiving waters – Gulf of Gdańsk, Baltic Sea (Poland)</td>
<td>Ewa Kotlarska, Poland</td>
<td>Poland</td>
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<td>11:45</td>
<td>Determining the minimal selective concentrations of antibacterial agents in complex aquatic bacterial communities</td>
<td>Sara Lundström, Sweden</td>
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<td>Antibiotic resistance genes in sewage and related water environment</td>
<td>Tong Zhang, China</td>
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Wastewater irrigation increases abundance of antibiotic resistance genes and potentially harmful Gammaproteobacteria in soils from Mezquital Valley, Mexico
Elisabeth Grohmann, Germany

Overview of meropenem and zinc resistance in biofilms – Rotating tubular reactors and river sediments in the River Tyne
Catherine Hands, United Kingdom

Antibiotic resistance in Pristine Red Sea Brine Pools
Ali H A Elbehery, Egypt

Persistence of resistance genes in sewage canalization networks of metropolitan areas – relationship between antibiotic selective pressure and antibiotic resistance genes
Thomas Berendonk, Germany

Environmental pollution from antibiotic manufacturing creates hotspots for resistance development
Joakim Larsson, Sweden

Microbial metabolic capabilities including antimicrobial biosynthesis during water infiltration revealed by metagenomic and transcriptomic analyses
Jörg Drewes, Germany

Exploring the effects of anthropogenic pollution on the environmental resistome of rivers and agricultural soils in Central Mexico using micro-biological and genomic approaches
Pablo Vinuesa, Mexico

Temporal dynamics and source-to-tap connectivity in a surface-water fed drinking water distribution system
Helmut Bürgmann, Switzerland

Free time
For recommendations, see page 28.

Conference Dinner
see page 28.
09:00–11:00

SESSION VI

MITIGATION STRATEGIES AND HOW TO EVALUATE THEM
(Round table and discussion groups)

Chairs
Mark Ibekwe, United States
Heike Schmitt, The Netherlands

09:00
Mitigation strategies for environmental sources of antibiotic resistance
I 13
Amy Pruden, United States

09:35
Artilyssins: Highly effective antimicrobial enzymes with a low risk of resistance formation for targeted elimination of bacterial pathogens
0 MIT 1
Stefan Miller, Germany

09:50
Thermophilic anaerobic digestion – an effective approach for blocking both horizontal and vertical gene transfer pathways of antibiotic resistance genes
0 MIT 2
Min Yang, China

10:05
Specific in silico tools for the detection of acquired antibiotic resistance genes
0 MIT 3
Gisle Vestergaard, Germany

10:20
Open discussion

11:00–11:20
Coffee Break

11:20
Closing Lecture – taking stock of ARGs as pollutants – past, now and future
11:20
James Tiedje, United States

12:05
Farewell and announcements
Kornelia Smalla, Germany
SESSION I  EVOLUTION OF ANTIBIOTIC RESISTANCE

P EVO 1  Antibacterial property of local soap (ncha nkota) sold in local markets in Nigeria, West Africa.  Hope Okereke, Nigeria

P EVO 2  Antimicrobial resistance of *Staphylococcus epidermidis* isolated in Tlemcen “Algeria”  Barka Mohammed Salih, Algeria

P EVO 3  Phenotypic and genotypic determination of mupirocin resistance among methicillin susceptibility and resistance in staphylococci isolated from noso-comial infection  Seyed Davood Hosseini, Iran, Islamic Republic of

P EVO 4  Evidence of continuous use of chloroquine in Ghana after its ban – Effect on antimalarial drug resistance  Johnson Nyarko Boampong, Ghana

P EVO 5  Investigating co-selection for antibiotic resistance in the environment  Aimee Murray, United Kingdom

P EVO 6  Multicenter study of resistance to antibiotics in *Enterobacter cloacae*  Souna Djahida, Algeria

P EVO 7  Co-selection for antibiotic resistant bacteria at sub-inhibitory concentration of biocides  Lihong Zhang, United Kingdom

P EVO 8  Modulation of microbial community permissiveness towards broad host range conjugal plasmid is metal specific  Uli Klümper, Denmark

P EVO 9  The role of the insertion sequence IS256 in genetic flexibility in *Staphylococcus aureus*  Gabriele Bierbaum, Germany

P EVO 10  The assessment of antimicrobial susceptibility of bacterial strains isolated from wild birds in the Danube delta biosphere reserve  Emoke Pall, Romania
P EVO 11  Studies on the correlation between metal content in polluted soil with bacterial resistance to antibiotics and metals
Geertje van Keulen, United Kingdom

P EVO 12  Natural transformation as a mechanism for the acquisition of extracellular DNA in bacteria
Kaare M. Nielsen, Norway

P EVO 13  Evolution of antimicrobial resistance
Elena Gómez-Sanz, Switzerland

SESSION II  MOBILIZING ANTIBIOTIC RESISTANCE GENES THROUGH ANTHROPOGENIC USE OF ANTIBIOTICS

P MOB 1  Horizontal gene transfer through plasmid transport: heavy metal and antibiotic tolerance in bacteria
Erum Shoeb Nasir, Canada

P MOB 2  Characterisation of bacterial populations and identification of antimicrobial resistance markers in the food production environment.
Christina Bronowski, United Kingdom

P MOB 4  Role of antibiotic use for resistance gene and integron carriage in Dutch pig farms
Heike Schmitt, The Netherlands

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SOCIAL PROGRAM

Welcome Reception at Wernigerode Castle
Our Welcome Reception of EDAR 3 will take place at the Wernigerode Castle. You are invited to join your colleagues and friends to tune into our 3rd International Symposium on the Environmental Dimension of Antibiotic Resistance. In this context the mayor of Wernigerode Mr. Peter Gaffert would like to welcome you personally to his town. Some snacks and drinks will be provided.

Date  Sunday, 17 May 2015
Time   18:30–20:00 hrs
Price  included for conference participants
       15 EUR for your accompanying person

The Castle is within walking distance from the HKK Hotel (20 minutes). Guides will accompany you from the hotel to the venue.

Meeting point: 18:15 hrs. after the last session, in the lobby of the HKK Hotel.

Social Evening at the “Fürstlicher Marstall Wernigerode”
Our conference dinner will take place at the “Fürstlicher Marstall Wernigerode”. The stable of the former estate house is now rebuilt as an event hall and exudes a rustic charm and cozy atmosphere. This enchanting atmosphere combined with excellent food and great music will give you the chance to end this evening dancing and reflecting on high-quality EDAR sessions.

Date  Wednesday, 20 May 2015
Start  20:00 hrs
Price  50 EUR

Shuttle: We are happy to provide a shuttle service to the venue by the traditional “Wernigeröder Bimmelbahn” (rambling train).

Meeting point: 19:30 hrs in front of the HKK Hotel
Please bring your name tag!
Please note that you will have to organize the return trip to the congress hotel by yourself. Phone numbers of cooperating taxi companies will be provided at the social evening venue.
To make your visit to Wernigerode even more impressive, we hereby propose a selection of sights, castles and parks to explore while you are in town. Please note that we do not offer organized tours and you need to plan your trips individually. If you have any questions or need assistance, please do not hesitate to contact us at the Check-In desk.

**Guided Tours**
The Wernigerode Tourismus GmbH daily offers guided tours which take you through the Old Town of Wernigerode and “Through Six Centuries” of town history. Highlights beside the historical Old Town are for instance Wernigerode’s half-timbered houses, the 16th century Town Square and the historic Baroque Town Hall.
Contact: Wernigerode Tourismus GmbH • Marktplatz 10
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+49 3943 55378-29 • info@wernigerode-tourismus.de
Fares:  Adults: 5 EUR • Students: 4,50 EUR • Children: 2,50 EUR

If you prefer a free self-guided walking tour, please do not hesitate to contact us at the Check-In desk and ask for a pocket-sized street map.

**Harz Narrow Gauge Railway**
The Harz Narrow-Gauge Railway Network with over 140 km of tracks running through exciting countryside locations is the longest unbroken narrow-gauge railway network in Europe with daily steam train services. The Trans-Harz railway line crosses the Harz Mountains from north to south. On the some 60 km track between Wernigerode and Nordhausen, passengers are treated to a kaleidoscopic journey along the numerous splendours of the Harz. For additional information on timetables and fares please do not hesitate to contact us at the Check-In desk.

**Wernigerode Castle**
The Wernigerode Castle was originally a medieval castle, which should secure the path of the German emperors of the Middle Ages on their hunting trips in the Harz mountains. Since 1930, the castle is open to the public. Two museum tours take guests through more than 40 original furnished rooms of the German nobility.
Opening hours:  daily 10:00–18:00 hrs
(last admission at 17:30 hrs)
Admission fee:  Adults 6 EUR • Pensioners/Students 5 EUR
Transfer service: Every 20 minutes from the town centre
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Antibiotics released in the environment by anthropogenic actions are frequently diluted in very large spaces, so that in most of cases, the concentrations to which bacteria will be exposed are expected to be very low. However, the "environmental pharmacokinetics" (Env-PK) of antimicrobial drugs is only superficially known (for instance the half-life of molecules), and the major difficulty is understanding the influence in Env-PK of the different compositions of complex environments at the microscale where bacteria should be affected. Antibiotics certainly have binding abilities for many environmental biological or mineral particulated components, they also might be degraded by living organisms, and obviously physical-chemical conditions, as pH, or even light exposure, might modify its local activity. Finally, antibiotics in the environment might influence the bacterial world in cooperation with other anthropogenic substances released in the environment, as biocides and heavy metals. In any case the expected amount of antibiotics in the environment seems to be very low, and most organisms will be exposed at sub-inhibitory concentrations. The question is if these concentrations could have effects on the biosphere, and particularly if they can influence the population biology of antibiotic resistant bacteria. Of course sub-inhibitory concentrations might influence bacterial physiology; in fact the natural role of antibiotics is based more on their role as "signals" than as "weapons". A frequent result of these effects is the slowing-down of growth rates. Antibiotic resistant variants will be selected over slow-growing wild-type organisms even at very low antibiotic concentrations. Sub-inhibitory concentrations of antibiotics (and also heavy metals), more than 100x times lower than those required for inhibiting visible growth in cultures, are able to provide enough bacterial harm to make profitable the maintenance of multi-resistance plasmids in spite of its intrinsic biological cost, and then assuring their continued selection propagation by lateral transfer. The smaller the changes in antimicrobial concentrations that are able to produce selective changes in the bacterial population structure, the bigger the size of the "environmental selective space" will be, and consequently the absolute number of bacterial cells exposed. An important point is that antimicrobials in the environment is forming complex concentration gradients, from the release-source sites until reaching the limit of non-biological-effect concentrations. The different concentrations along the gradient might result in discrete qualitative effects, as the selection of particular antibiotic resistant mutants at particular segments of the gradient, acting as (frequently narrow) selective compartments. Additionally, sub-inhibitory concentrations are not simply selective ones, as they can also increase genetic and phenotypic variability, offering novel chances to selective forces. In summary, there is clear evidence of the influence of sub-inhibitory antibiotic concentrations in shaping the evolutionary biology of resistance, including the emergence, invasion and occupation by antibiotic-resistant genes of significant environments for human and animal health.

**Antibiotics at Sub-Inhibitory Concentrations**

F. Saquet

1Ramón y Cajal University Hospital, Dpt. Microbiology, Madrid, Spain

The rapid rise of antibiotic resistant strains of bacteria in the last 70 years is the foremost example of natural selection in action, and has precipitated a worldwide crisis in disease control. At its heart, antibiotic resistance is an evolutionary and ecological phenomenon. Understanding the dynamics of this phenomenon would allow proactive rather than reactive responses, and could suggest strategies for minimising further erosion of our dwindling stock of antibiotics. Human attempts to control bacterial disease have resulted in the evolution of complex, mosaic DNA elements that can be thought of as xenogenetic, since their assembly has been driven by our use of disinfectants, antibiotics, heavy metals and pesticides. Individual xenogenetic elements can carry DNAs from a wide range of phylogenetic sources, and can include diverse resistance genes, integrons, transposons and insertion elements. The mosaic nature of these elements allows interaction with an ever-expanding range of other DNA elements, thus generating further complexity as an emergent property. Xenogenetic elements are being shed via human waste streams into aquatic ecosystems, along with significant concentrations of selective agents. Exposure to antibiotic pollution induces the bacterial SOS response, elevating the rates of point mutation, recombination and lateral transfer. This combination of factors promotes the secondary acquisition of novel resistance and pathogenicity determinants and selects for environmental organisms that acquire resistance elements by lateral gene transfer, thus generating new waves of potential opportunistic pathogens. We will give case examples, and show how exposure to sub-inhibitory concentrations of antibiotics causes genomic rearrangements and elevated resistance.
I 3
The Rifamycin Antibiotic Resistome
G. Wright
McMaster University, DeGroote Institute for Infectious Disease Research, Hamilton, Canada

Rifamycin antibiotics are mainstays of tuberculosis treatment, but are increasingly being used in combination therapy to treat multidrug resistant infections. Resistance to these antibiotics occurs frequently as a result of point mutations in the target RNA polymerase gene, however there is a unexpectedly rich diversity of genes that encode rifamycin modifying enzymes that is widespread in the clinic and the environment. Environmental bacteria in particular appear to be a deep reservoir of rifamycin resistance. The mechanisms, protein structures and distribution of these genes will be discussed along with the potential impact on clinical use of rifamycin antibiotics.

I 4
Antibiotic resistance gene discovery in the swine intestinal microbiome
H. Allen
National Animal Disease Center/USDA, Food Safety and Enteric Pathogens Research Unit, Ames, United States

Antibiotics have been administered to food-producing animals for over 50 years for the purposes of disease treatment, disease prevention, and growth promotion. Indeed, it has been estimated that the use of antibiotics in food animals now accounts for over half of antibiotic administration in the U.S. This extensive application has promoted the ubiquity and mobility of antibiotic-resistance genes in the animal gut microbiome. We are interested in the effects of in-feed antibiotic treatment on antibiotic resistance genes. In two separate studies, we analyzed feces from swine treated with or without antibiotics (ASP250 [chlorotetraacycline, penicillin, and sulfamethazine]) via metagenomics. The results showed that swine feces harbored abundant and diverse antibiotic resistance genes regardless of antibiotic treatment. In addition, some resistance genes increased in abundance following antibiotic treatment. Interestingly, one class of resistance genes, the aminoglycoside O-phosphotransferases (APH), increased in abundance despite conferring resistance to a class of antibiotic not administered. This is an important reminder of the potential to co-select for unpredicted resistance genes under a given antibiotic regimen. To further investigate this phenomenon, functional metagenomic libraries were built from swine fecal DNA and selected on various antibiotics, including the aminoglycoside antibiotics kanamycin. Seven resistant fosmids were isolated and the complete DNA insert was sequenced. The fosmids conferred resistance to chlorotetraacycline or kanamycin or both. Of the kanamycin-resistant fosmids, three encoded an APH, each in a different genetic context. Most fosmids contained evidence of horizontal gene transfer, with genes encoding seven transposases and five integrases, the latter of which represent the XerC family recombinases and the intA-like transposases. These results illustrate the extent to which antibiotic resistance genes have mingled and mobilized in the gut microbiome of contemporary agricultural animals. Explorations of antibiotic resistance gene ecology in the environment, including the host-associated environment, are essential for the development of resistance mitigation strategies and antibiotic usage policies.

I 5
Fate and effects of veterinary medicines in soil
J. Siemens
University of Bonn, Institute of Crop Science and Resource Conservation ¬ Soil Science and Soil Ecology, Bonn, Germany

Veterinary antibiotics are used worldwide for the treatment of farm animals, are excreted and reach agricultural fields with manure. In soils, veterinary antibiotics introduced with animal excreta might select bacteria resistant to these agents, leading to an increased abundance and transferability of antibiotic resistance determinants. The presentation will synthesize advances, limitations, and research needs in determining the fate of veterinary antibiotics and resistant bacteria applied with manure to soil and their effects on the structure and function of soil microbial communities with a focus on the findings of the research unit “Veterinary medicines in soil: Basic research for risk assessment” for the sulfonamide sulfamethazine and the fluoroquinolone difloxacin. There is strong evidence that manure and residues of antibiotics contained in manure synergistically promote the spreading of antibiotic resistance genes in soils. The sequestration of antibiotics in strongly bound forms that are not bio-accessible and their slow release back into soil water maintains low antibiotic concentrations in soil over extended periods of time (weeks to months). Plants can promote the degradation of antibiotics which was correlated with a reduced abundance of resistance genes in the soil surrounding their roots (rhizosphere). However, the selection of resistant bacteria may persist longer in the rhizosphere than in bulk soil. First mathematical models for linking the fate of antibiotics and their effect on the selection of antibiotic resistance genes in soil are available. Nevertheless, the heterogeneous distribution of bacteria and antibiotics in soil (“hot spots”), environmental factors like moisture, temperature, substrate and nutrient availability affecting concentrations of antibiotics and soil microbial activity, as well as the co-selection of resistant bacteria by various other pollutants like heavy metals or...
disinfectants complicate the identification of dose-response relationships for the selection of resistant bacteria in soil by veterinary antibiotics. Consequently, the use of antibiotics, heavy metals and disinfectants in animal husbandry should be minimized based on the precautionary principle to curtail the selection of resistant bacteria and the spreading of antibiotic resistance in agricultural soils.

16 Resistance and mobilome of fresh produce - the missing link
E. Topp
1 University of Western Ontario, Department of Biology, London, Canada

Food consumption is a potentially significant route of human exposure to antibiotic resistant bacteria. Most surveillance and research initiatives to evaluate the significance of the foodborne pathway have focussed on meat from terrestrial animal and poultry production, and the products of commercial aquaculture. Crop production systems will often be fertilized with animal manures and in some cases with sewage sludge. Furthermore, they may be irrigated with surface waters or reclaimed wastewater that may contain appreciable numbers of antibiotic resistant bacteria and antibiotic residues. Produce grown in irrigated or manured ground might carry an additional burden of antibiotic resistant bacteria, referenced to produce grown in ground without manuring or irrigation. Consumption of crops exposed to fecal material during production could then represent a route of exposure to antibiotic resistant bacteria and associated mobile genetic elements. This presentation will specifically deal with this concern. Drawing on our own research and that of others, it will give an overview of the fresh produce microbiome with respect to antibiotic resistance, and how the microbial composition of fresh produce can vary with commercial production practice. Key knowledge gaps will be identified, and some priority research recommendations will be provided.

17 The contribution of the mobilome in the dissemination of antibiotic resistance in rivers, sediments and farm slurries
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2 University of Birmingham, Institute of Microbiology and Infection, Biosciences, Birmingham, United Kingdom
3 Health Protection Agency, West Midlands Public Health Laboratory, Heart of England NHS Foundation Trust, Birmingham, United Kingdom
4 University of Exeter Medical School, Knowledge Spa, Royal Cornwall Hospital, European Centre for Environment and Human Health, Truro, United Kingdom

Multi-drug resistant Enterobacteriaceae pose a significant threat to public health. We aimed to study the impact of sewage treatment effluent on resistance reservoirs in a river, and the exposure risk caused to humans. River sediment samples were taken downstream and upstream of a WWTP over two years. Third generation cephalosporin (3GC) resistant Enterobacteriaceae were enumerated in sediment samples and PCR based techniques were used to elucidate mechanisms of resistance, with a new two-step PCR based assay developed to investigate \( \text{bla}_{\text{CTX-M-15}} \) mobilisation. Conjugation experiments and incompatibility replicon typing was used to investigate plasmid ecology and plasmids were sequenced to investigate mechanisms of co-selection and persistence of resistance genes. We report the first examples of \( \text{bla}_{\text{CTX-M-15}} \) in UK river water; the prevalence of \( \text{bla}_{\text{CTX-M-15}} \) was dramatically increased downstream of the WWTP. A range of plasmids in pathogens such as \( E. coli \) ST131 contained different genetic contexts for \( \text{bla}_{\text{CTX-M-15}} \) and also carried integrons. Transfer experiments proved biocides could select for transfer of plasmids carrying multiple resistance genes. Plasmid replicon typing revealed the plasmids responsible for transfer of the resistance genes belonged to a both broad and narrow host range plasmids such as incFIA, incFIB, incCI, incC2, incA, incK and incN. Plasmid sequencing gave in depth information on addiction systems, resistance genes and mobile genetic element. Subsequent work has followed plasmid stability in relation to selection and evaluated mechanisms of co-selection and gene recruitment.

The high diversity and host range of novel genetic contexts proves that evolution of novel combinations of resistance genes is occurring at high frequency and has to date been significantly underestimated. We have identified a worrying reservoir of highly resistant enteric bacteria in the environment which poses a measurable threat to human and animal health.
Transferrable antibiotic resistance in rivers and estuaries
I. Henriques 1, M. Tacão 1,2, A. Correia 1
1University of Aveiro, iBiMED & Department of Biology, Aveiro, Portugal
2University of Aveiro, CESAM & Department of Biology, Aveiro, Portugal

Rivers and estuaries, as main receptacles for contaminants, are probably reactors for the spread and evolution of antibiotic resistance. Consequences to public health and ecosystems may be dramatic but difficult to anticipate due to a lack of knowledge in this subject. We conducted research to uncover the role of rivers and estuaries as disseminators of antibiotic resistance. The AR gene pool in these aquatic systems has been characterized and factors contributing to AR persistence have been identified. Research has been conducted in rivers and an estuarine system located in the Northwest Coast of Portugal. We focused on resistance to beta-lactams encoded by transferable genetic determinants. A high diversity of beta-lactamase genes, similar to those found in clinical settings, was characterized using culture-dependent and culture-independent methods. Extended-spectrum beta-lactamase (ESBL) genes, particularly blaCTX-M-like genes, were detected in polluted rivers. Their genomic context was as previously described for clinical isolates. Differences between polluted and unpolluted rivers in terms of AR genes diversity were also uncovered using culture-independent methodologies. Co-selection of resistance to beta-lactams, aminoglycosides, quinolones and tetracyclines was frequently observed. Integrons and conjugative plasmids of groups IncF, IncK and IncI1 were implicated in these co-selection events. Our results favor the hypothesis that anthropogenic-driven selective pressures contribute to the persistence and dissemination of AR genes in aquatic systems, including genes conferring resistance to antibiotics that are critically important for human health. Mobile genetic platforms carrying multiple resistance determinants are clearly involved in the dissemination process. AR data may not only provide insights into water quality but may also allow to identify contamination sources.

Acknowledgements: I. Henriques acknowledges support from Fundação para a Ciência e Tecnologia (FCT) by Program Investigador FCT (IF/00492/2013), co-funded by the European Social Fund (ESF) through the Operational Program Human Potential (POPH). Authors thank FCT for financial support through the WATER JPI project StARE (WaterJPI/0002/2013).

Antibiotic resistance genes in an urbanizing watershed
Y. G. Zhu1, J. Q. Su1, F. Y. H. Zhao1, X. L. An1, W. Y. Ouyang1
1Chinese Academy of Sciences, Key Lab of Urban Environment and Health, Institute of Urban Environment, Xiamen, China

Jiulong River Watershed is located in the southwest of Fujian Province, a typical medium-size subtropical coastal watershed with the area of 14,742 km² and total length of about 1,285 km. The watershed has one main stream (north river) and two tributaries (west river and south river). In 2012, total population in the watershed is nearly 4 million and the average urbanization rate is about 55%. In this study, high throughput PCR was employed to investigate the temporal and spatial distribution of antibiotic resistance genes (ARGs) in the whole watershed. We selected 26 sampling sites, and four sampling times between Feb. 2013 and Dec. 2013. In this study, a total of 179 unique ARGs and 9 transposases were detected across all the samples. ARGs were detected with significantly higher abundance and diversity than that in source samples of Jiulongjiang river. Aminoglycosides, multidrug and tetracyclines resistance genes were the most prevalent. Normalized copy number and fold changes of ARGs showed obvious enrichment of ARGs in watershed samples. Our results demonstrated that urbanization has major impact on the abundance and diversity of AGRs within the watershed. Based on the copy numbers of ARGs detected at the estuarine samples and water flux, we estimated that at least 8.93x10²¹ copies of various ARGs were discharged into coastal water from Jiulongjiang river in 2013.

Acknowledgements: This study was financially supported by the Natural Science Foundation of China (21210008).

Antibiotic Resistance Genes in Sewage and Related Water Environment
T. Zhang1, Y. Yang1, B. Li1, X. X. Zhang1, L. Ma1, M. Zhang1
1The University of Hong Kong, Department of Civil Engineering, Hong Kong, China

Introduction: The occurrence, transformation and fates of antibiotics in sewage treatment plants (STPs) have been well studied [1-4]. But limited information is available on the emergence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) although ARB and ARGs have received great concerns [5], and been detected in various water environments and STPs [6,7]. STPs were regarded as the hotspots of ARB and ARGs. With the development of environmental molecular techniques and bioinformatics, some studies have been conducted and the role of STPs and the impact of human activity on the dissemination of ARGs became clearer. This study summarized some important works done in this field by our group.
Materials and Methods: DNA extracted from samples collected in STPs and related water environment were subjected for ARGs analysis using cloning-sequencing, quantitative PCR, or high-throughput-sequencing-based shotgun metagenomics. 

Results and Conclusions: (1) Occurrence and abundance of tetracycline and β-lactam resistance genes in activated sludge from 15 WWTPs across China and other global locations were determined using PCR and quantitative PCR [7,8]. Enrichment experiment revealed the major tetracycline resistant bacteria in activated sludge [9].

(2) The broad-spectrum profile of ARGs in activated sludge from a STP in a four-year period was investigated through metagenomic analysis using a structured ARGs database [10].

(3) HTS-based metagenomic approach was applied to investigate the profiles and fate of ARGs in a full scale STP. Totally, 271 ARGs subtypes belonging to 18 ARGs types were identified. Removal efficiency of ARGs in wastewater treatment is 99.82% while it was 20.70% in sludge treatment [11].

(4) PCR based molecular method and HTS-based metagenomics analysis revealed high levels of various ARGs in the plasmid metagenome as well as mobile genetic elements (MGEs) from activated sludge, including integrons, transposons and plasmids [12,13].

The abundance and diversity of ARGs and MGEs were also investigated through metagenomic analysis in aquaculture farm sediments, activated sludge, biofilm, anaerobic digestion sludge, and river water [14].

(5) Profiles of ARGs and MGEs were studied in sediment from deep ocean bed of South China Sea and Pearl River Estuary [15].

References

I 11
Wastewater irrigation increases abundance of antibiotic resistance genes and potentially harmful Gammaproteobacteria in soils from Mezquital Valley, Mexico

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Wastewater (ww) use for agriculture poses chances and risks at the same time. In some areas, e.g., semi-arid and arid areas, this practice is the only way to ensure farming over the entire year. In addition, the ww provides the necessary nutrients (C, P, N) for the cultivation of plants, making fertilizer application unnecessary. However, the release of pathogenic bacteria, antibiotics, antibiotic resistance genes, and other contaminants into the environment, might promote their accumulation and possibly also a dissemination of multi-resistant bacteria. In a study on one of the world’s largest irrigation fields, the Mezquital Valley in the North of Mexico City, quantitative real-time PCR analyses showed that large numbers of antibiotic resistance genes and nosocomial pathogens are released with the ww into the soils by irrigation. In particular, an increase of sul resistance genes of about two orders of magnitude (in relation to bacterial 16S rDNA copies) was observed in ww-irrigated soils compared to rain-fed soils.

tet(W), tet(Q) and aadA antibiotic resistance genes, class 1 integrons (intf1), quaternary ammonium compound resistance genes (qacE=+qacEd1f) and IncP-1 plasmids (korB) were also quantified by real-time PCR. Except for intf1 and qacE=+qacEd1f the abundances were below the detection limit in non-irrigated soil. The absolute abundance of 16S RNA genes in the ww-irrigated soils increased significantly over time suggesting an increase in bacterial biomass due to repeated ww-irrigation. Correspondingly, all resistance genes as well as intf1 and korB significantly increased in abundance over the period of 100 years of irrigation. In parallel, concentrations of the heavy metals Zn, Cu, Pb, Ni, and Cr significantly increased. However, no significant positive
correlations were found between the relative abundance of these genes and years of irrigation, indicating no enrichment in the soil bacterial community due to repeated ww irrigation or potential co-selection by increasing concentrations of heavy metals. ww irrigation has also a great impact on the bacterial soil community. In ww-irrigated soils of the Mezquital Valley a significant increase of γ-Proteobacteria was observed by amplicon sequencing compared to rain-fed soils. At genus level potentially pathogenic organisms could be detected, which were not found in rain-fed soils. These findings were confirmed by cultivation of bacteria from both irrigation regimes. 16S rDNA sequencing revealed an increased occurrence of γ-Proteobacteria (39 % Stenotrophomonas and 5 % Pseudomonas) among the isolates from ww-irrigated soils. These bacteria could not be isolated from rain-fed soils. Antibiotic resistance analyses of the isolates showed a greater incidence of antibiotic resistances, especially of multiresistances in the isolates from ww-irrigated soils. To determine potential health risks for consumers of the crops from ww-irrigation fields, total DNA from plants collected in irrigation fields was examined for enterococci and antibiotic resistance genes by PCR.

In summary, it was demonstrated that there was no uptake of resistance determinants by the plants. We conclude that major risks of ww irrigation are caused by direct contact with the ww and by consumption of insufficiently washed crops originating from ww-irrigation fields.

I 12
Environmental pollution from antibiotic manufacturing creates hotspots for resistance development
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Introduction: Antibiotic resistant bacteria and resistance genes occur in all environments. That, however, does not make all environments equal in terms of risks for recruitment of novel resistance factors from the environmental reservoir to human pathogens. On the contrary, risks will depend on selection pressures, abundances and nature of the resistance factor(s) present, as well as potential for gene transfer and further dissemination to humans. It is important to recall that emergence of resistance in a pathogen in principle only need to arise once, at one site. Thus, managing risks with “hotspots” are crucial regardless of where on earth they may be located.

Objective: To characterize known and novel antibiotic resistance factors in various environments polluted with high levels of fluoroquinolones (mg/L) from bulk drug manufacturing in Patancheru, India.

Methods: We have applied classical culturing, shotgun metagenomics, quantitative PCR, functional metagenomics, sequencing of integron cassettes, whole genome sequencing, plasmid capture experiments, and plasmid sequencing.

Results: To the best of our knowledge, the investigated industrial treatment plant harbours bacteria with the most extreme multi-resistance profile described in any environment. Similarly, the occurrence of integrons, analyzed both by community PCR and PCR of isolates, is unprecedented here. River and lake sediments are hosts for bacteria with resistance genes of principally all classes in very high numbers as assessed by metagenomics and quantitative PCR. Functional metagenomics revealed several novel resistance gene candidates for betalactams, quinolones, chloramphenicol and aminoglycosides. Interestingly, plasmids and genes involved in horizontal gene transfer are highly abundant as well. In accordance, we have captured several novel broad-host conjugative resistance plasmids, some of which contain the qnr VC1 gene, previously only known as a chromosomal gene.

Conclusion: These findings show that aquatic environments, exposed to exceedingly high levels of antibiotics for extended times, are incubators for multi-resistant bacteria with apparent risks for gene transfer to human pathogens. Actions are therefore urgently needed to reduce risks at such locations.

I 13
Mitigation strategies for environmental sources of antibiotic resistance
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Introduction: Antibiotic resistance likely represents one of the greatest health threats of this century, calling for a comprehensive and global strategy to combat its spread. Monitoring and quantifying the contribution of environmental sources of antibiotic resistance to human infections is an essential component of such a strategy. In tandem with such efforts, it is ideal to move forward in identifying effective mitigation strategies for limiting the spread of environmental sources of resistance and maximizing the lifespan of effective antibiotics for human health.

Objectives: The overall objective of this presentation is to synthesize the state of the knowledge of likely effective mitigation strategies that present barriers to the spread of environmental sources of antibiotic resistant bacteria and their antibiotic resistance genes (ARGs). Knowledge gaps, especially with respect to current trends in water and waste management, will be identified and an example of current research on recycled wastewater will be presented.
**Materials & Methods:** Examples will be presented across the agricultural, water, and wastewater sectors with emphasis on treatment technologies and methodology for their evaluation.

**Results:** Mitigation strategies should ideally be synergistic with other goals and considered holistically along with other relevant environmental and human health factors. Balance of cost and risk will have to be considered, and the most stringent (and also costly) technologies may be merited for likely “hot spots,” such as hospital waste. Evaluation of mitigation effectiveness presents a significant challenge given that there are no ideal methods for tracking the spread of antibiotic resistance, particularly with respect to the mobilization of ARGs. Methods such as culturing, q-PCR, q-PCR array, genomics/metagenomics, and their combination are valuable research tools, but need to be simplified and ideally standardized for monitoring and regulatory purposes.

**Conclusion:** The present time is opportune for advancing mitigation strategies for controlling the spread of environmental sources of antibiotic resistance. Considerations can be integrated into water and waste management strategies to present barriers to contamination of antibiotics, resistant bacteria, and ARGs.
Antibiotic resistance in bacteria is of major clinical and industrial concern, making a reliable method for prediction desirable. Contemporary metagenomic studies assume similarity to known resistance genes implies resistance, which is subsequently found throughout microbial niches. However, as many resistance genes evolved from housekeeping genes these analyses overestimate the prevalence of antibiotic resistance. A growing body of research today points to a link between antibiotic resistance and horizontal gene transfer. Given that few genes encoded by plasmids are housekeeping genes we hypothesize that by comparing gene synteny of putative resistance genes encoded by plasmids and chromosomes, we are able to separate housekeeping genes from resistance genes.

Using the penicillin resistance providing beta-lactamases as an example, we identify all putative beta-lactamase encoding genes using contemporary bioinformatics and combine them with a novel approach that utilizes gene synteny information to predict
antibiotic resistance from genomic scale sequencing data. The dataset we use is all fully sequenced bacterial and archaeal chromosomes and plasmids found in Genbank. To test our predictions we compare with experimental data forming part of this study and data found in the literature.

The contemporary annotation method identified 6145 putative beta-lactamase encoding genes, of which 4027 were annotated as such in just 1501 chromosomes. Analysis of the putative beta-lactamase gene synteny revealed that 90% of all plasmid encoded putative beta-lactamase encoding genes are proximal to horizontal gene transfer instigator, mainly transposases, while this is only the case for 8% of chromosomally encoded putative beta-lactamases. Finally we can trace all identified de facto beta-lactamase encoding genes back to 18 transitions from housekeeping genes. Our analysis shows a significant overestimation of beta-lactam resistance in Genbank and indicates that the origin of an acquired resistance gene is a rare occurrence.

In conclusion, by whole genome and plasmid analysis for gene synteny, we show that sequence homology is a poor measure for antibiotic resistance when analyzing metagenomic data.

O EVO 3
Insights into the resistome of bacterial communities from environments shaped by exceptional antibiotic selection pressures
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Introduction: Evolution has provided environmental bacteria with a plethora of genes that give resistance to antibiotic compounds. Under the selection pressure of antibiotics, a subset of these genes is believed to be recruited over time into pathogens by horizontal gene transfer. In pristine environments resistance genes are relatively rare; but their abundance, and also the risk for gene transfer events are likely to increase in antibiotic-contaminated environments. River sediment polluted with fluoroquinolones and other drugs discharged from bulk drug production in the Patancheru area in India, constitutes an environment with unprecedented, long-term antibiotic selection pressures. We hypothesize that previously unknown resistance genes have evolved and/or promoted here.

Objective: The objective was to explore the presence of novel antibiotic resistance gene candidates in highly fluoroquinolone-polluted river sediments.

Methods: We analyzed the river sediment resistome by a functional metagenomics approach, screening for DNA fragments providing resistance to different antibiotics in E. coli. DNA-inserts from E. coli with acquired resistance were sequenced using Sanger and PacBio RSII platform.

Results: Functional metagenomics recaptured the majority of the known antibiotic resistance genes previously identified from these river sediments by open shot-gun metagenomics. More interestingly, some of the resistance gene candidates were novel (less than 85% similarity to known genes). This included one novel qnr-like gene, a gene providing resistance to amikacin and a novel putative chloramphenicol acetyltransferase. We also found 12 novel β-lactamase-like genes, including one novel gene similar to class A carbapenemase. Functional metagenomics provide limited information on context, but some of the identified genes were associated with plasmid replication genes, integrons, transposons, and/or other resistance genes, suggesting a potential for mobility.

Conclusion: This study provides insight into a resistome shaped by an exceptionally strong and long-term antibiotic selection pressure. Understanding the mechanisms of resistance evolved under such conditions may make us better prepared for the resistance-challenges we will face in the clinics in the future.

O EVO 4
Bacterial antibiotic resistance can be induced by disinfection byproducts via mutagenesis
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Bacterial antibiotic resistance (BAR) in drinking water has become a global issue because it might threaten both environmental safety and human health. Usually the antibiotic concentrations in drinking water are too low to select antibiotic resistant strains effectively, suggesting that factors other than antibiotics would contribute to the emergence of BAR. In the current study, the impacts of mutagenic disinfection byproducts (DBPs) on BAR were explored, using seven typical DBPs, i.e., dibromochloroacetic acid (DBAA), dichlorocarboxonitrile (DCAN), trichlorocarboxonitrile (TCAN), bromoacetamide (BAcAm), tribromonitromethane (TBNM), potassium bromate (KBrO3) and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). After exposure to DBPs, Pseudomonas aeruginosa PAO1 gained increased resistance to both individual and multiple antibiotics. Norfloxacin and polymycin B resistances by treatment with MX, as well as ciprofloxacin and rifampin resistances by TBNM were enhanced even greater than tenfold compared with control. Obvious dose-response relationship was observed between most of the BAR increase and the
dosages of the selected DBPs. The increase of BAR was caused by the mutagenicity of DBPs, since antibiotic resistant mutation frequency declined by adding ROS scavenger. Mutagenesis was further confirmed by sequencing and aligning of the genes related to antibiotic resistance. Moreover, DBPs had the same effect on Escherichia coli, suggesting this effect is a common phenomenon that may contribute to the increase of BAR in drinking water. Our study revealed that, in addition to the known horizontal gene transfer pathway, mutagenesis caused by DBPs could also induce antibiotic resistance, even multidrug resistance, which may partially explain the lack of agreement between BAR and antibiotic levels in drinking water.

Poster Presentations

**P EVO 1**

**Antibacterial property of local soap (ncha nkota) sold in local markets in Nigeria, West Africa.**

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The local dark soap is one of the oldest soap known in the history of soaps in Nigeria. Local soaps were obtained from Nigerian cities of Lagos, Aba and Okigwe. Staphylococcus aureus and Staphylococcus epidermidis were obtained from the Microbiology Laboratory of Abia state University Uturu. The agar well diffusion and disc diffusion techniques were used for the susceptibility testing of these organisms to these local soaps. Their MIC’s were measured and results obtained showed that 0.278mg/ml was the value for these local soaps. The local soaps from Lagos gave the best antibacterial properties due to the method of preparation and the additives which they contain. The Aba soaps were effective against the S. epidermidis only while the Okigwe soaps did not give significant zones of inhibition against these test organisms.

**P EVO 2**

**Antimicrobial resistance of *Staphylococcus epidermidis* isolated in Tlemcen “Algeria”**

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Nosocomial infections are a real public health problem. We are interested in finding a particular species Staphylococcus epidermidis in the trauma unit knowing that it is more resistant to antibiotics and met more and more in a variety of infections in hospitals.

Therefore we conducted 265 samples in this unit at the University Hospital of Tlemcen, which consisted of nasal ports (48h before surgery) and surgical wounds (4 days after surgery) in operate patients. Using the API STAPH system 75 strains of *S. Epidermidis* were identified.

The drug resistance as NCCLS standards reveals a rate of 90% of multiresistant strains, mainly to ampicillin (100%), penicillin (100%), oxacillin (93.33%). We also found that 66.66% of strains were resistant to erythromycin and 33.33% resistant to vancomycin and only one strain was sensitive to oxacillin.

The study of the minimum inhibitory concentration for oxacillin showed a MIC <= 0.25 mg / l, which is consistent with the results of susceptibility testing.

**P EVO 3**

**Phenotypic and genotypic determination of mupirocin resistance among methicillin susceptibility and resistance in staphylococci isolated from nosocomial infection**

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Introduction: Mupirocin is a topical antibiotic. This antibiotic can inhibit most of gram-positive cocci. Shortly after consuming the antibiotic mupirocin, resistance has emerged. The main purpose of this study was to determine mupirocin resistance in Staphylococcus strains isolated from nosocomial infections in the city of Arak.

Material and Methods: A total of 150 Staphylococcus isolates (sensitive and resistant to methicillin S.aureus, coagulase negative staphylococcus) were subjected to the present study. In this study, 150 isolates of staphylococci were examined. PCR amplification of Sa442 gene was used as the identification marker for the confirmation of phenotypic diagnosis through biochemical and biological methods. In order to determine the presence of mecA gene in *S. aureus* isolates that were resistant to methicillin. All isolates were tested for mupirocin susceptibility by a disc diffusion method according
to CLSI guideline. The minimum inhibitory concentration (MIC) was determined by an E-test and they were also analyzed by a PCR for the presence of ileS-1, mupA and mupB genes.

**Results:** Among the 150 strains examined, 11 isolates were known resistant in disc diffusion and E-test. PCR result indicated that one isolate contains gene ileS-1, four isolates contain genes mupA and 6 strains containing both genes mupA and ileS-1. There was no strain that contained genes mupB and PCR results were fully consistent with the results of the E-test.

**Conclusion:** This is a report of low frequency of mupirocin resistance in the city of Arak. This result illustrated that the prescription of mupirocin by physicians in this geographical region is limited. However, incorrect use can lead to rise in resistant rate. In addition, the combination of phenotypic methods and PCR for the detection of resistance to mupirocin is recommended.

**References:**


**P EVO 4**

**Evidence of continuous use of chloroquine in Ghana after its ban: Effect on antimalarial drug resistance**

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**Introduction:** Lost of efficacy of Chloroquine (CQ) treatment resulted in the adaptation of artesunate-amodiaquine as the first-line drug for the treatment of uncomplicated malaria by the Ministry of Health, Ghana, in January, 2005. However, anecdotal evidence suggests that CQ is still being used in some communities in Ghana. This study was conducted to evaluate the continuous use of CQ and its effect on resistance markers in four communities in the Central Region of Ghana.

**Methodology:** Questionnaire, mystery buying method and urine CQ assay was used to survey continuous usage of CQ in the study communities. The prevalence of point mutations of Pfcrt and Pfmdr1 genes were assessed from *P. falciparum* isolates in blood samples of subjects employing the nested polymerase chain reaction (nested PCR) and restriction fragment length polymorphism (RFLP) techniques.

**Results:** A total of 618 subjects participated in the studies; 0.49% affirmed to be still using CQ; 16.9% of participants had CQ in their urine and 14.49% CQ stocking was obtained from communities. Of the 214 *P. falciparum* isolates; 53.74% had T76 mutation in Pfcrt. Also, 36.0%, 87.9%, 71.0%, 91.6% and 8.4% mutations at N86Y, Y184F, S1034C, N1042D and D1246Y of Pfmdr1 gene respectively, were found. Mutation at position 76 of Pfcrt gene was strongly associated with double mutations ($\chi^2=18.045, p=0.006$), triple mutations ($\chi^2=13.770, p=0.032$) and ($\chi^2=16.489, p=0.011$) of pfmdr1.

**Conclusion:** This study has revealed that the continuous use of CQ in Ghana has contributed to increased point mutations of pfmdr1 gene.

**P EVO 5**

**Investigating co-selection for antibiotic resistance in the environment**

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Antibiotic resistant bacteria present a major threat to human health and place an ever increasing financial burden on healthcare systems. Co-selection for antibiotic resistance could be occurring in the environment due to release of contaminants other than antibiotic residues, such as quaternary ammonium compounds (QACs). Found in many widely used products, they are released into the environment in large quantities and so may exert greater selective pressure than antibiotics.

The objective of the study was to characterise QAC resistance mechanisms in polluted matrices and determine if co-selection for antibiotic resistance was occurring. Metagenomic libraries constructed previously from polluted reed bed soil, sewage cake and ‘pristine’ grassland soil were screened on two QACs: benzalkonium chloride (BKC) and cetyltrimethyl ammonium bromide (CTAB). Unique inserts (determined by restriction digestion) from BKC resistant clones underwent transposon mutagenesis. Functional screening identified resistance gene knock outs, which were then sequenced. GenBank ORF Finder and BLASTp searches determined possible ORF functions. Cross-resistance was briefly investigated by comparing MICs of unique BKC resistant clones and corresponding knock outs to two antibiotics. Co-resistance was investigated by screening approximately equal numbers of CTAB resistant clones and clones isolated from the entire polluted libraries on two antibiotics at the clinical breakpoint (EUCAST), MIC and ~1.5x MIC of the host with empty vector.

Resistance to both QACs was markedly higher in libraries from reed bed sediment and sewage sludge than from grassland. Potential resistance mechanisms were diverse and the most common was an epimerase; similar genes have been shown to confer tetracycline resistance in a previous study [1]. Co-selection was not observed in the clones or for the antibiotics tested. Clinical resistance was rare (40%).

**References:**

Results to date suggest we are directly impacting evolution of environmental bacteria, increasing resistance to QACs. Further investigation of the epimerase-containing clones and complete sequencing of unique inserts is currently underway to determine if co-selection was occurring.


P EVO 6
Multicenter study of resistance to antibiotics in Enterobacter cloacae
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Method: Enterobacter cloacae is a major pathogen responsible of nosocomial infections. Pathogénicité is exacerbated by its resistance to antibiotics, acquired by extended spectrum β-lactamases (ESBL) and plasmid AmpC (pAmpC), often associated with resistance to aminoglycosides and quinolones. A multicenter retrospective cohort study was carried out to gain baseline information on antibiotics resistance of E. cloacae in tree hospitals in the west of Algeria.

Result: 158 strains were isolated between September 2009 and May 2012 from various units in the hospitals of Tiemcen, Sidi Bel Abbès and Oran. The analysis of resistance phenotypes to b-lactam has detected diversity phenotypic with dominance of strain producing extended spectrum β-lactamase (ESBL) or 51.3%. The pulsed field gel electrophoresis (PFGE) showed different clonal groups and confirm the epidemic nature of the strains studied. The most isolates produced ESBL CTX-M type, whereas only 5 produced SHV-type ESBLs. The blaTEM gene was found in all strains of E. cloacae. One isolate was found to produce plasmid-mediated AmpC β-lactamases (CMY-2), this gene was transferred from E. cloacae by electroporation. Conjugation experiments showed that blaCTX-M, blaTEM, and blaSHV were carried by conjugative plasmids of high molecular weight (≥ 70 kb).

Conclusion: These results show that the frequency of these multiresistant bacteria increasing dramatically in our hospitals and their emergence represents a serious therapeutic and epidemiological problem, hence the need for the establishment of a monitoring system of the microbial environment and strict application of hygiene measures.

P EVO 7
Co-selection for antibiotic resistant bacteria at sub-inhibitory concentration of biocides
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Antibiotic resistance (AR) poses an increasing threat to health care and to the environment. Gaining insights into the mechanisms of selection and enrichment of antibiotic resistant bacteria (ARB) in the gut of humans/animals and the natural environment is essential to tackle the problem. In addition to the use of antibiotics, excessive use of biocides has been hypothesised to result in co-selection for ARB due to genetic linkage between antibiotic and biocide resistance genes. To test this hypothesis, an isogenic pair of bacteria with and without biocide resistance genes, qacE or qacH that are commonly found in ARB, were constructed in low copy number plasmid under control of their natural promoters. The pair was competed in growth medium for 60 generations at sub-inhibitory concentrations of a biocide, benzalkonium chloride (BKC). In addition, sewage influent natural bacterial community was inoculated into medium and allowed to grow for 60 generations in the presence of BKC at sub-inhibitory concentrations. The proportion of the resistance strain to the isogenic susceptible strain and prevalence of class 1 integrons (Int1) in the complex community were quantified using Q-PCR. Minimal inhibitory concentration (MIC) and selective concentration (MSC) of BKC for the resistant strain were determined. It was shown that the biocide selected for qacE or qacH bearing bacteria at sub-inhibitory concentration in the dual strains system and enriched for Int1 bearing bacteria in the semi-natural system. The results indicated that biocides co-selects for ARB under laboratory conditions at sub MIC concentrations and suggests that such co-selection may occur in the natural environment.

P EVO 8
Modulation of microbial community permissiveness towards broad host range conjugal plasmid is metal specific
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Questions: The extent by which antibiotic resistance encoding conjugal plasmids transfer in microbial communities is of acute relevance in the age of massive antibiotic usage. When a plasmid newly enters a bacterial community, the community permissiveness towards plasmids is the key parameter to assess its spread. Transfer and maintenance of plasmids within the community are determined by specific genetic traits of the plasmid and of the donor and recipient strains. Apart from these genetic determinants, the occurrence of stressors might play a major role in altering the acute permissiveness of a bacterial community,
since plasmid transfer is considered a main process in immediate stress response and adaptation to environmental changes. One of the major sources of stress for soil communities is the introduction of metals through geological or anthropogenic sources like manure. We, therefore, aimed to answer the following questions: Does metal stress alter a soil community’s permissiveness towards broad host range plasmids and if so, can these changes be explained through the change in community diversity caused by the imposed stress? Do we observe a general stress response with regard to plasmid transfer for different metals introduced at equal inhibition levels or is this stress response metal specific?

Methods: We used a radiolabeled [3H]leucine incorporation approach to measure the 20% and 50% inhibition concentrations for 5 selected metals (Cu, Zn, Ni, Cd, As). A mCherry-tagged red fluorescent Escherichia coli donor strain carrying the gfp-tagged broad host range plasmid pJK5 was then mixed with a soil bacterial community and exposed to the metal stressor in a filter mating assay mimicking natural nutrient conditions, with maximized cell-to-cell contact. Plasmid transfer was observed and quantified by detecting green fluorescent transconjugant microcolonies using confocal laser scanning microscopy. Transconjugants were isolated using fluorescent activated cell sorting for bacterial size, gfp-based green fluorescence and exclusion of red fluorescence. Sorted transconjugants and stressed bacterial communities were subsequently analyzed by 16S rRNA gene amplicon pyrosequencing.

Results: The imposed metal stress lowered the transplasmid transfer frequency in the filter mating assays. The intensity of this effect was metal specific and could not be explained by the measured growth inhibition of the recipient community because the exposure was normalized to cause similar inhibition. Cadmium had a strong negative effect on the community’s permissiveness, while Zinc caused almost no detectable effect. A high diversity of transconjugants was retained within all transconjugal pools irrespective of stress exposure. Yet results revealed an effect on community permissiveness for the heavy metals Nickel or Copper, while Arsenic exposure caused no effect. The changes in transconjugal pool diversity could not be directly correlated to a change in community diversity through the introduced stress.

Conclusions: We demonstrate that metal stress changes the plasmid transfer ability within soil bacterial communities. Our results furthermore suggest that this effect is not general but corresponds to a metal specific stress response of the soil microbial community with regard to plasmid transfer.

P. EVO 9  
The role of the insertion sequence IS256 in genetic flexibility in Staphylococcus aureus

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Staphylococcus aureus is a pathogen that causes nosocomial and community-acquired infections. In recent years S. aureus has acquired resistance to nearly all antibiotics used in clinical practice. As a component of transposons, insertion (IS) elements are involved in the transfer of resistance genes between strains and species. Furthermore, the integration of a single IS element into a gene or its promoter may result in an inactivation or overexpression of the affected gene. IS256 is found frequently in CC8 and ST239 strains of S. aureus. As exposure to subinhibitory concentrations of antibiotics will not only select for resistant bacteria but may lead to an activation of mutational mechanisms, as for example the SOS response (1), the following questions were addressed in this work:

Questions: Is IS256 transposition activity affected by low concentrations of antibiotics? Is IS256 involved in generation of intermediate vancomycin resistance in S. aureus (VISA: vancomycin intermediate S. aureus)?

Methods: The transposition frequency of IS256 was monitored using a recombinant IS element in several host strains in the presence and absence of antibiotics and in the absence and presence of Sigma factor B. Insertions of IS256 were mapped by genomic sequencing in a clinical (SA137/93A) and a laboratory VISA isolate (SA137/93G).

Results: Low concentrations of antibiotics seem to activate transposition frequency whereas the activity of the stress sigma factor B inhibits the transposition of IS256 (2, 3). Furthermore, we identified the 3’ end of the rsbU gene, which encodes a positive regulator of sigma factor B, as a hotspot for IS256 insertion in the clinical isolate S. aureus SA137/93G as well as in the laboratory strain S. aureus HG001. Interestingly, subinhibitory concentrations of chloramphenicol in combination with heat stress, as well as linezolid and spectinomycin at physiological temperatures, selected for such rsbU:IS256 insertion mutants. In consequence of the inactivation of rsbU, the IS256 transposition frequency was increased 4-fold in the S. aureus HG001 mutant (4). Sequencing showed that the two VISA strains contained the so far highest number (SA137/93A: 44 insertions; SA137/93G 38 insertion) of insertions of IS256 of all sequenced strains harbouring this IS element. For two insertions an influence on resistance to vancomycin was demonstrated (5).

Conclusions: Low concentrations of antibiotics may activate transposition of IS256. As shown for the VISA strains the insertions may lead to an increase in resistance against antibiotics or - after insertion into a regulatory gene - an increase in transposition frequency.

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**Question.** A significantly increased level of antimicrobial resistance is demonstrated with regard to the bacteria present in the aquatic habitats. Thus, in such areas: a) migratory wild birds could pose serious risks to residents and tourists, being reservoirs/vectors for potentially MDR resistant pathogens and/or b) the sedentary birds should carry MDR bacteria, being in contact with domestic animals subjected to antibiotic therapy, and should represent a reservoir for the migratory birds. The research aimed to verify the truthfulness/overlap of the two hypotheses.

**Methods.** A total of 112 pharyngeal and cloacal swabs from 26 species of migratory and sedentary birds from the Danube Delta were processed by classical bacteriology methods. 113 strains were identified by use of Chromogenic UTI medium Brilliance™ and TCBS Cholera medium (Oxoid) as belonging to *Vibrio*, *Pseudomonas*, *Proteus*, *Escherichia*, *Staphylococcus* and *Enterococcus* genera. The antibiotic sensitivity patterns of 45 randomly selected bacteria (n=5-10 of each genus) to marbofloxacine, eritromycine, amikacyne, ampicilline, penicilline, enrofloxacin, ciprofloxacin, streptomycin were established based on the results of Kirby Bauer method, according to CLSI standards. MAR (multiple antibiotic resistance) (Krumperman, 1983) index for each strain and bacterial species as well as mean values for each bacterial genus were calculated.

**Results.** Out of 43 strains resistant to antibiotics, 17 partially or totally resistant strains were isolated in majority from sedentary *Parus major* and *Passer montanus*. More numerous migratory species such as: *Anas crecca*, *Tringa glareola*, *Embeliza schoeniclus*, *Tringa nebularia*, *Egretta garzetta*, *Falco subbuteo*, *Hippolais icterina*, *Gallinago gallinago* carried MDR resistant bacteria. Resistance to penicillin and ampicilline was present in the highest percent, when compared to other antibiotics. The strains sensitive to penicilline/ampicilline were sensitive to all tested antibiotics. The highest resistance was recorded for the emerging pathogen *V. mimicus*/metchnikovii, as specified by literature (Okada et al., 2010) followed by *Pseudomonas* aeruginosa and *Escherichia* spp. The most sensitive species were *V. alginoliticus*, isolated only from migratory birds, *E. faecalis* in sedentary and *Enterococcus* spp. in migratory birds (Fig. 1). Partial resistance (resistant colonies) was found in 21 of the isolated strains, of which 76.10 % were present in migratory birds. Calculation of the MAR index for the strains isolated from wild birds indicated a non-significantly (p=0.452) higher average value in migratory birds (0.258±0.08) when compared to sedentary (0.217±0.16) ones.

**Fig. 1.** MAR index for randomly selected bacterial species in sedentary and migratory birds from the Danube Delta Biosphere Natural Reserve

**Conclusions.** Both migratory and sedentary wild birds in our study were potential sources of MDR bacteria, with possible exchanges occurring between these two categories. The sanitary importance of MDR emerging bacteria in migratory birds is being stressed by the antibiotic resistance and pathogenic potential for contact categories, further studies being needed to establish the underlying transfer mechanisms.

**References**
Studies on the correlation between metal content in polluted soil with bacterial resistance to antibiotics and metals

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Studies on the correlation between metal content in polluted soil with bacterial resistance to antibiotics and metals Alyaa Abdelhameed1, Barry Rawlins2, Ricardo del Sol1, Geertje van Keulen1

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Microbes require some (trace) heavy metals for growth, while high concentrations may be toxic. Metal-resistant microbes have evolved in metal-polluted environments, some of which may also have (co-)developed antibiotics resistance. It is uncertain what the environmental risk is of (spread of) antibiotic resistances to humans from historically metal-contaminated areas. This study analysed the spatial variation of 16 metal concentrations in 375 topsoil samples from the Swansea/Neath/Port Talbot urban area, which contain some of the highest metal-polluted soils of the UK due mostly to metal working activities in the three previous centuries. Pairwise correlations showed mostly significant positive associations between 16 of the studied elements, e.g. copper and arsenic. Factor analysis showed that 68% of the observed variation in concentrations could be explained by four principal components. The archived soils were then subsampled over a range of metal concentrations, yielding 65 subsamples for further biomolecular analysis. Culture-dependent studies on dilute nutrient rich and humic acid agar media yielded many isolates with medium to high tolerance to several metals and antibiotics. Culture-independent studies are being performed to determine the bacterial diversity and abundance of resistance genes over the wide range of observed (mixed) metal concentrations.

Natural transformation as a mechanism for the acquisition of extracellular DNA in bacteria

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Several mechanisms of horizontal gene transfer (HGT) enable dissemination of antimicrobial resistance genes across bacterial populations, species and communities. These mechanisms provide a key basis for both short-term ecological adaptation and long-term evolution in bacteria. Natural transformation is one of these mechanisms that can for instance lead to the acquisition of antibiotic resistance after inter-genomic DNA recombination between both related and unrelated bacterial species. Here I present some results from experimental studies of how species-foreign DNA fragments can be acquired by the naturally transformable soil bacterium Acinetobacter baylyi. Moreover, we describe the fate of such DNA after initial recombination with the host genome. Finally, we present new models for how very short DNA fragments can be recombined with the genome of naturally transformable organisms.
SESSION I: Evolution of antibiotic resistance

P EVO 13
Evolution of antimicrobial resistance
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Introduction: Staphylococcus aureus (SA) and Staphylococcus pseudintermedius (SP) are nasal colonizers and opportunistic pathogens of humans and dogs, respectively. Interspecies transmission (IT) of SA/SP between owners and in-contact pets has been described. Concomitant carriage of SA/SP and methicillin-resistant coagulase-negative staphylococci (MRCoNS) might represent a risk for acquisition of antimicrobial resistance determinants.

Objectives: To analyze MRCoNS and SA/SP co-carriage among owners and co-inhabitant pets to evaluate (i) if owner-pet co-inhabitance may pose a risk for MRCoNS and SA/SP co-carriage and (ii) any possible transference of the β-lactams resistance determinant (mecA) between both bacterial groups.

Materials & Methods: In a former study (Gómez-Sanz; 36:83-94, 2013 CIMID) all nasal coagulase-positive staphylococci (CoPS) recovered from 133 individuals (67 owners, 66 pets -dogs, cats) at one sampling (T0) were studied. Here, MRCoNS from T0 were recovered and characterized. Co-carriage with the CoPS (36 SA, 18 SP) present was evaluated.

Results: Thirty-one MRCoNS were recovered (28.4% of humans, 18.2% of dogs). A total of 56.7% of owners and 45.5% of pets were positive for MRCoNS and/or CoPS. Carriage of MRCoNS as single species recovered was similar in owners and pets (11.9% and 12.1%). However, coexistence of MRCoNS and SA was more common among owners (13.4% versus 3.0%). Both populations predominantly carried only CoPS (>50% of positive individuals) followed by (i) co-carriage of MRCoNS+CoPS in owners (29.0%) and (ii) carriage of MRCoNS only in pets (26.7%).

Two individuals (dogs) carried more than one concomitant MRCoNS and/or CoPS, which were involved in cases of direct IT. Likewise, most individuals positive for more than one bacterial species originated from households with cases of IT. Two owners and their co-inhabitant pet carried methicillin-resistant SA, CoNS and SP, respectively, but different genetic backgrounds enclosing the mecA gene were observed.

Conclusion: Concurrent carriage of MRCoNS and SA/SP reveals common. Owners seem to be more prone to carry MRCoNS in concomitance with SA. Co-inhabitance with pets seems to increase the possibility to co-carry nasal staphylococci. Transmission potential of β-lactams resistance from MRCoNS to CoPS appears low.

P EVO 14
Antimicrobial resistance and integron carriage in Escherichia coli strains isolated from cattle slaughterhouse effluents
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Objectives: Cattle is a reservoir of antibiotic resistant E. coli strains. However, little information is available concerning the prevalence of these resistant strains in slaughterhouse effluents. The aim of this study was to evaluate the prevalence of antibiotic-resistant and integron-carrying E. coli in cattle slaughterhouse effluents.

Materials and Methods: Effluents of two French slaughterhouses were sampled: plant A, which only slaughtered adult cattle, and plant B which only slaughtered veal calves. Four types of samples have been collected: wastewater, treated effluent, recirculating sludge and waste sludge. The susceptibility of 40 E. coli isolates per sampling point was investigated by disc diffusion method testing 16 antibiotics. The screening of class 1, 2, 3 resistant integrons (RIs) has been performed by Real Time PCR on these strains.

Results: The wastewater treatment process eliminated about 99.9% of the E. coli. However, up to 103 CFU/mL E. coli were released into the river, and up to 104 CFU/mL E. coli were present in sludge before land application. The percentage of E. coli resistant to at least one of the tested antibiotics was significantly higher in plant B samples (90.6%) than in plant A (10.0%) (P<0.05). 57.5% and 9.4% of E. coli strains isolated from plant B harbored a class 1 and 2 integron, respectively versus 0.0% in plant A. Percentages of resistant E. coli and class 1 integron-carrying E. coli were not significantly different according to the sampling points.

Conclusion: The wastewater treatment had no selective effect on percentages of resistant and integron-carrying E. coli isolated in effluents from both slaughterhouses. The percentage of resistant and integron-carrying E. coli was significantly higher in calf slaughterhouse effluents.
O MOB 1

Class 1 integrons as markers of antibiotic resistance
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Class 1 integrons are genetic elements that are able to integrate mobile gene cassettes by site specific recombination. Most gene cassettes found in class 1 integrons confer either antibiotic or biocide resistance. This provides a theoretical mechanism for co-selection of antibiotic resistance by exposure to biocides. Class 1 integrons are also often carried on plasmids bearing other adaptive genes, such as metal resistance genes, providing further opportunities for co-selection.

We undertook analyses of class 1 Integron molecular prevalence in a variety of soil and aquatic environments, including from long term field experiments and from a long term sampling programme in the River Thames catchment. Next generation sequencing, functional metagenomic and statistical modelling approaches were used to relate class 1 Integron prevalence to changes in microbial diversity, relative abundance of clinically significant antibiotic resistance genes and to physico-chemical/spatial variables respectively.

Data showed that class 1 Integron prevalence correlated with changes in microbial diversity and relative abundance of key resistance genes. Prevalence also varied dramatically with degree of human impact as measured by land management practice, experimental treatment in field experiments and proximity to point and diffuse pollution sources in aquatic systems.

O MOB 2

Antibiotic use selects for strains with increased permissiveness for broad-host-range resistance plasmids within a local population
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Horizontal gene transfer through broad host-range plasmids can generate genetic variants within a population which might give it the chance to better respond to irregular environmental changes like antibiotic therapy. Thereby the population gains robustness. However, bacteria evolved many protective mechanisms against foreign DNA which often is deleterious. A local population might balance risk and benefit of foreign DNA uptake by modulating the ratio of individuals with high permissiveness to broad-host-range plasmids. We investigated whether heritable plasmid uptake rates varied among genetically indistinguishable isolates from a field population of Dickeya solani.

The transfer frequencies of broad-host-range IncP-1 plasmids pTH10 (IncP-1α) and pB10 (IncP-1β) from *Escherichia coli* to *D. solani* significantly differed among the isolates. Strains that reproducably differed in permissiveness for these plasmids by orders of magnitude were not distinguishable by other phenotypic traits, genomic fingerprints, or *hrpN* gene sequences. Such strains were isolated in close vicinity and from different plots of the field, indicating a reasonably fast genetic mechanism of switching between low and high permissiveness. Thus, the selection of transconjugants of these antibiotic resistance plasmids by antibiotic use will at the same time select for those strains within a population that are highly permissive for plasmid uptake.

In conclusion, a high frequency of permissive strains within a local population will enhance the adaptability of the population. The frequency of permissive strains will increase, if they have a selective advantage due to an acquired plasmid. This will typically occur in an irregular situation (e.g. antibiotic treatment, novel xenobiotics, improved host defence), while a regular environment will select against broad-host-range plasmids and permissivity. Consequently, the use of a particular antibiotic may increase the potential of the population to acquire plasmid-borne antibiotic resistance genes of any kind. This may foster the spread of antibiotic resistance among bacteria.

References


Recent advances in DNA sequencing have opened up possibilities to study the antibiotic resistance potential of bacterial communities in diverse environments. However, there are several methodological pitfalls that may hamper the usefulness of metagenomic resistance gene analyses, and can lead to gross misinterpretation of results. Many of these can easily be overcome by use of appropriate experimental designs, bioinformatics tools, databases and - obviously - taking a priori knowledge into account. In this talk, I will outline some best-practice bioinformatics methods that have enabled us to gain insight into the antibiotic resistome of different communities.

The talk will highlight the some common pitfalls related to interpretation of data, and exemplify how flawed analysis practices can result in misleading conclusions. I will particularly address how taxonomic composition influences the frequencies of resistance genes, the importance of knowledge of the functions of the genes in the databases used, and how normalization strategies influence the results. Furthermore, I will show how the context of resistance genes can allow inference of their potential to spread to human pathogens from environmental or commensal bacteria. All these aspects will be exemplified by data from our studies of environments subjected to pharmaceutical pollution in India, the effect of travel on the human resistome, and modern municipal wastewater treatment processes.

To facilitate analyses, we generally apply a protocol involving in-house developed, tailored resistance gene databases (Resqu and BacMet) and taxonomic analysis software (Metaxa2), as well as existing tools for read mapping (Vmatch and Bowtie) and assembly (Velvet, Ray and SPAdes). The latter often need further adaptation to be successfully applied in a resistance gene context, as illustrated by our work on the TrIMetAs assembler.

We conclude that in order to utilize metagenomic sequencing to gain knowledge of risks for resistance development and dissemination we need to move beyond simply reporting gene frequencies, and address complementary aspects of the data. Moreover, there is a critical need for use of resistance gene databases based on confirmed resistance functions rather than purely homology-based predictions.

Intensive animal productions are considered an important source of antibiotic resistance genes released into the environment, while information on farms with prudent antibiotic use or bio-farms is rare. Here we studied dissemination of tetracycline resistance (TC-r) genes at a dairy farm with prophylactic use of antibiotics. A middle-size dairy farm (300 animals, 800 ha of agricultural soil) in South Bohemia was studied. TC-r genes were screened in feces of cows that intrauterinally received 1 g of chlortetracycline (CTC) after each calving, and in feces of heifers and their first genetic elements responsible for the spread of TC-r genes, TC-r bacteria were isolated from feces, and conjugative plasmids were isolated either directly from feces (exogenous plasmid isolation) or from bacterial cultures carrying TC-r genes. Conjugation (mating pair) tests were used to confirm horizontal transfer of TC-r genes.

Results have shown that (i) the animals at the farm acquired the TC-r genes already 1-2 weeks after the birth; (ii) the TC-resistome of the herd consisted of stable 'core' genes present in virtually all samples (tetA, tetM, tetY and tetX), and variable genes occurring occasionally, but with no relation to the CTC-treatment (tetA, tetM, tetY and tetX); (iii) both soils in the farm and in the field were contaminated by fecal TC-resistome; (iv) the 'core' TC-r genes persisted in the field over 3 months; (v) the bacterial hosts of the 'core' TC-r genes remained unrevealed in this study, (vi) whereas tetA, tetM, tetY and tetX were shown to be harbored by Shigella, Lactobacillus and Clostridium, Acinetobacter and Wautersiella, respectively; (vii) the Shigella isolates were confirmed as active donors responsible for horizontal transfer of tetA gene via plasmids IncI2 and IncFIB; (viii) the genes tetA and tetY were on localized IncP-1IncH2Z and LowGC plasmids, respectively, as shown via exogenous plasmid isolation.

Our study thus showed that also smaller dairy farms with more prudent antibiotic use might be a source of antibiotic resistance gene contamination of their surroundings with potential risk for human health.
O MOB 5
What's the road to resistance? 15 years of antibiotic molecules application to farm soil do not significantly increase resistance genes abundance.

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In a context where pathogens acquire resistance to more and more antibiotic treatments in hospital settings a number of questions are raised regarding mobilization, acquisition and dissemination of antibiotic-resistance traits between and within bacterial populations. Since antibiotic resistance genes (ARG) are common features of many saprophytic soil bacteria [1] a significant ARG reservoir is readily accessible to previously sensitive pathogens by means of horizontal gene transfer (HGT) [2, 3]. It is known since decades [4] that environmental bacteria and pathogens share resistance genes hence the importance to clearly establish transmission routes of such traits in order to prevent their dissemination.

Crop fertilization using manure from animals previously treated with antibiotics is a major anthropogenic entry point of these pharmaceuticals in soils. A number of risk-assessment studies focused on the effect of manure fertilization on soil bacterial community and showed that such practice can indeed increase ARG abundance. Such studies are however most often depicting short- to mid-term impact and cannot distinguish what is leading increase in resistance genes abundance. Our objective was therefore to assess if long-term impact of repeated antibiotic molecules input would have a similar impact on soil bacterial communities, in absence of manure. Can similar concentrations of antibiotic select for resistance traits and increase their dissemination potential in absence of organic matter and intrinsic bacterial community added to soil via manure?

To test this hypothesis we analyzed environmental DNA extracted from soil samples under crop rotation and treated yearly with veterinary antibiotics (tylosin, chlortetracycline and sulfonamides) for 15 years (1999-present). Antibiotic molecule concentration added to soil each year represented similar amount to what is conventionally found in antibiotic-treated animal manure. Contaminated soil plots examined in this analysis represents a 12 years time frame (2001 - 2012) of this long-term experimental setup.

No correlation between increased antibiotic concentration added to soil and antibiotic-resistance gene abundance could be established with our results and the soil bacterial community is not significantly affected by such treatment. Investigation of ARG and mobile genetic element promoting gene transfer between bacterial lineages (i.e.: from environmental bacteria to pathogens) has also been analyzed. It shows that co-abundance is frequent in sequence datasets obtained from soil samples. However, new sequence analysis methods are needed to determine if co-abundance is indeed a sign of co-occurrence on the same molecule, explaining increased transfer potential and successful ARG dissemination.


Poster Presentations

P MOB 1
Horizontal gene transfer through plasmid transport: heavy metal and antibiotic tolerance in bacteria.

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Heavy metal tolerance in bacteria is a common finding when samples are collected from natural environment such as soil, water or air, due to high concentration of heavy metals in the atmosphere. Correlation has been observed between heavy metal tolerance and antibiotic tolerance in bacteria, indicating occurrence of tolerance against number of heavy metals and antibiotics in a single bacterial strain. Multiple stress tolerance in bacteria is a serious threat to the mankind, especially when revealed in pathogenic bacteria. The most common transmissible instrument for resistance among bacteria is the R-plasmid.

The aim of the present study is to monitor horizontal gene transfer of plasmid-determined stress tolerance under lab conditions. E. cloacae (DGE50) & E. coli (DGE57) were used throughout the study. Samples were collected from contaminated water and soil to
isolate bacterial strains having tolerance against heavy metals and antibiotics. We have demonstrated plasmid transfer, from Amp+Cu+Zn- strain (DGE50) to Amp-Cu-Zn+ strain (DGE57), producing Amp+Cu+Zn+ transconjugants (DGETC50→57) and Amp+Cu-Zn+ transformants (DGE TF50→57). DGE57 did not carry any plasmid, therefore, it can be speculated that zinc tolerance gene in DGE57 is located on chromosome. DGE50 was found to carry three plasmids, out of which two were transferred through conjugation into DGE57, and only one was transferred through transformation. Plasmid transferred through transformation was one out of the two transferred through conjugation. Though the results of transformation it was revealed that the genes of copper and ampicillin tolerance in DGE50 are located on separate plasmids, since only ampicillin tolerance genes were transferred through transformation as a result of one plasmid transfer.

By showing transfer of plasmids under lab conditions and monitoring retention of respective phenotype via conjugation and transformation, it is very well demonstrated how multiple stress tolerant strains are generated in nature.

P MOB 2
Characterisation of bacterial populations and identification of antimicrobial resistance markers in the food production environment.
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Introduction: Gastrointestinal disease is a huge socioeconomic problem around the world. Pathogens such as Campylobacter and Salmonella are widely are prevalent in food animals such as poultry, cattle, pigs and shellfish. Although the main route of transmission is thought to be foodborne, in ~50% of human Campylobacter cases, for example, the transmission pathway is unknown. Knowledge about the spread of foodborne pathogens and their antimicrobial resistance profile in the farm environment is important to the food production process. Antimicrobial resistance in these pathogens poses a threat to consumers and has cost and animal welfare issues in food production.

Objectives: We applied Next Generation Sequencing technology to the identification of antimicrobial resistance markers and the characterization of bacterial population structure in UK farm environment samples.

Materials & Methods: In order to whole genome sequence metagenomic DNA samples we used an unbiased approach whereby we extracted gDNA directly from poultry and dairy farm environments, samples were collected from water and by bootsock. Illumina TruSeq DNA libraries were constructed and sequenced these using an Illumina MSeq with v2 chemistry. An in-house bioinformatics pipeline was applied to characterize the microbial population present within the farm environments; in parallel we identified the presence of resistance markers in silico, using tools such as the CARD database.

Results: Our approach has allowed us to demonstrate the presence of pathogens, including Salmonella and Campylobacter down (Figure 1), alongside soil microbiota. Samples differed in their population structure both by type (bootsock or water sample) and by origin (poultry or dairy farm). We further characterized the resistome of these samples which indicates a larger abundance of resistance markers in poultry compared to dairy farm samples (Figure 2).

Conclusion: Our findings give an insight into the true distribution of pathogens in the farm environment; findings related to antimicrobial resistance markers may have implications for the consumer and treatment of food-animals. Using this approach, we will be able to establish how this composition changes over a season and in different niches around the farm, in order to identify transmission pathways into the food chain.

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Role of antibiotic use for resistance gene and integron carriage in Dutch pig farms

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While it is generally assumed that antibiotic use enhances the prevalence of antibiotic resistance in farm animal populations, there are few experimental studies quantifying this relationship, mostly due to the difficulties to obtain high quality antibiotic usage data. Here, we analyse the role of antibiotic selective pressure for resistance gene carriage in farm animals through quantifying tetracycline resistance and class 1 integrons in relation to antibiotic use and other farm parameters in 36 Dutch pig farms. 5 pooled samples of fecal material were taken per farm, and the tetW resistance gene and the intI1 gene were quantified by real-time PCR and normalized to the 16S gene. Animal management, biosecurity and hygiene practices were gathered from a questionnaire. Antibiotic usage was obtained from Dutch inventories of antibiotic product use and was converted to defined daily dosages per animal per year for each antibiotic class. The response of the genes was analysed through linear mixed models in univariate and multivariate models. Tetracycline resistance was relatively similar within and between farms reflecting the overall high usage of tetracyclines in the Netherlands, while the levels of IntI1 varied extensively within single farms and were higher in suckling piglets. The tetW concentration was associated with tetracycline use (doubling of tetracycline use increases tetW concentrations by 7%, p<0.0001). In contrast, among the antibiotic classes, intI1 showed the highest correlation with trimethoprim/sulfonamide use (p=0.003), in line with the presence of sul1 on class 1 integrons. In addition, lower tetracycline resistance was seen in farms with higher levels of external biosecurity (such as presence of a professional pest control program and of showers). IntI1 was negatively associated with internal biosecurity (for example, piglet groups of stable composition). To conclude, even though resistance to tetracyclines was present on all farms in high amounts, antibiotic usage further increased the concentration of tetracycline resistance genes and intI1 in pig feces. This points to the relevance of both reductions in antimicrobial use and changes in biosecurity practices in order to reduce resistance on animal farms.
P MOB 5
Multiple antibiotic resistances among shiga toxin producing Escherichia coli isolated from fecal samples of dairy cattle farms in Eastern Cape Province of South Africa
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Introduction: The use of antimicrobials in animal feed as growth promoters is common worldwide as a variety of antimicrobial agents are available for therapeutic use or as growth promoters in animals. Many studies have supported the claim that with the increased use of antimicrobial agents in animals and humans, an increased prevalence of resistant strains may be selected as a direct consequence of the antimicrobial use. The objectives of this study was to determine the prevalence of Shiga toxin-producing Escherichia coli (STEC) serogroups and antibiotic resistance patterns in feces of commercial dairy cattle.

Methods: During March to May 2014, fecal samples were collected from individual cattle (n = 400) in 2 commercial dairy farms comprising 800 and 120 heard each in the Eastern Cape Province of South Africa. Fecal samples were enriched in E. coli broth and subjected to 6-gene multiplex polymerase chain reaction (PCR) that identifies six O serogroups (O26, O103, O111, O121, O145, and O157) and singleplex PCR for virulence genes (stx1, stx2) on genomic DNA extracted by boiling method.

Results: Based on direct PCR detection, O157 (31.7%) was the only prevalent O serogroup detected as the other targeted serogroups were negative. Prevalence of Shiga toxin product capability among the isolates was 88.4% and distributed as 38.95%, 40% and 9.5% of Stx1, Stx2 occurring singly and 9.5% in combination respectively. Twenty five of the isolates did not harbor Shiga toxin producing genes and are thus nonpathogenic. Multiple antibiotic resistances was observed among the isolates and genetic profiling of resistance genes identified blaampC 90%, blaCMY 70%, blaCTX-M 65%, blaTEM 27% and blaTEM 27%, tetA 70% and strA 80% genes among the resistance isolates.

Conclusion: Results of this study indicate that of all six STEC serogroups profiled; only O157 serogroup is prevalent as they were identified while the other serogroups were not detected. Multiple antimicrobial resistances were observed among the isolates as majority of the isolates were resistant to all the drugs that are commonly used in the clinical management of the bacterial infections in humans. A substantial proportion of serogroup-positive samples did not harbor Shiga toxin genes.

P MOB 6
Pan-genomic identification of molecular correlates of antibiotic resistance in E. coli from children, animals and environment - a follow-up study in rural central India
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Introduction: Antibiotics have made a major contribution to human health but nowadays antibiotic resistance (ABR) is a serious & well established global health problem. The antibiotic resistant bacteria can be transmitted among individuals living in close contacts & to the environment in many ways. One of the obvious factors in the development & spread of ABR is the injudicious use of antibiotics, mostly, by the informal health-care providers (IHP) together with the users’ health seeking behaviour (HSB). Also, the repertoire of bacterial genes associated with ABR is continuously expanding with new genes being discovered regularly. Together with predominance of such IHPs in rural areas of India, it becomes imperative to study ABR in a more detailed & holistic way than it has been addressed till date in such settings so that the genomic attributes are explored in tandem with the social determinants.

Objectives: (1) To identify the epidemiological determinants of ABR pattern of E. coli isolated from stools of children’s cohort & animals sharing their environment over time (2) To determine the correlation of the coliform burden & ABR pattern over time between E. coli isolated from human, animal, environment & water samples (3) To explore the pan-genomic correlates of ABR pattern to selected antibacterial drugs in E. coli isolates using next generation whole genome re-sequencing.

Materials and methods: We are following 125 rural children (and their household members) between 1-3 years of age along with the water they drink, common animals & the waste water in their vicinity. Stool samples from the subjects & waters samples are being collected thrice a year for two years for isolating pure E. coli. Stool samples are processed directly on chromogenic media to morphologically differentiate coliforms while water samples are subjected to membrane filtration technique using same chromogenic media. Phenotypic & molecular analyses of ABR pattern to selected antibiotic classes (penicillins, cephalosporins, aminoglycosides, carbapenems, & fluoroquinolones) are done using standard methods. The E. coli DNA from ABR and susceptible isolates will be subjected to whole genome re-sequencing to reveal known and novel genetic changes associated with ABR.

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pattern. Simultaneously, the analysis of health seeking behaviour of the children's caregivers is also followed with respect to the pattern of antibiotic prescription predominantly by the IHPs to correlate the pattern of ABR with the HSB.

Results: Preliminary results, on the basis of CLSI breakpoints, reflect high proportion of isolates resistant to cephalosporins & fluoroquinolones. There is no resistance found to date for gentamycin & tigecycline among tested isolates. Also, fewer proportions of animal isolates showed phenotypic ABR. Drinking water results showed an average of 122 CFU/ml of water. Molecular studies are currently being done. The study is ongoing & detailed results will be presented at the conference.

Conclusion: Human health risks are associated with exposure to resistant bacteria via environment and contaminated drinking water ingestion. Drinking water appears to be a significant route of transmission of antibiotic resistance among peoples. The results of this study (to date) indicate healthy individuals carry bacteria harbouring resistance to a variety of antibiotics. Such holistic data might be very useful for assessing and designing public-health strategies that aim to reduce the development and spread of ABR.

P MOB 7
Antimicrobial usage in livestock and its implication on antimicrobial resistance spread in Germany
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Since 2009, in a systematic monitoring approach E. coli isolates from primary production, slaughterhouses and retail stores were collected. Minimum inhibitory concentration (MIC) was determined by the broth microdilution method according to CLSI guidelines (M07-A8) and using the plate format EUMVS (TREK Diagnostics Ltd., UK). Results were evaluated on the basis of the epidemiological cut-off values as fixed in Commission Decision 2007/407/EC or by EUCAST. Furthermore, in the research project VetCAb detailed data on antimicrobial usage in livestock were collected which allow the calculation of treatment frequencies (used daily doses) specifically for each livestock production group and antimicrobial class. Since 2011, based on a representative sample, data are collected and analysed for pigs, cattle and poultry. Overall resistance rates differed significantly between isolates from the different populations of fattening animals considered. The highest resistance rates were observed in isolates from broilers and fattening turkeys, followed by fattening pigs and veal calves. In fattened bovines, resistance rates decreased with age of animals. This ranking reflects very much the magnitude of antimicrobial usage (calculated as used daily doses) in the respective livestock populations. Even more worrying, most of the isolates were resistant to several antimicrobial classes. Resistance to the commonly used antimicrobial classes was frequently observed. More specific, resistance rates to tetracyclines were quite high in poultry, calves and pigs, reflecting usage patterns in these livestock species. In contrast, tetracycline resistance rates in beef cattle were below 20%. Resistance rates decreased in the bovine and porcine production chain, showing highest resistance rates in young animals at primary production and lower rates at slaughter. These patterns were quite similar for aminopenicillins, the second most frequently sold antimicrobial class in Germany. In contrast to tetracycline resistance, ampicillin resistance rates in broilers and chicken meat were much higher. Results demonstrate that the assessment of the implication of antimicrobial usage and antimicrobial resistance in livestock as regards to the spread into the environment should take into account the specific patterns observed. Whereas in young animals antimicrobial usage and prevalence of resistant bacteria is high, this is much lower at later stages in livestock species fattened for a longer period of time.

P MOB 8
Continuous assessment of health seeking behaviour in a cohort - Exploring demand side practice for utilization of health care services with emphasis on antibiotic use in rural India
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Introduction: Development of antibiotic resistance (ABR) is multifactorial with involvement of multiple stakeholders. Health seeking behaviour (HSB) of an individual is among the major factors. HSB defines the pathways to the health care system (HCS). In India, rural areas are particularly served by informal health care providers (IHCP), not legalized to practice allopathic medicine. IHCPs often use medicines inappropriately including antibiotics (AB) i.e. over-prescription, inappropriate drug choice and treatment regimen, which leads to the emergence and spread of ABR.

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SESSION II: Mobilizing antibiotic resistance genes through anthropogenic use of antibiotics

Objective: To identify, examine, analyze and interpret the main determinants of the HSB using Kroegers’ holistic framework approach and show association with the AB use.

Materials and Methods:
Study Design: Prospective follow up study.
Study setting: Study is undergoing in Ujjain district of Madhya Pradesh, India, with total population of 1.9 million (rural - 61%). Six villages of Demographic Surveillance Site of R.D.Gardi Medical College, Ujjain are selected purposively, within the 5km radius around a center point, owing to its maximum concentration of health care providers (HCP).
Sampling, participants and Data collection: The study cohort consists of all the members, in 125 households (HH) in 6 villages, selected by simple random sampling method. Participants are followed every alternate day for 2 years. Geographic information system (GIS) mapping of the HH and HCP is being done. GIS networks of health seeking routes were mapped and analyzed. Socio-economic background information of HH is collected using standardized HH questionnaire. A health seeking follow up form is designed; to record: history of illness, whether or not sought any treatment, their route through the HCS and reason for selection of this HCS. EpData software is used for capturing various components. Data is analyzed using STATA (STATA Corp Texas, USA) and spatial GIS software. Analysis of the factors associated with the HSB and health service utilization and their association with AB use is done using multinomial logistic regression.

Results: Preliminary results of the data collected till date, reflects that availability of health services, perceived severity of illness and access to HCS are 3 major factors affecting the HSB, second most is socio-economic status and health beliefs. In most of the cases the treatment was taken from the IHCP, 33% sought no treatment, 20% self medicated and 15% went to traditional healers. Illnesses mostly encountered among the cohort were cardio-respiratory (cough and cold, asthma etc.) followed by general illnesses such as fever, headache, body ache, etc. Seventy three percent of the prescriptions were with AB prescribed out of which 26% were with more than one AB. In 64% cases treatment was taken on 3rd day from the onset of the symptoms. Taking treatment from IHCP showed significant association with more AB prescription irrespective of the illness presented. The detailed results will be presented at the meeting.

Conclusion: HSB of an individual is a response of various events. Influence of these factors defines the pathway to HCS. In rural areas scarcity of formal HCP leads to IHCP. Such data on association of various factors affecting the HSB with AB use can be useful for designing strategies to reduce the spread of ABR.
Introduction: Antibacterial biocides and metals are widely used to reduce bacterial growth and transmission in clinical settings. Many of these compounds have the ability to co-select for antibiotic resistant bacteria. However, systematic data on which biocides and metals co-select for resistance to certain classes of antibiotics is lacking, as is knowledge on which environments and which bacteria tend to carry resistance genes to such compounds. This effectively prevents us from identifying risk scenarios.

Objectives: We investigated the co-occurrence of resistance genes to antibacterial biocides/metals and to antibiotics in all publically available, fully sequenced bacterial genomes and plasmids, thus identifying biocides and metals that are likely to co-select for resistance to certain classes of antibiotics. This also included the identification of environments and bacterial genera with high co-selection potential. Finally, we investigated to what degree resistance plasmids with co-selection potential tend to be conjugative.

Methods: Bacterial genomes (n=2539) and plasmids (n=4582) were retrieved from NCBI and characterized using our recently developed databases for biocide/metal (BacMet) and antibiotic (Resqu) resistance genes. Networks were built based on resistance genes co-occurrence patterns.

Results: A small fraction of the sequenced bacteria (17%) and plasmids (5%) showed co-selection potential based on co-occurrence of known resistance genes. The most common combination of resistance genes of two classes was chromosomal metal resistance genes together with plasmid-borne antibiotic resistance genes in the same cell. Interestingly, both the extent and nature of the co-selection potential differed widely between different biocides and metals, leading to the identification of chemicals of particular high concern. Several clinically important genera carried plasmids with co-selection potential, and these tended to be conjugative. Bacterial isolates from clinical and animal-associated environments showed the highest potential for co-selection.

Conclusion: This study provides a new conceptual framework for assessing co-selection potential between biocides, metals and antibiotics, and thereby it could provide some guidance on risk-reducing actions, in the clinics and other contexts.

O MOL 2
Phylogeny and comparative genomics unveil independent diversification trajectories of qnrB and genetic surroundings within Citrobacter sp.

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Question: To gain insights on the diversification trajectories of qnrB, the most common and diverse group of genes conferring decreased susceptibility to fluoroquinolones.

Methods: Phylogenetic and comparative genomics analysis of qnrB and its genetic surroundings were performed on sequences identified on twenty-one Citrobacter sp. isolates from environmental origins and also on those from available genomes of different Enterobacteriaceae species and origins harboring complete or truncated qnrB genes. The clonal relatedness among isolates, the location of qnrB genes and the characterization of their genetic surroundings were investigated by PFGE and hybridization, PCR mapping and sequencing.

Results: The phylogenetic analysis of qnrB genes revealed five main clusters (I-V) and one branch, sharing an overall 89% of identity. Six different qnrB surrounding regions, always comprising pspF and sapA and varying in synten or identity of other genes and intergenic regions, were identified, which were in most cases associated with a specific qnrB cluster. High similarities of qnrB surrounding regions with those identified in plasmids from different Enterobacteriaceae species were also observed. Despite we did not detect any known mobile genetic element, IRR-like sequences recognizable by ISEcp1-type insertion sequences or other mobile genetic elements were found, suggesting its involvement in the mobilization of qnrB and/or qnrB platforms to multidrug resistant plasmids widely spread in the clinical setting.

Conclusions: Our data unveil independent diversification trajectories of qnrB platforms within Citrobacter sp. and support for the first time a potentially relevant role of IRR-like sequences in the mobilization of chromosomally located qnrB genes and/or their surrounding regions.
Figure 1. Affiliation of qnrB genes and available genetic environments from *Citrobacter* spp. (A) Neighbor-Joining tree based on all *qnrB* gene sequences (http://www.lahay.org/qnrStudies/). Genetic distances were constructed using Kimura 2-parameter model. Numbers at branch points indicate bootstrap percentages (1000 replications) from NJ analysis and only values greater than 80% were shown. Horizontal bar: genetic distance of 0.05. Nucleotide sequence of *qnrD1* (GenBank accession number FJ228229) was used as outgroup. The *qnrB* genes for which the genetic environment was characterized in this study are represented in circles, whilst those available in the GenBank database are underlined. pl, plasmid-borne *qnrB*; cr, chromosomally-located *qnrB*; *, *qnrB* location not assessed. (B) Schematic representation of the available genetic contexts (GC) surrounding *qnrB* genes described in the chromosome of *Citrobacter* spp. Arrows represent incomplete open reading frames (ORFs) (dotted line) under our experimental conditions. Numbers between ORFs indicate the size of the intergenic region in base pairs (bp). Vertical black bars represent IRR2. Genetic environments are deposited at the GenBank database through the accession numbers KP339254 (*qnrB6*), KP339255 (*qnrB9*), KP339256 (*qnrB10*), KP339257 (*qnrB17*), KP339258 (*qnrB18*), JN173057 (*qnrB35*), JN173060 (*qnrB38*), ABWL02000005.1 (*qnrB39*), KP339259 (*qnrB56*), KP339260 (*qnrB57*), KP339261 (*qnrB58*), KP339262 (*qnrB59*), AB734055 (*qnrB60*), AB734053 (*qnrB61*), BBMW01000005.1 (*qnrB69*), KP339263 (*qnrB72*), and KP339264 (*qnrB73*).
Exploiting unique molecular signatures created during the evolution of complex resistance regions to track imminent threats by multiple antibiotic resistant pathogens.

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Background: Clustering of resistance genes on mobile genetic elements promotes the acquisition and spread of multiple antibiotic resistance. Multiple copies of IS26 are often found in complex antibiotic resistance gene loci (CRL) in Gram-negative bacteria. IS26 is known to mobilize clustered resistance genes and create unique molecular signatures that can be exploited for diagnostic purposes.

Objectives: To identify molecular signatures and evaluate tools to track movement of IS26 associated CRL.

Materials & Methods: PCR using primers located in int2 (L1) and IS26 (L-D2) can be used to identify unique molecular signatures created by insertion of IS26 in close proximity to class 1 integrons (Dawes et al., 2010 PLoS One 5(9):e12754).

Results: We successfully developed a PCR to track the movement of IS26-associated resistance CRL within E. coli of bovine and human origin (Dawes et al., PLoS One. 2010 5(9):e12754). BlastN analysis of the intervening sequence spanned by these primers identified a unique 2082 bp region in pASL01a from a commensal human strain of human origin (Dawes et al., PLoS One. 2010 5(9):e12754). Notably, the 2082 bp sequence shows 100% sequence identity to the CRL in genomic island 3 in O104:H4 enterohaemorhagic E. coli (EAHEC). From this we deciphered a plausible series of genetic events that led to the formation of the CRL in German outbreak strains of EAHEC.

Conclusions: Molecular signatures created by the insertion of mobile genetic elements can be used to track their movement and the bacteria that carry them and shed light on the micro-evolutionary events that form CRL.

Surveillance of ESBL producing Gram-negative bacteria in four dairy cattle farms in Egypt in 2014.

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Introduction: Industrial livestock farming is a possible source for multi-resistant Gram-negative bacteria, including producers of extended spectrum beta lactamases (ESBLs) conferring resistance to 3rd and 4th generation cephalosporins. Little information is currently available about the situation of ESBL producers in livestock farming especially outside of Western Europe. A surveillance study was conducted from January to May in 2014 in four dairy cattle farms in different areas of northern Egypt.

Materials and Methods: In 2014 266 samples were taken including rectal swabs from clinically healthy cattle (n = 210) and environmental samples from the stables (n = 56). After 24 hours of pre-enrichment in buffered peptone water, all samples were screened for ESBL producing Escherichia coli using MacConkey agar supplemented with 1 mg/L cefotaxime. These samples yielded 118 cultures of putatively ESBL producing E. coli on the selective medium that were subcultured and subsequently geno- and phenotypically characterized. Sensitivity testing using the VITEK-2 system confirmed ESBL production for 114 out of the 118 (97 %) isolates. All isolates were phenotypically analysed using two DNA-microarray based assays: CarbDetect AS-1 and E.coli PanType AS-2 Kit (ALERE). These tests allow detecting of a multitude of genes and alleles thereof associated with carbapenem, cephalosporin and other common antibiotic resistance. Serotype was determined using the E. coli SeroGenotyping AS-1 Kit (ALERE).

Results: All isolates were geno- and phenotypically identified as E. coli. 113 of 114 phenotypically cephalosporin-resistant isolates harboured at least one of the ESBL resistance genes covered by the assays [blaCTX-M15 (n=105), blaCTX-M9 (n=1), blaTEM (n=90), blaSHV (n=11)]. Alarmingly, the carbapenemase gene blaOXA-48 was found in six isolates that also were phenotypically resistant to imi- and meropenem. Using the array-based serogenotyping method, 66 of 118 isolates (55 %) could genotypically be assigned to O-types.
SESSION III: Molecular Studies reveal a link between the hospital and the environment

Conclusion: Our data show that ESBL producing E. coli isolates with different underlying resistance mechanisms are common in investigated dairy cattle farms in Egypt. The global rise of ESBL and carbapenemases producing Gram-negative bacteria is a big concern and demands intensified surveillance.

P MOL 2
Resistance genes in Staphylococcus aureus from European wildlife
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Introduction: There is increasing evidence for a zoonotic background of MRSA harbouring mecC encoding an alternative gene for a penicillin-binding protein.

Objective: In this study, wildlife isolates were screened for mecA, mecC and other resistance genes.

Materials and Methods: Wildlife isolates of S. aureus from Germany, Austria and Sweden were characterised by microarray analysis (Alere Technologies GmbH), VITEK-2 and MLST.

Results: The mecC gene was identified in CC136-MRSA-XI from two diseased Swedish hedgehogs (Erinaceus europaeus), from a fox (V. vulpes), two hares (Lepus europaeus) and a fallow deer (D. dama) from Germany. One CC599-MRSA-XI was found in a road-killed hedgehog from Germany. These isolates also harboured the SCCmec Xi-associated beta lactamase and heavy metal resistance genes.

The mecA gene was found in CC398-MRSA-IV from a mallard (Anas platyrhynchos), CC398-MRSA-V from a marten (Martes sp.) and ST3/ST225-MRSA-II (Rhine-Hesse/New York-Japan Clone) from a hare, all from Austria. These isolates also carried blaZ+aadA-aphA2-dfrD, blaZ+ermR+tetK and blaZ+ermA+aadD, respectively.

S. aureus CC1 from fallow deer and mouflon (Ovis aries), CC133 from a mute swan (Cygnus olor), ST425 from roe and red deer (C. capreolus, Cervus elaphus), boars (Sus scrofa) and badgers (Meles meles), CC692 from several bird species, ST2279 from lynx (L. lynx) and reindeer (Rangifer tarandus), ST2425 from hare, ST2691 from moose (Alces alces) and a new sequence type, ST2963, from a wild cat (Felis silvestris) were negative for all resistance genes tested.

Conclusion: MecC appears to be common in wildlife and mecC MRSA can be found in several host species. MecA MRSA can be expected in urban wildlife living in proximity to humans and their offal. More systematic studies on S. aureus in wildlife are needed as wild animals might serve as reservoir for resistant S. aureus with potential for transmission to domestic animals and humans.

P MOL 3
Isolation and Characterization of Bacteria from Selected Day-care Centres in Ile-Ife
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Objectives: The study identified and characterized bacterial contaminants in selected day care centres in Ile-Ife, Nigeria; determined the susceptibility of the isolates to antibiotics; and detected possible carriage of plasmid DNA among the multiple antibiotic resistant isolates.

Methods: Ninety swab samples collected from day-care children’s hands, attendants’ hands, and fomites in nine selected crèches centres were cultured on both Nutrient and MacConkey agar plates incubated at 37°C for 24 hours. Isolates were identified by standard procedures. Antibiotic susceptibility of isolates was determined by Kirby Bauer’s antibiotic disk diffusion method. Detection of Plasmid DNA in representative multiple antibiotic resistant isolates was by alkaline lysis.

Results: One hundred and forty eight bacteria comprising 108 Gram negative and 40 Gram positive bacteria were recovered. Escherichia coli 45 (30.4%) was the predominant bacteria isolated followed by Staphylococcus aureus 24 (16.2%), Salmonella sp...
SESSON II: Molecular Studies reveal a link between the hospital and the environment

17 (11.5%), Proteus sp 16 (10.8%) and Bacillus sp 16 (10.8). Resistance to antibiotics varied greatly. The highest resistance was against ampicillin (85.2%), amoxycillin (79.6%), cotrimoxazole (61.1%), and ciprofloxacin (5.6%) being the least. The incidence of multiple antibiotic resistance was high in Gram-negative and -positive bacterial isolates with diverse MAR patterns. Forty eight (83.2%) of the 59 representative MAR isolates harboured plasmid DNA with estimated molecular weights ranging from 9.47 to 20.72 kb.

Conclusion: Day-care centres were found to harbour potentially pathogenic bacteria that are highly resistant to some commonly used antibiotics. These centres are therefore reservoirs of clinically important pathogens.

P MOL 4
Clones of the ESBL- E. coli lineages ST131, ST410 and ST617 are present in avian environmental and human clinical isolates from the same area
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Background: Within the last years the occurrence of extended-spectrum beta-lactamase (ESBL)-producing E. coli in antimicrobially non-treated wildlife has been reported. To understand possible transmission scenarios and to evaluate an indicator function of wild birds for an environmental pollution with multi-resistant bacteria we screened avian wildlife from the Berlin area (Germany) and compared ESBL-producing strains to human clinical isolates from the same area.

Methods: A total of 320 wild birds were screened for ESBL-producing E. coli during entrance examination in a small animal clinic using cloacal swabs and selective plating (4µg/ml cefotaxime). Isolate characterization included ESBL confirmatory testing, MIC-testing, PCR screening for ESBL-resistance determinants like \( \text{bla}_{\text{CTX-M}}, \text{bla}_{\text{PER}} \) and \( \text{bla}_{\text{TEM}} \) as well as non-beta-lactam resistance genes including tetA-C, aadA-3, and strA/B. The phylogenetic background was determined via Multilocus sequence typing (MLST) and structure analysis. All ESBL-producers were additionally screened for clonal relatedness via pulsed field gel electrophoresis (PFGE).

Results: Overall, 26 (8%) of the sampled birds carried an ESBL- or AmpC- producing E. coli. The ESBL-resistance was always encoded on the genes \( \text{bla}_{\text{CTX-M}} \) or \( \text{bla}_{\text{PER}}, \text{bla}_{\text{TEM}} \). Besides the wide range of genes encoding for non-beta-lactam resistances high rates of virulence genes related to extra-intestinal pathogenic E. coli were detected. MLST analysis found that almost all avian ESBL-producers belonged to typical ESBL-sequence types like ST131, ST648, ST617, ST224 or ST167. PFGE detected identical clones within several STs, including ST131, ST410 and ST617 among isolates from wild birds and humans.

Conclusions: Wild birds carry substantial numbers of ESBL-producing E. coli resembling clinical relevant strains from human and veterinary medicine, pointing towards an environmental pollution by multi-resistant E. coli clones from medical facilities.

P MOL 5
Genetic diversity and antibiotic resistance of clinical and non-clinical isolates of Escherichia coli in Grenada
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E. coli exhibits an increasing resistance to broad spectrum antimicrobial agents as well as the subsequent generations of these drugs. Previous genetic diversity studies indicated transmission of food-borne and environmental E. coli strains to human patients. Therefore it is important to study the genetic diversity and antibiotic-resistance patterns both in clinical and environmental E. coli to predict potential of transmission of organisms and genes for antibiotic resistance.

The aim of this study was to compare the genetic diversity and the antibiotic resistance patterns of uropathogenic E. coli (UPEC), to strains from freshwater, seawater and iguanas.

Multiple strains of E. coli were isolated from human urine (25), iguana gut (39), fresh (11) and marine waters (10) in Grenada. E. coli isolates were identified using API20E E.coli ATCC 25922 as a reference strain, followed by Rep-(GTG) 5 PCR and BOX-PCR extragenic DNA fingerprinting. The antibiotic resistance was assessed using the Kirby-Bauer disc diffusion against the eleven most commonly prescribed antibiotics in Grenada. Excel I-Test and StatisticaTM were utilized for comparison of patterns of drug resistance among four ecotypes.

Both DNA fingerprinting methods targeted non-protein coding or extragenic DNA and demonstrated enormous diversity within the population of the studied bacteria. In compliance with DendroUPGMA comparison of DNA fingerprints based on the Pearson’s coefficient, we found that 56% of clinical UTI E. coli isolates were unique for human hosts, while 24% were related to iguana E. coli isolates (Figure 1). The co-clustering relatedness analysis of the (GTG) 5dendrograms confirmed the results of the fingerprinting of dominating ecotypes, indicating that clinical isolates were most often related to the iguana isolates, since they shared more pairs than any other ecotype tested. Forty eight percent of UPEC were resistant to at least one antibiotic, 16% were single drug resistant (SDR) while 32% were multidrug resistant (MDR). About 3% of iguanas (SDR 18%), 20% of marine (SDR 10%) and 64% of freshwater (none SDR) were multidrug resistant. Ciprofloxacin resistance was identified in the seawater (10%) while resistance to
gentamicin was found in freshwater (9%). Resistance primarily to carbenicillin, ampicillin and tetracycline was observed among the isolates. There was moderate susceptibility to sulphamethaxazole/trimethoprim. Tetracycline and carbenicillin presented resistance. Most of the compared isolates were susceptible to fluoroquinolones, to third generation cephalosporins and aminoglycosides (Figure 2). In accordance with t-test analysis of resistant/susceptible patterns among four ecotypes the most similar patterns (83.3%) were observed between freshwater and marine water isolates, as well as in marine and iguana isolates. Clinical E. coli was similar to marine (50%), iguana (72.7%) and fresh water (72.7%) . There were only three drugs which showed significant difference between the UPEC and the freshwater isolates, namely ampicillin, amoxicillin/clavulanate and nitrofurantoin (results are not shown). We identified resistance to β-lactam antibiotics and lack of resistance to ciprofloxacin and gentamycin both in clinical and most of non-clinical strains of E.coli. The fractions of drug resistance strains and resistance patterns of the UPECs were similar to E.coli isolated from natural sources in Grenada.

Clinical E. coli was similar to marine (50%), iguana (72.7%) and freshwater (72.7%). There were only three drugs which showed significant difference between the UPEC and the freshwater isolates, namely ampicillin, amoxicillin/clavulanate and nitrofurantoin (results are not shown). We identified resistance to β-lactam antibiotics and lack of resistance to ciprofloxacin and gentamycin both in clinical and most of non-clinical strains of E.coli. The fractions of drug resistance strains and resistance patterns of the UPECs were similar to E.coli isolated from natural sources in Grenada.

**Figure 1:** Dendrogram based on UPGMA analysis based on comparison of normalized distances of the (GTG)5 PCR amplified DNA markers

**Figure 2:** Percentage of resistant strains per ecotype per drug tested.
Multidrug resistant *Rhizobiales* in an urban river nearby a regional hospital in Montpellier, France

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*Rhizobiales* are environmental bacteria often multidrug resistant (MDR) as other members of the rhizosphera. Some sub-populations of *Rhizobiales* are known as human-associated (in *Rhizobium pusense* and *Ochrobactrum anthropi* for instance). We aimed to evaluate the impact of runoff water from the hospital of Montpellier (2700 beds) on the presence of MDR and/or human-associated *Rhizobiales*. We therefore studied waters, sediments, epilithic and epiphytic biofilms upstream and downstream the hospital in an urban river running nearby during 5 weeks. A cultivation-based approach on a selective medium containing antibiotics was combined with a cultivation-independent method, i.e., *Rhizobiales*-specific 16S rRNA gene PCR and Temporal Temperature Gel Electrophoresis (TTGE), in order to describe the MDR cultivable community.

A higher frequency of TTGE signals was observed in samples downstream than upstream the hospital (92% and 47%, respectively). The 10 detected OTUs belonged to the genera *Ochrobactrum/Brucella*, *Pseudochrobactrum*, *Agrobacterium* and *Rhizobium*. The groups « *O. anthropi/Brucella* » and « *Pseudochrobactrum lubricantis* » were the major OTUs, each representing 32% of the TTGE bands and they were mostly present downstream. Sediment community profiles seemed more stable over time than water communities. *P. lubricantis* group was mainly epiphytic while *O. anthropi* group was more often in epilithic biofilms.

Among major OTUs, twenty-one *O. anthropi* strains were isolated and studied by MultiLocus Sequence Typing and compared to a collection of strains including 70 strains isolated in patients of the Montpellier hospital. The river strains were divided into 14 sequence types (ST), some of them being undescribed, confirming the high genetic diversity of this species. Six STs included both river and clinical strains (mainly from cystic fibrosis patients). Finally, 4 river strains (1 upstream and 3 downstream the hospital) belonged to the human-specific clonal complex CC4, which had never been previously isolated outside of clinical settings.

These results suggested the dissemination of MDR and/or human-associated *Rhizobiales*, mainly downstream the hospital. This result worths to be confirmed in order to propose the follow-up of autochthonous particular populations of *Rhizobiales* as markers of anthropisation.
Oral presentations

O FAT 1
Manure fertilization increases resident antibiotic-resistant bacteria in soil
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Introduction: The increasing prevalence of antibiotic resistant bacteria is a serious public health threat. There is growing evidence that agricultural use of antibiotics contributes to the spread of antibiotic resistance genes in the environment, including to human pathogens. Manures from farm animals are commonly used as soil fertilizers, and they are also important reservoirs of antibiotic resistance genes, even when the animals have not been treated with antibiotics. However, we know very little about the mechanisms by which manure fertilization influences the behavior of resistance genes in the environment.

Objectives: The objective of this study was to assess the impact of cow manure on the β-lactam resistance profile and composition of bacterial communities in soil.

Materials & Methods: We treated soil with either inorganic fertilizer (NPK) or manure from dairy cows with no history of antibiotic treatment. Total and beta-lactam resistant bacteria in soil before and after fertilization were monitored by culturing. Functional metagenomics was used to identify resistance genes in soil and manure and quantitative PCR (qPCR) was used to quantify these genes in soil over time. 454 tag sequencing of the 16S rRNA genes was used to assess how microbial communities change in the soil after manure application.

Results: Culturing showed that the soil treated with manure contained a higher abundance of β-lactam resistant bacteria than soil treated with NPK. Functional metagenomics indicated that the higher levels of resistant bacteria in manured-amended soil was attributable to an enrichment of resident soil bacteria that harbor β-lactamases. qPCR showed that manure treatment enriched the bla(CEP-04) gene, which is highly similar (96%) to a gene found previously in a Pseudomonas sp. Analysis of 16S rRNA genes indicated that the abundance of Pseudomonas spp. increased in manure-amended soil. Populations of other soil bacteria that commonly harbor β-lactamases, including Janthinobacterium sp. and Psychrobacter pulmonis, also increased in response to manure treatment.

Conclusion: Our results indicate that manure fertilization may lead to blooms of certain antibiotic resistant subpopulation of the soil community, even when the manure comes from cows that have not been treated with antibiotics. These data demonstrate the importance of nonintuitive impacts of agricultural practices on the intrinsic resistance of soil microbial communities.

O FAT 2
Antibiotic resistance gene concentrations in agroecosystems following beef manure or poultry litter deposition
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Antibiotics are commonly used in livestock production to promote growth and combat disease. However, as much as 80% of the drug may pass through the animal and into the soil where selective pressure may favor survival of antibiotic resistant bacteria. Recent studies have shown that there is also a potential for spread of antibiotic resistance genes (ARG) to the environment following application of livestock manures. In this study, concentrations of bacteria with ARG in soils with applied poultry litter (PL; 2 years) or from a beef cattle backgrounding operation (BB; 3 year) were determined. Samples were taken (1) following PL application to soils under conventional or no till management and (2) from soils taken from the BB while livestock were on-site and to identify intervention strategies to limit their impact outside of the agricultural environment.

Materials & Methods: The BB landscape was determined. Samples were taken (1) following PL application to soils under conventional or no till management and (2) from soils taken from the BB while livestock were on-site and following their removal. Total and beta-lactam resistant bacteria in soil before and after fertilization were monitored by culturing. Functional metagenomics was used to identify resistance genes in soil and manure and quantitative PCR (qPCR) was used to quantify these genes in soil over time. 454 tag sequencing of the 16S rRNA genes was used to assess how microbial communities change in the soil after manure application.

Results: Culturing showed that the soil treated with manure contained a higher abundance of β-lactam resistant bacteria than soil treated with NPK. Functional metagenomics indicated that the higher levels of resistant bacteria in manure-amended soil was attributable to an enrichment of resident soil bacteria that harbor β-lactamases. qPCR showed that manure treatment enriched the bla(CEP-04) gene, which is highly similar (96%) to a gene found previously in a Pseudomonas sp. Analysis of 16S rRNA genes indicated that the abundance of Pseudomonas spp. increased in manure-amended soil. Populations of other soil bacteria that commonly harbor β-lactamases, including Janthinobacterium sp. and Psychrobacter pulmonis, also increased in response to manure treatment.

Conclusion: Our results indicate that manure fertilization may lead to blooms of certain antibiotic resistant subpopulation of the soil community, even when the manure comes from cows that have not been treated with antibiotics. These data demonstrate the importance of nonintuitive impacts of agricultural practices on the intrinsic resistance of soil microbial communities.
O FAT 3
Antibiotic resistant bacteria from livestock husbandry are released with biogas plant digestate into the environment

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Intensive application of antibiotics in livestock husbandry can lead to an increasing occurrence of antibiotic-resistant bacteria in manure. Beside the application as biofertilizers, manure is a major input material for biogas plants. It was not investigated so far, whether or not antibiotic resistant bacteria are eliminated by the biogas plant process or transferred through biogas plants and released into the environment when biogas plant digestate is used as biofertilizer. The aim of our study was to monitor the presence of antibiotic resistant and potentially pathogenic bacteria in input (mixed manure) and output samples of German biogas plants. The study was part of the BMBF founded project RiskAGuA.

Two biogas plants were investigated in an annual cycle in 2013/2014 with respect to the presence of extended-spectrum beta-lactamase (ESBL)-producing E. coli, vancomycin resistant Enterococci (VRE) and methicillin resistant Staphylococci (MRS) using selective pre-enrichment procedures combined with subsequent cultivation approaches on CHROMagar media and resistance-gene-screenings. ESBL-producing E. coli and VRE were cultured from input and output-, MRS only from input material. ESBL-producing E. coli carried mainly blaCTX-M and blaTEM genes and belonged to several ST types (MLST analysis). VRE were most closely related to E. gallinarum-E. casseliflavus, E. viikiiensis and E. lemani and were shown to carry vanA or vanB genes; MRS carried mecA genes and were most closely related to S. haemolyticus, S. lentus, and S. sciuri.

In addition, the microbial communities of input and output samples of 15 German biogas plants were analyzed once in 2012 using molecular 16S rRNA gene based approaches. Community fingerprinting by denaturing gradient gel electrophoreses (DGGE) showed diverse community pattern in input, but more similar patterns in output samples. Pyrotag sequencing showed a relatively low abundance of E. coli, Enterococci, and Staphylococci in input and output samples and a high abundance of uncultured bacteria that originate from livestock husbandry are released into the environment in case of application of biogas plant digestates as biofertilizers.
Figure 1 Bacterial communities (most abundant taxa) of input and output samples of 15 German biogas plants (BGA 001 to BGA 015) investigated in 2012 using a 16S rRNA gene Pyrotag sequencing approach. Numbers in boxes represent the relative abundance of taxa within the analyse samples.

O FAT 4
Metals as co-selective agents in soil bacterial communities
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Agricultural practices such as application of manure create favorable conditions for the environmental dissemination of antibiotic resistance. This may happen in part due to the dissemination of antibiotic resistant bacteria from manure to soil and due to high levels of antibiotic residues in manure. However, toxic levels of antibiotics are rarely attained in agricultural soils. By contrast metals widely used as animal growth promoters (Cu or Zn) and as pesticides (Cu) may accumulate in agricultural soils and provide persistent co-selective agents for antibiotic resistance. We previously demonstrated that long-term (>65 years) exposure to Cu co-selected for antibiotic resistance in soil receiving no known inputs of antibiotics. Here, we extend our previous work by studying the co-selection phenomenon and its underlying mechanisms in more detail. Phenotypic antibiotic resistance profiling was performed at the community level using the pollution-induced community tolerance (PICT) approach complemented by phenotypic resistance plating of soil bacterial isolates. Antibiotic resistance genes in soil were enumerated using a high-capacity quantitative PCR array. Plasmid prevalence was determined in a large set of soil bacterial isolates and plasmid DNA from selected isolates was sequenced (Illumina MiSeq). Our results suggest that the potential for metal-induced co-selection is a general phenomenon in Danish agricultural soils, but not in ‘pristine’ permafrost. However, co-selection of antibiotic resistance is difficult to predict and requires higher Cu levels than required for development of Cu resistance. The detection of a novel plasmid carrying two copper resistance gene clusters along with various efflux pump encoding genes likely to be involved in antibiotic resistance suggest co-resistance as a likely co-selection mechanism in polluted soils, but such mobile genetic elements may be missing in permafrost harboring ‘pre-antibiotic era’ bacterial communities.

O F AT 6
Effects of advanced treatment on the removal of antibiotic resistance genes in wastewater treatment plant
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The concentrations of eight antibiotics in all samples were measured using liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS), and results showed that Oxytetracycline (OTC) had the highest concentration in manure reaching up to 138.7mg/kg. Ten tet genes (tetA, tetB, tetC, tetG, tetL, tetM, tetO, tetQ, tetW, and tetX), two sul genes (sulI and sulII), and the integrase gene (intI1) were quantified by real-time polymerase chain reaction. Results showed that the abundance of ARGs fluctuated within EAS. TetQ had the highest relative abundance and the relative abundance of tetG had the least variation within the system, which indicated that tetG is persistent in the agricultural environment and requires more attention. Compared to the relative abundance in manure, tetC and tetM increased in the biogas residue, tetO, tetQ, tetW decreased (P < 0.05), with other genes showing no significant change after anaerobic digestion. Most ARGs in downstream components (soils and fishpond) of the EAS showed significantly higher relative abundance than the conventional agricultural system (P < 0.05), except for tetG and sulI. The bacterial resistance rates of sulfonamide (46.19%) is much higher than that of tetracycline (8.51%) on average in the studied system, which indicated that tetG is persistent in the agricultural environment and requires more attention.
system, most likely because the use of sulfonamide is more prevalent than tetracycline in this area. A total of 114 ARB isolates were selected to assess the overall antimicrobial resistance. 30 genus of ARB were identified among all the isolates. The genera of sulfamethoxazole resistant (SMXr) bacteria were different from tetracycline resistant (TCr) bacteria. Staphylococcus and Acinetobacter were the most dominant bacteria genus in SMXr bacteria (19.3% of the total resistant bacteria) and TCr bacteria (14.0% of the total resistant bacteria), respectively. Several strains of resistant opportunistic pathogens (e.g., Pantoaea sp.) were found from vegetable samples, which may increase the risk of antibiotic resistance genes to public health. This is the first study to investigate the behavior of antibiotics and ARGs in circular agricultural system in China and provides insight into potential options for prudent use of antibiotics in agricultural activities.

O FAT 7
The role of wildlife in disseminating antibiotic-resistant bacteria to and from livestock facilities
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Introduction: Bacterial pathogens causing food-borne illnesses in humans are known to occur in many species of avian and mammalian wildlife, including synanthropic (peri-domestic) species found at livestock facilities in the U.S. While wildlife are increasingly implicated in transmission of antimicrobial-resistant (AMR) bacteria, the degree of this transmission is poorly understood. Thus, understanding the role of wildlife as reservoirs and disseminators of AMR bacteria in livestock facilities is crucial for assessing their importance in food safety strategies.

Objectives: We addressed whether synanthropic wild mammals maintain and move AMR bacteria in livestock facilities by 1) estimating the prevalence of AMR strains in different species of wild mammals on and near livestock facilities in the U.S., and 2) estimating movement and visitation rates of wild mammals to sources of livestock contamination.

Materials & Methods: We sampled wild mammals for AMR bacteria at livestock facilities in Colorado, USA. We trapped wild mammals both within and outside livestock facilities, sampled them for AMR bacteria, and marked them with PIT tags. From sampled individuals, we examined four classes of AMR: extended-spectrum cephalosporin, fluoroquinolone, macrolide, and methicillin resistance and used MALDI-TOF to identify AMR bacteria to species.

Results: We captured 65 raccoons (Procyon lotor), 131 deer mice (Peromyscus spp.) and 39 individuals of other species at livestock facilities. Raccoons had higher prevalence (36.7-87.7%) of AMR bacterial strains relative to other wildlife species. Most raccoons (72.7%) were infected with multiple strains of AMR bacteria, with 49.0% infected with >3 AMR strains. Based on PIT tag sensors, raccoons frequently moved from surrounding areas into feed troughs used by cattle; most individuals frequented troughs 75-100% of the days that they were tracked.

Conclusions: Synanthropic wild mammals are important disseminators of AMR bacteria on livestock facilities in the U.S. Because raccoons are also associated with aquatic habitats, they may act as important traffickers of AMR bacteria between livestock facilities and water sources that could extend AMR bacterial contamination beyond livestock facilities.

O FAT 8
Antibiotic resistance genes in manure-amended soil and vegetables at harvest
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Introduction
Antibiotics are poorly absorbed by treated animals and high concentrations of residual antibiotics have been commonly detected in animal manures. China produced an estimated 1900 million tons of livestock manure annually. When manure were used as fertilizer, residual antibiotics and ARGs could disperse into agricultural soil, which may exert selection pressure on antibiotic resistance. More importantly, the enrichment of ARGs in manure-amended soil can potentially disseminate resistance to vegetables, particularly that are eaten raw or subjected to minimal processing, which representing an important vehicle for ARGs transmission into human and posing potential threat to human health.

Objectives: To explore the influence of plants on the abundance of ARGs in manure-amended soil, and the occurrence of ARGs on harvested vegetables.

Patients & methods or Materials & methods: In this study, we sampled long-term manure-amended soils and conducted a pot experiment with or without vegetables. A nylon mesh bag was placed in the central of each pot to create the rhizosphere (rhizosphere soil), and the remaining soil out of the bag was taken as the non-rhizosphere (bulk soil). After the potting soil were thoroughly watered and pre-incubated overnight, the lettuce and endive seeds were sown in the root bags next day. Eight replicates for each plant and additional four pots without plants were used as control. All pots were incubated in a greenhouse and
watered every two days with deionized water to maintain at 70% of water-holding capacity. Soil samples were taken on 30 d and 60 d (after vegetables harvested) and stored at -80°C before DNA extraction. The vegetable samples were divided into three parts, that is, root endophytes, leaf endophytes, and phyllosphere microorganisms. The ARGs that were frequently detected and intI1 gene were quantified by quantitative PCR (Q-PCR) using a SYBR Green approach.

**Results:** Twelve ARGs and one integrase gene (intI1) were frequently detected in all soil samples, and among them, sulI, tetG, tetC, tetA, and tetM genes had lower abundance in planted soil than in control soil. ARGs and intI1 gene were also detected on harvested vegetables grown in manure-amended soil, including endophytes and phyllosphere microorganisms. No significant difference was found between rhizosphere and bulk soil samples for most detected genes.

**Conclusion:** The results demonstrated that planting had an effect on the distribution of ARGs in manure-amended soil, and ARGs were detected on harvested vegetables after growing in manure-amended soil, which had potential threat to human health.

**Poster presentations**

**P FAT 1**

Screening of antibiotic resistant bacteria in livestock manure using as a soil fertilizer in Tatarstan Republic (Russia)

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Tetracycline is broad-spectrum antibiotic widely used for treating infections in humans and animals. Oxytetracycline (tetracycline derivative) is used as veterinary antibiotic and as growth promoter. It was reported, that antibiotics cause development of antibiotic resistance among bacteria of livestock gut as well as of soil while manure containing antibiotics is used as soil fertilizer.

The objective of this study is screening of antibiotic resistant bacteria in livestock manure using as a soil fertilizer in Tatarstan Republic that is one of the major agricultural regions of Russia.

Seven types of animal manure were studied: chicken, bovine, goat, rabbit, fresh swine manure, stored swine manure and swine manure with sawdust. Manure samples were obtained from biggest farms at Tatarstan Republic. Colony forming units (CFU) of bacteria were determined on meat-peptone agar containing 100 mg L⁻¹ oxytetracycline. Besides, three groups of tetracycline resistant genes were determined with PCR reaction: efflux pump genes - tet(A), tet(B), tet(C), tet(E); the ribosome protecting genes - tet(M), tet(O), tet(S); and the degradation enzyme gene - tet(X).

The oxytetracycline resistant cultivable bacteria were revealed in all seven manures. The total bacterial CFUs were estimated to be 9.4×10⁴, 4.4×10³, 2.1×10³, 1.3×10², 1.1×10², 1.5×10² and 4.1×10² per gram of chicken, bovine, goat, rabbit, fresh swine manure, stored swine manure and swine manure with sawdust, respectively (Fig. 1). It must be underlined that the diversity of the resistant bacteria was quite low: we observed one or two different species on the agar plates. Using PCR with tet-primers, it was shown that tet(E) genes are presented in chicken and bovine samples; tet(M) gene - in chicken manure; tet(S) gene - in chicken and bovine manure; tet(X) gene - in chicken, rabbit, goat, fresh swine manure and swine manure with sawdust (Fig. 2).

Thus, tet(X) genes were found to be the most predominant. Tet(A), tet(B) and tet(C) genes were not detected in manure samples. In the future estimation of microbial species diversity by 454-pyrosequencing is planned.

**Fig. 1.** Colonies grown on meat peptone agar with 100 mg L⁻¹ oxytetracycline: 1 - from bovine manure water extract, 2 - from chicken manure water extract.

**Fig. 2.** Amplicons obtained by using primers to oxytetracycline resistant genes: efflux pump genes - tet(A), tet(B), tet(C), tet(E); the ribosome protecting genes - tet(M), tet(O), tet(S); and the degradation enzyme gene - tet(X). Template DNA was obtained from chicken manure (1), bovine manure (2), rabbit manure (3), swine manure with sawdust (4), fresh swine manure (5), goat manure (6), stored swine manure (7).
SESSION IV: Fate and Effects of antibiotics in agro-ecosystems

P FAT 2
Speciation and Antimicrobial Activities of Enterococcus Species from mixed Agricultural and Urban Ecosystems
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Introduction. Enterococcus species are sometimes used as indicator bacteria for water quality assessments. However, their speciation and antimicrobial activities in a mixed watershed with different contaminant sources have rarely been done.

Method. To address these problems, we investigated levels of diversity and antimicrobial activities of Enterococcus species from surface water and sediment in a region of southern California (United States) with a history of high concentration of cattle in a mixed urban setting. Quarterly water samples were collected from seventeen stations in the middle Santa Ana River Watershed for two years. Enterococcus Species were enumerated using the most probable-number (MPN) assay.

Results. The most prevalent were Enterococcus faecium (32 % of isolates), Enterococcus faecalis (31%), Enterococcus mundii (26%), and others (11%). The gelatinase (geiE), collagen-binding protein (ace), and aggregation substance (asa1) were detected in 95%, 30%, and 57% of Enterococcus isolates, respectively. Isolates were resistant mainly to tetracycline (>70% of E. faecium, E. faecalis), erythromycin (22%), kanamycin (13.0%), and ampicillin (9%). E. faecium exhibited the highest resistant phenotype and genotype, and resistance to TET was most prevalence in agricultural sediment.

Conclusion. Continuing investigations are required to help understand and mitigate the impact of AR Enterococcus species on human and animal in urban environments.

P FAT 3
Development and validation of a multi-component analysis of veterinary antibiotics in swine manure using UPLC-MS/MS
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The last years, concerns about the occurrence and dissemination of antibiotic residues in the environment have emerged. Antibiotic residues give rise to the development of antibiotic resistance in microbial communities. This includes resistance in pathogens which can be induced directly or indirectly by transfer of resistance elements from non-pathogenic to pathogenic microorganisms. However, little is known about the concentrations and fate of antibiotics in manure and soil and no regulations exist for concentrations. Although several measures have already been taken, the use of antimicrobials in swine farming is still high. It has been estimated that about 75% of the overall administered antibiotics are not absorbed by the animals but are excreted in the urine or the faeces. These residues may enter the environment directly by spreading of manure and can either accumulate there, leach into surface- and groundwater or be taken up by plants intended for human or animal consumption.

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The aim of this study was to develop a method for the quantification of different classes of antibiotics in swine manure in one run using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), in order to screen swine manure on the presence of antibiotic residues. Chromatographic conditions and mass spectrometric parameters were optimized on a Xevo TQ-S mass spectrometer coupled to an Acquity UPLC H-class system. The optimal extraction solvent was found to be a mixture of acetonitrile and trichloroacetic acid. After extraction by shaking and centrifugation, an evaporation and reconstitution step was followed by ultrasonication and filtration.

The final results of this validation study as well as some preliminary results on the antibiotics detected in swine manure will be presented during the symposium.

P FAT 4
Conjugal gene transfer to enteropathogenic bacteria in the phyllosphere
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E. coli and Salmonella species have been recognized as dangerous contaminants on plant leaf surfaces, also referred to as phyllosphere, of fresh produce with sometimes dramatic consequences for the consumer. To add to these worries, the phyllosphere has been shown to be a hotspot for horizontal gene transfer by plasmid conjugation and therefore potential exchange of antibiotic resistance (AR) genes. However, the prevalence of conjugal plasmid transfer to potential enteropathogenic bacteria has not been investigated. With a growing pool of antibiotic resistant environmental bacteria due to anthropogenic activities, the frequency of plasmid-borne AR increased in all environmental niches including crop irrigation water. This increases the probability of antibiotic resistance transfer to enteropathogenic bacteria in the phyllosphere.

Here, we investigate the frequency and site of conjugal gene transfer into model enteropathogenic bacteria in the phyllosphere. To investigate phyllosphere conjugal gene transfer toward model E. coli and Salmonella spp., we are using a diverse set of typical phyllosphere inhabitants including Alphaproteobacteria, Bacteriodetes, Betaproteobacteria, and Gammaproteobacteria species, as well as a broad spectrum of different plasmid backbones. Plasmid backbones and receiving enteropathogens are tagged with different AR as well as differentially colored fluorescent proteins, allowing for the determination of conjugation frequency and the site of conjugation in planta. To that end, a laboratory scale, medium throughput system, employing a 24-well plate assay is used. The model plant Arabidopsis thaliana is precultivated in those 24-well plates and inoculated with donor and receiver bacteria. Conjugation frequency is determined by plate counting and the site of conjugation by using epifluorescence microscopy.

With this study we provide a comprehensive picture of the ecology of enteropathogenic bacteria on plant leaves at a micrometer resolution. The ability of those bacteria to survive in the phyllosphere while acquiring AR genes from environmental bacteria is a finding with great importance that has to be taken into account in crop irrigation source choices and irrigation regimes and future antibiotic resistance mitigation policies.

P FAT 5
Detection of antimicrobial resistance genes in avian manure, pig slurry and agricultural and natural soils.
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The usage of antibiotics in animal husbandry has promoted the abundance of antimicrobial resistance genes (ARGs) in manure. The information of ARGs presence in agricultural soil amended with animal manure is scarce. The aim of this study was to determine the presence of ARGs in animal waste and in soils amended and non-amended with animal manure. A total of 32 samples were analyzed (8 avian manure, 8 pig slurry, 8 agricultural soil and 8 natural soil). 17 ARGs (armA, bl8TEM1, bl8KPC1, bl8KPC1, bl8INT1, cat, catl, mecA, qnrB, qnrS, tetA, tetB, tetC, tetM, tetQ, sul1, vanA) were determined for real time PCR detection. Statistical analysis was performed in order to detect statistical significance between sample types by chi-square test. 13 out of 17 ARGs analysed were detected in at least one sample. In avian manure thirteen different ARGs were present with a mean of 9 genes per sample. The most prevalent were sul1, sull, tetA, tetB, tetQ and bl8TEM1. In pig slurry eleven ARGs were detected with a mean of 7 genes per sample. SulI, sulll, tetA, tetB, tetC, tetM and tetQ were found in more of the 80% of the samples. Nine ARGs were found in amended soil, including all tet and sul genes and also bl8TET1 and qnrS. In contrast only sul1 was found in one sample of non-amended soil. In conclusion, animal waste carry ARGs that can be spread into the environment through manure application to agricultural soil. The more common ARGs, as tet and sul, but also relevant ARGs such bl8TET1 and qnrS were present in manured agricultural soil analysed in this study, suggesting the impact of this human activity.
Detection of MRSA and ESBL producing bacteria in biogas plants

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Introduction: Studies concerning the occurrence and fate of antibiotic resistant bacteria in biogas plants are rather scarce. Antibiotic resistant bacteria might be present in different substrates for biogas plants like corn silage and poultry or cattle manure. To what extent these microorganisms are usually reduced during the different stages in the biogas production is not yet clear. It could also be possible that some microorganisms will multiply if conditions are favourable for bacterial growth in these plants. It is important to gain more information on these topics to evaluate the hygienic quality of the digested residues which are often used as fertilizers.

Objectives: In a former project nine Bavarian biogas plants were sampled at different stages of biogas production - from original substrates to digested residues. The samples were analyzed for pathogens like STEC, Campylobacter spp. and Clostridium difficile with combined cultural and real-time-PCR methods. A high percentage of samples was tested positive for viable Clostridium difficile and STEC genes. In the current project new samples are taken in these biogas plants and the occurrence of MRSA and ESBL producing Enterobacteriaceae is examined.

Materials and methods: Substrates, fermenter contents and digested residues are analyzed for MRSA and ESBL producing bacteria with combined cultural and PCR methods plus an automated system for antibiotic susceptibility testing (VITEK®, bioMérieux).

Briefly, for detection of MRSA the sample is incubated in Mueller Hinton Broth. After enrichment a portion is transferred to Trypticase Soy Broth and incubated again. Nuc (specific for S. aureus), mecA (specific for MRSA) and the PVL gene (encoding Panton- Valentine leukocidin) are then detected with real-time-PCR. If PCR results are positive, portions of the Trypticase Soy
Broth are streaked on MRSA selective agar plates. After incubation and subcultivation the cultures are characterized with the VITEK®-system. Finally the results are confirmed by real-time-PCR.

For detection of ESBL producing Enterobacteriaceae the sample is incubated in peptone broth. A portion of the broth is then streaked on MacConkey agar with cefotaxime. After incubation and subcultivation the cultures are characterized with the VITEK®-system. The results are confirmed by real-time-PCR (CTX-M) and conventional PCR (ampC).

**Results and conclusions:** Testing of samples has started in January 2015. First results will be presented and discussed.

**References:**


**P FAT 8**

Impact of fertilizer type on the abundance and proportion of antibiotic resistance genes in grassland soil

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The common practice of soil fertilization with animal manure is the major route of antibiotic residues and antibiotic resistance genes (ARGs) from farm to the environment where they are likely to persist and spread. Anaerobic digestion of manure before land application has been suggested as a measure to minimize the spread of resistance from manure to soil. However, the data showing the ability of mesophilic anaerobic digestion to reduce ARG levels in manure has so far been inconclusive (Chen et al., 2010; Ghosh et al., 2009).

The aim of this study was to assess both the short-term and long-term effect of three fertilizer types (cattle slurry, cattle slurry digestate, and mineral fertilization) on the abundance and proportion dynamics of five ARGs (blaCTX, blaOXA2, tetA, sul1, and qnrS) in agricultural grassland soil.

Fertilization was performed three times with seven week intervals on three replicate zones for each treatment type and 11 soil samplings were performed during the study period from April to September 2013. DNA from soil and organic fertilizer samples was extracted and quantitative PCR (qPCR) was applied for the quantification of bacterial 16S rRNA gene and five ARGs (blaCTX, blaOXA2, tetA, sul1, and qnrS) encoding resistance to several major antibiotic classes (β-lactams, tetracyclines, sulfonamides, fluoroquinolones). ARGs were also normalized against 16S rRNA gene, representing the proportion of ARGs in the bacterial community of soil.

Anaerobic digestion of cattle slurry resulted in significant reduction of blaCTX proportion in bacterial community while the abundance and proportion of sul1, qnrS and blaOXA2 genes increased significantly compared to untreated cattle slurry. Grassland treatment with organic fertilizers resulted in significantly higher abundance and proportion of blaCTX and sul1 genes in soil bacterial community throughout the study period compared to mineral fertilizer treatment and no-treatment control zones. However, the abundance and proportion of tetA genes were significantly reduced in zones treated with organic fertilizers.

The type of fertilizer significantly affects the antibiotic resistant portion of microbial community in treated soil.

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SESSION IV: Fate and Effects of antibiotics in agro-ecosystems

P FAT 9
Multi drug resistant ESBL E. coli from cattle slurry in a dairy unit in Nottingham
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A significant amount of antibiotics is used in treating infections in farm animals, and as prophylactics against infection. Antimicrobial resistance is a crucial problem that is now of great concern in public health, with food and food producing animals as a potential route for spread of these resistances, especially resistance to cephalosporins which is increasing. In this study E. coli isolated from farm cattle slurry was used as an indicator for the persistence of antibiotic resistance genes in the environment. TBX agar, MacConkey Agar with and without Cefotaxime (2mg.L⁻¹) and CHROMagar ESBL medium were used to isolate and enumerate the total coliforms, and E. coli from slurry samples collected from different areas in a Dairy Research Unit. 160 isolates from TBX plates were confirmed as E. coli by phenotyping (indole, oxidase and API20E). ERIC PCR was used to investigate the genotypic diversity of these isolates. Antibiotic sensitivity testing using the disc diffusion method was used to investigate the antibiotic resistance profile of E. coli isolates to a range of antibiotics (17 antibiotics). A confirmation for the ESBL/AmpC multiresistance E.coli was done using total ESBL/AmpC confirmation kit. PCR was also used to detect ESBL and AmpC genes CTX-M, SHV, TEM and CMY. More than 55% of the isolates showed multiple resistances to the tested antibiotics, with isolates resistant to between 2 and 13 antibiotics. The highest percentage of resistance was to Ampicillin and the lowest resistance showed against Imipenem. In respect to Extend Spectrum Cephalosporins, the highest resistance was against Cefotaxime.

P FAT 10
Do toxic metals constitute stronger selective agents for development of bacterial community tolerance to tetracycline than tetracycline itself?
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The use of veterinary antibiotics for animal production is likely to decline in the future due to concern about antibiotic resistance. By contrast, metals such as copper (Cu) and zinc (Zn) are routinely used as animal growth promoters and can accumulate in agricultural soils receiving animal manures or metal-based pesticides. We hypothesized that Cu and Zn can exert stronger and more persistent selection pressures for development of antibiotic resistance in soil as compared to the antibiotics themselves. Specifically, we here compare the ability of Cu, Zn, and tetracycline to co-select or select for tetracycline resistance of a bacterial community in an agricultural soil. Model laboratory microcosms were set up by spiking agricultural soil with different levels of Cu, Zn or tetracycline. Pollution-induced community tolerance (PICT) to tetracycline was measured in all microcosms using the [3H]leucine incorporation technique after 2 months of toxicant-induced community succession. Bacterial growth ([3H]leucine incorporation), soil pH (pHwater) and toxicant bioavailability (whole-cell bacterial bioreporters specific for either Cu, Zn or tetracycline) were monitored during the whole PICT development. Bacterial community tolerance to tetracycline increased significantly in soils spiked with environmentally relevant levels of Cu (≥333.3 mg/kg) and Zn (≥500 mg/kg), but not in soil spiked with unrealistically high levels of tetracycline (up to 100 mg/kg). Furthermore, the resistance levels to tetracycline in microcosms were correlated to the initial toxicity or growth inhibition induced by metals. One explanation for the weak selection posed by tetracycline could be that tetracycline is less bioavailable in soil (bioreporter measurements pending). In conclusion, our study indicates that toxic metals in soil can exert a stronger selection pressure for resistance development to a specific antibiotic than the antibiotic itself.

P FAT 14
Understanding the fate of antibiotics applied with animal manure in agriculture field causes risks for soil and human health
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In recent years a major worldwide problem has arisen with regard to infectious diseases caused by antibiotic resistant microbes. Widespread use of veterinary antibiotics as an additive in animal feeds for their growth promotion and improvement of feed efficiency which is reaching in the agricultural fields by application of farm yard manure as organic fertilizers. Agricultural use of antibiotics is believed to contribute to the spread of antibiotic resistance, but the mechanisms by which many agricultural practices influence resistance remain obscure. Although manure from dairy farms is a common soil amendment in crop production, its impact on the soil microbiome and resistome is not known. Where, it has raised concerns about soil health, the fate of antibiotic
that affects on structure and functional activities of beneficial microbial communities like plant growth promoting microorganism (PGPM) and the development and transferability of antibiotic resistant microorganism and associated with mobile genetic element. It is estimated that approx 75% antibiotics are not absorbed by animals and are excreted in waste. Tetracyclines and penicillins are most commonly used antibiotics in animal husbandry. Antibiotic resistance selection occurs among gastrointestinal bacteria, which are also excreted in manure and stored in waste holding systems. The increased abundance and mobilization of antibiotic-resistance genes (ARGs) might contribute to the emergence of multi-resistant human pathogens that increase the risk against to antibiotic treatment of bacterial infections. Currently, needs to research focus on determining the fate of veterinary antibiotics resistant bacteria applied with manure to soil, and their effects on the structure and function of soil microbial communities in bulk soils in the rhizosphere. Soil is a large reservoir of microbial diversity and the majority of antimicrobial compounds used today in human and veterinary health care have been isolated from soil microorganisms. Soil harbours a large genetic diversity at small spatial scale, favouring exchange of genetic materials by means of horizontal gene transfer (HGT). This type genetic transformation causes the development of antibiotic resistant strains of microbes which is highly pathogenic for human being. As expert of sustainable agriculture, we isolated 200 to 500 microbial strains from different soil sample of eastern Uttar Pradesh and we was found that so many strains have showed antibiotic resistance properties. We have isolated some effective microbial strains (e.g. Mesorhizobium sp. BHR202, Pseudomonas fluorescens BHUPS806, P. aeruginosa BHUPS801, P. putida BHUPS804, Burkholderia cepacia BHUPS803, Bacillus megaterium BHUPS14, Bacillus subtilis BHUPS813, Paenibacillus polymyxa BHUPS817). These strains have very good properties of plant growth promoting properties. We have used as bio- fertilizers for multiple crop productions. According to my knowledge, we should think some alternative practices for overcome the uses of higher dose of antibiotic against pathogen or develop healthy human and animal, they have potential ability against fight the pathogens. This kind of practices may be help for overcome the application of antibiotic for disease caring. Others alternative, regarding antibiotic resistance, we can develop some genetically modified stains that can degrade the antibiotic in to free inorganic nutrient for plant growth and yield.

Keywords: Antibiotic-resistance genes; Plant growth promoting microorganism, Soil health, Sustainable Agriculture

Do organic fertilizers enhance resistance genes and mobile genetic elements in field soils?

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The extensive use of antibiotics in livestock is assumed to contribute to the rising emergence of antibiotic resistant pathogens. Substantial proportions of antibiotics applied to farm animals may be excreted unchanged or as metabolites and thus are present in manures. In addition, manures are a reservoir of various antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) such as plasmids or integrons that might foster ARG spread among different bacteria. Recently, IncP-1 plasmids were captured based on antibiotic resistances conferred from digestates of biogas plants fed with manure as co-substrate, indicating that these might as well contribute to the spread of ARGs and MGEs. Thus, by applying manures or digestates on field soil, ARGs might be transferred to bacterial communities associated to soils or plants. We performed a field plot study to analyze the effect of different organic fertilizers (manure, digestate) vs. anorganic fertilizer on the abundance of ARGs, disinfectant resistance genes, integrons and plasmids in agro-ecosystems (bulk soil, rhizosphere, roots of maize plants). Quantification of the genes \( m, suI, suI2, tetA, tetM, tetQ, tetW, intI1, intI2, qacE \Delta1 \) and of IncP-1 (\( \text{korB} \)), IncP-1 (\( \text{trfA} \)) and LowGC (\( \text{traN} \)) plasmids in total community DNA was done by real-time PCR assays. Manure application was found to increase temporarily the relative abundance of integrons and all tested resistance genes in bulk soil, whereas digestates only increased the abundances of \( \text{intI2} \). These effects disappeared in bulk soil until harvest but at that time less pronounced differences were detectable for some genes in rhizosphere and root samples. Remarkably, at harvest relative abundances of \( \text{tetW} \) were highest in rhizosphere samples fertilized with digestates. Abundances of specific sequences for the subgroup of IncP-1 (\( \text{trfA} \)) and LowGC (\( \text{traN} \)) plasmids were below the detection limit in the majority of samples and no differences in relative abundance in bulk soil were observed over time for IncP-1 (\( \text{korB} \)) plasmids. Our data suggest that spreading of manures on field soil is more likely to contribute to the spread of ARGs in agro-ecosystems than BGP digestates and that the persistence of ARGs and integrons might be extended in root-influenced soil compartments.
SESSION IV: Fate and Effects of antibiotics in agro-ecosystems

P FAT 16
Evaluation of qPCR for the determination of antibiotic effects in manured soil
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By manuring, antibiotics and antibiotic resistance determinants located on mobile genetic elements are applied to agricultural soils where they favor the development and dissemination of antibiotic resistance. Ultimately, antibiotic resistance genes in the environment could contribute to adverse effects on human and animal health, if transferred to pathogens. However, threshold concentrations of antibiotics in manure that are relevant for the development and dissemination of antibiotic resistance in soils are rarely reported.

We aimed to evaluate quantitative real time PCR (qPCR) as a method to assess threshold concentrations of antibiotics in manure affecting the abundance of antibiotic resistance genes in soil. Furthermore, class 1 integrons in soil bacterial communities were quantified as a marker for selective pressure by antibiotics applied with manure.

Therefore, a sandy and a loamy soil were mixed with manure and five concentrations of streptomycin or doxycyclin in a microcosm experiment (four replicates). Soil was sampled immediately after mixing as well as after 28 and 92 days. Total community DNA was extracted and the abundance of resistance genes (aadA, strA, tet(A), tet(M), tet(W), tet(Q), sul1, qacE+qacEd1) and class 1 integron integrase genes (intI1) was analyzed by qPCR relative to 16S rRNA genes.

The application of manure to soil increased the relative abundance of all antibiotic resistance genes to a detectable level. On day 28, streptomycin concentrations were positively correlated with the relative abundance of aadA and qacE+qacEd1 in the sandy soil, while no correlations were found in the loamy soil. Doxycyclin concentrations were positively correlated with the relative abundance of tetracycline resistance genes tet(A), tet(M), tet(W), tet(Q) as well as with intI1, aadA, sul1, and qacE+qacEd1 in the sandy but not in the loamy soil. Significant correlations were still detectable after 92 days in the sandy soil.

Preliminary results indicate that qPCR can assist to define threshold concentrations of antibiotics in manure that will affect the abundance of antibiotic resistance genes in soil. However, threshold concentrations might depend on antibiotic compound, soil type and hot spots present in soil.

P FAT 17
Determination of streptomycin residues on fruits after treatment against fire blight- a worst case scenario
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Question: Following devastating fire blight damages in pome fruit production an exceptional compromise for agricultural application of the antibiotic streptomycin for control of Erwinia amylovora in Germany was stipulated. Based on regulations for imminent danger provided by the plant protection act, a framework of strict prerequisites and control measures was agreed upon as basis for any potential permission of streptomycin application. Given the precautionary residue limit for streptomycin of formerly 20µg/kg, now 10µg/kg, products from treated orchards were monitored. Contrary to previous data, in 2008 the AGES reported streptomycin residues in individual apple batches determined with a newly established LC/MS-MS technique. Regulations for field application had to be revised accordingly.

To facilitate a save application scheme individual determinants for residue formation were tested. Different application time points and frequencies of streptomycin treatment on apple were compared. Residue formation on directly treated tissue was compared against untreated plant sections.

Methods: Fruits and leaves of apple were collected in an experimental orchard where streptomycin had been applied from 1-3 time points during bloom. To enhance formation of detectable residues the application was five or twenty-five times higher concentrated than in recommended field application. Collected plant tissue was stored at -20°C and analyzed via LC/MS-MS.

Results: The highly overdosed application of streptomycin had a visible phytotoxic effect. Leaves showed growth defects and large chlorotic areas. In contrast to the physiological response observed, residue levels on fruits at harvest time points were close to the detection limit. As expected, later treatments and increasing number of treatments facilitated higher residue levels. No quantitative evidence for active transport or accumulation in fruit tissue was observed.

Conclusions: Individual reports of low-level streptomycin residues in apple fruit after treatment against fire blight led to a revised application strategy. Maximum number of applications had been reduced to two applications and application was limited strictly to bloom. We conducted an exaggerated ‘worst case’ application experiment to trace streptomycin residues and exclude risk of enhanced residue formation due to transport and accumulation. No evidence for streptomycin accumulation in fruits was observed.
Characterization of tetracycline resistance in feedlot runoff applied to a vegetative treatment system

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Introduction: In U.S. beef cattle operations, manure management systems effectively reduce survival and transport of zoonotic pathogens, and previous studies show that the taxonomic composition of a microbial community changes once feces is excreted from the animal. Our hypothesis is that since manure management systems change the community structure, the same manure management strategies that can reduce pathogens will also impact the number and types of antibiotic resistant (AR) bacteria.

Objectives: The objectives were to evaluate whether a vegetative treatment system, where feedlot runoff is applied to fields contacting cool season grasses, reduced the number of bacteria resistant to selected antibiotics, characterize these AR bacteria (ARB) spatially and temporally, and describe the relationship between phenotypic and genotypic resistance profiles observed in the isolates.

Methods: The VT system consisted of a field that was divided into 8 application cells, separated from each other with earthen berms (Figure 1), allowing for replication of our measurements on a field scale. Measurements were taken in the spring and summer of three consecutive years from feedlot runoff applied at the top of the cells, and excess wastewater that collected at the bottom of each cell. ARB survival in soil was tracked, and the vertical transmission of ARB was evaluated. Baseline data were collected from ground deposited feces, rain water runoff, and the untreated berm areas. Tetracycline resistance was chosen because it is commonly used as a target in environmental samples. Isolates were screened for resistance to 12 antibiotics using standard disk diffusion methods, and characterized for the presence or absence of 11 tetracycline resistance genes.

Results: There were significantly fewer tetracycline resistant bacteria (TRB) in the VT rainwater samples, compared to feedlot runoff and excess wastewater, with feedlot runoff and excess wastewater samples typically 2 to 3 orders of magnitude higher in concentration of resistant bacteria. No discernible annual or seasonal trends were observed. Bacteria carrying tetracycline resistance could be cultured from soil samples immediately following feedlot runoff application, and numbers decreased by 0.5 log over two weeks. Berm samples were negative for TRB. Both single and multiple resistant E. coli strains were more likely to be found in the runoff, compared to excess wastewater and rain. Of 467 isolates screened for resistance to 12 antibiotics, 96% were resistant to more less than three of the antibiotics assayed. Over 12,000 isolates were screened for 11 tetracycline resistance genes (TRG). Of these 77% had at least one, and 26% carried multiple TRGs. The maximum number of TRGs found in a single isolate was three. Low concentrations of several antibiotics, including tetracyclines, were measured in feedlot run-off and in soil samples collected from areas receiving wastewater from the feedlot.

Conclusion: Although tetracycline resistance was routinely detected in bacteria from beef cattle feedlot runoff, soil sampling indicated that these microorganisms did not survive long-term in the soil. The VT was therefore shown to be effective at reducing the number of tetracycline resistant bacteria originating in manure, when evaluated over the course of a one year application time.

Mathematical model for spread of antibiotic resistance in a dairy unit in the UK: the importance of horizontal gene transfer

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Antimicrobial resistance is a crucial problem that is now of great concern in public health, with food and food producing animals as a reservoir and route for spread of these resistances. In this study, we use a mathematical modelling approach to investigate the emergence of antimicrobial resistant microbes in a slurry tank from a dairy farming unit in Leicestershire, UK. Specifically, we wanted to know which parameters most influence the proportion of antimicrobial resistant microbes at the end of a 5 month period of filling the tank, prior to spread of slurry onto land. The model has been parameterized using realistic parameters relevant to the specific dairy unit under study (200 cows yielding circa 10,000 litres, housed indoors and a mechanical slurry management system) and microbial-specific parameters. Through conducting a thorough sensitivity analysis of parameters within realistic ranges, we have found that the single most important factor influencing the proportion of antimicrobial resistant organisms in the slurry tank is the rate of horizontal gene transfer between resistant and sensitive bacteria. The level of antibiotic use, the proportion of resistant microbes resident in cattle intestines and the fitness changes of carrying resistance (whether positive or negative) all have relatively smaller impacts. The model concludes that efforts to control emergence of antimicrobial resistance in dairy systems might be best focussed on reducing opportunities for gene exchange between microbes.
Production-scale biogas plants commonly receive energy crops, manure and fecal matter from farm animals as substrates. Since considerable amounts of antibiotics are administered in animal husbandry, selected antibiotic resistant microorganisms from the animal’s gut microbiome end up in biogas reactors where they may proliferate or exchange resistance determinants. On a regular basis, digestates from biogas plants are spread as fertilizer on agricultural areas involving the risk of resistance genes being disseminated in the environment. To analyze agricultural biogas plants as reservoirs for β-lactamase genes, corresponding deeply sequenced and assembled metagenome data (403 Gb) were screened for the occurrence of β-lactamase genes. A new bioinformatics pipeline applying profile Hidden-Markov-Models (pHMMs) deduced from sequences of the ARG-ANNOT database (Antibiotic Resistance Gene-ANNOTation) for each of the 31 different β-lactamase groups, representing the four β-lactamase Ambler classes A, B, C and D was developed. Identified hits were compared to the NCBI protein database by BLAST, to interpret obtained results. If accessible, PubMed publications comprising predefined key words linked to the BLAST hits are also presented. The pipeline can be applied to any type of metagenomic dataset.

With an e-value cutoff of 1e-15 referring to the pHMMs, 207 putative β-lactamase genes were identified. These are unequally distributed across the four β-lactamase classes. About 8% matched class A, 37% class B, 51% class C and only 4% class D. Among these hits, some correspond to known β-lactamases. However, the majority represent putative variations of so far undiscovered β-lactamases. As a proof of concept, one candidate gene for each class will be synthesized to verify its antibiotic resistance properties.

Hence, obtained results provided evidence that biogas plants have to be considered as reservoirs for various β-lactamase genes and since digestates are used as fertilizer in agriculture, resistance determinants may be disseminated in the environment.

**P FAT 20**

A bioinformatics pipeline for the detection of β-lactamase genes in metagenome sequence data and its application to production-scale biogas plants

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In agriculture, copper (Cu) is extensively used for its antimicrobial properties. For example, copper sulfate is used in dairy cattle as a feed additive and footbath disinfectant. The generated wastes (e.g. manure, foot bath water) contain high copper concentrations eventually introduced into agricultural soil by fertilization and/or irrigation. Several studies have highlighted the coselection of antibiotic resistance by either high or sub-lethal concentration of metals in the environment. Plasmids involved in antibiotic resistance dissemination often harbor genes encoding resistance to metals such as Cu. The hypothesis of this study is that Cu resistance properties.

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**P FAT 21**

Does the extensive use of copper in agriculture promote antibiotic resistance spread?

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In agriculture, copper (Cu) is extensively used for its antimicrobial properties. For example, copper sulfate is used in dairy cattle as a food additive and footbath disinfectant. The generated wastes (e.g. manure, foot bath water) contain high copper concentrations eventually introduced into agricultural soil by fertilization and/or irrigation. Several studies have highlighted the coselection of antibiotic resistance by either high or sub-lethal concentration of metals in the environment. Plasmids involved in antibiotic resistance dissemination often harbor genes encoding resistance to metals such as Cu. The hypothesis of this study is that Cu resistance is disseminated in the environment. To analyze agricultural biogas plants as reservoirs for various β-lactamase genes and since digestates are used as fertilizer in agriculture, resistance determinants may be disseminated in the environment.

Production-scale biogas plants commonly receive energy crops, manure and fecal matter from farm animals as substrates. Since considerable amounts of antibiotics are administered in animal husbandry, selected antibiotic resistant microorganisms from the animal’s gut microbiome end up in biogas reactors where they may proliferate or exchange resistance determinants. On a regular basis, digestates from biogas plants are spread as fertilizer on agricultural areas involving the risk of resistance genes being disseminated in the environment. To analyze agricultural biogas plants as reservoirs for β-lactamase genes, corresponding deeply sequenced and assembled metagenome data (403 Gb) were screened for the occurrence of β-lactamase genes. A new bioinformatics pipeline applying profile Hidden-Markov-Models (pHMMs) deduced from sequences of the ARG-ANNOT database (Antibiotic Resistance Gene-ANNOTation) for each of the 31 different β-lactamase groups, representing the four β-lactamase Ambler classes A, B, C and D was developed. Identified hits were compared to the NCBI protein database by BLAST, to interpret obtained results. If accessible, PubMed publications comprising predefined key words linked to the BLAST hits are also presented. The pipeline can be applied to any type of metagenomic dataset.

With an e-value cutoff of 1e-15 referring to the pHMMs, 207 putative β-lactamase genes were identified. These are unequally distributed across the four β-lactamase classes. About 8% matched class A, 37% class B, 51% class C and only 4% class D. Among these hits, some correspond to known β-lactamases. However, the majority represent putative variations of so far undiscovered β-lactamases. As a proof of concept, one candidate gene for each class will be synthesized to verify its antibiotic resistance properties.

Hence, obtained results provided evidence that biogas plants have to be considered as reservoirs for various β-lactamase genes and since digestates are used as fertilizer in agriculture, resistance determinants may be disseminated in the environment.
Human activities play major roles in the dissemination of antibiotic resistance. Growing evidence have emphasized anthropogenic effluents as player of the environmental spread of antibiotic resistance. Integrons, bacterial genetic element involved in bacterial adaptation, have been used in numerous studies as marker to assess anthropogenic impact on the environment. In this study integrons have been used as marker of the multidrug resistance in hospital effluents. In addition characterization of the bacterial communities in those effluents was carried out to identify putative player in this integron dissemination.

Wastewater samples from 8 European hospital centers (HE), 2 geriatric units (GE) and 3 urban areas (UE) unaffected by significant clinical activities were sampled. Total genomic DNA was extracted and class 1, 2 and 3 integrons were quantified using a multiplex qPCR. Results were normalized to the 16S rRNA-encoding gene copy number so as to calculate the normalized copy number abundance of each classes of integrons. Molecular profiling of the bacterial communities of each samples was performed by a 16S rRNA gene based approach by 454 pyrosequencing.

Significantly higher normalized copy number of integrons were present in all studied HE (t-test, p-value <0.05). Class 1 integrons were the most representative class, followed by the class 3 or the class 2 integrons. The normalized copy number of integrons in the GE were similar to the UE. These results highlighted that hospital effluents contain bacterial communities more enriched in integron. Gamma-Proteobacteria, Firmicute and Bacteriotales where the most dominant phyla represented in all effluents investigated. Bray-Curtis similarity index point out similarities between certain groups of samples. Although some HE clustered together no specific pattern clustering the HE from the UE and GE were observed.

This survey suggests that hospital environment selects for bacterial strains harboring integrons, raising the question whether or not HE should receive a dedicated treatment before release in the sewage network? Further depiction of the microbiome data should help to determine if any phyla might be related to the integron normalized data and highlight factors of integrons dissemination in HE.
O DIS 1
Exploring plasmid-based dissemination of antibiotic resistance genes in environmental matrices
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Plasmid transfer has been shown repeatedly as playing an important role in antibiotic resistance gene dissemination and considerable efforts are given to identify permissive environments and/or conditions driving plasmid transfer in microbial communities.

The objectives of our work were to investigate the permissiveness of various environments to plasmid transfer in order to identify conditions affecting plasmid transfer in environmental microbial communities.

We recently developed a sensitive molecular approach based on qPCR allowing the detection of rare transfer events of known plasmids in natural microbial communities. In brief, this approach consists in inoculating microcosms with a donor bacterium and to quantify the relative abundance of both the plasmid and the initial host DNAs over time. As conjugative transfer is an intercellular mode of DNA replication, the plasmid to donor DNA ratio increases in the community total DNA when the plasmid transfers into the indigenous population.

Using this approach, we showed that the transfer of the IncP-1b plasmid pB10 in complex environments was surprisingly rare and strongly matrix dependent. On the one hand, in some instances, the transfer of pB10 appeared to be influenced by eukaryotic predation, which could either promote or inhibit plasmid transfer depending on the environmental matrix considered. But, on the other hand, classical ecology approaches such as TTGE-based analyses appeared to be too poorly resolutive to point out relevant community members/structures supporting pB10 transfer. However, further analyses by qPCR of the IncP-1α/β plasmids abundance demonstrated that pB10 transfer tends to be supported by environmental matrices exhibiting a higher initial content of IncP-1α/β plasmids, which by itself could be seen as a community trait.

In conclusion, the present work tends to show that the relative abundance of IncP-1 plasmids in a given microbial community reflects its permissiveness to the transfer of plasmids belonging to the same incompatibility group and that the phenomenon apparently prevails over transfer limitation due to the superinfection immunity. Detecting environmental communities presenting a high content of a plasmid family could be used to identify putative hot spots supporting conjugative gene transfer.

O DIS 2
Distribution and fate of antibiotic resistance bacteria and genes in enhanced anaerobic digestion of sewage sludge with microwave pretreatment
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Introduction: Sewage sludge treatment is one of the key control processes for antibiotic resistance spreading to the environment. For the purpose of better methane production and antibiotic resistance control during sludge treatment, the fate of tetracyclines and β-lactam antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in the enhanced anaerobic digestion with microwave pretreatment were investigated in this study.

Methods: Three combined microwave pretreatments, microwave-acid (MW-H), microwave (MW) and microwave-H2O2-alkaline (MW-H2O2) were used for enhancing methane production from sludge anaerobic digestion. The performance of methane production of different pretreated sludge were determined by biochemical methane potential (BMP) assays. The anaerobic ARB were enumerated by samples dilution and plating on wilkins-chalgren anaerobe agar containing an antibiotic at defined concentration. Quantitative real time PCR (qPCR) was used to quantify the ARGs.

Results: More soluble COD was released from sludge pretreated by the MW-H2O2 and then resulted in the maximum methane production in the anaerobic digestion. The combined MW sludge pretreatment could reduce 0.55–5.04 logs ARB, while only the MW-H pretreatment could reduce all ARGs and intI1 (0.62–2.82 logs reduction), which had the best ARB and ARGs removal performance. However, the oxytetracycline (OTC) and cephalothin (CEP) resistance rates increased in all pretreatments. Moreover, the pretreated sludge had better ARB removal effect than the control during the subsequent enhanced anaerobic digestion, with all resistance rates and ARB concentration of pretreated sludge tending to decline (0.01%–24.16% reduction vs. 0.56–2.88 logs removal). Even though intI1 and most ARGs rebounded after the enhanced anaerobic digestion especially for the MW-H pretreated sludge, the intI1 and ARGs of digested sludge after pretreated were less than or close to those of unpretreated sludge. Furthermore, only MW-H2O2 pretreatment could reduce all ARGs abundance in sludge by 15.38%–83.12%, implying that the MW-H2O2 pretreatment for enhanced anaerobic digestion of sludge was conducive to inhibit the horizontal gene transfer (HGT).
SESSION V: Dissemination of antibiotics and antibiotic resistance genes through aquatic ecosystems/sewage treatment plants

Conclusions: The enhanced anaerobic digestion with the MW-H\textsubscript{2}O\textsubscript{2} pretreatment was considered to be the optimal sewage sludge treatment method for its best soluble COD release during pretreatment process, maximum methane production and good antibiotic resistance control.

DIS 3

Multidrug-resistant human-associated bacteria from wastewater treatment plants and their receiving waters - Gulf of Gdansk, Baltic Sea (Poland).

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Introduction: The safe, economical and reliable way of wastewater disposal is a principal problem in ecosystems, which are highly anthropogenically impacted, like the Gulf of Gdansk and its shallow western branch - Puck Bay. Due to the limited water exchange between these water bodies and open Baltic Sea, the introduced pollutants remain there for a long time. Nowadays, antibiotic resistant bacteria and antibiotic resistance genes are considered as novel emerging pollutants in marine environments.

Methods: In this study bacteria associated with the human intestine were isolated from two local wastewater treatment plants (Gdansk-Wschód and Gdynia-Dębogórze) and their receiving waters: Gulf of Gdansk (Baltic Sea). Bacteria were isolated according to the procedure dedicated for fecal coliforms. All isolates were biochemically identified and their drug susceptibility was determined. Isolates resistant to at least one antibiotic were tested for prevalence of integrons and antibiotic resistance genes using PCR.

Results: In all samples Escherichia coli was a dominant species. However, members of other genera (Aeromonas, Citrobacter, Enterobacter, Klebsiella, Shigella, Plesiomonas and Vibrio) were also isolated. Vibrio cholerae strains were detected only in Puck Bay waters. They were non-pathogenic, lacked the genes involved in cholera enterotoxin production, but they all possessed intIAVch.

Among 445 tested Escherichia coli isolates 36\% (n=161) were resistant to at least one antibiotic. Among resistant strains 26\% were multidrug-resistant (MDR) (resistant to 3 classes of antibiotics). In 55 isolates (31 \%) we detected integrons (class 1 in 49 isolates, class 2 in 5 isolates, class 2 integrons were detected only in multidrug-resistant isolates). Isolates with resistance to β-lactams were the most frequent (n=134, 83\% of all resistant isolates). Among them, the bla\textsubscript{TEM} gene was dominant (found in 78\% of isolates resistant to β-lactams). Other genes coding β-lactamases were found only occasionally (bla\textsubscript{OXA} in 7 isolates, bla\textsubscript{SHV} in 5 isolates and bla\textsubscript{CTX-M} in 2 isolates). Among isolates resistant to sulphonamides (n=50) sul2 gene was predominant (found in 42 isolates), followed by sul1 gene (in 29 isolates). sul3 gene was found in two isolates from wastewater samples. In tetracycline-resistant isolates (n=87) we found mostly tetA and tetB genes (in 51 and 35 isolates, respectively). tetD and tetG genes were found only in two isolates, from marine waters collected near marine outfall of WWTP Gdynia-Dębogórze. In isolates resistant to quinolones (ciprofloxacin and levofloxacin) we tested only the presence of plasmid-mediated quinolone resistance genes (PMQR genes). We detected qnrS, qnrA, qepA and aac(6\')-Ib genes. In aminoglicoside-resistant strains (n=10) strAB and aac(3)\textsubscript{II} genes were the most frequent.

Also two isolates of E. coli were found to produce extended-spectrum β-lactamases (ESBL). Their ESBL type was characterized as CTX-M-15 β-lactamase type, that is produced by nosocomial and community strains of Enterobacteriaceae in different countries.

Conclusions: Data obtained in this study indicated that in general applied wastewater treatment level together with effective dilution of treated wastewater by marine outfalls were sufficient to protect coastal water quality from sanitary degradation (low number of E. coli detected in marine waters). Detailed analyses showed however, that human-associated bacteria, even potential pathogens and bacteria carrying antibiotic resistance genes of clinical significance can survive in wastewater and marine water conditions. These findings highlight that further studies are needed to understand the dissemination, stability and transmission of resistance genes in water ecosystems.
Determination of the minimal selective concentrations of antibiotics in complex aquatic bacterial communities


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Introduction: Sewage contains a mixture of antibiotics and other co-selective agents such as biocides and metals. It is unknown to what extent antibacterial agents select and co-select for resistance in aquatic ecosystems e.g. wastewater treatment plants and receiving waters. To comprehend the environmental impacts of these pollutants we need to know the concentration at which resistance increases and to what extent co-selection occurs.

Objectives: The aim was to determine the minimal selective concentration (MSC) of an antibiotic (tetracycline) and the minimal co-selective concentrations (MCCs) of a common biocide (oxytetracycline) in complex aquatic bacterial communities.

Materials & methods: To contrast meropenem and Zn resistance in communities in Zn polluted and pristine reaches in the River Tyne, United Kingdom.

Materials & methods:

Six aerobic rotating tubular reactors were used to treat domestic sewage amended with the following: no amendment (control), 2 mg/L meropenem (Reactor 2), 2 mg/L Zn (Reactor 3), 2 mg/L meropenem and 2 mg/L Zn (Reactor 4), 2 mg/L meropenem and 20 mg/L Zn (Reactor 5), 2 mg/L meropenem and 100 mg/L Zn (Reactor 6). Samples for microbiological, metal and molecular analysis were collected from a common influent tank, six effluent flasks and from biofilm at the top (upper biofilm) and bottom end (lower biofilm) of each reactor. Results were contrasted with microbiological and metal analytical results from monthly sediment samples from sites with high (South Tyne; ST) and low (North Tyne; NT) levels of Zn pollution.

Results: Reactor 2, which was dosed with 100 mg/L Zn, had combined resistance to meropenem and Zn in 51% of collected isolates, while 24% of isolates from the 20 mg/L Zn reactor displayed combined resistance. Moreover, only 2 mg/L Zn additions caused combined resistance up to 5% of isolates, and meropenem plus 2 mg/L Zn resulted in 9%. Elevated Zn resistance was seen in sediments that have high Zn (ST). In addition soluble and total Zn levels significantly correlated with Zn resistance across sites (r² = 0.40, p<0.01; r² = 0.85, p<0.01). Further, 3% combined meropenem and Zn resistance was detected in isolates at low Zn sediment sites, compared to 8% at high Zn sites.

Conclusions: Elevated Zn levels in domestic sewage clearly increased meropenem resistance levels in treated effluents.

- Resistance to Zn and combined meropenem and Zn increased in sites with high Zn pollution.
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References:

O DIS 6
Antibiotic resistance in Pristine Red Sea Brine Pools
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Introduction: Antibiotic resistance (AR) is a complex problem with global clinical impact. Understanding the origin and evolution of AR will not only provide us with a fundamental scientific inquiry, but will also shed light on how we can prevent and deal with resistance.

Objectives: The aim of this study is to assess the origin and evolution of AR by investigating the abundance and diversity of AR in pristine Red Sea samples and compare them with human-impacted marine environment and sewage sample.

Materials & methods: Metagenomic sequences of water and sediments from Red Sea brine pools and overlying water column, representing pristine environments were analyzed. More than 89 million sequence reads were aligned by BLASTx to antibiotic resistant polypeptides contained in the Comprehensive Antibiotic Resistance Database (http://arpcard.mcmaster.ca). Reads with more than 90% identity over at least 25 amino acids were considered AR positive. Reads that were assigned to mutational AR genes were further filtered by alignment to reference non-resistant genes and checked for the presence of one or more literature-reported mutations using a customized script. The presence of mobile genetic elements (MGEs) was also inspected and correlated to AR (Fig. 1). Red Sea AR reads were compared to samples from the Gulf of Mexico (from Global Ocean Sampling) and wastewater treatment plant (WWTP) sequences retrieved from the Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis (CAMERA:http://camera.calit2.net/) and Sequence Read Archive (SRA:http://www.ncbi.nlm.nih.gov/sra), respectively.

Results: Several AR genes were detected in Red Sea brine samples (Fig. 2). The Gulf of Mexico showed slightly higher relative abundance, while WWTP had extremely higher levels of AR. A similar trend was observed with MGEs, which showed strong linear correlation with AR abundance.

Conclusion: This study provides new evidence on the role of environmental organisms as reservoirs for AR genes. Moreover, it highlights the augmented contribution of human activity on the abundance, diversity and evolution of AR. Our results suggest a potential role of wastewater in the dissemination of AR. Further analysis of candidate AR genes identified here may shed light on the evolution of AR in clinical practice.

Figure Legends:
Figure 1. Methodology and study layout. QC, quality control; CARD, Comprehensive Antibiotic Resistance Database; aa, amino acid; nt, nucleotide; IS-Finder, database for insertion sequences; INTEGRALL, database for integrons and integron-related gene cassettes.

Figure 2. Relative abundance and types of antibiotic resistance. Relative abundance is defined in part per million (ppm)-one positive read per million reads. AT, Atlantis II Deep. Samples with numbers directly following AT are water column samples from overlying the brine pool. Numbers indicate depth in meters. DD, Discovery Deep; KD, Kebrit Deep; BR following Deep abbreviation, brine; INF, interface; UCL, upper convective layer; LCL, lower convective layer; UINF, upper interface; LINF, lower interface; S following deep name, sediment; number following S, sediment section; NBI, non-brine I site; WWTP, wastewater treatment plant.

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Figure 1

Figure 2
The occurrence of antibiotics in natural and urban environments seems to favor the development and spread of antibiotic resistance. At the same time the key factor for the evolution of new antibiotic resistances is the ability of an organism to adapt quickly to new environmental conditions. One of the environments where the selective pressure of antibiotic pollution could exert an effect on microbial communities is the urban water system. The sewage canals and waste water treatment plants in particular are known to carry elevated concentration of pollutants and microorganisms. The co-existence of these two factors could lead to a gradual increase in the prevalence of resistance within the indigenous microbial community.

In our study raw waters originated from the sewage pipes of the main neighborhoods of Dresden metropolitan area (Germany) were seasonally collected along the years 2012-13 and screened for the presence and amount of antibiotic resistance genes (ARG). The genes of interest belonged to the chemical classes of β-lactams, quinolones, macrolides, sulphonamides and tetracyclines. Concentrations of antibiotic residues in the raw waters and effluent were measured and the correlation between antibiotic residues and antibiotic resistance genes revealed via multivariate statistical analysis. Moreover the diversity and species distribution of microbial communities inhabiting sewage waters and effluent (MiSeq) will help us to explain the seasonal variation of ARGs content. Overall, despite the sanitation processes the effluent did not show any reduction in ARGs pointing out the need of newer wastewater technologies capable to limit the spread of ARGs in freshwater ecosystems.

Microbial metabolic capabilities including antimicrobial biosynthesis during water infiltration revealed by metagenomic and transcriptomic analyses

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Introduction and Objective: Freshwater can infiltrate through subsurface layer and augment groundwater. By combing metagenomic and metatranscriptomic analyses, the objective of this study was to reveal the metabolic potential and biosynthesis and resistance of antibiotic properties expressed by the microbial community during infiltration of water through porous media.

Materials and Methods: To investigate microbial community characteristics during water infiltration, a series of soil-columns simulating subsurface infiltration zone and receiving different organic matter compositions and trace organic chemicals were established. Microbial biomass, phylogenetic composition, metabolic potential and properties expressed were resolved along the vertical depth of infiltration zone by using qPCR, 16S rRNA sequencing, metagenomic and metatranscriptomic analyses.

Results and Conclusions: Distinct differences were observed between microbial phylogenetic structures in shallow (0-30 cm) versus deeper (30-120 cm) sediments. A unique microbial community with significant metabolic capacities of biodegrading xenobiotics including numerous chemical pollutants as well as antibiotic synthesis and resistance developed naturally as a function of available carbon. In accordance with metagenomic results, penicillin and cephalosporin biosynthesis (EC3.5.2.6) as well as streptomycin biosynthesis (EC1.1.1.133) were overrepresented in deeper sediments (Figure 1). Beta-lactamase (EC1.5.2.6) for penicillin and cephalosporin biosynthesis and resistance also increased with depth. These results suggest that microbial groups specifically associated with shallow sediments are well adapted to comparably nutritionally rich environments such as mesotrophic conditions. In contrast, the overrepresentation of many secondary metabolite metabolisms and particularly xenobiotic biodegradation and biosynthesis of antibiotics such as penicillin, cephalosporin, streptomycin became became more important for microbes adapted to oligotrophic conditions in the deeper infiltration zone. The availability of the primary substrate or biodegradable organic carbon seems to be mainly responsible for this expression rather than the presence of trace organic chemicals.
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Figure 1

**O DIS 9**

Exploring the effects of anthropogenic pollution on the environmental resistome of rivers and agricultural soils in Central Mexico using microbiological and genomic approaches

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Introduction:
In Mexico rivers are systematically used for the disposal of untreated waste waters, and then for agricultural field irrigation. We hypothesize that the massive release of human gut bacteria into rivers promotes the dispersal of these bacteria on broad geographic scales, and the horizontal transfer of their resistance determinants to native bacteria.

Objectives
To analyze large collections of Proteobacteria recovered from heavily polluted and almost pristine rivers, sediments and agricultural soils irrigated with clean or contaminated waters to determine the effects of anthropogenic pollution on their diversity patterns, antibiotic (AB) resistance gene (ARG) content and resistance profiles (ARP).

Materials & methods:
Proteobacteria were isolated at 10 sampling points from the water column and sediments of 4 rivers, and 5 agricultural soils from Morelos, Mexico, using different media and ABs (Amp, Tm, CTX, IMI, CIP). All isolates were classified at the genus level by 16S rDNA sequence analysis. Their ARPs against 11 ABs in 5 families were determined. MLSA and MLST were performed for selected genera along with class-1 integron detection. Statistical analyses were performed in R.

Results:
Our collection currently contains 1685 environmental isolates classified at the genus level by 16S sequencing. Here we focus on two groups. For 252 Enterobacteriaceae (6 genera) recovered from the water column of nearby Apatlaco (APA=contaminated) and Tembembe (TBB=pristine) rivers, we found that the APA was strongly dominated by *Escherichia* isolates, while in the TBB the abundance of *E. coli* and 4 other genera was equivalent. 52% of the APA isolates carried class-1 integrons, but only 17% of the TBB strains. A very significant association was found between IntI1 presence and MDR (Fig. 1). 120 cassette arrays were sequenced (Fig. 1), uncovering three novel class-1 integrons (In1112, In1113, In1114); Ramos-Madrigal et al. 2015 (submitted).

All *Stenotrophomonas* strains (n=268) in our collection are MDR, dominating the collections from river sediments. The mean no. of resistances was significantly higher in contaminated sites (p-val = 0.0075). MLSA analysis revealed a high diversity of novel *S. maltophilia* STs (>95%), as well as novel species. 12 strains were subjected to WGS sequencing (Illumina 2x300 bp) for comparative genomics. We determined core and accessory sets of efflux pumps and ARGs (Fig. 2).

Conclusion:
We found compelling evidence for the impact of human contamination on the diversity and resistomes of Proteobacteria.

Acknowledgements
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Figure Legends

Figure 1. (A) Prevalence of class-1 integrons and diversity of cassette arrays in Enterobacteriaceae recovered from the Apatlaco and Temembe rivers in Morelos, Mexico. (B) Box-plot and formal statistical analyses showing the significant association between the presence of intI1 and multiresistance.

Figure 2. Hierarchical clustering of presence-absence patterns showing the distribution of efflux pump gene families and their copy numbers in 18 Stenotrophomonas genomes, 12 of which were generated in this study. The analysis reveals the presence of a set of shared core efflux pumps and associated proteins, as well as strain-specific ones.
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O DIS 10
Temporal dynamics and source-to-tap connectivity in a surface-water fed drinking water distribution system
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Introduction: Surface waters receiving wastewater can be contaminated with antimicrobial resistance factors (ARF: resistant bacteria, resistance genes, mobile genetic elements). To assess the risk associated with such situations, the connectivity of the ARF in the environment with human and animal populations needs to be better understood. One potential contact point is drinking water. In developed countries, drinking water is frequently disinfected by chlorination, but it is not well known to which extent ARF traverse the treatment. It is also not well understood to which extent ARF in treated drinking water are determined by the quality of the source water versus processes internal to the drinking water treatment and distribution system. In this work, we have studied the environmental ARF contamination originating from a Swiss City using Lake Geneva as the receiving water for its treated wastewater and as a drinking water source.

Objectives: Our objective was to study the dissemination of ARF into the receiving lake water and to determine if this potential contamination affected the quality of the raw water pumped for drinking water production. We aimed to assess the impact of the raw water quality on the abundance of ARF in treated drinking water. To this end, we wanted to determine the temporal dynamics of ARF abundance in the source water as well as the drinking water production and distribution system.

Materials & methods: We studied the prevalence of ARF in wastewater, in the lake, and at various stages of the drinking water treatment process and across the distribution network for a full year. Antibiotic resistant heterotrophs were analyzed by plate counts in the presence of different combinations of antibiotics. Total cell counts were determined by flow cytometry and live-dead staining. Antibiotic resistance genes were detected and quantified using qPCR assays. Assimilable Organic Carbon and other water quality parameters were determined.

Results: ARF are disseminated with the treated wastewater at relatively high concentrations, and a tributary urban stream was identified as an additional source. The contamination has affected the ARF abundance in the lake sediment, indicating that contamination may be transported towards the drinking water intake. ARF concentrations increased in winter, during holomixis. Drinking water treatment by chlorination was found to be a largely effective barrier against ARF, only low numbers of cultivable resistant bacteria were found in the distribution system or tap water. A selective effect of chlorination was not observed. However, a considerable amount of dead cell material and amplifiable resistance gene fragments are transported with the chlorinated water. Therefore, resistance gene abundance in tap water appeared more strongly coupled to source water quality, while the cultivable fraction, impacted by regrowth in the system, appeared decoupled.

Conclusion: The work indicates that ARF dissemination with treated wastewater can affect the quality of surface water pumped for drinking water production. However, the treatment process largely uncouples the ARF load from what is found in the source. The potential for uptake of ARF with finished drinking water was judged to be minor compared with other likely uptake routes.

Poster Presentations

P DIS 1
Abundance of different antibiotic resistances in treated sewage and receiving water bodies
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To date, little is known about the fate and the behavior of antibiotic resistant bacteria in the aquatic environment. To address this, the resistance behavior of 1236 Escherichia coli (against ampicillin, cefotaxim, ciprofloxacin, cotrimoxazole), 1166 enterococci strains (against ampicillin, ciprofloxacin, chloramphenicol, erythromycin, vancomycin) and 2259 staphylococci strains (against ciprofloxacin, clindamycin, erythromycin, oxacillin) isolated from sewage after different treatment steps as well as from receiving rivers was tested.

The respective sewage treatment steps had different effects on the abundance of the respective resistance patterns: Whereas the percentage of ampicillin resistant E. coli increased during ozonation (16 %), the percentage of antibiotic resistant enterococci decreased (25.4 %) corresponding with a decreased number of E. faecium. The percentage of antibiotic resistant E. coli in mechanical and biological treated sewage and river water was similar (~17 %), while that of resistant enterococci and staphylococci was lower in receiving water (21 and 7.5 % respectively). The fact that extended-spectrum-ß-lactamase producing E. coli strains could be isolated indicates that they are present in relevant numbers. Even if no methicillin resistant Staphylococcus aureus was isolated, mecA expressed by coagulase-negative staphylococci could be detected in the aquatic environment.

Interestingly, the percentage of inducible clindamycin resistant staphylococci on erythromycin resistant isolates was higher in river water (53.6 %) than in raw sewage (17.5 %). In comparison to hospital, the erm-gene diversity detected in the aquatic environment
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was broader. Whereas in hospital erm-mediated MLSβ-resistance is connected with erythromycin MICs ≥ 256 mg/L, e.g. erm(44)-expressing staphylococci isolated from aquatic environments revealed low-level erythromycin resistance (MICs: 4-8 mg/L). The regulation of antibiotic resistance genes of environmental strains seemed to be an adaption to their habitat facing antibiotic-concentrations in the ng/L range. These isolates might be misclassified interpreting MICs based on clinical breakpoints. Therefore, the antibiotic resistance status in aquatic ecosystems might be underestimated.

P DIS 3

Constitutive presence of antibiotic resistance genes within the bacterial community of a large subalpine lake

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Although of diverse origin, the persistence of ARGs in aquatic environments is highly influenced by anthropic activities, allowing potential control actions in well-studied environments. However, knowledge of the abundance and space-time distribution of ARGs in ecosystems is still scarce. We investigated the presence and the abundance of twelve ARGs (against tetracyclines, β-lactams, aminoglycosides, quinolones and sulphonamides) at different stations, depths, and seasons, in Lake Maggiore, a large subalpine lake. In order to compare seasonal dynamics to long term changes we additionally sampled one station daily over the course of a week. Dynamics of ARG were determined by qPCR and correlations of each gene with the microbial community composition (Illumina sequencing of 16S rDNA) and a number of other ecological parameters were evaluated. Our results suggest the constitutive presence of at least four ARGs (tet(A), sul1, blaCTXM & str(B)) within the bacterial community with a high proportion of bacteria potentially resistant to tetracyclines and sulphonamides. The dynamics of tet(A) and sul1 genes were positively correlated to dissolved oxygen and negatively to chlorophyll, suggesting that the resistant microbes inhabit specific niches. We, however, also observed short-lived peaks of blaCTXM, suggesting point pollution, and sudden disappearance of str(B) coinciding with a significant change of the bacterial diversity. These observations indicate that the lake is a reservoir of antibiotic resistances, highlighting the need of a deeper understanding of the sources of ARGs and the factors allowing their persistence in waters.

P DIS 4

Occurrence of antibiotic resistance genes and faecal indicator bacteria at a karst spring

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Introduction: In recent years, the aquatic ecosystem was recognised as reservoir for bacteria carrying antibiotic resistance genes (ARGs). However, in particular knowledge with respect to ARGs in raw water used for drinking water production is limited.

Objectives: Karst systems are important sources of drinking water, but are known to be vulnerable to human and animal waste contamination. In our study, the occurrence of ARGs and the origins of fecal pollution were investigated.

Materials & methods: The occurrence of eleven antibiotic resistance genes (tet(A), tet(B), tet(C), tet(K), sul, sul1, dfrA1, dfrA12, ermB, aadA, and blaSHV) was investigated using PCR. Faecal indicator bacteria, turbidity, electrical conductivity, and karst spring discharge were determined. In addition, culture-independent Microbial Source Tracking (MST) tools for identification of faecal pollution sources (human, ruminant) were applied.

Results: The ARGs most frequently detected were sul1 (48.2 %), sul2 (37.5 %), dfrA1 (37.5 %), tet(B) (33.9 %) and ermB (28.6 %) which encode for resistance to sulfonamide, trimethoprim, tetracycline, and macrolides. In particular during storm events resulting in high discharge rates (up to 0.78 m³/s), maximum numbers of ARGs, E. coli and enterococci were observed. ARG copy numbers increased up to 2.8 × 10² and 1.27 × 10³ gene copies per mL for sul1 and ermB, respectively. Studies into MST revealed the presence of human and ruminant gene markers.

Conclusion: The study demonstrates that ARGs are present in the karst spring water used for drinking water supply, ARGs, faecal indicator bacteria, and MST markers indicated raw water pollution by wastewater (retention basin overflow) and animal farming in the catchment area. The ARGs sul1, sul2, dfrA1, tet(B) and ermB are suggested as key ARGs for a long-term monitoring.

Acknowledgement: The study was funded by the German Federal Ministry of Education and Research (BMBF) as part of the funding program Sustainable Water Management (NaWaM-RISKWa, grant number 02WRS1277C).
Antibiotic resistance genes (ARGs) as emerging contaminants are posing a growing risk to human health and global ecosystem. Estuarine environment is believed to be a reservoir of antibiotic resistance. This study provides a panorama profile of ARGs in Chinese estuarine sediments through a high-throughput quantitative PCR approach. 286 ARGs and 10 MGEs were targeted including class 1 integron and transposens genes. A total of 259 genes including 248 ARGs, 9 transposase genes and 1 class 1 integrase gene were detected in all samples. Multidrug and beta-lactam were the two most dominant types of ARGs. The total copy numbers of ARGs per gram of sediments ranged from 8.81E+05 (ZJ-QTJ) to 1.75E+08 (FJ-JLJ) with an average of 2.50E+07. The total ARGs abundance was found to be significantly correlated with both transposases and integrase ($r$ as high as 0.976 for integrase and 0.967 for transposases), suggesting the potential horizontal gene transfer. Correlation analysis revealed that human activities (total population, GDP, municipal domestic sewage and aquaculture) were found to be significantly correlated to the total abundance of aminoglycoside/multidrug/sulfonamide resistance genes, demonstrating the impact of human activities on the selection for antibiotic resistance in estuaries, and also implies that considerable amounts of ARGs were imported through municipal domestic sewage and aquaculture discharges.

Can chlorination co-select antibiotic resistant genes?
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The widespread of antibiotic resistant genes (ARGs) represents a considerable public health concern. Some factors other than antibiotics such as metals, organic compounds, and disinfectants, were reported to show selective pressure on antibiotic resistant bacteria (ARB) and their relative genes. Thus, the tolerance of bacteria to antibiotic and contaminant simultaneously increases, that is co-selection. Chlorination was commonly used in water treatment to guarantee microbial security, as well as to control the ARB and ARGs, because of its moderate cost and simple application. Although several published surveys have reported that chlorination might select some ARGs, yet other studies indicated chlorination would rather remove or induce the antibiotic resistance. So there is still limited evidence showing if ARB and ARGs could be co-selected by chlorination, and the results are still largely unclear.

In this study, high capacity qPCR was applied to focus on 282 ARGs and 13 mobile genetic elements (MGEs) in the secondary effluents of a municipal WWTP after chlorination. Considering the relative abundance of ARGs, the average fold change of 120 ARGs among the 125 detected ones was below 1. However, only 5 ARGs, i.e. tetA-02, tetPA, ampC-04, tetPB-03, and dfrA1, were
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potentially selected by 4.69, 8.24, 9.62, 7.50, and 11.25 folds, respectively. Moreover, the average relative abundance of 11 detected MGEs was all below 1 fold after chlorination. Therefore, ARGs and MGEs were more likely to be removed by chlorination rather than be co-selected. It was the first comprehensively research on the effect of chlorination on ARGs using high capacity qPCR.

Key words: Antibiotic resistant genes, chlorination, co-selection, water treatment

P DIS 7
Quinolone resistance mechanisms among extended-spectrum β-lactamase (ESBL) producing Escherichia coli isolated from rivers and lakes in Switzerland
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Sixty extended-spectrum β-lactamase (ESBL)-producing Escherichia coli isolated from rivers and lakes in Switzerland were screened for individual strains additionally exhibiting a reduced quinolone susceptibility phenotype. Totally, 42 such isolates were found and further characterized for their molecular (fluoro)quinolone resistance mechanisms. PCR and sequence analysis were performed to identify chromosomal mutations in the quinolone resistance-determining regions (QRDR) of gyrA, gyrB, parC and parE and to describe the occurrence of the following plasmid-mediated quinolone resistance genes: qepA, aac-6'-Ib-cr, qnrA, qnrB, qnrC, qnrD and qnrS. The contribution of efflux pumps to the resistance phenotype of selected strains was further determined by the broth microdilution method in the presence and absence of the efflux pump inhibitor phe-arg-β-naphthylamide (PAβN).

Almost all isolates with reduced susceptibility to (fluoro)quinolones, except two isolates, showed at least one mutation in the QRDR of gyrA. Ten strains showed only one mutation in gyrA, whereas thirty isolates exhibited up to four mutations in the QRDR of gyrA, parC and/or parE. No mutations were detected in gyrB. Most frequently the amino-acid substitution Ser83 → Leu was detected in GyrA followed by Asp87 → Asn in GyrA, Ser80 → Ile in ParC, Glu84 → Val in ParC and Ser458 → Ala in ParE. Plasmid-mediated quinolone resistance mechanisms were found in twenty isolates bearing QnrS1 (4/20), AAC-6'-Ib-cr (15/20) and QepA (1/20) determinants, respectively. No qnrA, qnrB, qnrC and qnrD were found. In the presence of PAβN, the MICs of nalidixic acid were decreased 4- to 32-fold. (Fluoro) quinolone resistance is due to various mechanisms frequently associated with ESBL-production in E. coli from surface waters in Switzerland.

P DIS 8
A novel Tn3-like composite transposon harbouring blaVIM-1 in Klebsiella pneumoniae isolated from river water
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We present a new plasmid (pOW16C2) with a novel Tn3-like transposon harbouring blaVIM-1 from a Klebsiella pneumoniae strain isolated from river water in Switzerland. Complete nucleotide sequence of pOW16C2 was obtained using a Pacific Biosciences SMRT sequencing approach and coding sequences were predicted. The 59,228 bp sequence included a typical IncN-like backbone and a mosaic structure with blaVIM-1, aacA4, aphA15, aadA1, catB2 and qnrST conferring resistance to carbapenems, aminoglycosides, chloramphenicol, reduced susceptibility to quinolones and trimethoprim, respectively. Most of these resistance genes were inserted in a class 1 integron that was embedded in a novel Tn3-like composite transposon.

IncN plasmids carrying carbapenemases are frequently isolated from K. pneumoniae strains in clinical settings. The dissemination of K. pneumoniae harbouring blaVIM-1 in surface water is a cause for increased concern to public health.
P DIS 9

Enterobacteriaceae with extended-spectrum- and plasmid-associated AmpC-type β-lactamase-encoding genes isolated from freshwater fish from two lakes in Switzerland

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The progressive global spread of Enterobacteriaceae harbouring plasmid-mediated beta-lactamases conferring resistance to modern beta-lactam antibiotics is worrisome, particularly dissemination into the general human public - as observed since roughly 10 years. This raises questions about bacterial reservoirs not linked to hospitals. Since extended-spectrum beta-lactamase (ESBL) producers had already been reported from healthy humans and along the food chain as well as in animal husbandry, the aim of the present study was to screen wild fish for carriage of enterobacterial producers of beta-lactamases compromising higher generation cephalosporins.

The intestines from 139 freshwater fish (Coregonus lavaretus, Abramis brama, Rutilus rutilus, Perca fluviatilis, Esox lucius, Tinca tinca, Centrarchidae species and Salmo trutta) were enriched overnight in EE broth. Subsequent selection was on chromogenic Brilliance ESBL agar (Oxoid Ltd., Basingstoke, UK) to gain ESBL-producers. Oxidase-negative colonies recovered from these media were characterized by species identification, antibiotic susceptibility testing, phylogrouping, multi-locus sequence-typing (MLST), and sequencing of identified beta-lactamase (bla) genes.

Seven isolates were identified as member of the worldwide pandemic multiresistant clone phylotype B2 sequence type 131 (B2:ST131) which is strongly associated with potentially severe infections in humans and animals. Thirteen other isolates were grouped within the pathogenic phylogroups B2 (other ST than ST131) and D whereas fifteen belonged to the apathogenic phylogroups A and B1.

P DIS 10

Human recreational exposure to antibiotic resistant bacteria in coastal bathing waters

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Questions: Infections caused by antibiotic resistant bacteria are associated with poor health outcomes and are recognised as a serious health problem. Much research has been conducted on the transmission of antibiotic resistant bacteria (ARB) to humans. Yet the role the natural environment plays in spreading ARB to humans is not well understood. Antibiotic resistant bacteria have been detected in natural aquatic environments, and ingestion of seawater during watersports is one way many people could be directly exposed.

The aim was to estimate the prevalence of resistance to a type of antibiotic (third-generation cephalosporins (3GCs)) among Escherichia coli in coastal waters in England and Wales and to use this to quantify ingestion of these ARB by people participating in watersports in seawater. Another aim was to use this value to derive a population-level estimate of exposure to these bacteria during recreational use of coastal waters in 2012.

Methods: The prevalence of 3GC resistance amongst E. coli isolated from coastal waters was estimated using culture-based methods. This was combined with the density of E. coli reported in designated coastal bathing waters along with estimations of the volume of water ingested during various watersports reported in the literature to calculate the mean number of 3GC-resistant E. coli (3GCREC) ingested during participation in several watersports.

Results: 0.12% of E. coli isolated from surface waters were resistant to 3GCs. This value was used to estimate that in 2012 in England and Wales over 6.3 million watersport sessions occurred that resulted in the ingestion of at least one 3GCREC.

Conclusions: Despite low prevalence of resistance among E. coli, there is an identifiable human exposure risk for water users, which varies among different watersports. Millions of watersports sessions occurred in 2012 that were likely to have resulted in people ingesting 3GCREC However, this is expected to be a significant underestimate of recreational exposure to ARB in coastal waters.

This is the first study to use volumes of water ingested during a variety of different watersports to estimate human exposure to ARB. Further work needs to be done to elucidate the health implications and clinical relevance of exposure to ARB in aquatic environments in order to fully understand the risk to public health posed by this type of exposure.
SESSION V: Dissemination of antibiotics and antibiotic resistance genes through aquatic ecosystems/sewage treatment plants

P DIS 11
Impact of wastewater from different sources on the prevalence of antimicrobial-resistant Escherichia coli in sewage treatment plants in South India

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Introduction: The antimicrobial-resistant bacteria excreted by patients flow into a hospital sewage system or directly into a municipal wastewater stream, which is then treated in a sewage treatment plant (STP). After treatment in STP, the water is discharged into surface waters or is used for irrigation. Thus, STP is one of the most important interfaces of environmental contamination with antimicrobial-resistant bacteria. Little is known about the contamination of wastewater by antimicrobial-resistant bacteria in India to date.

Objectives: In the present study, systematic sampling of wastewater was performed in three different STPs in South India for the investigation of the distribution of antimicrobial-resistant Escherichia coli. The objective of this study is to identify factors affecting the prevalence of antimicrobial-resistant E. coli in wastewater.

Materials and methods: Water samples were collected from three different STPs. The sources of sewage for STP1, STP2, and STP3 are domestic wastewater, combination of hospital and domestic wastewaters, and exclusively hospital wastewater, respectively. The water samples were collected at three intermediate treatment steps and the final treatment step after filtration. The sampling was conducted in three different seasons, during post-monsoon (February), pre-monsoon (May), and monsoon (September), in 2013. E. coli was isolated from the water samples and the antimicrobial susceptibility was examined. Pulsed-field gel electrophoresis was performed to investigate genotype distribution of E. coli isolates from different STPs.

Results: The results of logistic regression analysis suggest that the hospital wastewater inflow significantly increased the prevalence of antimicrobial-resistant E. coli, whereas the treatment processes and sampling seasons did not affect the prevalence of these isolates. A bias in the genotype distribution of E. coli was observed among the isolates obtained from STP3.

Conclusion: Hospital wastewaters should be carefully treated to prevent the contamination of Indian environment with antimicrobial-resistant bacteria.

P DIS 12
Increased in abundance of antibiotic resistance genes is associated with bacterial community succession during in-vessel urban sewage sludge composting

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Introduction: Composting is common used for recycling of urban sewage sludge to arable land to improve soil properties, which represents a potential pathway of spreading antibiotic resistant bacteria and genes to soils. While, the dynamics of antibiotic resistance genes (ARGs) and the mechanisms during sewage sludge composting was not fully explored.

Objectives: The objective of the present study is to investigate the dynamics of ARGs and bacterial communities during lab-scale in-vessel composting of urban sewage sludge.

Patients & methods or Materials & methods: Dewatered sewage sludge was collected from the Tong’an Sewage Treatment Plant, Xiamen, China. A Lab-scale municipal sewage sludge composting was conducted, high-throughput quantitative PCR and 16S rRNA gene based illumina sequencing were used to investigate the dynamics of ARGs and bacterial communities respectively.

Results: A total of 156 ARGs were detected encoding resistance to almost all major class of antibiotics, with increasing numbers of ARGs during composting, ARGs were detected with significantly increased abundance and diversity, and distinct patterns during composting. Aminoglycosides, tetracycline and Macrolide-Lincosamide-Streptogramin B (MLSB) resistance genes were the most abundant ARGs. Normalized copy number of ARGs was also increased during compost (Figure 1), indicating that the increase in ARGs abundance was not due to the propagation of overall bacterial population but to the enrichment of ARGs in bacterial communities. Obvious shift in bacterial community structures and compositions were observed during composting, with Actinobacteria existing as dominant phylum at the late phase of composting. The large proportion of Actinobacteria may partially explained the increase of ARGs abundance during composting, ARGs patterns were significantly correlated with bacterial community structures (Figure 2).

Conclusion: Dynamic of ARGs was strongly affected by bacterial phylogenetic and taxonomic compositions. The increase of ARGs abundance during composting implied that direct application of sewage sludge compost on field may lead to the release of abundant ARGs in soils.

Key words: urban sewage sludge, composting, antibiotic resistant genes, bacterial community,
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Figure 1: Normalized copy numbers of ARGs during urban sewage sludge composting.

Figure 2: Procrutes test showed significantly correlation between ARGs patterns and bacterial community structures.
Increased levels of antibiotic resistance in urban river: a case study in Jiulongjiang River, China

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The rapid global urbanization and other extensive anthropogenic activities exacerbated the worldwide human health risks induced by antibiotic resistance genes (ARGs). Knowledge of the origins and dissemination of ARGs is essential for understanding modern resistome in the urban environment, while little information is known for regarding the overall resistance levels in urban river.

To evaluate the impact of urbanization on the resistance pattern in urban river, the abundance of multi-resistant bacteria (MRB) and ARGs were investigated using culture dependent method and high throughput quantitative PCR (HT-qPCR). Water samples were collected from human-impacted Jiulongjiang river passing through the center of Longyan, China (C9, C11) and the source of it (C8) representing relatively pristine areas free of human activities (Fig.1). The abundance of MRB (conferring resistance to four combinations of antibiotics), ranging from $7.14 \times 10^3$ CFU·L$^{-1}$ to $5.86 \times 10^7$ CFU·L$^{-1}$, were significantly higher in urban samples, which is two orders of magnitude of that in source water. HT-qPCR were performed using Wafergen SmartChip real-time system, which enabled simultaneous qPCR reactions targeting for 285 unique resistance genes, 8 transposases genes and 2 integrons genes. A total of 212 ARGs were detected among all the water samples, which encoded resistance to almost all major class of antibiotics ($\beta$-lactamase, Macrolide-lincosamide- streptogramin B (MLSB), Aminoglycosides, Tetracycline, Fluoroquinolone-quinolone-florfenicol-chloramphenicol and amphenicol (FCA), Vancomycin, and Sulfonamides) and encompassed major species of mechanisms. Detected ARGs numbers (161) in urban samples were twice that (79) in pristine samples, and nearly all the ARGs detected in control sample also appeared in urban samples, indicating the input of extraneous ARGs into urban river. Shannon diversity index showed significant higher diversity in urban sites. The total abundance of ARGs in urban samples (ranging from $9.72 \times 10^{10}$ to $1.03 \times 10^{11}$ copies·L$^{-1}$) was over two orders of magnitude higher than that in pristine ones (ranging $7.18 \times 10^8$ copies·L$^{-1}$), and the normalized copy number of ARGs also surpassed 2.4 (each bacterial cell could harbor at least 2.4 ARGs) in urban rivers compared with 0.26 in the pristine source. Among these ARGs, up to 182 ARGs were enriched 53-fold (median) up to 1157-fold (maximum) and mobile genetic elements (MGEs) were enriched 491-fold (median) up to 2447-fold in urban samples. The elevated levels of ARGs were accompanied with significant high abundance of MGEs, furthermore, significant correlations were observed between the abundance of ARGs and MGEs, implicating the potential of horizontal gene transfer of ARGs. Both principle component analysis and cluster analysis showed distinct ARGs patterns between urban and pristine samples ($P<0.05$, Fig. 2). The newly emerging ARGs and enrichment of ARGs and MGEs deepen our understanding of the impact of human activities on the spread of ARGs. HT-qPCR enabled rapid, quantitative and comprehensive profiling of highly diverse ARGs in environment. Further quantitative analysis of ARGs from source to river will provide a robust basis for management of antibiotic resistance in the future.
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P DIS 14
Development of a method to quantify transfer events of antibiotic resistance plasmids in complex microbial communities

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Introduction: Dissemination of antibiotic resistance in bacterial communities by means of plasmid-associated horizontal gene transfer (HGT) has been recognized as a global concern. Comparative analyses of bacterial genomes and between metagenomic datasets provide evidence of numerous HGT events that have happened in the past, although straightforward monitoring of the spread of antibiotic resistance genes in natural habitats remains demanding due to the complexity of the environment and existing methodological weaknesses. Both culture dependent and independent approaches have been used to detect plasmid transfer in environmental samples. However, most assays fail short as both the number of transfer events and subsequent selection of transconjugants contribute to the result.

Objectives: Our aim was to develop a quantitative method for plasmid transfer in complex communities that minimizes the possible additional effect of selection. We also aimed to use this method to assess the minimal concentration of selected antibiotics that induces HGT.

Methods and Results: Time optimization is important to balance sufficient time for induction of HGT events with increased risk for selection. Furthermore, the role of donor bacteria to carry plasmids with resistance genes for the same agent as used for the HGT induction is questionable and needs to be evaluated. We have therefore analysed different combinations of transfer inducers and plasmids with different resistance profiles. A single donor - single recipient model was used for these purposes. In order to quantify conjugation events in a complex community, experiments with traceable recipient bacteria has also been performed. Effort on reduction of high background level, which is a population of natural multi-resistant bacteria already present in the environment, has been made to improve the detection of transfer events.

Conclusion: Taken together, our ongoing work on improvement and optimization of HGT detecting procedure will hopefully provide us with a better methodological toolkit in order to understand the specific environmental conditions and factors that trigger dissemination of mobile resistance elements resulting in the formation of drug-resistant pathogens.

P DIS 15
Antifouling paint co-selects for antibiotic resistant bacteria in the marine environment

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Introduction: Heavy metals can contribute to the development of antibiotic resistance via co-selection. Many antifouling boat paints contain significant amounts of heavy metals and are used extensively throughout the world. Marine environments harbor a wide variety of antibiotic resistant bacteria and may therefore serve as reservoirs for resistance genes.

Objectives: To investigate if heavy-metal based antifouling paints can pose a risk for co-selection of antibiotic resistant bacteria and, if so, identify the underlying genetic basis.

Materials & methods: Three independent experiments were performed. In each of these, painted and unpainted plastic panels were placed one meter under the water surface in a marina on the Swedish west coast and biofilms were harvested after 2.5-4 weeks. Total DNA was isolated from the marine biofilms and subjected to metagenomic sequencing on the Illumina HiSeq platform. Biofilm bacteria were also cultured on marine agar supplemented with tetracycline, gentamicin, CuSO4, or ZnSO4 and the resulting growth was compared to that seen on control agar plates without antimicrobials.

Results: Bacteria sampled from surfaces painted with copper and zinc-containing antifouling paint showed, in addition to increased heavy metal resistance, decreased susceptibility to tetracycline. No significant increase in gentamicin resistance was observed. Metagenomic sequencing revealed enrichment of RND efflux systems with documented ability to confer decreased susceptibility to antibiotics, including such with potential to also pump heavy metals, in bacterial communities from painted surfaces. Increased levels of specific integrase and ISCR transposase genes were also observed, which might indicate a genetic basis for beneficial adaptive capacity among bacteria colonizing panels with antifouling paint. Further bioinformatics analyses will provide deeper insight into the genetic context of the detected genes tentatively involved in metal and antibiotic resistance.

Conclusion: Heavy metal-based antifouling paint can co-select for antibiotic resistant bacteria and bacterial communities from painted surfaces show genetic profiles suggesting good adaptive capacity that might confer decreased susceptibility to a variety of antimicrobials.
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P DIS 17
Microbiological characterization of aquatic microbiomes targeting taxonomical marker genes and antibiotic resistance genes of opportunistic bacteria

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The dissemination of medically relevant antibiotic resistance genes (ARG) (blaVIM-1, vanA, ampC, ermB, and mecA) and opportunistic bacteria (E. faecium/faecalis, P. aeruginosa, Enterobacteriaceae, S. aureus, and CNS) was determined in different anthropogenically influenced aquatic habitats in a selected region of Germany. Over a period of two years, four differently sized wastewater treatment plants (WWTP) with and without clinical influence, three surface waters, four rain overflow basins, and three groundwater sites were analyzed by quantitative Polymerase Chain Reaction (qPCR). Results were calculated in cell equivalents per 100 ng of total DNA extracted from water samples and per 100 mL sample volume, which seems to underestimate the abundance of antibiotic resistance and opportunistic bacteria. High abundances of opportunistic bacteria and ARG were quantified in clinical wastewaters and influents of the adjacent WWTP. The removal capacities of WWTP were up to 99% for some, but not all investigated bacteria. The abundances of most ARG targets were found to be increased in the bacterial population after conventional wastewater treatment. As a consequence, downstream surface water and also some groundwater compartments displayed high abundances of all four ARG. It became obvious that the dynamics of the ARG differed from the fate of the opportunistic bacteria. This underlines the necessity of an advanced microbial characterization of anthropogenically influenced environments.

P DIS 18
Assessing the potential of rural and urban wastewater treatment plants to introduce antibiotics into the environment in Western Australia

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An important step towards understanding long-term water security options in Australia, is gaining knowledge of the environmental value and the risks (e.g. human health and eco-toxicological risks) associated with wastewater (WW) discharge. Treated WW is normally discharged to the environment requiring proper management to protect public health and the environment. Compliance monitoring of WW discharge in Australia is predominantly based on physical-chemical assessments of bulk parameters (e.g. pathogens, nutrients, total suspended solids, BOD, oil and grease content). However, there is also an increasing concern about the potential eco-toxicological adverse effects of mixtures of antibiotics ending up in the environment as result of wastewater discharge. Once they are released in the environment, these chemicals may potentially cause detrimental effect such as the development of antibacterial resistance genes (ARG) within the indigenous microbial community. The nature and characteristics of the receiving environment are playing a major role in antibiotics and ARG (“naked” DNA or associated antibiotic resistant bacteria) transport and development of antimicrobial resistance. A comprehensive understanding of potential environmental impacts related to antibiotic and related ARG discharges requires the integration of both chemical and microbial (i.e., targeted genes) profiles along the migration pathways of these contaminants in the receiving environment. This is particularly relevant for WW effluents as they contain complex mixtures of antibiotics, as total availability and activity can be underestimated if concentrations are close to or below chemical detection limits or if uncommon antibiotics are not included in chemical monitoring.

In this work, we are undertaking a screening survey on a number of antibiotics as well as on the presence of some targeted antibiotic resistant genes in rural wastewater (used for irrigation) and the receiving environment (i.e., groundwater, and seawater near-by the WW point of discharge) from a WWTP operating in WA. Results on antibiotics occurrence and removal will be compared to existing information in large-scale urban WW treatment plants from the city of Perth, WA.
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P DIS 19
Susceptibility to 15 antimicrobial agents of Aeromonas strains isolated from freshwater
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Aeromonas species are globally distributed gram-negative, oxidase-positive fermentative rods found in aquatic environment (freshwater, saline water and wastewater). First described as fish pathogen, it is now also considered as an emerging human pathogen, in both immuno-competent and immuno-compromised patients. Up to date, at least 26 species have been reported in the genus Aeromonas. In the present study, 240 Aeromonas spp. isolates were collected from freshwater in 16 rivers of western France during winter (Feb.-March) and summer (June-July) 2014. All isolates were identified at species level using Maldi-Tof. Homemade 96-well microplates containing different concentrations of 15 antimicrobials, namely cefotaxime, ceftazidime, chloramphenicol, colistin, enrofloxacin, erythromycin, florfenicol, fluomquine, gentamicin, nalidixic acid, oxolinic acid, streptomycin, tetracycline and trimethoprim-sulfamethoxazole, were prepared according to ISO 20776-1. The broth micro-dilution method (CLSI - VET04-A) was used to determine antimicrobial susceptibility of each isolate. No epidemiological cut-off for Aeromonas is currently available, clinical breakpoints available for Enterobacteriaceae were adapted when available to interpret Minimum Inhibitory concentration (MIC). At least 10 different species were identified. Aeromonas bestiarum was the dominant one. So this study provides a first set of MIC distributions of various Aeromonas spp. to 15 antimicrobials. These distributions might represent a first step in defining the intrinsic resistance profiles of these Aeromonas species and will enable clear delineation of the wild-type population from the one with acquired resistance. Further experiments are still needed, but here is the foundation stone of a surveillance program on circulation of resistance mechanisms in the aquatic environment where Aeromonas could be the antimicrobial resistance sentinel bacteria such as E. coli in the terrestrial environment.

P DIS 20
Effect of metal contamination on antibiotic resistance traits in epiphytic bacteria
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Heavy metals are allegedly among the main co-selectors of antibiotic resistance. The phytosphere in heavy metal contaminated salt marshes may constitute a niche for antibiotic resistance co-selection. The main goal of this study was to assess co-selection for antibiotic resistance traits in epiphytic bacteria from the plant Halimione portulacoides in salt marshes with and without contamination. Metal quantification demonstrated high levels of metals, particularly mercury, in contaminated sites. Most strains from the control site affiliated with Vibrio spp. (62.5%) and Enterobacteriaceae (18.8%). On contaminated sites, the majority affiliated with Enterobacteriaceae (34.5%) and Pseudomonas spp. (23.6%). Antibiotic multiresistance was higher in contaminated sites (43.6% of the total number of strains) than in control sites (28.1%). Principal component analysis of the susceptibility levels to 16 antibiotics and 6 heavy metals showed a clear separation between contaminated and control sites. The presence of antibiotic resistance genes was investigated by PCR but common acquired genes were not detected, suggesting the predominance of intrinsic resistance mechanisms. Plasmid restriction analysis showed a distinct pool of plasmids in contaminated and control sites. Replicon typing allowed to detect 3 drug resistance associated replicon types (IncF1, FrepB and FIB), common among Enterobacteriaceae. Whilst all 3 Inc types were found in bacteria from control site (7 plasmids), only one plasmid, from IncFIB group, was identified from contaminated sites. This suggests for unrelated plasmid pools in both sites and that multiresistance in Enterobacteriaceae from contaminated site is not supported by common drug resistance plasmids. The co-occurrence of heavy metal and antibiotics resistance genetic determinants in these plasmids is currently being investigated. Overall results suggest that antibiotic resistance in epiphytic bacteria is selected by heavy metal contamination in salt marshes. This work was supported by Fundação para a Ciência e a Tecnologia through project Phytomarsh (PTDC/AAC-AMB/118873/2010) program COMPETE: FCOMP-01-0124-FEDER-019328, and through grants to S Araújo (BI/UI88/5111/2013), C Oliveira (SFRNBFD/96620/2009).
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P DIS 21
Dissemination of ESBL genes within microbial communities in river sediments
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Enterobacteriacaeae isolates from river sediment upstream and downstream of a wastewater treatment plant (WWTP) in the Finham catchment area possessing 3rd generation cephalosporin (3GC) resistance, were investigated with respect to multidrug resistance containing plasmids and the transmissibility of these plasmids under different selection pressures. Biparental mating experiments were conducted with selection for biocide resistance, specifically to quaternary ammonium compounds (QAC) (which are widely used both agriculturally and industrially and consequently found consistently in the environment) and metal ion resistance to determine whether antibiotic resistance genes (ARG) could be indirectly selected for. We hypothesised that environmentally induced selection pressures could co-select for ARG and propagate the environmental antibiotic resistome indirectly. Our results showed that QACs could indirectly select for ARG, and that the F-type plasmids were the most likely to transfer. The most likely ARG to transfer were bla TEM and aac 6’ conferring resistance to 3GC and aminoglycoside and fluoroquinolones respectively.

Experiments were conducted using biparental matings with the same Escherichia coli isolates with multidrug resistant plasmids as donors, and diverse host and environmental Enterobacteriaceae isolates (including Klebsiella, Aeromonas and Citrobacter) as recipients. Our aim was to determine host range of these F-type plasmids. We hypothesised transfer is likely to occur from E. coli to other environmental bacteria under selection for 3GC and QAC resistance.

Growth curve experiments were done to determine fitness costs associated with possession of these large conjugative plasmids. Experiments tested plasmid stability and maintenance under a range of conditions. Preliminary results indicate possession of these plasmids has a significant fitness cost with an increased doubling time of approximately 1.5 times that of wildtype E. coli.

In conclusion these preliminary results indicate that ARG can be transferred via indirect selection and consequently the use, and release of biocides in to the environment may be a key factor with respect to the spread of the environmental resistome.

P DIS 23
Human exposure to ESBL producing E. coli in recreational waters - hospital wastewater as a source
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While ESBL producing E. coli have been found in several surface waters in Europe, the role in recreational waters for ESBL uptake is yet unknown. We investigated human exposure to ESBL through swimming in fresh water, and possible sources of ESBL producing E. coli in aquatic environments. During the summer season, samples were obtained from fresh water bathing sites (n=66). At two locations repeated samples (n=12) were taken to map temporal variability. Samples were analysed for the presence of ESBL producing E. coli through filtration and cultivation on commercial media and on TBX amended with cefotaxime. In addition, an urban water chain was investigated to examine the role of human wastewater as source of ESBL. Samples included hospital wastewater, influents and effluents of the urban wastewater treatment plant (WWTP) connected to the hospital as well as its receiving surface water, and influents and effluents of a suburban WWTP neither treating hospital nor elderly people’s homes wastewater. Urban water chain samples were also analysed using nanoliter-scale quantitative PCR targeting 68 resistance genes.

While hospital wastewater contained the highest concentrations of ESBL producing E. coli (around 6 log CFU/1000 ml), the influence of hospital wastewater on the urban WWTP influent was limited, mainly due to the presence of ESBL in the general population (i.e. suburban WWTP, around 5 log CFU/100 ml). Although WWTP treatment decreased concentrations of ESBL by 2-3 log units, the influence of the WWTP on the receiving surface water was clearly measurable; ESBL concentrations were higher than concentrations in recreational surface water (E. coli intake was modelled to a mean of 0.8 ESBL during a typical swimming event, intake being higher for flowing waters and after rainfall events. Nanoliter-scale quantitative PCR confirmed the presence of diverse resistance genes including ESBL resistance in hospital wastewater. Urban and suburban WWTP influent had similar resistance gene profiles, again pointing to a limited influence of this particular hospital on the overall communal wastewater. To conclude, human wastewater is a source of ESBL in the aquatic environment, and recreation can lead to ESBL uptake especially in flowing waters and shortly after rainfall events.

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P DIS 24
Monitoring of antibiotic resistances in influent and effluent of Dresden wastewater treatment plant and a European perspective
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The occurrence of antibiotics in natural and urban environments seems to favor the development and spread of antibiotic resistance. At the same time the key factor for the evolution of new antibiotic resistances is the ability of an organism to adapt quickly to new environmental conditions. One of the environments where the selective pressure of antibiotic pollution could exert an effect on microbial communities is the urban water system. The sewage canalizations and waste water treatment plants in particular are known to carry elevated concentration of pollutants and microorganisms. The co-existence of these two factors could lead to a gradual increase in the prevalence of resistance within the indigenous microbial community.

In our study raw waters originated from the sewage pipes of the main neighborhoods of Dresden metropolitan area (Germany) were seasonally collected along the years 2012-13 and screened for the presence and amount of antibiotic resistance genes (ARG). The genes of interest belonged to the chemical classes of β-lactams, quinolones, macrolides, sulphonamides and tetracyclines. Concentrations of antibiotic residues in the raw waters and effluent were measured and the correlation between antibiotic residues and antibiotic resistance genes revealed via multivariate statistical analysis. Moreover the diversity and species distribution of microbial communities inhabiting sewage waters and effluent will help us to explain the seasonal variation of ARGs content. Overall, despite the sanitation processes the effluent did not show any reduction in ARGs pointing out the need of newer wastewater technologies capable to limit the spread of ARGs in freshwater ecosystems. Results are compared to an ongoing European sampling campaign on wastewater treatment plant effluents.

P DIS 25
Polluted Tunisian rivers are a reservoir of multidrug resistant bacteria by production of IMP-8, IMP-10, and IMP-13 metallo-β-lactamases located on class 1 integrons and other extended-spectrum-β-lactamases
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Background: Antibiotic-resistant bacteria have been surveyed widely in water bodies, but few studies have determined the diversity of antibiotic-resistant bacteria in river waters. This study was undertaken to investigate the origin of resistance among polluted river bacterial isolates in Tunisia.

Methods: In this study 128 isolates resistant to β-lactam antibiotics were obtained from 2 polluted rivers in the north of Tunisia. Isolates were identified using Phoenix phenotyping criteria. The occurrence of blaTEM, blaSHV, blaCTXM, blavIM, and blaimp was studied by polymerase chain reaction (PCR) amplification and sequencing, and the genetic relatedness of the 16 IMP-producing Klebsiella pneumoniae isolates was analyzed by comparison of Xba I pulsed-field gel electrophoresis (PFGE) profiles.

Results: Using Phoenix phenotyping criteria, diverse genera of bacteria were identified with different rates of prevalence and with different minimum inhibitory concentrations against different antibiotics. The occurrence of bla TEM, bla SHV, bla CTXM, bla CMY, bla VIM, and bla IMP genes was confirmed. The DNA sequences upstream and downstream of blaIMP genes were determined, revealing that all IMP-encoding genes constituted the first cassette of class 1 integrons, followed by aacA gene cassettes encoding aminoglycoside resistance. Comparison of PFGE profiles showed that only 2 of the isolates were clonal, the other 14 displaying unique profiles. The blaCTXM gene was the most dominant of the extended spectrum-β-lactamase (ESBL) genes, while the blaTEM gene was the second-most dominant.

Conclusion: The discovery of highly diverse ESBL-producing bacteria and metallo-β-lactamases, particularly blaimp, in polluted river water raises alarms with regard to the potential dissemination of antibiotic-resistant bacteria in communities through river environments.
 SESSION V: Dissemination of antibiotics and antibiotic resistance genes through aquatic ecosystems/sewage treatment plants

P DIS 26
Effects of lethal and sublethal levels of antibiotics on sediment microbial community
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In aquaculture, antibiotic treatment in fish farms leads to high local concentrations of antibiotics in the water compartment and in the adjoining sediments. Antibiotics in aquatic ecosystems act as an ecological factor that could potentially affect microbial community. The effects include phylotypic structure alteration, resistance expansion, and ecological function disturbance in micro-ecosystem. A number of studies have demonstrated that microbial community structure altered upon addition of antibiotics in sediment and water environment by molecular fingerprinting techniques. However, the information provided by molecular fingerprinting techniques is limited. In this study, we used a microcosm approach and applied next generation sequencing to understand the effect of antibiotics on microbial community. The antibiotics we used is florfenicol, that developed initially for aquaculture applications and used worldwide, including China.

Lethal (50 and 3 mg florfenicol kg⁻¹ sediment) and sublethal levels of antibiotics (0.3 mg florfenicol kg⁻¹ sediment) were selected to stress the bacteria in sediment. Bacterial phylotypes shifted after 3 d of lethal level antibiotic treatment comparing to non-treated samples, with the treated sediments showing an increase in Campylobacterales, in which more than 90% OTUs that belonged to genus Arcobacter. Bacterial communities were maximally perturbed after 7d treatment and showed a trend to converge to the non-treatment on 14d. After 28 day, communities with treatment and non-treatment clustered again. Interestingly, the change of communities showed very similar pattern even they were stress by different concentration florfenicol. Sublethal levels of antibiotics also lead to alteration of communities after 7 day with the treated sediments showing an increase in Oceanospiralaceae. Antibiotic tolerance analysis revealed that the communities with lethal dose treatment have developed high antibiotic resistance to florfenicol, more than 128 fold comparing to non-treatment after 3 day. The communities kept high tolerance till 60d. Sublethal dose treatment also resulted in increase of tolerance but not as high as lethal dose. Meanwhile, time-course quantitative PCR analysis also showed the abundance of florfenicol resistance genes (floR, cmlA) and intI1 spiked sharply after 3 day with treatment of lethal dose antibiotics and kept the high level till 60 day. The abundance of three genes showed similar increased trend with sublethal dose, but not as high as lethal dose. The results suggested that the effect of florfenicol on bacterial community could fade away, however, the effect on resistance would maintain a long time. Our study provides new insights into the response process of microbial community adaptation to antibiotic exposure and has implications for the antibiotic lasting impact to the environment.

P DIS 28
Behavior of antibiotic resistant bacteria during oxidative treatment of wastewater with ozone
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Introduction: Ozonation is one of the key technologies for removing micro pollutants from conventionally treated wastewater. Due to its reactivity towards various biomolecules, including DNA, ozone has also great potential for eliminating antibiotic resistant bacteria from sewage.

Objectives: The study aimed on investigating the dynamics of multiresistant bacteria in Switzerlands first full scale ozonation plant, including conventional biological treatment (BT), ozonation and post-ozonation treatment. Of particular interest was the efficiency of eliminating multiresistant bacteria at ozone doses optimized for micropollutant-removal (~0.55 g O₃/g DOC) at this plant. Moreover, differences in the potential for regrowth during two post-ozonation treatments, sand filtration (SF) or moving bed (MB), were evaluated.

Materials and Methods: Total and membrane-intact cell counts (IC) were measured by flow cytometry, whereas total culturable and multiresistant bacteria were determined by plate counts on AQ dry plates, supplemented with or without two different combinations of antibiotics.

Results: IC decreased about 1 log-unit each after BT and ozonation, but re-increased to 20% of pre-ozonation levels after SF or only 12.5% after MB. The strongest reduction in total culturable numbers (2-3 log-units) occurred after BT. However, multiresistant bacterial numbers dropped only less than 1 log-unit, resulting in a strong increase of relative multiresistant numbers and suggesting selection for these traits during BT. Ozonation led to 1.5-log-unit reduction of both total culturable and multiresistant bacteria, resulting in nearly multiresistant bacteria-free water. After SF, total cultivable and multiresistant bacteria nearly reached pre-ozonation levels again, while however proportions of multiresistant bacteria decreased. In comparison, MB effluents contained 1.5 log-units lower total culturable bacteria. However, proportions of multiresistant bacteria in WB effluents reached 20-50 %, whereas they were comparatively low (0.01-0.35%) within the biofilms formed on the carriers.

Conclusions: Ozones doses optimized for micropollutant removal, efficiently reduce multiresistant bacteria in treated sewage in a full-scale-ozonation plant. However, this effect seems to be undermined by post-ozonation treatment and more so by moving bed treatment than by sand filtration.
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P DIS 29

The genetic basis of allicin resistance in a highly resistant *Pseudomonas fluorescens* isolate

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In 1944 Cavallito & Bailey purified the substance responsible for the antimicrobial activity of garlic and named it allicin [1]. Allicin is a thiosulfinate and a reactive sulfur species (RSS) [2] and reacts with free thiol groups in thiol-disulfide exchange reactions, e.g. with cysteine residues in proteins and glutathione [3]. The resulting protein modification can affect its structure and function and this is thought to be the basis of allicin’s antimicrobial action. Additionally, allicin causes oxidative stress leading to alterations in cellular redox homeostasis which is associated with the induction of apoptosis in eucaryotes [4]. Allicin has potential for use in medicine [5] as well as in agriculture [6,7]. Despite its multi-target mode of action, which makes it difficult for cells to adapt to allicin, we have isolated an allicin resistant *Pseudomonas fluorescens* strain (PIAR1) from garlic cloves. PIAR1 is also resistant to ampicillin, carbenicillin and nalidixic acid. In order to identify the genes which condition resistance to allicin in PIAR1 we have 1) sequenced the PIAR1 genome 2) prepared a genomic library for conjugation into a susceptible pseudomonad 3) developed a transposon mutagenesis strategy for PIAR1. We report here on our progress.


P DIS 30

Investigation of culturable antibiotic resistant bacterial communities in a Mediterranean karstic hydrosystem

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Waters play a crucial role in interconnecting different ecosystems like humans, animals, soils and hydrosystems, contributing to the circulation of antibiotic-resistant bacteria. Karstic hydrosystems are commonly known to be vulnerable to anthropogenic contaminations due to direct connections between surface water and groundwater and to be heterogeneous due to the contrast between waters stored in some compartments (with long residence time) and waters with fast transit. Considered as integrator ecosystems, natural waters are of great interest to survey the antibiotic resistance in the environment, which is relevant to human health. Among natural hydrosystems, karstic aquifers are poorly investigated for emergence and dissemination of antibio-resistance. The question of their role as reservoir for antimicrobial resistance according their hydrogeological characteristics remains open.

The Lez karst hydrosystem supplies drinking water to the metropolitan region of Montpellier, in southern France. The aim of this study is to evaluate the resistance level and the biodiversity of bacterial communities in the Lez aquifers in contrasted hydrogeological conditions and seasonality.

For this purpose, we develop an original mixed method associating i) selective culture, using culture media containing tetracycline, vancomycin, ofloxacin, amoxicillin, cefadroxime or cefazidime at increasing concentrations, and ii) molecular approaches, implementing 16S rRNA gene PCR-Temporal Temperature Gradient Gel Electrophoresis (TTGE) and sequencing.

Minimal antibiotic concentrations inhibiting 50%, 70% and 90% of the whole culturable community were determined. Resistance to beta-lactams were clearly fluctuant between both compartments and periods of sampling, whereas differences obtained with the others antibiotics were not so marked. Then, the taxonomic diversity and the dynamics of the resistant sub-communities were described.

These results show that hydrogeological conditions and thus the speed of water circulation in the karst aquifer can impact the resistance of bacterial communities. Then, the potential risk of bacterial resistance will be discussed in term of resistance transferability and relevance in clinical epidemiology.
SESSION V: Dissemination of antibiotics and antibiotic resistance genes through aquatic ecosystems/sewage treatment plants

P DIS 31
Monitoring of tetracycline resistance bacteria and genes in municipal and hospital wastewaters in Iran
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Raising the resistance of microorganisms to antibiotics represents a serious international concern. Increase of ARB in the environment may pose a threat to public health. Wastewater is a major source of ARB and ARGs in the environment which acts as a pathway for introduction of ARB and ARGs from anthropogenic sources into natural system. The present study was conducted to determine the abundance of tetracycline resistant bacteria (TCr) and gene (tet(W)) in hospital and municipal waste-waters. The influence of wastewater treatment plants (WWTPs) on the fate of TCr bacteria and tet(W) gene was also investigated. A total of 66 samples from raw and final effluent of hospital and domestic WWTPs were analyzed. TCr bacteria as a major part of heterotrophic plate count (HPC) bacteria were found in the raw wastewater and final effluent samples in a concentration ranged from 5.19×10⁶-1.28×10⁸ to ND (not detected)-4.60×10⁶ CFU/100ml, respectively. Tet(W) gene concentrations in the raw wastewaters were found to be in the range of 3.87×10⁷-6.23×10¹³ copies/100ml and WWTPs did not contribute in effective reduction of tet(W) gene. No significant correlation was found between the levels of tet(W) gene and TCr bacteria in either raw wastewater or final effluent. The results of this study showed that TCr bacteria and tet(W) gene were present in relatively high levels in both municipal and hospital wastewaters. The results also indicated that conventional wastewater treatment plants didn’t contribute in effective reduction of tet(W) gene and wastewater effluents are a potential source for dissemination of tet(W) gene into the natural environment.

Keywords: TCr bacteria, tet(W), Municipal wastewater, Hospital wastewater, wastewater treatment plant

P DIS 32
Development and preliminary validation of a molecular method for quinolone resistance prediction of Piscirickettsia salmonis
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Piscirickettsia salmonis is the major threat for the sustainability of the Chilean salmon industry, and causative agent of piscirickettsiosis. The disease is highly prevalent and disseminated in the main rearing areas of the country. The most important control measure has been the use of antibiotics, being quinolones the former first line drugs. Nowadays, a large proportion of circulating strains displays a quinolone-resistant phenotype. As a facultative intracellular pathogen with a fastidious and slow growth, antimicrobial resistance profiles of P. salmonis are difficult to obtain. In this regards, the aim of this study was to validate a molecular method that will be able to reliably predict the quinolone-resistant phenotype among P. salmonis field isolates. 292 field isolates collected between 2010 and 2014 from marine farms located in southern Chile were phenotypically characterized for susceptibility to quinolones using a microdilution method. Two subpopulations were obtained. A sample of 10 isolates from both groups were sequenced using the Illumina HiSeq platform. From drafted genomes, the sequences of well-known molecular markers associated to quinolone resistance were extracted and aligned. The analysis disclosed that a unique single nucleotide polymorphism (SNP) on the quinolone resistance-determining region (QRDR) of a gene that codes for the DNA gyrase A (gyrA) was involved in a non-synonymous mutation. For this candidate marker, a TaqMan real time PCR was designed and tested on the entire strain collection. Remarkably, a correlation near to 100% at genotypic level was obtained by comparison with the corresponding phenotypic data.

Our results show that the current situation of resistance to quinolones in the P. salmonis population in Chile is linked to a unique SNP on the QRDR of gyrA, and that the prediction of a relevant phenotype from the genotypic characterization of this marker in this bacterium is feasible.

Funding: INNOVA-CORFO 12BPC2-13471 and 14IDL2-30005 projects.
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P DIS 33
Effects of sub-inhibitory levels of antibiotics on nutrient utilization, niche overlap and competitive interactions among freshwater bacterioplankton isolates
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Bacterial community assembly is to a large extent governed by competitive interactions favoring some bacteria over others depending on the environmental conditions present. Increasing amounts of antibiotics reach the aquatic environment. We hypothesized that sub-inhibitory concentrations of antibiotics (SICA) have the potential to modify the strength and outcome of competitive interactions and that such environmentally relevant concentrations of antibiotics have the potential to disturb bacterial community assembly and functionality.

Bacteria were isolated from a mesotrophic lake just north of Copenhagen (Lake Fure) on artificial lake water (ALW) media and isolates were characterized by 16S rRNA sequencing and genomic PCR fingerprinting (UP-PCR). Isolated bacteria belonged to the genera Aeromonas, Agrobacterium, Rheinhermera, Brevundimonas, Bacillus, Mitsuaria and Asticcacaulis and four unique strains were selected and subjected to different concentrations of tetracycline to determine minimal inhibitory concentrations and levels of SICA to be used for subsequent ongoing experiments. For these ongoing experiments, isolates are tested for nutrient use in Biolog plates with or without SICA to determine the effects of tetracycline on competitive interactions and niche overlaps. The isolates are tested both individually and as various dual culture combinations under strictly defined conditions. It is hypothesized that SICA will have an effect on niche space (i.e. number of substrates being used) and competitive interactions.

Key words: competitive interactions, freshwater bacteria, niche overlap, tetracycline

P DIS 34
Mixture toxicity of antibiotics and biocides in aquatic bacterial communities.
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Organic pollutants commonly exist as mixtures in the environment and consequently it is important to study the ecotoxicity of chemicals not only as individual compounds, but also as their mixtures. Antibiotics and biocides frequently co-exist in contaminated freshwater environments, but little is known about their joint effects on bacterioplankton communities and associated ecosystem services. We hypothesized that environmentally relevant concentrations of antibiotics and biocides may collectively affect bacterial growth dynamics, selection for antibiotic resistance and taxonomic composition of freshwater bacterioplankton communities.

Aquatic bacterial communities were sampled from Lake Fure, a mesotrophic lake situated just north of Copenhagen, Denmark. Microcosms were prepared with a mixture of 0.7 µm filtrated lake water containing bacteria and 0.2 µm sterile filtrated lake water in the ratio 1:4 in order to stimulate the ability of bacterioplankton communities to respond to the following test chemicals: tetracycline (TET), sulfadiazine (SDZ), methylisothiazolinone (MIT), dichlorooctylisothiazolinone (DCOIT), and linear alkylbenzene sulfonate (LAS). Growth dynamics and pollution-induced community tolerance (PICT) were measured using the [3H]leucine incorporation technique, and samples have been taken for analysis of test compound dissipation and bacterial community composition by high-throughput 16S rRNA amplicon sequencing.

The antibiotics and biocides tested all inhibited bacterial growth as individual compounds within the first 24-48 hours, but only at relatively high concentrations (10 mg/L for TET and SDZ, 1 mg/L for MIT and 0.6 mg/L for DCOIT). After 72 hours, bacterial growth rates completely recovered or even increased relative to the control treatment. This recovery of bacterioplankton productivity coincided with PICT responses to both to SDZ and DCOIT (data for TET is in process). In ongoing experiments, mixtures of TET, SDZ, and DCOIT are being tested and will show if there is an additive, synergistic or antagonistic effect of these compounds. Finally, we plan to do 16S rRNA amplicon sequencing in order to document PICT-associated bacterioplankton community shifts.

Key words: antibiotics, biocides, mixture toxicity, PICT
O MIT 1
Artlysins: Highly effective antimicrobial enzymes with a low risk of resistance formation for targeted elimination of bacterial pathogens
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Broad use, but especially misuse of antibiotics is resulting in increasing levels of antibiotics present in the environment, leading to a growing antibiotic resistance. Thus, there is a clear need for effective biodegradable antimicrobial alternatives, which ideally show a targeted mode of action. Artlysins constitute a novel class of efficient enzyme-based antibacterials with a new mode of action. They are recombinant fusion proteins consisting of a bacteriophage-encoded endolysin, which degrades the peptidoglycan, combined with a targeting peptide that transfers the endolysin through the outer membrane of Gram-negative bacteria. Artlysins® Art-175 was applied against P. aeruginosa, a Gram-negative pathogen, well known for being highly resistant to antibiotics and being responsible for re-occurring infections.
Art-175 passes the outer membrane and kills P. aeruginosa, including multidrug-resistant strains, in a rapid and efficient (~5 log) manner. Time-lapse microscopy confirms that Art-175 punctures the peptidoglycan layer within a minute, inducing a bulging membrane and complete lysis. Minimal inhibitory concentration (MIC) experiments show Art-175 to be highly effective on P. aeruginosa. The MIC of 10μg/ml was independent of the strains being highly resistant to antibiotics. Resistance development against Art-085 and Art-175 is not observed within 20 experimental cycles on all strains investigated. Whereas a significant resistance development against ciprofloxacin occurred already within 7 cycles of the MIC experiments. The strains PAO1p and Br257 showed a significant resistance development against ciprofloxacin with a more than 30-fold increase on Br257. Art-175 does not require an active bacterial metabolism for its antibacterial activity, thus it shows a superior bactericidal effect against P. aeruginosa persisters (up to more than 4 log). Systemic infections by P. aeruginosa can be successfully treated with Art-175 in a mouse model.

In summary, Art-175 is a novel antibacterial protein targeting P. aeruginosa and well suited for a broad range of applications and with a unique potential to target persister-driven chronic infections in an environmentally friendly way.

O MIT 2
Thermophilic anaerobic digestion: an effective approach for blocking both horizontal and vertical gene transfer pathways of antibiotic resistance genes
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Introduction: Modern sludge treatment processes are expected to attenuate the quantities of antibiotic resistance genes (ARGs) in sewage sludge, and recent studies found that thermophilic anaerobic digestion (AD) is more effective than the more widely applied mesophilic one in terms of ARGs control. It is important to understand why the two AD processes are so different in the reduction of ARGs, which could provide solid scientific support for selection of AD processes.

Objectives: The purpose of this study was to elucidate the mechanisms of ARGs reduction during AD.

Materials & methods: One lab-scale digester was transformed from mesophilic to thermophilic. Twenty-four resistance genes (tetA), tet(C), tet(G), tet(L), tet(Q), tet(O), tet(M), tet(W), tet(X), erm(B), erm(F), erm(T), erm(X), mef(A), mef(A), Mph(B), aac(3)-II, aacA4, aadA, aadB, aadD, aphA1, strA and strB, three mobile elements (intI1, ISCR1 and Tn916/1545) and bacterial 16S rRNA gene were tracked during the transition period using quantitative PCR.

Results: The total quantity of the twenty-four ARGs decreased 38.82 % and 64.99 % after mesophilic and thermophilic AD, respectively. Thermophilic AD led to better reductions in the total quantities of tetracycline and macrolide resistance genes, while aminoglycoside resistance genes were more effectively decreased under mesophilic condition. Except for mph(B), mef(A) and aadE, other ARGs got similar or better removal under thermophilic condition. Thermophilic digestion also had a better reduction of intI1 and tet(A), tet(G) and tet(O) displayed a positive correlation with intI1 occurrence (P < 0.05) during the transition period. The total abundance of the reported hosts of the target ARGs in digested sludge decreased from 16.16 % to 7.77 % after temperature increase, and tet(X) and strB genes exhibited significantly positive correlation with their reported hosts.

Conclusion: The overall results suggest better attenuating the HGT potential through the remove of mobile elements and better blocking VGT of ARGs by eliminating their mesophilic hosts are the two main reasons for the observed advantage of the thermophilic digestion on the reduction of ARGs.
Specific in silico tools for the detection of acquired antibiotic resistance genes

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Metagenomic studies have shown natural environments to harbour a vast diversity of predicted antibiotic resistance genes and raised the two linked questions: What is the intrinsic function of these genes and what is the selective forces at play which could explain their significant abundance and diversity (1–3). We believe this high amount and diversity of genes is partly linked to the tools commonly employed for functional annotation. Contemporary bioinformatical antibiotic resistance detection methods rely on sequence similarity to known resistance genes ignoring genetic context. These approaches lead to overprediction as exemplified by Genbank containing 4027 annotated beta-lactamases in 1501 bacterial and archaeal genomes. A recent bioinformatical study which took genetic context into account indicated that gene synteny can be used to separate de facto beta-lactamases from proto-resistance genes. Proto-resistance genes which share common ancestry and thus sequence similarity with resistance genes, but provide little or no antibiotic resistance themselves (4).

The objective of this study was to increase the specificity of bioinformatical antibiotic resistance detection without losing significant sensitivity with the long term goal of developing a method specific for high throughput sequencing. Furthermore we believed the method would provide indications on the origin and evolution of antibiotic resistance determinants as well as give answers as to the intrinsic function of some putative proto-resistance genes.

We investigated if overprediction of resistance caused by proto-resistance genes is unique to beta-lactamases or if it extended to resistance genes for other antibiotics such as sulfonamide and tetracycline. This was done using a bioinformatical approach encompassing gene synteny on all fully sequenced bacterial chromosomes and plasmids found in Genbank. Secondly we started developing a method specific for metagenomic data sets and established the quality of metagenomic data necessary for extracting adequate gene synteny information for utilization of our pipeline. This was done on various datasets of both environmental and clinical nature.

References

Figure 1: Sequence conservation between beta-lactamases, proto-resistance genes and paralogs makes contemporary sequence based methods overpredict resistance.
P MIT 2
Application the electrooptical analysis of microbial cells for estimation of antibiotic resistance
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4EioSystem GbR, Berlin, Germany

Introduction: Study of the adaptation of microbes to antibiotic action is an important problem that is of theoretical and applied significance.

Objectives: Obtaining the effect of ampicillin and kanamycin on the electrophysical characteristics of ampicillin- and kanamycin-sensitive and ampicillin- and kanamycin-resistant Escherichia coli cells.

Materials & methods: All experiments were conducted by ELBIC EO analyzer at a wavelength of 670 nm.

Results: We examined the effect of ampicillin and kanamycin on the electrophysical characteristics of ampicillin- and kanamycin-sensitive and ampicillin- and kanamycin-resistant Escherichia coli cells. Substantial changes in the orientational spectra (OS) of suspensions of cells incubated with various ampicillin and kanamycin concentrations took place only at the first five frequencies of the orienting electric field (10—1000 kHz). The maximal change in the magnitude of the electro-optical signal occurred at 50 mg/ml of ampicillin and 10 mg/ml of kanamycin. The suspension-OS changes did not depend on the antibiotic-action period. Under the action of ampicillin and kanamycin, sensitive and resistant E. coli strains gave different electro-optical (EO) effects. It follows that the sensitive and resistant E. coli strains exhibit different of the EO effect on the action of ampicillin and kanamycin.

Conclusion: We are thinking that by recording the OS changes taking place under the effect of ampicillin and kanamycin, one can study the action of the antibiotic on microbial cells and to draw conclusions about the existence of resistance to this antibiotic in the cells studied. Thus, the suspension-OS changes occurring under the effect of ampicillin and kanamycin may be used as a test for resistance to this antibiotic in the cells studied.

P MIT 3
Development of DNA-extraction methods to determine prevalence of antibiotic resistances on pig farms
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Several studies have evaluated the occurrence of antibiotic resistances in farm animals. Today, the focus is on the spread of antibiotic resistances from the farm to the external environment. Antibiotic resistant bacteria already present within farms, e.g. in water, feed, dust or animal excreta, are considered as important vectors in the transmission of antibiotic resistances to the environment. Investigations addressing this aspect mostly rely on traditional microbiological methods. In the present study, we employ molecular biological methods to analyze presence and levels of genes encoding for commonly used antibiotics (e.g. Tetracycline, Sulfonamide and Streptomycin) in samples collected at a pig farm. These samples include swabs of water pipes, swabs of feed troughs, dust samples and feces samples.

The objectives are development of DNA-extraction methods from samples of various origins (characterized by different bacterial loads, texture, and matrix effects) within a farm and development of polymerase chain reaction (PCR) methods for detection of resistance genes. Of special interest is the comparison of fecal samples to other samples (water, dust, ...) from the same farm to determine the distribution of certain genes within a farm and whether distribution of genes within the farm is important for transmission to external environments.

Development of suitable DNA-extraction methods is based on procedures described in literature. Such procedures include protocols from commercial kits as well as protocols using polyvinylpolypyrrolidone, silica solution, Chelex 100, or heated sodium hydroxide solution (HotShot).

Because this study was only recently initiated, a limited amount of data is available. However, preliminary results indicate presence of antibiotic resistance genes not only in animal feces but also in the surrounding farm environment. Molecular determination of antibiotic resistance genes in fecal samples is a fast method to detect and quantify resistance genes / resistant bacteria in farms and helps to evaluate risks for transmission of these genes / bacteria to the external environment.
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