7th Symposium on Antimicrobial Resistance in Animals and the Environment

26-28 June 2017 | Braunschweig, Germany

ARAE 2017 - Proceedings

Contents

Welcome to ARAE 2017

Welcome from Dr. Maria Flachsbarth

Parliamentary State Secretary

German Federal Ministry of Food and Agriculture (BMEL)

The global rise of antimicrobial resistance threatens valuable therapeutic approaches in modern human and veterinary medicine. Antimicrobial resistance is not a future threat looming on the horizon. It is present today and the consequences are devastating. The international community is aware of the danger posed by especially multiresistant pathogens. Joint efforts at national, European and international level are necessary to combat this danger. Antibiotic resistance is therefore a priority topic for the German Federal Government.

Major progress in developing new antimicrobial compounds or even classes cannot be expected in the near future; the appropriate use of available antimicrobials and the preservation of their effectiveness are therefore more essential than ever. We need efficient strategies to this effect with a view to reducing the use of antimicrobials in all relevant sectors. In Germany, one important milestone was the launch of the antibiotics minimisation concept for animal husbandry in 2014 that was legally established in the 2013-Medicinal Products Act. So far, this new concept has proved to be a full success and has helped to considerably reduce the use of antibiotics in animal husbandry in Germany.

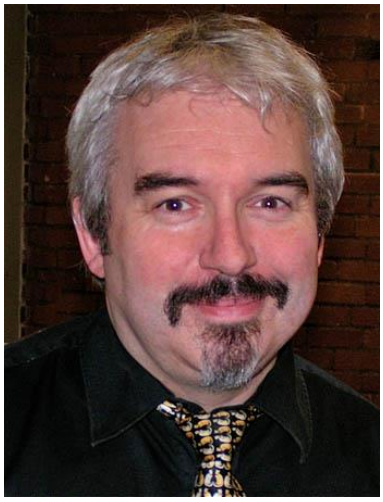
Human, animal and environmental health is inextricably linked. The development of antimicrobial resistance can therefore only be tackled by taking a cross-sectoral approach: the One Health approach. Building a bridge between public health, health care, animal health and the agricultural sector is essential. The Federal Government has been pursuing the One Health approach for some time. In 2015, the German Federal Cabinet adopted the German Antimicrobial Resistance Strategy (DART 2020). DART 2020 is a joint strategy of the Federal Ministry of Health, the Federal Ministry of Food and Agriculture and the Federal Ministry of Education and Research pooling the measures required to reduce antimicrobial resistance. As a horizontal strategy, DART 2020 applies to human medicine, veterinary medicine and agriculture equally.

Since antimicrobial resistance can spread beyond borders via commercial and passenger traffic, cooperation must not be limited to the national level. The framework for joint measures at the global level is provided by the Global Action Plan on Antimicrobial Resistance developed by the Word-Health Organisation (WHO) in conjunction with the World Organisation for Animal Health (OIE) and the UN Food and Agriculture Organisation (FAO). With antimicrobial resistance already a focal point of Germany´s G7 presidency, the subject has also been placed on the agenda of Germany´s G20 presidency. This underlines the importance of the issue and makes a contribution to closer international cooperation. Germany has thus adopted a pioneering role in the fight against antimicrobial resistance.

What else can we do to combat multiresistant pathogens or prevent such multiresistance in the first place? Researchers can make valuable contributions by working to produce new effective antibiotics that are needed to combat multiresistant bacteria. At the same time, we need to improve our understanding of how resistance develops and spreads among bacteria. This is the only way to develop effective strategies to prevent increasing resistance.

I wish to extend my sincere gratitude to everyone who contributes to meeting this demanding challenge!

Welcome from Kristina Kadlec, PhD, Prof. Lothar Kreienbrock and Prof. Stefan Schwarz



Kristina Kadlec, PhD

Head of the working group Molecular Microbiology and Antibiotic Resistance, Institute of Farm Animal Genetics

Friedrich-Loeffler-Institut

Prof. Lothar Kreienbrock

Director of the Department of Biometry, Epidemiology and Information Processing

University of Veterinary Medicine Hannover

Prof. Stefan Schwarz

Managing director of the Institute of Microbiology and Epizootics

Freie Univeristät Berlin

Antimicrobial resistance (AMR) is a problem of global concern that has worsened in recent decades. In general, the selection pressure as imposed by the use of antimicrobials is one of the major driving forces in the development of AMR and thus contributes to increasing rates of AMR among bacteria. This situation is true for human as well as veterinary medicine, but also for aquaculture and horticulture. In addition, effluents from hospitals, farms and factories that produce antimicrobial agents can cause a contamination of the environment with antimicrobial agents, thereby increasing the selection pressure on environmental bacteria. The mechanisms and epidemiological risks related to AMR are a fast-evolving field in which there are numerous activities including ongoing research, training and management options to fight AMR. This is reflected by the constantly increasing number of publications related to AMR over the last 30 years with approximately 2,000 publications in 1986 and > 8,000 publications listed in 2016 in PubMed.

By the decision of the World Health Assembly of the World Health Organization (WHO), a Global Action Plan on AMR (GAP AMR) was published in 2016, recognizing the urgent need for cross-sectoral action to address AMR. The GAP AMR was therefore accompanied by resolutions of the Food and Agriculture Organization of the United Nations (FAO) as well as the World Health Organisation for Animal Health (OIE). Although Germany was not the first promoter in resistance research and politic activities in recent years, Germany tried to change its habits. As an example, the G7 summit in Germany in 2015 was the first political event where G7 officially addressed this health issue on a prominent scale.

This Symposium on Antibiotic Resistance in Animals and the Environment (ARAE) is a very important cornerstone in the research on AMR. The ARAE, which has been held in 2-years intervals since 2005, provides an important venue for networking between and discussion among scientists working on different aspects in the field of AMR and in finding ways to mitigate the impact of resistance. In 2017, the 7th ARAE is hosted for its first time in Germany.

It is a great pleasure and honour for us and all other people involved in hosting this conference. More than 150 scientists from more than 25 countries and 6 continents will gather in Braunschweig to present and discuss the latest findings in the field of AMR. We wish all participants a scientifically excellent and inspiring meeting, in which they can exchange ideas and start new collaborations in a friendly and relaxing atmosphere. Braunschweig and its surroundings welcome you with a picturesque Old Town, local food and drinks and a venue in a quiet and rural environment.

Welcome to everybody,

Kristina Kadlec, Neustadt-Mariensee

Lothar Kreienbrock, Hannover

Stefan Schwarz, Berlin

Committees

Scientific Committee

Stefan Schwarz, Berlin
Lothar Kreienbrock, Hannover
Kristina Kadlec, Neustadt-Mariensee
Annemarie Käsbohrer, Berlin/Wien
Guido Werner, Wernigerode
Heike Kaspar, Berlin
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Katja Hille, Hannover
Nicole Werner, Hannover
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Margit Fink, Braunschweig

Keynote Speakers

Séamus Fanning, Dublin
Thomas U. Berendonk, Dresden
Laurent Poirel, Fribourg
René Hendriksen, Lyngby
Scott McEwen, Guelph
Engeline van Duijkeren, Bilthoven

Acknowledgements

The Organising Committee thanks all those who have generously contributed to the success of the 7th Symposium on Antimicrobial Resistance in Animals and the Environment.

Förderverein für angewandte Epidemiologie und Ökologie e.V.

ARAE 2017 – General Information

Venue

The ARAE Symposium will take place in the Forum of the Johann Heinrich von Thünen Institute.

Johann Heinrich von Thünen Institute

Federal Research Institute for Rural Areas, Forestry and Fisheries

Bundesallee 50

38116 Braunschweig

Germany

Forum, Building No. 227

Übersichtsplan

Johann Heinrich von Thünen-Institut
Bundesforschungsinstitut für Ländliche Raume, Wald und Fischerei

Präsident, Präsidialbüro und Forum	227
Institut für Agrarklimaschutz	246 202 254
Institut für Agrartechnologie	213 249
Institut für Betriebswirtschaft	203
Institut für Biodiversität	246
Institut für Ländliche Räume	255
Institut für Marktanalyse	203
Zentrum für Informationsmanagement - Fachinformation	255
Fahrbereitschaft	221
Gästehaus	228
Zentrum für Informationsmanagement - Informationstechnik	205
Personalvertretung	229
Verwaltung	208

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)

Leitung und Verwaltung	247
Baustelle	224

Julius Kühn-Institut (JKI)
Bundesforschungsinstitut für Kulturpflanzen

Institut für Pflanzenbau und Bodenkunde	230 253 219 250

Friedrich-Loeffler-Institut (FLI)
Bundesforschungsinstitut für Tiergesundheit

Institut für Tierernährung	252
Versuchsstation	251

Deutscher Wetterdienst (DWD)

Agrarmeteorologische Forschung Braunschweig	649

Bundesanstalt für Immobilienaufgaben (BImA)

Technischer Dienst	201
Tischlerei	206

Allgemeine Einrichtungen

Haupteingang/Pförtner	223
Kantine	210
Kindertagesstätte (Krümelkiste)	245

Bei Fragen zu Anwohnern auf dem Gelände wenden Sie sich bitte an den Pförtner.

Johann Heinrich von Thünen-Institut

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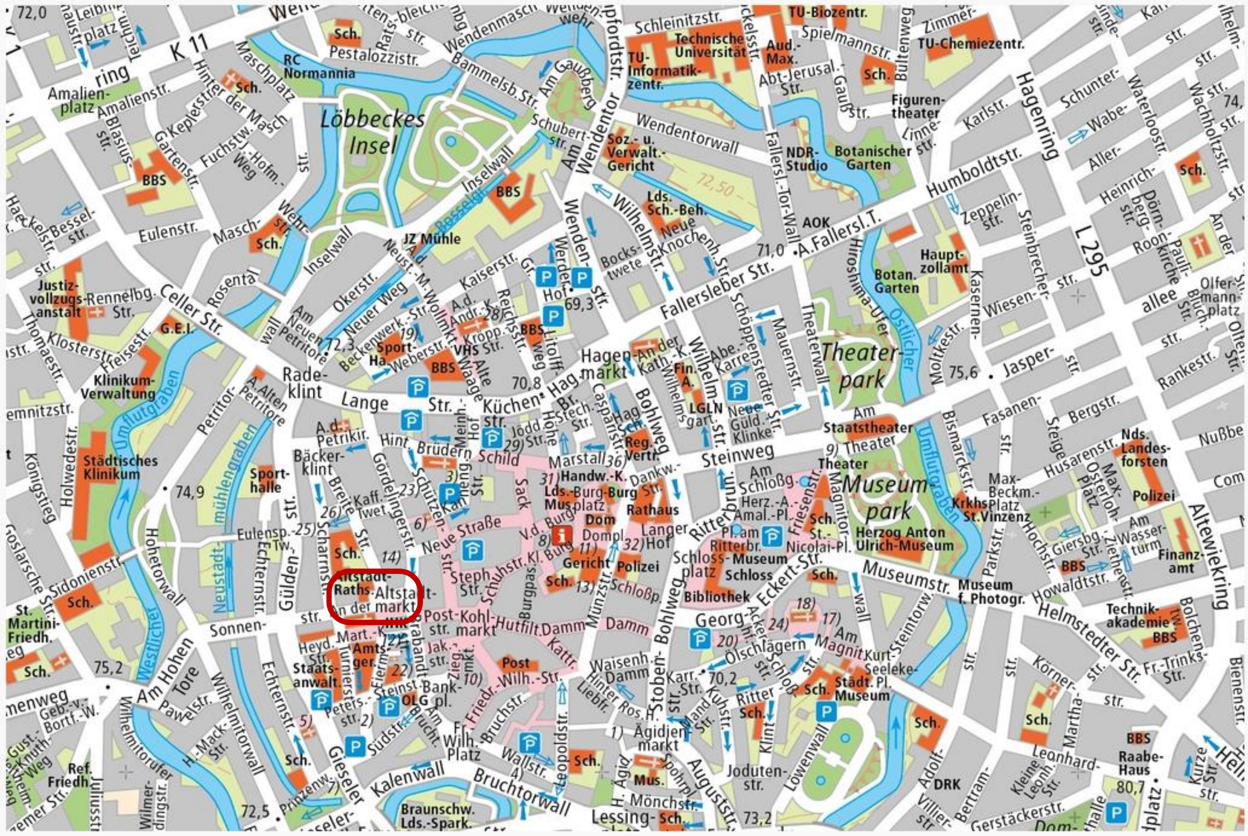
THÜNEN

General plan of the Johann Heinrich von Thünen Institute with the Forum and the canteen marked.



Map of the old town of Braunschweig with the Altstadtrathaus or Dornse marked where the Welcome Reception will take place on Sunday evening. (Source: Stadt Braunschweig - Open GeoData, 2017, Lizenz: dl-de/by-2-0)

Picture of the Altstadtrathaus called "Dornse" in the old town of Braunschweig. Source: Commons

Welcome Reception

The symposium starts with a Welcome Reception on Sunday, 25 June 2017, in the old town hall (Altstadtrathaus or Dornse, Altstadtmarkt 7, Braunschweig). This venue is located in the old town and in walking distance from the hotels.

Registration

Registration will be open from 18.00 until 20.00 during the Welcome Reception on Sunday evening at the old town hall.

At the ARAE conference venue, the registration desk will be located in the Foyer outside the lecture hall. Opening hours of the registration desk are:

Monday, June 26: 8.30-18.15

Tuesday, June 27: 9.00-17.00

Wednesday, June 28: 9.00-14.00

WLAN

For internet access at the venue, please sign the WLAN sheet at the registration desk. WLAN will be provided without charge.

Badges

Your name badge is included in the conference materials which you receive during registration. Please wear your badge during conference hours.

Lunch

In your conference materials, you will find two lunch vouchers for Monday and Tuesday, respectively. Each voucher is valid for a menu consisting of a main dish, two side dishes and a soft drink. You can purchase additional dishes or drinks.

Lunch will be served at the canteen of the Institute (Nr. 210 on the site map, page 10). A choice of dishes will be available.

Social Dinner

The Social Dinner will take place on Tuesday, 27 June 2017, at the International museum of wind and water mills in Gifhorn. A bus transfer is organised for all participants directly from the conference venue and back to the hotels in Braunschweig.

Continuous Veterinary Education

The ARAE symposium is accredited with 16 ATF hours for continuing veterinary education by the Federal Chamber of Veterinarians.

Oral Presentations

Please provide your oral presentation on a USB stick in the lecture hall. The lecture hall is equipped with a PC and a projector. Please make sure you use either a power point or a pdf file format.

Keynote presentation: time slot is 45 minutes, presentation of about 35 minutes + about 10 minutes for discussion
Oral presentation: time slot is 15 minutes, presentation of 12 minutes + 3 minutes for discussion.

Posters

Please refer to the poster programme in order to find the board number assigned to your poster. Mount your poster on Monday morning on the respective board. Please remove your poster at the end of the conference.

Speed poster presentation: Presentation in the auditorium, very strict time limit of 3 minutes, no discussion,and a poster as described below.

Poster: the size of the poster should be 841 x 1189mm / 33.1 x 46.8 in (width x hight); the poster boards have a width of 900mm. Please be present at your own poster(s) in the indicated one of the two poster sessions and use the other poster session as a visitor.

Veterinary Microbiology – ARAE 2017 Special Issue

The international peer-reviewed journal Veterinary Microbiology (Impact factor 2.564; https://www.journals.elsevier.com/veterinary-microbiology) will prepare an ARAE 2017 special issue. Reviews, original papers and short communications prepared from data presented as either oral presentations or posters during the ARAE 2017 meeting may be submitted after the meeting via the journal's website.

Regular publication in Veterinary Microbiology is free of charge. Open access publication is possible subject to payment of a fee. All submissions will undergo a regular peer-review process. Once a manuscript has been accepted, it will appear in ScienceDirect and will receive a DOI and a PII number, so that it can be cited immediately.

All articles from ARAE 2017 will be summarized in a special volume of the journal about 6 months after the ARAE symposium.

Travel Information

Public Transport: Bus 411 from Braunschweig city to the bus stop “Bundesallee”
Duration: 30 Minutes

Shuttle Service: A bus shuttle is organised for each of the conference days between the hotels and the conference venue.

Monday, 26.06.

Bus 1		Bus 2		Bus 3	
7.30h	Steigenberger Parkhotel	7.30h	Deutsches Haus	7.30h	Mercure Hotel
7.40h	BEST WESTERN City-Hotel & BEST WESTERN Hotel Stadtpalais	7.40h	Hotel Fourside	7.40h	Intercity Hotel
				7.55h	Pentahotel

18.30h: Back to the hotels with the same bus numbers.

Tuesday, 27.06.

Bus 1		Bus 2		Bus 3	
7.45h	Steigenberger Parkhotel	7.45h	Deutsches Haus	7.45h	Mercure Hotel
8.00h	BEST WESTERN City-Hotel & BEST WESTERN Hotel Stadtpalais	7.55h	Hotel Fourside	7.55h	Intercity Hotel
				8.10h	Pentahotel

17.15h: three busses to the the International Museum of wind and water mills.

22.00h / 22.30h / 23.00h: busses to old town/city centre of Braunschweig and Braunschweig train station.

Wednesday, 28.06.

Bus 1		Bus 2		Bus 3	
7.45h	Steigenberger Parkhotel	7.45h	Deutsches Haus	7.45h	Mercure Hotel
8.00h	BEST WESTERN City-Hotel & BEST WESTERN Hotel Stadtpalais	7.55h	Hotel Fourside	7.55h	Intercity Hotel
				8.10h	Pentahotel

14.00h: two busses to Braunschweig train station and old town/city centre of Braunschweig.

Programme

ARAE 2017

Sunday, 25 June 2017			Page
18:00	Registration and Welcome Reception at the old town hall (Altstadtrathaus) in Braunschweig		
19:00	Opening	Kristina Kadlec Lothar Kreienbrock Helmut Blöcker (Mayor of Braunschweig)	

Monday, 26 June 2017			Page
8:30	Registration		
9:00	Welcome Note	Stefan Schwarz	
1st Session: Monitoring and Epidemiology of AMR		Chairs: Axel Cloeckart, Stefan Schwarz	
9:15	Key Note: Pandoras box- full of eastern (AMR) delights!	Séamus Fanning Dublin, Ireland	24
10:00	QnrD in fluoroquinolone-resistant *Proteae* of animal origin	Marisa Haenni Lyon, France	34
10:15	Human health risk of dietary intake of antibiotic residues in beef in Maroua, Cameroon	Ronald R. B. Vougat Ngom Maroua, Cameroon	35
10.30	WGS-based elucidation of linezolid resistance locus *optrA* in clinical *Enterococcus* spp. isolates from Germany	Jennifer Bender Wernigerode, Germany	36
10:45	Coffee break		
2nd Session: AmpC & carbapenemase-producing *Enterobacteriaceae*		Chairs: Anne Käsbohrer, Yvonne Pfeifer	
11:00	Prevalence of extended-spectrum β-lactamase- and AmpC-producing *Escherichia coli* in dairy calves: does age matter?	Maike Gonggrijp Deventer, The Netherlands	37
11:15	NGS-based analysis of AmpC-β-lactamase CMY-2-producing *Escherichia coli* from humans, livestock and food in Germany	Michael Pietsch Wernigerode, Germany	38
11:30	Fecal carriage and characterization of extended-spectrum β-lactamase- and AmpC β-lactamase-producing *Escherichia coli* from healthy horses in France	Benoît Doublet Nouzilly, France	39
11:45	Faecal carriage of ESBL- and AmpC–producing *Escherichia coli* from co-habiting people and pets	Leah Toombs-Ruane Massey, New Zealand	40
12:00	Epidemiology of carbapenemase-producing *Enterobacteriaceae* in a farrow-to-finish swine production system in the United States	Dixie F. Mollenkopf Columbus, USA	41
12:15	Carbapenemase producing *Escherichia coli* isolated from German retail seafood	Nicole Roschanski Berlin, Germany	42
12:30	Lunch		
3rd Session: AMR in the Environment		Chairs: René Hendriksen, Kristina Kadlec	
14:00	Key Note: Wastewater treatment plant effluents and their implications for antimicrobial resistance in surface water and water reuse	Thomas U. Berendonk Dresden, Germany	26
14:45	Antibiotic resistance prevalence of *Pseudomonas* spp. and identification of metallo-β-lactamase producing *Pseudomonas* spp. in wastewater treatment plant effluent	Thi Thuy Do Maynooth, Ireland	43
15:00	Frequent detection of CRE in Dutch municipal wastewater	Hetty Blaak Bilthoven, The Netherlands	44
15:15	*Poster Speed Presentations*		
15:45	*Poster Session*		
16:15	Coffee break		

4th Session: Use of Antimicrobial Agents		Chairs: Lothar Kreienbrock	
16:45	Monitoring antibiotic usage in animals: Do existing systems provide comparable data?	Nicole Werner Hannover, Germany	45
17:00	Used Daily Dose vs. Defined Daily Dose – Advantages and disadvantages of different dosage assumptions for the monitoring of antimicrobial usage in livestock	Svetlana Kasabova Hannover, Germany	46
17:15	Association between veterinary antibiotic use and resistance in commensal *Escherichia coli* from livestock in Belgium between 2011 and 2015	Bénédicte Callens Brussels, Belgium	47
17:30	The effect of antibiotic use on resistance in commensal *Escherichia coli* from pre-weaned calves on a large commercial dairy farm in Washington State	Josephine A. Afema Pullman, USA	48
17:45	The use of antimicrobial drugs in fattening pigs in Italy	Rosanna Desiato Torino, Italy	49
18:00	Monitoring antimicrobial resistance in the Norwegian environment using wild red foxes as an indicator	Solveig S. Mo Oslo, Norway	50
18:15	Closing of Day 1		
18:30	Departure to Braunschweig City / Hotels		

Tuesday, 27 June 2017			Page
5th Session: Colistin Resistance		Chairs: Geovana B. Michael	
9:00	Key Note: Plasmid-mediated resistance to polymyxins; animals as reservoirs for humans?	Laurent Poirel Fribourg, Switzerland	27
9:45	Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study	Yingbo Shen Beijing, China	51
10:00	The environmental contamination of NDM and MCR-1 in food animal production chain	Yang Wang Beijing, China	52
10:15	Coffee break		
6th Session: more about the Colistin Resistance Gene *mcr*		Chairs: Laurent Poirel, Yang Wang	
10:45	The dissemination of *mcr-1* among *Escherichia coli* of human and animal origins	Shaolin Wang Beijing, China	53
11:00	Occurrence of colistin resistance gene *mcr-1* in *Escherichia coli* isolates associated with diarrhea and edema disease in piglets in Europe	Christa Ewers Gießen, Germany	54
11:15	Detection of plasmid-mediated colistin-resistance genes in *Enterobacteriaceae* isolates from food-producing animals and meat. Identification of the novel variant *mcr-3*, Portugal, 2010-2015	Manuela Caniça Lisbon, Portugal	55
11:30	*Poster Speed Presentations*		
12:00	*Poster Session*		
12:30	Lunch		
7th Session: Novel Diagnostic Tools		Chairs: Patrick Boerlin, Heike Kaspar	
14:00	Key Note: Metagenomics analysis – surveillance of all AMR genes from sewage	René Hendriksen Lyngby, DK	29
14:45	Validation of a Novel Susceptibility Testing Method for *Haemophilus parasuis*	Sandra Prüller Hannover, Germany	56
15:00	Biocide susceptibility testing – development of a new testing method	Andrea T. Feßler Berlin, Germany	57
15:15	ARIBA: a new high throughput tool to identify antimicrobial resistance determinants from short read sequencing data	Alison E. Mather Cambridge, UK	58
15:30	Coffee break		

8th Session: MRSA		Chairs: Engeline van Duijkeren, Marisa Haenni	
16:00	Variability of SCC*mec* elements in livestock-associated CC398 MRSA	Stefan Monecke Jena, Germany	59
16:15	Unexpected occurrence of MRSA in Swedish wild hedgehogs (*Erinaceus europaeus*) - A pilot study	Stefan Börjesson Uppsala, Sweden	60
16:30	Carriage dynamics of methicillin resistant *Staphylococcus aureus* and changes in the nasal microbiome after long- and short-term exposure to the pig farm environment.	Md Zohorul Islam Copenhagen, Denmark	61
16:45	Closing of Day 2		
17:15	Departure to dinner and social event at the Mühlenmuseum Gifhorn		

Wednesday, 28 June 2017			Page
9th Session: Novel and Alternative Approaches in Fighting AMR		Chairs: Alison E. Mather, Nicole Werner	
9:00	Key Note: CIA list of the WHO	Scott McEwen Guelph, Canada	31
9:45	Reduction in antimicrobial usage following veterinary intervention in dairy herds	Scott McDougall Morrinsville, New Zealand	62
10:00	Testing the susceptibility of bacterial mastitis isolates to host defense peptides: technical challenges and data output for clinical isolates	Maren von Köckritz-Blickwede Hannover, Germany	63
10:30	Coffee break		
10th Session: ESBL-producing *E. coli* in animals		Chairs: Guido Werner	
11:00	Longitudinal study of ESBL-carriage on an organic broiler farm: horizontal plasmid transmission	Angela H.A.M. van Hoek Utrecht, The Netherlands	65
11:15	Plasmid and chromosomal location of $bla_{CTX\text{-}M\text{-}15}$ genes detected in *Escherichia coli* from diseased cattle	Geovana Brenner Michael Berlin, Germany	66
11:30	Antimicrobial resistance prevalence in Harbour (*Phoca vitulina*) seal pups stranded in the Netherlands and antibiotic treatment effect in their gut microbiome during rehabilitation	Ana Rubio-Garcia Pieterburen, The Netherlands	67
11:45	Extended spectrum β-lactamase producing *Enterobacteriaceae* in imported and domestic food products purchased at retail in Canada	Michael Mulvey Guelph, Canada	68
12:00	Key Note: Epidemiology of ESBL-*E.coli* from the One Health perspective	Engeline van Duijkeren Bilthoven, The Netherlands	33
12:45	Closing of the 7th ARAE		
13:15	Coffee break		
14:00	Departure to Braunschweig City / Main station		

Posters

Monday 26 June			
No.	15:15h Poster Speed Presentations 1	Presenting author	Page
71	Antimicrobial usage and risk of extended-spectrum β-lactamase producing *Escherichia coli* in animal-rearing households of selected rural and peri-urban communities, Nigeria	Ojo	138
45	Motivations for treatment decisions made by calf care workers on western United States dairies	Moore	113
43	Extended-spectrum cephalosporin resistance in *Escherichia coli* from Alberta beef cattle	Cormier	111
13	NDM-1 producing *Vibrio parahaemolyticus* isolated from imported shrimps	Briet	81
17	An *in vitro* chicken gut model demonstrates transfer of a multidrug resistance plasmid from *Salmonella* to commensal *Escherichia coli*	Card	85
59	Extended-spectrum beta-lactamase (ESBL) of faecal *Escherichia coli* isolate recovered from European free-tailed bat (*Tadarida teniotis)* in Portugal	Caniça	127
55	Airborne colonization of piglets with livestock-associated MRSA	Rosen	123
49	MRSA carriage among human volunteers visiting a swine farm	Angen	117
No.	15:45h Poster Session 1	Presenting author	Page
1	Adaptation of *Salmonella* Typhimurium strains to organic acids and consequences on antibioresistance	Bridier	70
3	Characterization of SGI1 multidrug resistance gene cluster in ciprofloxacin-resistant *Salmonella enterica* serotype Kentucky from human and poultry sources in Belgium	Doublet	71
5	Vigilance for *Salmonella* in Feedstuffs in Costa Rica: Prevalence and Tetracycline Resistance	Molina	73
7	Pig faecal bacteria exhibiting colistin and imipenem resistance	Joyce	75
9	Prevalence and anti-microbial resistance (AMR) profile of non-typhoidal *Salmonella* of pigs in Kenya and Malawi	Wilson	77
11	Characterization of extended-spectrum β-lactamase- and AmpC-producing *Escherichia coli* from legally and illegally imported meat	Müller	79
13	NDM-1 producing *Vibrio parahaemolyticus* isolated from imported shrimps	Briet	81
15	Wastewater is a reservoir for clinically relevant carbapenemase and 16S rRNA methylase producing *Enterobacteriaceae*	Zurfluh	83
17	An *in vitro* chicken gut model demonstrates transfer of a multidrug resistance plasmid from *Salmonella* to commensal *Escherichia coli*	Card	85
19	Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* in Latvian broiler chickens	Kovalenko	87
21	Personalized medicine - Fast detection schemes towards antibiotic susceptibility testing	Rösch	89
23	Use of on-farm bacterial culture and decision support tools to reduce antimicrobial usage in cases of mild to moderate clincial mastitis in cows	Heuer	91
25	Identification of a novel multiresistance integrative and conjugative element ICE*Pmu2* in a bovine *Pasteurella multocida* isolate from Germany	Kadlec	93
27	First extended-spectrum β-lactamase in *Mannheimia haemolytica*	Kadlec	95
29	Definition of national Defined Daily Doses (DDDch) and Defined Course Doses (DCDch) for antimicrobial preparations for pigs in Switzerland	Kümmerlen	97
31	Quinolone resistant *Escherichia coli* from broiler in Norway - characterization, comparison to human isolates and molecular risk assessment	Slettemeås	99
33	Quinolone resistance despite low antimicrobial usage: comparison of occurrence in different species in Norway the last ten years	Kaspersen	101
35	Antimicrobial susceptibility and genetic relatedness of respiratory tract pathogens before and after antibiotic treatment	Niemann	103
37	Antimicrobial resistance after fluoroquinolone treatment	Käsbohrer	105
39	Cross-sectional study on the prevalence of ESBL-/AmpC-producing *Eschericha coli* in food in Germany	Käsbohrer	107
41	Long term effects of antimicrobial use in feedlot cattle early in the feeding period on *Salmonella enterica* spp. population, distribution and antimicrobial resistance profiles in feces during feeding period and on hide and in lymph nodes at slaughter	Levent	109
43	Extended-spectrum cephalosporin resistance in *Escherichia coli* from Alberta beef cattle	Cormier	111
45	Motivations for treatment decisions made by calf care workers on western United States dairies	Moore	113
47	Improved DNA extraction and purification with nano-magnetite beads facilitates highly sensitive detection of MRSA directly from swabs	Schwarz	115

49	MRSA carriage among human volunteers visiting a swine farm	Angen	117
51	Identical genotypes of community-associated MRSA (ST59) and livestock-associated MRSA (ST9) in both humans and pigs in rural China	Wang	119
53	Novel pseudo-staphylococcal cassette chromosome *mec* element (φSCC*mec*T55) in methicillin-resistant *Staphylococcus aureus* ST9	Wang	121
55	Airborne colonization of piglets with livestock-associated MRSA	Rosen	123
57	Vegastudy: Do vegetarians less often carry ESBL-producing *Escherichia coli*/*Klebsiella pneumoniae*?	van Duijkeren	125
59	Extended-spectrum beta-lactamase (ESBL) of faecal *Escherichia coli* isolate recovered from European free-tailed bat (*Tadarida teniotis)* in Portugal	Caniça	127
63	Characterization of clinical bovine and human CTX-M-15-producing *Escherichia coli* isolates by biocide susceptibility testing	Brenner Michael	130
65	Detection of plasmid-mediated AmpC β-lactamase CMY-2 in *Escherichia coli* isolated from diseased food-producing animals	Brenner Michael	132
67	Occurrence and characterization of ESBL-encoding plasmids among *Escherichia coli* isolates from fresh vegetables	Freitag	134
69	Crystal structure of the multidrug resistance regulator RamR complexed with bile acids	Nishino	136
71	Antimicrobial usage and risk of extended-spectrum β-lactamase producing *Escherichia coli* in animal-rearing households of selected rural and peri-urban communities, Nigeria	Ojo	138
73	Antimicrobial resistance: a sheep mastitis treatment problem or a sheep in wolf's clothing?	Silva	140
75	Prevalence and distribution of ESBL and AmpC β-lactamases producing *Escherichia coli* in food-producing animals and meat in Latvia	Streikiša	142
77	High prevalence of cephalosporin-resistant commensal *Escherichia coli* in calves in Latvia	Terentjeva	144
79	Antimicrobial susceptibility of *Avibacterium paragallinarum* isolates from outbreaks of infectious coryza in Dutch commercial poultry	Heuvelink	146
81	First report of bla_{OXA-58} positive *Acinetobacter pittii* isolates from pet animals	Ewers	148
83	Monitoring of antimicrobial susceptibility of poultry pathogens in The Netherlands, 2014-2016	Wiegel	150
85	Detection of OXA-181-carbapenemase, colistin resistance determinant MCR-1 and AmpC β-lactamase CMY-2 genes in an *Escherichia coli* strain from swine	Pulss	152
87	Early detection of polymyxin-resistant Gram-negative bacteria using chromID® Colistin R agar, a new chromogenic medium	Marchand	154
89	Active screening for *mcr-1* in faecal samples from livestock in the Netherlands by non-selective enrichment and PCR	Veldman	156
91	Occurrence of MCR-1-producing *Enterobacteriaceae* in pigs, Portugal	Poirel	158

Tuesday 27 June			
No.	11:30h Poster Speed Presentations 2	Presenting author	Page
88	Insights in the genetic diversity of *Escherichia coli* from livestock and food harboring *mcr*-1	Grobbel	155
28	Understanding the behavioural influences and perceptions on antimicrobial resistance and antimicrobial use of UK Veterinary Surgeons: A mixed methods study	Coyne	96
40	Quality of veterinary pharmaceuticals and their use by pastoralists in the Far North Region of Cameroon	Vougat Ngom	108
78	WGS and plasmidome-analysis of related broiler and human cephalosporin-resistant *Escherichia coli* isolates to study possible transmission events	Visser	145
68	OXA-23 and OXA-58 carbapenemase-genes in *Acinetobacter indicus* isolates from cattle in Germany	Klotz	135
58	Extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing *Enterobacteriaceae* in water sources in Lebanon	Haenni	126
64	Diversity of VIM-1 producing *Escherichia coli* from German livestock	Hammerl	131
8	F33: A-: B-, IncI1/ST136, and IncN plasmids accelerate the emergence of the fosfomycin resistance gene *fosA3* in *Escherichia coli* from pigs, chickens and dairy cows in Northeast China	Zhang	76
No.	12:00h Poster Session 2	Presenting author	Page
4	Characterization of ESBL/AmpC-producing *Salmonella enterica* from the Colombian poultry chain using whole genome sequencing	Castellanos	72
6	Characterisation of a colistin resistance plasmid isolated from the gastrointestinal tract of broiler chickens	Delaney	74

8	F33: A-: B-, IncI1/ST136, and IncN plasmids accelerate the emergence of the fosfomycin resistance gene *fosA3* in *Escherichia coli* from pigs, chickens and dairy cows in Northeast China	Zhang	76
10	Antibiotic resistance patterns in bacteria isolated from slaughterhouse worker faeces in western Kenya	Kemp	78
12	Extended spectrum β-lactamase producing *Enterobacteriaceae* in local and imported poultry meat in Ghana	Eibach	80
14	Minimum inhibitory concentrations and antibiotic resistant genes in the freshwater cyanobacteria *Microcystis aeruginosa*	Caniça	82
16	Agricultural soils harbor high levels of potentially mobile antibiotic resistance genes	Caniça	84
18	Multiple drug resistance identified from a soil microbiome using functional metagenomics	Murphy	86
20	Effects of different floor designs in fattening turkeys on the development of antibiotic resistance in commensal *Escherichia coli*	Keller	88
22	Probing novel natural products for antibiotic activity against zoonotic bacteria	Pietschmann	90
24	CXC chemokines exhibit antimicrobial activity against multidrug-resistant Gram-negative pathogens	Lomonaco	92
26	Resistome of multidrug resistant *Klebsiella pneumoniae*	Lomonaco	94
28	Understanding the behavioural influences and perceptions on antimicrobial resistance and antimicrobial use of UK Veterinary Surgeons: A mixed methods study	Coyne	96
30	Development of antimicrobial resistance of fecal *Escherichia coli* in growing pigs	Abukabar	98
32	Antimicrobial susceptibility to critically important antibiotics of *Enterococcus faecium* and *Enterococcus faecalis* recovered from healthy cattle, pigs and chickens in nine EU countries (EASSA Study)	de Jong	100
34	Characterization of quinolone resistance mechanisms in *Enterobacteriaceae* isolated from companion animals in Europe (ComPath II study)	de Jong	102
36	Monitoring of antimicrobial susceptibility of major PPDS (MMA) pathogens recovered from acute cases in sows across Europe: VetPath results	El Garch	104
38	Monitoring of antimicrobial susceptibility of enteric pathogens isolated from diseased cattle and pigs across Europe: VetPath results	El Garch	106
40	Quality of veterinary pharmaceuticals and their use by pastoralists in the Far North Region of Cameroon	Vougat Ngom	108
42	Monitoring of antibiotic resistance in bacteria in pigs	Meißner	110
44	More than 50% reduction of antibiotic sales in German livestock farming between 2011 and 2015	Wallmann	112
46	Antibacterial activity and mechanism of action of dryofragin against methicillin-resistant *Staphylococcus aureus*	Hua	114
48	*Staphylococcus aureus* from zoo animal and wildlife	Feßler	116
50	Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* from employees and the environment of a small animal clinic	Feßler	118
52	Characterization of two novel small plasmids in *Staphylococci sciuri* of animal origin	Du	120
54	Methicillin-resistant *Staphylococcus aureus* in raw cow milk and soft cheese (wara) sold in Abeokuta, Nigeria	Omoshaba	122
56	ESBL and colistin resistance dynamic during veal calves fattening in France	Haenni	124
58	Extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing *Enterobacteriaceae* in water sources in Lebanon	Haenni	126
60	Spread of CTX-M-9-producing *Enterobacteriaceae* among rescued birds in France	Haenni	128
62	Molecular characterisation of CTX-M-15 producing isolates from food in Germany	Irrgang	129
64	Diversity of VIM-1 producing *Escherichia coli* from German livestock	Irrgang	131
66	Molecular analysis of plasmids coding for cephalosporin resistance in *Escherichia coli* from broilers	Kempf	133
68	OXA-23 and OXA-58 carbapenemase-genes in *Acinetobacter indicus* isolates from cattle in Germany	Klotz	135
70	Antibiotic resistance profile of bacteria isolated from mobile phones in Yaba College of Technology, Lagos, Nigeria	Ogundipe	137
72	Mastitis and antimicrobial resistance in Austrian dairy cows	Schabauer	139
74	Diffusion of antimicrobial resistance across management niches on dairy farms	Sischo	141
76	Characterization of antimicrobial resistant *Escherichia coli* from wild reindeers in Norway and Svalbard	Sunde	143

Abstracts – Oral Presentations

Pandoras box – full of eastern (AMR) delights!

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In China an estimated 30 million incidents of food-borne illness are attributed to *Salmonella* species. It ranks as the fourth most common aetiological agent of food-borne disease in China. Consequently, salmonellosis represents a major public health challenge. On-going surveillance has identified isolates of this bacterial genus from a range of sources that elaborate a resistance phenotype to several antimicrobial compounds. In some of these cases, bacteria express an extended spectrum b-lactam (ESBL)-resistant phenotype that is concurrent with one to fluoroquinolones (FQ) and in others resistance to carbapenems can be identified. This presentation will describe a number of related studies that identified and characterised *Salmonella* species from across the food chain.

***Salmonella* recovered from whole chicken carcases at retail outlets-** in this study 2,210 non-typhoidal Salmonella (NTS) were recovered and their susceptibilities to a panel of antimicrobial compounds determined. *Salmonella* Enteritidis, *S.* Indiana and S. Infantis were common. Most (80.18%) of these isolates were found to be resistant to at least one antimicrobial compound and 54.6% were defined as multi-drug resistant (MDR). Resistance to nalidixic acid (NAL) was common (70.6%) among the 11 tested compounds and no isolate was found to be resistant to carbapenems. There were 119 antimicrobial resistance profiles identified. Two-hundred eighty-four isolates including 99 *Salmonella* Indiana were resistant to seven or eight classes of antimicrobial compound. One-hundred eighty-three *Salmonella* Indiana isolates were found to be co-resistant to ciprofloxacin and cefotaxime and 179 of these were confirmed as extended-spectrum β-lactamase producers.

***Salmonella* Indiana in poultry-** a second study was carried out in 2012 that identified and characterised 133 *Salmonella* Indiana from poultry production environments along with an additional 21 clinical isolates. A multi-drug resistant (MDR) phenotype was confirmed and all of these isolates studied, bar one, were found to be resistant to ESBLs. Twelve different AMR profiles were recorded and none were resistant to imipenem nor tigecycline. Among the 154 isolates studied, four-point mutations were identified in the quinolone resistance determining region (QRDR) in 152 isolates of which 80 possessed amino acid substitutions in GyrA (S83F; D87G); ParC (T57S; S80R) as the dominant types. Several PMQR determinants were identified and 98 of the 154 possessed more than one of these marker types.

Five $bla_{\text{CTX-M}}$ genotypes were identified among the 153 positive isolates and of these $bla_{\text{CTX-M-65}}$ was found to be present in 131 *S*. Indiana. The latter were recovered from isolates cultured from poultry slaughterhouses and from clinical cases.

Comparisons of the corresponding pulsotypes as determined by PFGE, between isolates cultured from food-producing animals and patients exhibited diversity among the strains.

Characterisation of a large molecular weight plasmid containing *bla*$_{NDM-1}$ from a *S.*Indiana poultry isolate- an extensively drug resistant (XDR) *Salmonella enterica* serotype Indiana C629 (*S.* Indiana C629) was cultured from a slaughtered chicken carcass and found to express a metallo-b-lactamase activity. Whole genome sequencing (WGS) detected a large plasmid denoted as plasmid pC629 to which a *bla*$_{NDM-1}$-encoding gene was mapped. Interestingly the latter was contained within a composite transposon, flanked by IS*26* elements. Other determinants mapping to this plasmid included; *aac(6')-Ib-cr*, *aac(3)-VI*, *aadA5*, *aph(4)-Ia*, *arr-3*, *blmS*, *brp*, *catB3*, *dfrA17*, *floR*, *fosA*, *mph(A)*, *mphR*, *mrx*, *nimC/nimA*, *oqxA*, *oqxB*, *oqxR*, *rmtB*, *sul1* and *sul2.*

Characterisation of the genetic basis for resistance to various antimicrobial compounds identified plasmids as a major contributing factor. On-going surveillance by regulatory agencies is an important step required to identify routes of transmission, as these MDR- bacteria pose an important threat to animal and public health.

Wastewater treatment plant effluents and their implications for antimicrobial resistance in surface water and water reuse

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Due to the worldwide health impacts of antibiotic resistant pathogens, scientists are increasingly interested in the role of wastewater treatment plants (WWTPs) as a sink and source for antibiotic resistant bacteria and their genes. To date, the dynamics of resistant bacteria and associated genes in municipal WWTPs remains relatively unexplored, but there is clear evidence that antibiotic resistant organisms and genes are released with WWTP effluents to receiving environments. Studies have demonstrated that the absolute quantity of antibiotic resistance gene copies are reduced during conventional wastewater treatment, but it is also apparent that relative abundances of key resistance genes normalized by 16S rRNA copy numbers frequently show no significant reduction and sometimes even increase. This pattern appears to be WWTP specific, however, and there are also cases where the relative abundances of some resistance genes have reportedly decreased from WWTP influent to effluent. In this context it is also important to investigate the microbial composition of WWTP influents and effluents, and existing data suggests that the rarer species of WWTP microbial communities may have a relatively large impact on the abundance of antibiotic resistance genes. In this presentation I summarize these results and discuss their implications for the spread of resistance genes within the wider environment.

Plasmid-mediated resistance to polymyxins; animals as reservoirs for humans ?

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Transferable polymyxin resistance in gram negative rods has recently been reported either from human and animal enterobacterial isolates. Colistin is an old drug that was first introduced in 1959 but remaining on the shelf in human medicine for many years due to renal and neurotoxicity. However, it has been and is still being extensively used in veterinary medicine. We are experiencing a renewed interest for that drug due to the rapid emergence of multidrug-resistant gram negatives in human medicine.

The plasmid-borne *mcr*-1 gene encodes a phosphoethanolamine transferase that mediates addition of phosphoethanolamine to the lipid A moiety of the lipopolysaccharide, consequently conferring resistance to polymyxins. This gene is so far being mainly identified in *Escherichia coli*, and to a lesser extent in *Klebsiella* spp. and *Salmonella* spp.

The *mcr*-1 gene from human *E. coli* isolates in many different countries, in all continents. It was also identified from imported food products in Denmark (meat) and Switzerland (vegetables). MCR-1-producing *Salmonella enterica* isolates of different serotypes were identified from food samples in Portugal and France. Noteworthy, an epidemiological survey conducted in France from a collection of extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates recovered from feces of diarrheic veal calves at farm from 2005 to 2014 showed a very high rate of MCR-1-positive isolates (20.5%).

Taken together, those informations indicate that (i) the spread of *mcr-1* is not recent, it has already occurred worldwide at least due to its location on conjugative plasmids, and (ii) *E. coli* is so far the main reservoir of this resistance trait either among human, animal, or environmental isolates. This is a source of concern since *E. coli* isolates are easily exchanged from the environment to humans in which it may stay as a commensal state in the gut flora; also, it is the number one pathogen for humans.

MCR-1 is one of the few and clear examples of the animal origin of a resistance trait that may later hit the entire human health system, along with the examples of some methicillin-resistant *Staphylococcus aureus* clones (such as CC398), the serotype O104:H4 CTX-M-15 ESBL-producing enteroaggregative *E. coli*, and ESBL-producing *Salmonella* spp. strains.

The heavy usage of colistin in veterinary medicine is therefore of concern. By standardising the sales of antimicrobials in relation to the total weight of animals « at risk » treatment across Europe in 2011, it was estimated that polymyxins are the fifth most sold group of antimicrobials (7%). Colistin and polymyxin B are used for treating infections caused by Enterobacteriaceae in rabbits, broilers, veal, beef cattle, dairy cattle, and mostly in pigs in Europe. In addition, in other parts of the world, polymyxins are used as growth promoter, a usage that has been banned in Europe since 2006.

The occurrence of polymyxin resistance in animal isolates has been very likely underestimated and unrecognised for years, since determination of polymyxin susceptibility is difficult. The impact of the use of polymyxins in agriculture was not seriously taken in account as long as there was no critical need for colistin in human medicine. However, the time has changed and a coordinated reevaluation of polymyxin usage in agriculture is urgently needed to prevent selection of polymyxin-resistant isolates in veterinary medicine, that might subsequently be transferred to humans. Nevertheless, decisions to ban polymyxins in agriculture is far to be simple and might be a matter of balancing risk against benefits.

Global surveillance and Ecology of antimicrobial resistance in sewage

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Background: Human and animal populations are increasingly confronted with novel, emerging or re-emerging infectious, zoonotic, and communicable diseases including those that are multi-drug resistant (MDR). Many of these events can be attributed to increased globalization, urbanization, poverty, lack of sanitation, climate change, population growth, and intensive farming. This calls for new and innovative ways to monitor large populations in a global context.

Surveillance of pathogens and antimicrobial resistance (AMR) are essential in disease control and prevention strategies. Exposure to human waste is a well-established risk factor, why sewage has been suggested as an alternative to clinical or individual human samples for population based surveillance.

The rapid developments in high-throughput sequencing and metagenomic analysis offers the potential to simultaneously determine the presence and prevalence of a large number of DNA sequences and thus, greatly enhance our ability to rapidly detect emerging pathogens and related AMR genes.

If monitoring of pathogens and AMR in sewage can provide timely information on pathogens of concern, this information can be used to assist risk managers with information on appropriate prevention and treatment strategies and potential needs for environmental remediation.

Here, we present our vision and current efforts in establishing genomics-based global surveillance of AMR and infectious pathogens. We present results from pilot and proof of concept projects (i.e. airplanes, slum city, and global large cities) where we monitor AMR and pathogens using metagenomics and associated epidemiology.

Methods: Arriving airplanes offer a potentially ideal opportunity for global surveillance collecting toilet waste from passengers. Toilet waste from 18 international airplanes arriving in Copenhagen, Denmark, were collected and investigated by whole community sequencing (WCS) combined with bioinformatics analysis quantifying the occurrence of all known AMR genes and a number of selected pathogens.

Challenging settings, such as urban slums and other informal settlements have proven to be disease hotspots and ideal locations for early detection and warning of novel or re-emerging infectious, zoonotic, and communicable diseases. Thus, provide another unique opportunity to utilize

wastewater and urban sewage to monitor large human populations. Urban sewage was collected from Kibera, an urban slum in Nairobi, Kenya over a period of three months and investigated based on WCS. To evaluate the performance, the results of the metagenomics analysis were compared with the data of a conventional syndromic surveillance program performed on site in Kibera.

Lastly, we launched a Global Sewage Surveillance study, collecting urban sewage from 77 cities across 63 countries including all inhabited continents. The samples were treated in a similar way as earlier explained using high-throughput sequencing and metagenomic analysis determining AMR gene distribution. The MGmapper tool mapped paired-end sequence reads to various databases including the ResFinder database.

Findings: In the airplane study, we found that tetracycline, macrolide and beta-lactam resistance genes were the most abundant in all samples including more critical important AMR (e.g. $bla_{\text{CTX-M}}$) carried on airplanes from South Asia compared to North America.

The metagenomics analysis of the urban sewage from Kibera was able to detect various human disease pathogens and associated AMR i.e. a 60-fold increased relative abundance of *Shigella* within one week. In the same week, considerably higher relative abundances of AMR classes i.e. tetracycline (*tet*A, *tet*40) and fluoroquinolone were observed. This result was supported and in concordance with data of a conventional surveillance. One sample was spiked with a MDR *Salmonella* Typhi containing several resistance genes. The spiked strain was detected as expected leading to high relative abundances of aminoglycoside, sulphonamide, trimethoprim, beta-lactam, and phenicol resistance genes in the respective samples.

Lastly in the Global Sewage Surveillance study, we found a very high abundance of different AMR genes and profound strong regional effects representing a snapshot of the world resistome. African countries were found to have a high prevalence of tetracycline, phenicol and sulfonamide resistance in contrast to more industrialised countries. Thus, in samples from Europe and North American higher macrolide resistance was observed. The diversity of antimicrobial resistance was higher in Asian countries than in the rest of the world with a high degree of quinolone resistance. We identified the specific AMR genes that differentiate the regions and are exploring whether country attribute data can help explain the observed patterns.

Conclusion: The most important outcome of these studies will be a proof-of-concept of “real-time” large-scale population surveillance combining state-of-the-art technology and analytic facilities that provide better and faster detection and control of health risks. The impact from these kinds of projects could establish the foundation for the first surveillance of a large, healthy human population and possibly animal populations. Thus, it could reduce morbidity and mortality through rapid disease detection, reduce the development of antimicrobial resistance through proper drug adherence and enable earlier clinical treatment, and ultimately improve treatment outcome and minimize disease spread. The outcome could lead to a complete paradigm shift in the way infectious disease surveillance of nationwide or disease hot spots are conducted.

World Health Organization Ranking of Antimicrobials According to their Importance in Human Medicine: The WHO CIA List and Guideline Development Process

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The goal of the World Health Organization (WHO) Action Plan on Antimicrobial Resistance is to ensure continuity of successful treatment and prevention of infectious diseases with effective and safe medicines that are used in a responsible way. The Global Action Plan includes strategic objectives that seek to improve awareness and understanding of antimicrobial resistance, strengthen knowledge through surveillance and research, and optimize the use of antimicrobial agents. The Action Plan highlights the need for a "One Health" approach that includes coordination among international sectors and organizations, including human and veterinary medicine, agriculture and environment.

WHO has categorized antimicrobials with respect to importance to human health for use in developing risk management strategies related to antimicrobial use in food-producing animals. The list was first developed in Canberra, Australia in 2005 and is regularly revised by the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR). Antimicrobial classes used in human medicine are categorized into three groups: critically important, highly important, and important, based on two criteria. The critically important classes are further categorized into highest priority and high priority, based on three additional criteria. In the recently released 5th revision of the WHO CIA List, the highest priority critically important antimicrobials are cephalosporins (3rd generation and higher generation), glycopeptides, macrolides and ketolides, polymyxins, and quinolones[1].

Purpose: The WHO CIA List has been used by national authorities, and food and agricultural industries for prioritizing the development of risk management strategies for antimicrobials importance to human health in order to preserve their effectiveness in human medicine. Other uses include: ensuring that critically important antimicrobials are included in antimicrobial susceptibility monitoring programmes; development of prudent use and treatment guidelines in humans and animals; and communicating risks to the public.

[1] http://www.who.int/foodsafety/publications/antimicrobials-fifth/en/

Criteria: Criterion 1 (C1): The antimicrobial class is the sole, or one of limited available therapies, to treat serious bacterial infections in people. Criterion 2 (C2): The antimicrobial class is used to treat infections in people caused by either: 1) bacteria that may be transmitted to humans from non-human sources, or 2) bacteria that may acquire resistance genes from non-human sources. Prioritization criterion 1 (P1): High absolute number of people, or high proportion of use in patients with serious infections in health care settings affected by bacterial diseases for which the antimicrobial class is the sole or one of few alternatives to treat serious infections in humans. Prioritization criterion 2 (P2): High frequency of use of the antimicrobial class for any indication in human medicine, or else high proportion of use in patients with serious infections in health care settings, since use may favour selection of resistance in both settings. Prioritization criterion 3 (P3): The antimicrobial class is used to treat infections in people for which there is evidence of transmission of resistant bacteria (e.g., non-typhoidal *Salmonella* and *Campylobacter spp*.) or resistance genes (high for *E. coli* and *Enterococcus spp*.) from non-human sources.

Guideline development: To further preserve the effectiveness of antimicrobials used in human medicine, WHO is currently in the process of developing an official WHO Guideline based on the WHO CIA List. A WHO guideline contains recommendations about health interventions. WHO uses standards and methods to ensure that guidelines are free from bias, meet a public health need and contain recommendations that are based on a comprehensive and objective assessment of the available evidence. The rigorous guideline development process includes: (i) identification of priority questions and critical outcomes; (ii) retrieval of the evidence; (iii) assessment and synthesis of the evidence, including specifically commissioned systematic reviews using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach; (iv) formulation of recommendations; and (v) planning for the dissemination, implementation, impact evaluation and updating of the guideline. It is anticipated that the guideline development process will be completed in late 2017.

Epidemiology of ESBL-E.coli from the One Health perspective

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Antibiotic resistance in animals becomes a public health concern when there is transmission of antibiotic resistant bacteria, or their resistance genes, from animals to humans. Third and 4th generation cephalosporins are critically important for the treatment of certain human bacterial infections and resistance to this class of antibiotics, mediated by extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases, has emerged in Gram-negative bacteria, especially Enterobacteriaceae. Human infections due to ESBL-producing Enterobacteriaceae are increasing worldwide and are often preceded by asymptomatic carriage. Transmission of these bacteria or their resistance genes from animals to humans might occur via the food chain, by direct contact with animals or via the environment. The relative contribution of each of these routes to carriage among humans remains to be elucidated. Attribution is the process of determining how much of a given „infection“ (or in this case carriage) is due to particular sources and pathways. Attribution is a useful tool to prioritise intervention strategies, but in order to determine the impact of these measures it is necessary to understand transmission dynamics and ultimately quantify transmission in human and animal populations. In this lecture, an overview of recent research on the epidemiology of ESBL-*E. coli* from a OneHealth perspective will be given, addressing animals, humans and the environment. In addition, examples of approaches that might be used to attribute ESBL/AmpC-producing Enterobacteriaceae to different sources and routes, and factors that might hamper the development of these approaches will be discussed.

QnrD in fluoroquinolone-resistant *Proteae* of animal origin

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Background and objectives: Fluoroquinolones (FQ) are widely used in veterinary medicine and numerous *Enterobacteriaceae* are now resistant to these molecules. The major mechanisms refer to chromosomal mutations in the *gyrA/B* and *parC/E* genes, but plasmidic genes (*qnr*) can also confer low-level resistances. In *Proteus* spp, the *qnrD* gene is located on a small plasmid that is readily disseminating. Since few data exist on *qnrD* of animal origin, our goal was to characterize the *Proteae* isolates collected from diseased animals and France and presenting this gene.

Materials and methods: Between 2008 and 2015, 454 clinical *Proteus* spp were collected. Resistances to enrofloxacin (ENR) and ciprofloxacin (CIP) were identified on antibiograms performed by disk diffusion, and the presence of *qnr* genes (*qnrA*/*B*/*S*/*D*) was detected by PCR. MICs to FQs were determined by E-test on all *qnrD*-positive isolates and mutations in the *gyrA*/*B* and *parC*/*E* genes were detected. Plasmids carrying the *qnrD* gene were sequenced by chromosome walking and transformed in the TG1 donor strain. Clonality was assessed by PFGE.

Results: A total of 80 (17.6%) isolates were resistant to ENR, and 14 (12 *P. mirabilis*, 1 *P. penneri*, 1 *P. vulgaris*) carried the *qnrD* gene. These 14 non-clonal isolates were collected from dogs (n=11) and bovines (n=3). MICs of 0.125-4 mg/L and 0.5-32 mg/L were observed for ENR and CIP, respectively. No mutation was detected in the *gyrB*/*parE* genes whereas 11 isolates were mutated in *parC* and 9 in *gyrA*. The *qnrD* gene was carried by small plasmids (2657bp to 2683bp), of which 11 were identical or similar to the prototypic pDIJ09-518a plasmid and 3 were related to the pEAD1-2 palsmid. TG1 transformants carrying the different plasmids showed a 4-fold increase in the MIC of nalidixic acid and a 8- to 16-fold increase in the ENR MIC, irrespective of the detected mutations on the plasmid.

Conclusion: *qnrD* gene was detected for the first time in animal isolates in France, and in 17.5% of the isolates presenting a resistance or a decreased susceptibility towards FQ. This suggests an efficient dissemination of this gene. Plasmid characterization showed that *qnrD* is preferentially carried by pDIJ09-518a-like plasmids, but plasmids related to pEAD1-2 were also detected. Interestinlgy, mutations in these plasmids did not seem to affect their contribution to FQ MIC. Finally, chromosomal mutations were detected in isolates presenting a high-level resistance to FQ.

Human health risk of dietary intake of antibiotic residues in beef in Maroua, Cameroon

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Background and objectives: The contamination of food by chemical hazards is a worldwide public health concern and is a leading cause of trade problems internationally. Based on former work describing the prevalent use and misuse of antibiotics in cattle in the Far North Region of Cameroon, we designed a study to evaluate the risk of antibiotic (penicillin G and oxytetracycline) intake via beef consumption amongst population in Maroua (Far North Region of Cameroon).

Materials and methods: To determine the mean concentration of antibiotic residues in beef, 404 samples of liver and muscle were collected from 202 cattle selected randomly in all the slaughterhouses of Maroua and Godola and analyzed using Liquid Chromatographic tandem Mass Spectrometry (LC-MS-MS). However beef consumption patterns for different populations were determined using a nutrition survey conducted in 202 households selected using a gridded map and random selection method.

Results: Results found revealed that out of 202 cattle 41 (20.30%) showed positive results in one or more of their organs. The average concentration of residues in beef determined was 17.58 µg/kg for penicillin G and 240 µg/kg for oxytetracycline. The estimated daily intakes of penicillin G and oxytetracycline through consumption of beef were 0.048±0.012 µg/Kg and 0.651±0.164 µg/Kg respectively. Based on the estimated intake and comparison with the acceptable daily intake, we assessed the risk in Maroua as acceptable (2.17%) for oxytetracycline and greater (9.6%) for penicillin G.

Conclusion: The findings of the present study could be alarming for the legislative authorities in food security and safety. This highlights a very serious problem, both for the consumers of Maroua and the herders of the region as for the whole economy of Cameroon. It would therefore be important that measures be taken at several levels by the actors of the sector (public authorities, veterinary auxiliaries, etc.) to guarantee the safety of the food from animal origin.

WGS-based elucidation of linezolid resistance locus *optrA* in clinical *Enterococcus* spp. isolates from Germany

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Background and objectives: Linezolid (LZD)-resistant *Enterococcus* spp. (LRE) are generally detected at low prevalence. However, the National Reference Centre (NRC) for Staphylococci and Enterococci in Germany has received an increasing number of clinical LRE isolates in recent years. Resistance to this last resort antibiotic can be achieved, amongst others, by acquisition of the methyltransferase Cfr or the ABC-transporter OptrA. Both genes were described to reside on plasmids or other mobile genetic elements. It has been hypothesized that these elements were co-selected by antibiotic use in livestock and emerged in staphylococci and enterococci from food-producing animals. Little is known about the prevalence of OptrA in clinical *Enterococcus* (E.) isolates from Germany. Thus, we aimed to comprehensively asses the prevalence, loci organization and transferability of *optrA* in LRE received by the NRC from 2007 until 2016.

Materials and methods: In total, 540 LRE isolates were screened by PCR for the presence of *optrA*. Twenty *optrA*-positive LRE were subjected to whole genome sequencing (WGS). Localization of *optrA* was additionally examined by S1-PFGE and Southern hybridization. Transferability of *optrA* was assessed by filter-mating to *Enterococcus* spp.

Results: A high proportion of LZD-resistant *E. faecalis* were tested positive for *optrA*. It is worth mentioning that within the collection of isolates an increase of *optrA*-positive *E. faecalis* isolates from 0 % to 90.9 % (2007 to 2016) was observed. In total, 9 different *optrA* variants were detected. Phylogenetic analyses clearly demonstrated that multiple introduction events of the resistance locus had occurred and that this was independent of the date or federal state of isolation. Bioinformatics analyses produced plasmid sequences identical to already described *optrA*-containing vectors as well as novel *optrA* insertion sites. Successful transfer of the resistant determinant to susceptible recipients was achieved.

Conclusion: Our analyses suggest that highly plastic *optrA*-encoding mobile genetic elements emerged especially in *E. faecalis* clinical isolates in recent years. This represents a worrisome situation with respect to resistances against last resort antibiotics and demonstrates the One Health dimension of a putative co-selective effect by antibiotic use in either sector. As transfer of the resistance locus is possible, immediate attention and thorough examination of *optrA* transmission is required in order to prevent further dissemination of multi-drug resistant pathogens.

Prevalence of extended-spectrum beta-lactamase- and AmpC-producing *Escherichia coli* in dairy calves: does age matter?

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Background and objectives: Extended-spectrum beta-lactamase (ESBL)- and plasmid-mediated AmpC beta-lactamase (AmpC)-producing *Enterobacteriaceae* show resistance to beta-lactam antimicrobials including third generation cephalosporins, which are categorized by the WHO as 'critically important for human medicine'. These bacteria are still emerging worldwide and are therefore of great importance for public health. Several studies have been conducted on ESBL/AmpC-producing *E. coli* in dairy cattle. However, little is known about the prevalence and the moment of first colonization of ESBL/AmpC-producing *E. coli* in young dairy calves.

Materials and methods: A cross-sectional study was conducted followed by a longitudinal study. The cross-sectional study included 196 randomly selected conventional dairy farms. From these herds, faecal samples of all calves (≤21 days) were collected. Next, ten of these 196 farms were selected and revisited three times with an interval of three months. Of all the calves (≤21 days, n=73) present during the cross-sectional study faecal samples were collected again during these three visits. Molecular typing was used for further determination of phenotypically confirmed ESBL- and AmpC-producing *E. coli*.

Results: The animal prevalence of ESBL/AmpC-producing *E. coli* in calves (≤21 days, n=681) was 33% (95% CI:30-37%). Per day of age the median of the number of sampled calves was 32 (range: 7-48). The highest prevalence of ESBL-producing *E. coli* was found in the cohort of calves aged three to four days (n=79, 24% (95% CI: 15-33%)). In the cohort of calves aged five to six days (n=72) the prevalence was 6% (95% CI: 3-11%), which was significantly lower (proportion test, $p<0.01$). The prevalence of ESBLs remained below 14% in the following cohorts of calves aged seven to 21. The calves that were followed for one year and sampled at all four sampling moments (n=45), showed that the persistence of ESBLs/AmpCs is limited. Of the 28 calves (62% , 95% CI:47-76%) that were positive at the first sampling, none (one-sided 97.5% CI:0-12%) tested positive again after four months, 14% (95% CI:4-33%) after eight months and 4% (95% CI:1-18%) after twelve months.

Conclusion: This study showed that calves can become colonized with ESBL/AmpC-producing *E. coli* in the first days after birth. However, the prevalence reduces with increasing age suggesting that most positive calves stop shedding ESBL/AmpC-producing *E. coli* within the first year.

NGS-based analysis of AmpC-β-lactamase CMY-2-producing *Escherichia coli* from humans, livestock and food in Germany

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Background and objectives: Resistance to third-generation cephalosporins in *Escherichia coli* is mainly mediated by extended-spectrum beta-lactamases (ESBLs) and AmpC-beta-lactamases. Overexpression of the naturally, chromosomal-located *ampC* gene of *E. coli* causes cephalosporin resistance, but more common are plasmid-encoded AmpC enzymes (e.g. CMY, ACC, DHA) that were acquired from other species. The most frequent AmpC enzyme is CMY-2. It is produced by ca. 1% and ca. 30% of the third-generation cephalosporin-resistant *E. coli* from humans and poultry, respectively.

To identify possible pathways of transmission of the bla_{CMY-2} gene or CMY-2-producing *E. coli* clones, we performed whole-genome sequencing of 170 isolates collected between 2008 and 2016 all over Germany in the scope of different studies of the national research project “RESET”.

Materials and methods: CMY-2 positive *E. coli* from different sources (humans n=51, healthy broilers n=51, chicken meat n=56, turkey meat n=7, diseased pigs/chickens n=5,) were sequenced using the Illumina MiSeq platform. Resistance genes and phylogenetic markers, such as multi-locus sequence type and plasmid replicon types were identified (CGE Finder series).

Results: The 170 sequenced isolates showed a highly diverse distribution of sequence types (STs) and replicon types. Fifty-nine different STs were identified; most prevalent types were ST38 (n=19) as well as ST131 (n=16) and ST117 (n=13). The highest intersection of STs between the different reservoirs were found for ST131 (human n=8/food n=2/animal n=6) and ST38 (3/9/7). Frequent plasmid replicon types were FIB (n=138) and FII (n=90), IncI1 (n=87) and IncK (n=80). Analyses of the bla_{CMY-2} containing contigs revealed the replicon types IncK (n=74) and IncI1 (n=62) as the gene bearing plasmidic backbone for most of the isolates. Additional beta-lactamase genes (bla_{TEM}, bla_{CTX-M}, bla_{OXA}, bla_{SHV}) were detected in 50% of the isolates.

Conclusion: The results showed clonal relatedness for CMY-2-positive *E. coli* from different origins for the clonal lineages ST131 and ST38. Frequent correlation of a plasmid replicon type to distinct STs was shown for IncK and ST58, ST429 and ST38. However, the majority of isolates belonged to various clones and harboured different bla_{CMY-2}-bearing plasmids. This indicates a more likely plasmid-mediated spread rather than a clonally driven spread of bla_{CMY-2} across the *E. coli* host populations.

Fecal carriage and characterization of extended-spectrum β-lactamase- and AmpC β-lactamase-producing *Escherichia coli* from healthy horses in France

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Background and objectives: Companion animals in close contact with humans should be regarded as a potential reservoir of extended-spectrum cephalosporins (ESC)-resistant bacteria. The objective was to determine the fecal carriage of ESC-resistant *Escherichia coli* isolated from healthy horses in France and to characterize the genetic determinants responsible for ESC resistance.

Materials and methods: Fecal samples from 738 randomly selected healthy adult horses were collected in 41 horse stables during the summer of 2015 in France and screened for the presence of ESC-resistant *E. coli* strains. The ESC resistance genes among non-redundant *E. coli* strains were determined using PCR and sequencing. ESC phenotypes were horizontally transferred by conjugation or transformation. Extended-spectrum-β-lactamase (ESBL)- or AmpC-carrying plasmids were typed by PCR-based replicon typing, restriction fragment length polymorphism, and multilocus sequence typing. The ESC-resistant *E. coli* strains were typed by *Xba*I macrorestriction analysis, phylogroup determination, and virulence genes profile.

Results: In 16/41 (39%) of the stables, at least one horse carrying ESC-resistant *E. coli* isolates was identified. ESC-resistant *E.coli* isolates were found in 26/328 (7.9%) of the horses screened individually. Fifty-one non-duplicate ESC-resistant *E. coli* isolates were included in the molecular resistance analysis. All these isolates showed a great diversity of *Xba*I macrorestriction profiles, belonged mainly to phylogroup B1, and were negative for major *E. coli* virulence genes in animals (*eae*, *stxA*, *stx2A*, *iutA*, *eltB*, *estA*, *estB*) suggesting that they are commensal, non-pathogenic isolates. The ESBL $bla_{CTX\text{-}M}$ genes were dominant ($bla_{CTX\text{-}M\text{-}1}$, n=35; $bla_{CTX\text{-}M\text{-}2}$, n=8; $bla_{CTX\text{-}M\text{-}14}$, n=2). The ESBL/AmpC genes were identified on various conjugative plasmids belonging to the IncHI1, IncI1, IncN, and IncY groups and with different additional non-β-lactam resistance phenotypes. Interestingly, the most prevalent ESBL genes, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}2}$, were mainly located on large conjugative IncH1 plasmids. RFLP and MLST plasmid analysis are underway to assess the relatedness of these ESBL plasmids.

Conclusion: For the first time, a large-scale survey revealed a significant carriage of ESBL-producing *E. coli* and plasmids carrying ESBL genes in faeces from healthy horses in France. Presence of ESBL plasmids in the intestinal microflora of horses may have significant implications for horses and public health in France.

Faecal carriage of ESBL- and AmpC –producing *Escherichia coli* from co-habiting people and pets

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Background and objectives: Companion animals often live as members of a household, and may be a risk for human carriage or infection with extended-spectrum ß-lactamase (ESBL) producing Enterobacteriaceae. The objective of this study is to determine whether pets play a role in the transmission of antimicrobial resistant (AMR) bacteria within households.

Materials and methods: People selected as part of an on-going prospective case-control study of community acquired ESBL - or AmpC ß-lactamase - producing *E. coli* urinary tract infections (UTI) in Auckland, New Zealand were asked for faecal samples from themselves and any other family members and/or family pets. Faecal samples from these households were screened for ESBL- and AmpC ß-lactamase- producing Enterobacteriaceae isolates using selective and differential agar, where up to eight colonies from each sample were selected for further evaluation. ESBL or AmpC ß-lactamase production by these faecal isolates was confirmed using a disk-diffusion assay according to EUCAST recommendations. Sixty-nine ESBL- or AmpC- producing *E. coli* isolates from five households were whole genome sequenced and their genetic relatedness compared using MLST and SNP analysis.

Results: Nineteen faecal samples were collected (11 humans, 8 animals) across five households. Two of the 8 animal samples were positive for ESBL or AmpC ß-lactamase producing bacteria. Of the human samples, 10/11 were positive for ESBL or AmpC ß-lactamase producing bacteria, including five samples from people previously identified as having an ESBL-producing UTI. From these 19 faecal samples, 68 ESBL-producing *E. coli* (including 12 isolates that were also AmpC ß-lactamase producers) and one AmpC ß-lactamase producing *E. coli* were isolated. Using the results of SNP analysis, the genetic relatedness of these 69 isolates revealed evidence of clustering within households. Multilocus sequence type ST-131 accounted for 37/69 isolates, all from humans, and the remaining isolates were distributed between eight other MLST types. One animal was found to be carrying the same ST type as a human within the same household, and the isolate was also clustered with other human isolates on SNP analysis.

Conclusion: While animals may play a role in transmission or risk of acquisition of AMR bacteria to humans, we provide evidence of transmission between humans within households. The risks animals and humans provide to each other requires further investigation.

Epidemiology of carbapenemase-producing *Enterobacteriaceae* in a farrow-to-finish swine production system in the United States

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Background and objectives: Carbapenemase-producing *Enterobacteriaceae* (CPE) present an urgent threat to public health. While carbapenem antimicrobials are restricted in food-producing animals, other β-lactams, such as ceftiofur, are frequently applied in livestock. This use may provide selection pressure favoring the amplification of carbapenem resistance but this relationship has not been established. Recently reported in US livestock, plasmid-mediated CPE have also been reported from livestock in Europe and Asia.

Materials and methods: We previously reported the presence of the rare carbapenemase gene, $bla_{\text{IMP-27}}$, in the environment of a large farrow-to-finish swine environment. To gain a better understanding of CPE in this swine production system, we followed a cohort of 350+ market pigs from late sow gestation to the final finishing phase. Environmental and fecal samples were collected during 8 visits over a 5-month period in 2016. Samples were screened using selective media for the presence of CPE, with resulting carbapenemase-producing isolates further characterized.

Results: Of 55 environmental and 109 sow fecal samples collected from a farrowing barn on our initial visit, 35 (64%) environmental and 15 (14%) sow fecal samples yielded isolates of multiple *Enterobacteriaceae* species carrying the metallo-β-lactamase gene $bla_{\text{IMP-27}}$ on an IncQ1 plasmid. The frequency of IMP-27 positive environmental (n=32), sow fecal (n=30), and piglet fecal swab (120) samples was highest for all groups when the market pig cohort was between 1 and 10 d, with observed prevalence of 97%, 28%, and 18%, respectively. After weaning, $bla_{\text{IMP-27}}$ was detected in only a single environmental sample from the wall of a nursery pen, with no CPE recovered from pigs in the finishing phase.

Conclusion: Frequently used in US swine production to treat and control disease, piglets on this farm receive ceftiofur at birth, with males receiving a second dose at castration (≈day 6). This selection pressure may favor the dissemination of $bla_{\text{IMP-27}}$-bearing *Enterobacteriaceae* in this farrowing barn. The absence of this selection pressure in the nursery and finisher barns likely resulted in the loss of the ecological niche needed for maintenance of this carbapenem resistance gene.

Carbapenemase producing *Escherichia coli* isolated from German retail seafood

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Background and objectives: Within the last couple of years it was shown that the occurrence of carbapenem resistant bacteria is no longer limited to clinical settings. Carbapenemase-producing bacteria have been isolated from environmental surroundings as well as wild birds, companion- and food-producing animals all over the world. This situation is worrying and depicts an important issue for the public health sector. In 2013, the EU legislation implemented the monitoring of carbapenem-resistance in *Salmonella* and *E. coli* in food-producing animals as well as meat samples derived thereof (2013/652/EU). However, vegetables, fruits or sea food, are frequently consumed raw and might also be a source of antimicrobial resistant bacteria. In the here described study, seafood samples derived from retail markets in Berlin, Germany (sampled from December 2015 to August 2016) were investigated for the presence of carbapenemase-producing *Enterobacteriaceae*.

Materials and methods: A set of 45 *Enterobacteriaceae* isolated from clams and shrimps were investigated for the presence of the carbapenemase genes bla_{VIM}, bla_{NDM}, bla_{OXA-48}, bla_{KPC} and bla_{GES} using real-time PCR. Positive isolates were further investigated by MIC determination, plasmid transformation and whole genome sequencing, followed by a subsequent data analysis using the CGE platform (http://www.genomicepidemiology.org/).

Results: Out of the 45 investigated isolates, one *E. coli* (ST10) derived from a Venus clam - harvested in the Mediterranean Sea - contained bla_{VIM-1}. This gene was part of the variable region of a class I integron accompanied by the resistance genes *aac*A4, *aph*A15, *aad*A1, *cat*B2 as well as *sul*1. Whole genome analysis showed that beside the class 1 integron, several additional resistance genes including the extended-spectrum beta-lactamase bla_{SHV-12} and the fluoroquinolone resistance gene *qnrS1* were co-located on the same IncY plasmid. In addition bla_{ACC-1} was detected within the bacterial chromosome.

Conclusion: The presence of carbapenemase-producing *Enterobacteriaceae* in German retail seafood is worrisome and emphasises the importance of further monitoring programs as well as the admittance of seafood samples into the national surveillance programmes. In the same way intervention strategies including the prevention of an environmental spread of the resistant bacteria are crucial in human- as well as veterinary medicine.

Antibiotic resistance prevalence of *Pseudomonas* spp. and identification of metallo-β-lactamase producing *Pseudomonas*. spp. in wastewater treatment plant effluent

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Background and objectives: Antibiotic resistant bacteria are an emerging environmental concern with a potential impact on human health. Wastewater treatment plants (WWTP) have been recognized as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. The studies on antibiotic resistance in aquatic environments are mainly performed on *Escherichia coli* and *Enterococcus* spp. However, there are few studies on antibiotic resistance in other pathogens such as *Pseudomonas* spp. The objectives of this work were to determine the antibiotic resistance pattern of *Pseudomonas* spp. in WWTP effluent and to identify the possible mechanism(s) mediating imipenem resistance.

Materials and methods: Cultivable *Pseudomonas* spp. were enumerated and isolated from two WWTP effluents (WWTP A and B) on cetrimide agar with supplements of amoxicillin (32 mg/L), tetracycline (16 mg/L) and ciprofloxacin (1 mg/L) by a membrane filtration method. The antibiotic resistance profile of these bacteria were characterized using the agar dilution method. Imipenem resistant strains were identified by a disk diffusion method. These strains were screened for metallo-β-lactamase by a double disk synergy test using Imipenem and Imipenem EDTA**.**

Results: Among all *Pseudomonas* spp. enumerated from WWTP A and B respectively, the most prevalent resistance phenotype was amoxicillin (44%, 39%), followed by ciprofloxacin (10.4 %, 19.3 %) and tetracycline (7.8 %, 9.8 %). Multidrug resistant strains of *Pseudomonas* spp. were detected from both WWTPs at a low rate (2%). Thirty-one strains were imipenem resistant, from which 20 showed positive results by the double disk synergy test using Imipenem and Imipenem EDTA.

Conclusion: The resistant *Pseudomnas* spp. including MDR strains and imipenem resistant strains in WWTP effluent presented a problem of the dissemination of these bacteria in the environment. Our results suggest that *Pseudomonas* spp. should be monitored as a possible source of mobile antibiotic resistance determinants from WWTP effluent, which can be transferred between bacterial communities including human and animal pathogens. Currently there are no legislative requirements relating to treatment of or concentration of antibiotics, antibiotic resistant bacteria or ARGs in treated effluents. Conventional WWTPs are not specifically designed to treat or remove these contaminates.

Frequent detection of CRE in Dutch municipal wastewater

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Background and objectives: In the Netherlands, reports of infections with carbapenem-resistant *Enterobacteriaceae* (CRE) are still scarce. A preliminary study in 5 Dutch wastewater treatment plants (WWTP) demonstrated a discordantly high prevalence of CRE in municipal wastewater. Since municipal wastewater reflects carriage in the population, this finding suggested a higher level of dispersion of CRE than previously assumed. The goal of the present study was to establish Dutch prevalence of CRE based on a representative set of 100 WWTP, and identify risk factors associated with prevalence.

Materials and methods: Selected WWTP varied in size and with respect to supply of wastewater from health care institutions. CRE from influents and effluents were isolated and enumerated on ChromIDCARBA and ChromIDOXA-48 agar. Isolates were characterized with respect to carbapenemase genes (KPC, NDM, OXA-48-like), ESBL-genes, and sequence type.

Results: CRE were detected in 90% of the WWTP, and in 55% of the effluents. The most prevalent species were $bla_{OXA\text{-}48\text{-}like}$ positive *E. coli* (OXA-48-like-EC; 88%). Influent concentrations of these bacteria were on average 1.9×10^3 cfu/l, and 500 times lower than those of ESBL-producing *E. coli* (ESBL-EC). Given the approximate prevalence of ESBL-EC of 5%, this extrapolates to 1 in 50,000 people carrying OXA-48-like-EC. Carriage in the community was supported by a relatively small effect of WWTP size and presence of health care institutions on OXA-48-like-EC concentrations. Other carbapenemase-producing *Enterobacteriaceae* (CPE) were $bla_{OXA\text{-}48\text{-}like}$ carrying *K. pneumoniae* (33%) and *Enterobacter* spp. (2%), bla_{NDM} carrying *E. coli* (18%) and *K. pneumoniae* (6%), and bla_{KPC} carrying *K. pneumoniae* (8%) and *Enterobacter* cloacae (1%). Detected carbapemenase genes included OXA-48 (82%), OXA-181 (34%), OXA-162 (1%), NDM-1 (8%), NDM-5 (15%), NDM-7 (1%), and KPC-2 (9%). Of all CRE isolates, 39% additionally carried a $bla_{CTX\text{-}M}$ type ESBL gene. NDM- and KPC-type CPE were most prevalent in, but not restricted to, the most densely populated areas. Concentrations of CRE-types other than OXA-48-like-EC, in particular CR *K. pneumoniae* and *Enterobacter* spp., appeared to be strongly associated with WWTP size and presence of health care institutions.

Conclusion: CRE are more common than assumed based on clinical reporting. They are emitted to the aquatic environment with discharge of treated wastewater. Dissemination of CRE may pose a great risk to public health.

Monitoring antibiotic usage in animals: Do existing systems provide comparable data?

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Background and objectives: In order to assess the risk of antibiotic resistance (AMR), it is crucial to know how many and which antimicrobials are used in veterinary medicine. Hence, monitoring of antimicrobial use (AMU), especially in livestock, is an important instrument in understanding and combatting the development of AMR. Monitoring systems exist in many countries, but there is no global consensus on the documentation of AMU, thus hampering comparability of data between countries and the human and veterinary sector. The main objective of this study is to analyse how AMU monitoring systems are set up and conducted in different countries, which key figures and variables are used to describe and evaluate antimicrobial consumption in animals, and whether they facilitate integration with antimicrobial resistance data.

Materials and methods: The most relevant systems of monitoring AMU are analysed and the different methods for estimating AMU are compared in order to evaluate their fitness for purpose. Differences in data collection, analysis and documentation are described.

Results: Most countries collect overall sales data on antimicrobials, but some do also provide data on the used level of farms or animal species and production type. In that cases, key figures and variables differ a lot between countries. Additionally, some systems aim at benchmarking farmers and/or veterinarians. Antimicrobial consumption data should be contrasted to the respective animal population, and the choice of the denominator also influences the outcome.

Conclusion: It is not recommended to use sales data to evaluate antibiotic consumption if the purpose is to analyse the impact on selection of antimicrobial resistance. In this regard, most existing AMU monitoring systems are not sufficient yet. Moreover, the differences in systems that collect more detailed information, e.g. on use at species level, do not allow for direct comparison. An integrated One Health approach to monitor AMU is needed. At international level, several organizations (e.g. EU, OIE, WHO) have initiatives to support the development of antimicrobial consumption data collection and reporting. However, these initiatives are still in process. Up to now, existing monitoring systems for antibiotic consumption in animals are lacking harmonisation, and most of them do not provide enough information to evaluate the risk of AMR emerging from use of antimicrobials in veterinary medicine.

Used Daily Dose vs. Defined Daily Dose – Advantages and disadvantages of different dosage assumptions for the monitoring of antimicrobial usage in livestock.

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Background and objectives: Antimicrobial resistance is a serious threat for public health globally and the reason why antibiotic usage in livestock is becoming more and more subject of public debate. Tackling the problem of rising resistances requires amongst others valid data, which as a consequence requires harmonized monitoring of antibiotic use and a benchmarking system on farm level.

Up to date there is no harmonized monitoring of antibiotic use and system for assessment of these data Europe-wide, which may facilitate a direct comparison between different European countries. Most of the monitoring systems are based on sales data. Therefore, to assess the number of animals treated, overall assumptions about the defined daily doses and also about the weights of the treated animals have to be made. Only few monitoring systems are collecting data that makes the calculation of used daily doses possible.

Materials and methods: Within the VetCAb research project (Veterinary Consumption of Antibiotics; see vetcab-s.de) data about the use of antibiotics in livestock in Germany in the last several years were collected and evaluated. The data collection is based on official application and delivery forms, voluntarily provided by veterinarians and farmers. Up to now there are more than 200 000 records for the years 2011, 2013, 2014 and 2015 in the database, providing the basis for detailed evaluations. The aim of the study is to analyze how often food producing animals were treated with antibiotics during a given time period.

Results: Compared to other antibiotic monitoring systems, the VetCAb-database maintains not only data about amounts of each antimicrobial delivered to the farmers, but also detailed information about the number of animals treated, the treatment duration, application route and also the indication. Therefore the calculation of the used daily dose for every treatment is possible. In this evaluation we calculated the treatment frequency for each farm on the basis of UDD and in contrast the treatment frequency based on the DDD published by ESVAC in April 2016. Results showed that there are differences between both outcomes, which may have serious implications for the benchmarking of farms. Furthermore, it reflects that the calculation procedure also has an impact on the comparison between populations which needs further reflection.

Conclusion: In our presentation we are going to present the results of these evaluations for pigs, cattle and poultry and to argue the advantages and disadvantages of these two calculation methods.

Association between veterinary antibiotic use and resistance in commensal *Escherichia coli* from livestock in Belgium between 2011 and 2015.

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Background and objectives: Antibiotic use is a significant factor in the selection and spread of antibiotic resistance in commensal and pathogenic bacteria. Bacteria are frequently found resistant to many antibiotics to the point that both animal and public health are now seriously challenged. Exploring the possible association between antibiotic use and resistance is a highly desirable exercise that was tentatively completed in the present study, focusing on commensal *Escherichia coli* (*E. coli*) from livestock in Belgium.

Materials and methods: Annual data on antibiotic use and resistance from 2011 to 2015 were retrieved from the Belgian veterinary surveillance program on antibiotic use and resistance of *E. coli* from veal calves, young beef cattle, pigs and broiler chickens. Different algorithms were explored to perform a temporal trend analysis on veterinary antibiotic use on the one hand and on resistance to 11 antibiotics in *E. coli* from livestock species on the other hand. The correlation between the average antibiotic resistance prevalence per antibiotic for all livestock species and the use of the corresponding antibiotic class and the total use were investigated. Additionally, a logistic regression analysis intended to quantify the effect of antibiotic use on the prevalence of resistance.

Results: A continuous decreasing trend in antibiotic use was observed for all classes, except for the phenicols. Antibiotic resistance of commensal *E. coli* significantly decreased for several of the tested antibiotics in all livestock species. A more rapidly reverted resistance was seen to $3^{th}/4^{th}$ generation cephalosporins and fluoroquinolones. Moderate to strong correlations between antibiotic use and resistance were found, except for antibiotic resistance to chloramphenicol and gentamicin and the use of the corresponding antibiotic class. Yet, total antibiotic use was positively correlated with chloramphenicol resistance, showing the potential importance of co-selection of chloramphenicol resistance. Odds ratios (>1) were higher when associating antibiotic resistance to the use of the corresponding class than to the total use.

Conclusion: The continuous decrease in antibiotic use appears to have a positive effect on the levels of resistance of most antibiotic classes. Analyses were performed on small datasets, though, and care must be taken while making inference. Also, for more meaningful discussions, antibiotic use data at animal species and farm level are needed.

The effect of antibiotic use on resistance in commensal *Escherichia coli* from pre-weaned calves on a large commercial dairy farm in Washington State

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Background and objectives: Antibiotics are commonly used in pre-weaned calves to treat diarrheal and respiratory diseases. Evaluating the impact of antibiotic use in food animals on development and dissemination of resistance is important for developing strategies to mitigate resistance in zoonotic bacteria. We evaluated the impact of antibiotic treatments on resistance in fecal commensal *Escherichia coli* from 1–11 week old pre-weaned dairy calves.

Materials and methods: This repeated cross sectional study commenced in June 2016 with antibiotic use data collected daily and fecal samples collected at 6 week intervals. From August 2016 through January 2017, fecal samples were collected biweekly. During each fecal sampling, a total of 9–11 samples were collected across age categories. We cultured 127 fecal samples from 121 calves and obtained 504 isolates and determined minimum inhibitory concentration to 12 antibiotics using agar microdilution assay. Latent class regression analysis was performed to determine association between age, antibiotic treatments, and resistance class.

Results: Calves received a combination of antibiotic treatments 1–2 times (n = 68), 3–4 times (n = 34), or 5–6 times (n = 5), while 14 calves were untreated. Forty one calves (33.9%) received enrofloxacin treatment and isolates from these calves were more likely to be resistant to ciprofloxacin (odds ratio 2.3, 95% CI: 1.5–3.5) and nalidixic acid (odds ratio 2.7, 95% CI: 1.8–3.9) than isolates from calves untreated with enrofloxacin. Whereas only 3 calves received ceftiofur treatment, 27.4% of the isolates were resistant to ceftiofur. Latent class analysis of resistance data revealed five main resistance classes with 23.2% of the isolates having high probability of resistance to 11 antibiotics. Latent class regression analysis revealed an association between age, treatment intensity, enrofloxacin treatment, and resistance class. Particularly, 2–3 week old calves were more likely to be in a class with resistance to ciprofloxacin, nalidixic acid and ceftiofur than 1 week old calves. Likewise, calves that received 3–4 treatments were more likely to be in highly resistant classes than untreated calves.

Conclusion: Enrofloxacin use was associated with fluoroquinolone resistance while ceftiofur resistance could be attributed to co-selection resulting from use of other antibiotics. These findings could be useful in informing policies to mitigate antimicrobial use in food animals.

The use of antimicrobial drugs in fattening pigs in Italy

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Background and objectives: Antimicrobial resistance is a global threat causing annually almost 25 thousands deaths in EU and costs of 10s of billions of Euros. One main risk factor is the indiscriminate use of antimicrobials both in humans and in animals. In particular the antimicrobial consumption in veterinary medicine has been associated with the spread of resistant microorganisms from animals to humans. In Italy, the only existing data collection on veterinary antimicrobials refers to sales data. To make available consumption data, an ad hoc study was carried out in fattening pigs herds to collect data on drug prescription and administration and to evaluate the appropriate use of the antimicrobials.

Materials and methods: Over the period between January 2013 and December 2015 five fattening pig farms were recruited to the study in a small area of the Piedmont Region. Data on veterinary antimicrobial prescription and administration were collected and entered in a database to calculate: the animal Defined Daily Doses (nDDDvet), the animal Defined Course Doses (nDCDvet), the number of DDDvet/100animals/day and the appropriate use. The latter was calculated as the ratio of the used daily dose (UDD) to the DDDvet. Overall, 22,989 pigs and 221 veterinary prescriptions were considered.

Results: The most often applied oral antimicrobial drug groups were tetracycline (23%), macrolides (21%), lincosamides (18%), beta-lactams (12%), associations of active ingredients (10%), fluoroquinolones (6%), amphenicols (6%), pleuromutilins (4%). Large differences were found in antimicrobial use between farms, probably depending on herd-specific diseases, differences in herd management systems and/or veterinarian prescription habits. When the use was considered appropriate, differences in the UDD/DDDvet ratio were found between animal categories, with a peak of 45% underdosed administration at the end of the fattening cycle.

Conclusion: nDDDvet, nDCDvet, UDD, DDDvet and their ratio are valuable indices to use in evaluating antimicrobial administration at farm level. In this study, the nDDDvet, nDCDvet indices showed large differences in antimicrobial drug consumption between herds. Based on the UDD/DDDvet ratio, we have been able to show that antimicrobial drug treatments are often not administered at the correct dose, as they revealed a high number of underdosage. However as the UDD/DDDvet ratio may also be affected by the animal weight at treatment, care is needed in the collection of accurate data.

Monitoring antimicrobial resistance in the Norwegian environment using wild red foxes as an indicator

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Background and objectives: Wild animals can be reservoirs of antimicrobial resistant (AMR) bacteria. Red fox is widely distributed throughout Norway, and a good indicator species for monitoring AMR in its habitat. In urban areas, red foxes may interact with human waste and infrastructure. Red fox is a top predator species, possibly accumulating AMR bacteria from consumption of prey. The red fox preferred habitat is highly adaptive, ranging from non-inhabited, remote areas to large cities. This enables us to study possible alterations in the occurrence of AMR bacteria in foxes living in areas with different human population densities. The aim was to assess the occurrence of AMR bacteria in Norwegian red foxes and investigate possible associations with human population densities.

Materials and methods: Faecal samples from 287 foxes in the Norwegian monitoring programme for *Echinococcus multilocularis* were investigated. Two strategies for detection of AMR bacteria were used; 1) selective screening for *Escherichia coli* resistant to cephalosporins, carbapenems, quinolones or colistin, and enterococci resistant to vancomycin and 2) non-selective culturing and susceptibility testing of one indicator *E. coli* from each sample. Broth microdilution was used for susceptibility testing. PCR and sequencing was done to detect resistance genes. The isolates were divided in three different risk groups based on population density; low exposure (<five inhabitants per km^2), medium exposure (five-200 inhabitants per km^2) and high exposure (>200 inhabitants per km^2).

Results: Preliminary results showed that *E. coli* resistant to carbapenems, colistin or vancomycin were not detected. *E. coli* resistant to cephalosporins and quinolones were detected with low to moderate frequencies. The selective screening indicates a difference in the occurrence of quinolone resistance in low exposure areas with 9% as compared to medium and high exposed areas with 18% and 21%, respectively. The occurrence of cephalosporin resistance was 1% in low exposure areas, while it was 4% and 5%, in medium and high exposed areas, respectively. For indicator *E. coli*, the occurrence of AMR was 20% in the high risk areas while it was 13% and 9% in low and medium exposed areas.

Conclusion: *E. coli* resistant to important antimicrobials occur with low to moderate rates in red foxes in Norway. Furthermore, our results indicate that human population density is a driver for the prevalence of AMR in Norwegian wildlife.

Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study.

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Background and objectives: *mcr-1*-positive *Enterobacteriaceae* (MCRPE) have attracted substantial medical, media, and political attention; Herein, we report the prevalence of MCRPE in human infections and carriage, clinical associations of *mcr-1*-positive *Escherichia coli* (MCRPEC) infection, and risk factors for MCRPEC carriage.

Materials and methods: We undertook this study at two hospitals in Zhejiang (ZJ) and Guangdong (GD), China. We did a retrospective cross-sectional assessment of prevalence of MCRPE infection from isolates collected from 2007 to 2015. We did a retrospective case-control study of risk factors for infection and mortality after infection, using all MCRPEC from infection isolates and a random sample of *mcr-1*-negative *E. coli* (MCRNEC) infections from the retrospective collection from 2012 to 2015. We also did a prospective case-control study to assess risk factors for MCRPEC from inpatients and collected in 2015, compared with MCRNEC from inpatients. Strains were analysed for antibiotic resistance, plasmid typing, and transfer analysis, and strain relatedness.

Results: We identified 21621 isolates of *Enterobacteriaceae* and other species from 18698 inpatients and 2923 healthy volunteers. Of 17498 isolates associated with infection, *mcr-1* was detected in 76 of 5332 *E. coli*, 13 of 348 *K. pneumonia*, one of 890 *Enterobacter cloacae*, and one of 162 *Enterobacter aerogenes*. For the infection study, we included 76 MCRPEC and 508 MCRNEC. Overall, MCRPEC infection was associated with male sex (p=0.011), immunosuppression (p=0.011), and antibiotic use, particularly carbapenems (p=0.002) and fluoroquinolones (p=0.017), before hospital admission. For the colonisation study, we screened 2923 rectal swabs from healthy volunteers, of which 19 were MCRPEC, and 1200 rectal swabs from patients, of which 35 were MCRPEC. Antibiotic use before hospital admission (p<0.0001) and living next to a farm (p=0.03) were associated with MCRPEC carriage in 35 patients compared with 378 patients with MCRNEC colonisation. *mcr-1* could be transferred between bacteria at high frequencies (10^{-1}-10^{-3}), and plasmid types and MCRPEC MLSTs were more variable in GD than in ZJ and included ST131.

Conclusion: Infection with MCRPEC is associated with sex, immunosuppression, and previous antibiotic exposure, while colonisation is also associated with antibiotic exposure. MLST and plasmid analysis shows that MCRPEC are diversely spread throughout China and pervasive in Chinese communities.

The Environmental Contamination of NDM and MCR-1 in Food Animal Production Chain

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Background and objectives: Antimicrobial resistance is now recognized as one of the most serious global threats to human and animal health. These concerns are exemplified by the rapid increase in carbapenem resistance Enterobacteriaceae (CRE) and *mcr-1*-positive Enterobacteriaceae (MCRPE). We carried out an extensive and systematic sampling regime in food-animal production chain to understand the prevalence of CRE and MCRPE across the farming sectors.

Materials and methods: From November 2014 to August 2015, we collected non-duplicate samples of cloaca/caeca/retail meat of chicken from poultry industry chain, including hatchery farms, commercial farms, slaughterhouse and supermarkets. Specifically in nearby environment of commercial farms, we further collected faecal samples of dogs, farmers, birds, as well as the bird nests and flies. The microbiology, detection and location of carbapenemase genes and *mcr-1* were conducted for the collected samples. We carried out the conjugation, whole genomic sequencing and phylogenetic analysis of CRE and MCRPE isolates of various origin in food production chain and its surrounding environment.

Results: We show that *mcr-1* but not bla_{NDM} is prevalent in hatcheries yet bla_{NDM} quickly contaminates chicken flocks through dogs/flies/wild birds. Direct sample testing (DST) for bla_{NDM} and *mcr-1* on commercial farms, slaughterhouse and supermarkets revealed considerably higher levels of positive samples than the bla_{NDM}- and *mcr-1*-positive *E. coli* indicating a substantial segment of the unseen resistome - a phenomenon we have termed “phantom resistome”. WGS identified common bla_{NDM}-positive *E. coli* shared among farms, flies/dogs/farmers, providing direct evidence of carbapenem-resistant *E. coli* transmission and environmental contamination.

Conclusion: It is evident from our data that bla_{NDM} is the dominant mechanism of CRE in both animals and humans in China. The Chinese farming environment contains a considerable phantom resistome carrying bla_{NDM} and/or *mcr*-1 genes, suggesting that the level of environmental contamination is underestimated. In particular, flies showed the biggest difference between bla_{NDM} detection by DST (62/120) and bla_{NDM}-positive strains (31/120). This is the first published study linking flies to the spread of carbapenem resistance. Given that they carry a “phantom” bla_{NDM} and *mcr-1* gene pool, their ability to contaminate the environment has immense public health concerns.

The dissemination of *mcr-1* among *Escherichia coli* of human and animal origins

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Background and objectives: Since the discovery of the mobile colistin resistance gene *mcr-1* in early 2016, numerous publications have reported the presence of *mcr-1* all over the world. However, few reports studied the impact of *mcr-1*-positive *Escherichia coli* (MCRPEC) from the clinical settings. Recently, two publications ascertained the prevalence of MCRPEC, clinical associations and outcomes of MCRPEC in the patients hospitalized with infections in China. Multi-locus sequence typing (MLST) suggested the MCRPEC of human origin are extremely divergence. So far, very few reports have studied the diversity of MCRPEC from animal origins. Here, we report the similarity and divergence of MCRPEC from animals along with humans.

Materials and methods: Deep sequencing was performed for 83 *mcr-1* positive and 29 *mcr-1* negative *E. coli* isolates of chicken origin. Subsequently, the sequencing data was retrieved for the MLST analysis using SRST2 and PubMLST database. We further combined the MLST data of animal origin and 126 *mcr-1* positive and 506 *mcr-1* negative *E. coli* isolates of human origin from our recent publication, and then conducted the phylogenetic analysis using Minimum Spanning Tree algorithm by BioNumerics.

Results: The combined data indicated the significant horizontal dissemination of *mcr-1* through *E. coli* isolates from both human and animal origins (Figure 1). In total, *mcr-1* was found in 85 of 189 ST clades. Specifically, *mcr-1* was found in 73 and 27 ST clades of *E. coli* isolates of human and animal origins, respectively. Of which, 15 ST clades were shared by MCRPEC of human and animal origins, which revealed an evidence of transmission route of mcr-1. Even though the size of samples is limited, MCRPEC still could be observed in a variety of ST clades in the *E. coli* of human and animal origins. Moreover, a total of 18 ST clades harbor ten or more isolates, of which, 15 ST clades possessed at least one MCRPEC isolate, and the proportions of MCRPEC were over 50% in five ST clades, including ST 48 (10/11; 90·9%), ST101 (14/19; 73·7%), ST46 (11/15; 73·3%), ST156 (12/18; 66·7%), ST10 (19/36; 52·8%), which suggested the prevalence of MCRPEC is unevenly distributed among the ST clades.

Conclusion: Considering this plasmid borne *mcr-1* gene is highly transmissible, we believe that *mcr-1* could be disseminated into more strains with new ST clades among animal, food-borne, and even human pathogens. Taken together, we should pay attention to the spreading of the *mcr-1* to the pathogenic *E. coli*.

Occurrence of colistin resistance gene *mcr-1* in *Escherichia coli* isolates associated with diarrhea and edema disease in piglets in Europe

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Background and objectives: Colistin is a polypeptide antibiotic that is extensively used for group treatments of pigs, poultry and veal calves to control economically important infections caused by virulent strains of *Escherichia coli*. This practice raises many concerns particularly since plasmid-borne colistin resistance gene *mcr-1* has been discovered worldwide in Gram-negative bacteria of humans, animals, food, and environment. In this study the occurrence of *mcr-1* in *E. coli* associated with disease in pigs was assessed.

Materials and methods: *E. coli* isolates (7,138) originated from cases of diarrhoea or edema disease in piglets in Germany and in 15 other EU countries. Isolates had been archived due to the possession of distinct adhesin/toxin genes. The PCR assay of Liu *et al.* (2016) was used to screen bacteria for the *mcr-1* gene. MIC data of *mcr-1*-positive isolates were determined according to ISO standard 20776-1:2006. Whole genome sequence (WGS) analysis was performed using an Illumina MiSeq sequencer and software services provided at the Center of Genomic Epidemiology.

Results: A total of 638 isolates (8.9 %) proved *mcr-1* positive with considerable variation from 0 to 70.4 % dependent on the country of origin. Noteworthy, two colistin susceptible isolates harboured a *mcr-1* gene that was disrupted by an IS26-like insertion element. Among isolates classified as enterotoxic *E. coli* (ETEC), edema disease *E. coli* (EDEC), ETEC/EDEC or attaching & effacing *E. coli* (AEEC) the *mcr-1* gene was present in 4.9 %, 12.9 %, 17.7 %, and 5.5 % of the isolates, respectively. In Germany, all isolates recovered in 1999-2007 (1,679) tested *mcr-1* negative. However, *mcr-1* was present in each of the following years, first at constantly increasing rates from 2008 (1.6 %) until 2013 (17.7 %), and also in 2014 (10.9 %) and 2015 (14.8 %). WGS analysis of 17 selected *mcr-1*-positive AEEC isolates assigned them to 8 genoserotypes including O26:H11 (3 isolates) and O103:H2 (2) and to ST20 and ST29, which are well-known characteristics of atypical enteropathogenic *E. coli* (aEPEC) found in humans and other animals.

Conclusion: Our results suggest that *mcr-1* circulates among pigs in Germany at least since 2008 and is now common in those *E. coli* that are regarded pig-adapted pathogens. However, distinct EPEC strains may have a broader host spectrum and may serve as a vehicle for transmission of *mcr-1*-bearing plasmids particularly between pig and human intestinal microbiotas.

Detection of plasmid-mediated colistin-resistance genes in *Enterobacteriaceae* isolates from food-producing animals and meat. Identification of the novel variant *mcr-3*, Portugal, 2010-2015.

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Background and objectives: Following the original report of plasmid-mediated colistin resistance (PMCR) in China, several studies in different countries reported a worldwide distribution of the *mcr-1* gene in *Enterobacteriaceae*. A novel variant, *mcr-2*, was also detected in colistin-resistant *Escherichia coli* isolates, from sick calves and piglets in Belgium; since that several other *mcr-1* variants has been identified. In this study, we analysed colistin-resistant *E. coli* and *Salmonella enterica* isolates from different animal origins, for the presence of PMCR encoding genes. Thus, our aim was to understand the extension of the problem of colistin resistance and PMCR, as colistin is the last resort to treat human infections caused by Gram negative bacteria resistant to all antibiotics, namely carbapenems.

Materials and methods: The antimicrobial susceptibility of 1206 *E. coli* and 634 *S. enterica* isolates from food-producing animals, meat and animal feed was determined by Minimum Inhibitory Concentrations (MIC) and interpreted according to Ecoffs (EUCAST). All isolates with colistin MIC>2µg/mL were considered as colistin-resistant and screened for the presence of PMCR-encoding genes (*mcr-1* and *mcr-2*), using a multiplex PCR, followed by sequencing for identification. All isolates harbouring *mcr* genes and exhibiting an ESBL or PMAβ phenotype were amplified by PCR and sequenced for the respective *bla* genes.

Results: Among 138 colistin-resistant isolates 100 were *mcr*-1-like genes: 94.2% (97/103) detected in *E. coli* plus 8.6% (3/35) in *S. enterica*. All but one amplicon exhibited a sequence with 100% homology to *mcr-1*; that amplicon differed from *mcr-1* by one point mutation (T1238C), hereafter named *mcr-3*, leading to the amino acid substitution Val413Ala; 42 *E. coli* isolates were ESBL/PMAβ co-producers: $bla_{\text{CTX-M-32}}$, *n*=14; $bla_{\text{CTX-M-1}}$, *n*=13; $bla_{\text{CTX-M-14}}$, *n*=5; $bla_{\text{CTX-M-8}}$, *n*=1; $bla_{\text{CTX-M-27}}$, *n*=1; $bla_{\text{SHV-12}}$, *n*=3; $bla_{\text{CMY-2}}$, *n*=3; $bla_{\text{ESAC,}}$ *n*=2.

Conclusion: We observed a high frequency (72.5%) of colistin-resistant isolates with *mcr-1*-like genes, 42% of which were ESBL/PMAβ co-producers. Selection pressure exerted by broad-spectrum cephalosporins and other antimicrobials may select and enhance the rapid dissemination of PMCR; e.g. the new *mcr-3* positive isolate co-harboured a $bla_{\text{CTX-M-8}}$ gene. Of note is the high frequency of *mcr* positive *E. coli* isolates from turkeys, when comparing with other European countries. Globally, these results seem alarming in terms of public health.

Validation of a Novel Susceptibility Testing Method for *Haemophilus parasuis*

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Background and objectives: We recently developed a suitable method for susceptibility testing of the fastidious organism *Haemophilus parasuis*, which causes high economic losses on pig farms worldwide. As the method needs further validation and to examine the susceptibility status of German *H. parasuis* isolates, a larger number of *H. parasuis* field isolates were tested. Furthermore, an interlaboratory comparison trial examined the reproducibility of the results.

Materials and methods: In total, 123 *H. parasuis* field isolates from different geographical regions in Germany collected between 2013 and 2016 were tested with the newly developed method. Isolates belonging to seven different serotypes and with different growth capacities were included in the study. For broth microdilution susceptibility testing, 24 antimicrobial agents and combinations were tested. For the interlaboratory comparison, *H. parasuis* type strain DSM 21448 and one field isolate were tested by six laboratories. For this, MIC determinations were conducted in two replicates with 14 antimicrobial agents and two antimicrobial combinations.

Results: Susceptibility testing with the newly developed medium could be performed for all *H. parasuis* field isolates. A classification of the isolates into susceptible, intermediate and resistant was not possible as no breakpoints are available for *H. parasuis*. A bimodal distribution of MIC values, which is indicative for a non-wild-type population, was detected for some antimicrobials such as aminoglycosides, β-lactams, fluoroquinolones, tetracyclines and trimethoprim/sulfamethoxazole. Broad distributions comprising 9-13 dilution steps were detected for the antimicrobial agents tiamulin, tetracycline, tilmicosin, tulathromycin and for the antimicrobial combination trimethoprim/sulfamethoxazole. For ampicillin and penicillin, eight and seven isolates, respectively, exhibited distinctly higher MICs of 64 or 128 µg/ml as the remaining isolates and may be considered as resistant.

Conclusion: The study demonstrated the suitability of the recently proposed method for susceptibility testing of *H. parasuis* by testing a larger number of field isolates. The interlaboratory comparison trial indicated a good agreement of results between laboratories experienced in working with *H. parasuis.* The MIC values determined in this study allow an estimate of the susceptibility status of German *H. parasuis* isolates and may help to establish breakpoints for this pathogen.

Biocide susceptibility testing – development of a new testing method

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Background and objectives: Biocides are of high relevance for cleaning and disinfection not only in hospitals, but also in food production, animal stables and animal clinics. So far, there is only little information about susceptibility of bacterial pathogens to biocides. This is mainly due to the fact, that only approved methods for testing the activity of biocides are available, but not for biocide susceptibility testing of bacterial isolates. The latter method was developed during this study.

Materials and methods: For the method development, the *Staphylococcus aureus* reference strain ATCC® 6538 was comparatively investigated six times at independent occasions for its susceptibility to benzalkonium chloride (BAC), glutaraldehyde (GLU) (2-fold dilution series) and isopropanol (ISO) (2 % steps) in amounts of 2 and 10 mL. The inoculum preparation was performed either directly from glycerol stock cultures on TSA plates or from further subcultures, and by either direct colony suspension method (DCS) or the use of glass beads (GB). The results were read after 24 h, 48 h and 72 h of incubation at 37°C. The preparation of the biocide dilutions was performed in 1 mL and 5 mL of double distilled water and mixed with 1 mL and 5 mL of double-concentrated tryptic soy broth (TSB) resulting in the normal TSB concentration. These tubes were inoculated with 20 µL inoculum for 2 mL dilutions or 100 µL inoculum for 10 mL dilutions.

Results: Per biocide 144 MIC values were obtained. Deviations of more than +/- one dilution step were seen once for BAC and for ISO after 72 h. Evaporation of ISO, resulting in higher MICs, could be avoided by additional sealing. The most common value was 0.0001 % for BAC (n=82) and GLU (n=119) and 6 % for ISO with additional sealing (n=101) and 8 % for ISO without additional sealing (n=51). Based on the high reproducibility, we propose a protocol for biocide susceptibility testing as follows: Two-fold-dilution series are used for biocide concentrations ≤ 1 % and 2 %-steps for concentrations > 1 %. A fresh overnight culture is used and the inoculum can be prepared either by DCS or GB in 2 mL amounts to reduce the biocide waste. The incubation shall be performed at 37°C for 24 h, which can save time and space in the incubators.

Conclusion: This method will facilitate the biocide susceptibility testing of bacterial isolates and shall contribute to a harmonization of the biocide susceptibility testing of bacterial pathogens in routine diagnostics.

ARIBA: a new high throughput tool to identify antimicrobial resistance determinants from short read sequencing data

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Background and objectives: Antimicrobial resistance (AMR) is one of the major threats to health, and with the increasing use of whole genome sequencing, our understanding of the mechanisms and diversity of AMR is growing. However, few high-throughput bioinformatics tools exist to analyse and predict the resistance of a bacterial isolate from directly from sequencing data. The available methods are limited in the types of AMR mechanisms they can detect and/or are not scalable to high-throughput environments. Rapid and accurate identification of AMR is an important component of any strategy to tackle AMR, and we present a new tool, ARIBA, which provides such an approach that can be applied to the increasing numbers of bacterial sequences available.

Materials and methods: ARIBA uses a combined mapping/alignment and targeted local assembly approach to identify AMR genes and variants efficiently and accurately from paired sequencing reads. It can easily be provided with custom reference datasets, and supports the use of a number of public AMR databases. It distinguishes between coding and non-coding sequences; provides details on each sequence present in the sample; verifies whether or not identified genes are complete, truncated or fragmented; and reports single nucleotide polymorphisms (SNPs) and indels within sequences with interpretations of their effect, such as non-synonymous changes. We benchmark ARIBA against SRST2 and KmerResistance, two available command line tools that can use custom reference data, using three bacterial datasets.

Results: We demonstrate a number of ways in which ARIBA improves upon existing tools and provides extra functionality, where it: 1) verifies completeness of acquired AMR genes; 2) identifies SNPs known to be associated with AMR; 3) allows exploration of the association of AMR determinants with user-provided phenotypic AMR data; and 4) identifies SNP frequency in multicopy genes.

Conclusion: ARIBA is a fast, computationally efficient, and accurate method of identifying AMR determinants from sequence reads. Moreover, ARIBA reports significantly more details than existing tools, particularly variant calls, enabling a deeper understanding of the resistance associated with each isolate.

Variability of SCC*mec* elements in livestock-associated CC398 MRSA

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Background and objectives: During the last decade, *Staphylococcus aureus* strains from clonal complex (CC) 398 emerged as livestock-associated MRSA and can now be found in most Western European countries. They are common among pigs and turkeys, and their prevalence in pets, in horses and in humans appears to be increasing. CC398 MRSA can harbour a variety of different resistance markers including genes associated with heavy metal resistance. The aim of the study was to assess the variability of such isolates with special emphasis on SCC*mec* elements.

Materials and methods: About 60 CC398 MRSA isolates were collected from humans and animals in Germany and Austria. They were genotyped using DNA microarrays as described previously. This allowed assignment to the CC398 lineage as well as detection of 85 SCC-associated markers. In addition, 12 published genome sequences were analysed for comparison.

Results: The most common SCC*mec* element in CC398 MRSA was a VT (or 5C2&5) composite element that additionally harboured *czrC* (zinc and copper resistance), as represented by the genome sequence of SO385 (GenBank accession no. AM990992.1). It was found in nearly half of the analysed isolates and sequences. In addition to that, another fourteen different variants of SCC*mec* elements were identified. These included four SCC*mec* IV elements, three SCC*mec* V elements, six SCC*mec* VT elements and one class C pseudo-SCC-element. Most of these harboured markers associated with resistance to arsenic, copper or zinc. Six of these SCC*mec* elements did not match published sequences and thus warrant further studies.

Conclusion: CC398-MRSA-IV and V/VT are not homogeneous strains but differ in carriage of a variety of distinguishable SCC*mec* elements. This could be attributed to an ongoing evolution including acquisition of heavy metal resistances and/or to a polyphyletic origin, i.e., to multiple incorporations of different SCC*mec* elements by CC398-MSSA. This phenomenon can be exploited for typing purposes.

Unexpected occurrence of MRSA in Swedish wild hedgehogs (*Erinaceus europaeus*) - A pilot study

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Background and objectives: Meticillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of infections in humans and animals. In Sweden, MRSA is still rare both in humans and animals. In fact, the first case in animals was first described in 2006 and through 2015 only a total of 126 cases in animals has been confirmed. Interestingly enough during 2014 three unrelated cases of *mec*C-MRSA was described post mortem in wild hedgehogs at the National Veterinary Institute (SVA), Sweden. Furthermore, *mecC*-MRSA had previously been described in two hedgehogs in 2003 and 2011 respectively. Since MRSA are rare in Swedish animals these random findings of *mec*C-MRSA were remarkable. The aim of the present study was therefore to investigate the occurrence of MRSA in Swedish hedgehogs.

Materials and methods: Samples from 55 hedgehogs were collected on arrival at wild-life rescue centers or at postmortem examination at SVA. Isolation was performed using pre-enrichment broth with NaCl and aztreonam, which was then sub-cultivated on MRSA 2 Brilliance agar (Oxoid, UK). Suspected MRSA was confirmed by PCR and confirmed MRSA was further characterized by *spa*-typing and antibiotic susceptibility testing.

Results: In total, MRSA was isolated from 64% of the sampled animals (n = 55), with all isolates carrying the *mec*C gene. The Isolates belonged to eight different *spa*-types and the majority (n = 28) showed reduced susceptibility only to β-lactam antibiotics.

Conclusion: This limited study indicates that *mec*C-MRSA are common in Swedish hedgehogs, which can point to hedgehogs being a reservoir of these bacteria.

Carriage dynamics of methicillin resistant *Staphylococcus aureus* and changes in the nasal microbiome after long- and short-term exposure to the pig farm environment.

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Background and objectives: Pigs and the pig farm environment are known sources of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA). Long-term occupational exposure and short visits to pig farms may have different effects on LA-MRSA colonization and the composition of the nasal microbiome in human. We aimed to investigate carriage dynamics of LA-MRSA and changes in the nasal microbiome in pig farm workers during a work week and in human volunteers after short-term exposure to the pig farm environment.

Materials and methods: We conducted two prospective cohort studies with pig farm workers and human volunteers, respectively. In study 1, nasal swabs were collected twice daily before and after work for an entire work week from 31 pig farm workers on eight farms. In study 2, nasal swabs were collected from eight volunteers before, immediately after, and 48 h after a 1-hour farm visit. MRSA and *S. aureus* was quantified in all samples, and the microbiome diversity was determined in selected samples from pig farm workers and in all samples from volunteers based on 16s *rRNA* gene sequencing.

Results: Twenty-nine out of 31 pig farm workers carried high level of LA-MRSA (1-7 $\log_{10}$ CFU/swab) during the entire week, including the weekend. Only two were almost always negative for LA-MRSA but carried high levels of *S. aureus*. There was no correlation between the number of working hours and the level of LA-MRSA carriage. On the other hand, all volunteers carried low levels of LA-MRSA (2 $\log_{10}$ CFU/swab) and only immediately after the farm visit, but were negative before and 48 h after the farm visit. The pig farm workers' nasal microbiome did not change over the 1-week study period and contained a variety of bacteria usually found in the pig farm environment. In contrast, the volunteers' nasal microbiome changed significantly during the farm visit but reverted to its original composition within 48 h.

Conclusion: Pig farm workers harbor a stable "farm microbiome" in their nose, including LA-MRSA, during the length of a working week and even over the weekend, whereas the microbiome in short-term visitors changes only transiently. The observed difference in nasal microbiomes between farm workers and visitors may have implications for the overall risk for transmission to household members and the public. The transmission of the microbiome, e.g., LA-MRSA, is probably limited to the first few hours after the farm visit and is absent after 48 hours.

Reduction in antimicrobial usage following veterinary intervention in dairy herds

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Background and objectives: The aim of the study was to assess antimicrobial usage following implementation of a novel veterinary intervention programme designed to focus on stewardship of antimicrobial usage on dairy farms.

Materials and methods: 67 dairy farms located in the North and South Islands of New Zealand were enrolled in a two year prospective intervention study. In the initial 12 months of the study (Year 1), standard on-farm prescribing and treatment protocols were used by all farms. Total antimicrobial usage was calculated from records of sales from the servicing veterinary businesses, using the population corrected unit (PCU) approach. Usage (mg/kg live weight/year) was calculated as [total mass of active antimicrobials sold]/[number of cows in the herd x 450 kg]. Within island, herds were ranked on antimicrobial usage, and within sequential pairs, randomly assigned to either a control group or a veterinary intervention group. Veterinarians servicing the herds attended a workshop outlining issues associated with antimicrobial resistance and antimicrobial usage, were provided with a series of open ended questions for use with each selected herd owner, and asked to undertake a 90 minute visit to each of the treatment herds. At the end of this visit, the herd owner and/or manager and the veterinarian were asked to define three or more specific goals around reducing antimicrobial usage on farm. Antimicrobial sales to the farms were monitored over the subsequent 12 month period (Year 2). The change in antimicrobial usage (that is Year 2 - Year 1) was analysed using linear regression with treatment group and farm location (Island level) as the main effects and with herd size as a covariate.

Results: Prior to the veterinary intervention the antimicrobial usage was 7.18 (95%CI=6.40-7.95) mg/kg live weight, and did not differ between groups (P=0.62). Veterinary intervention was associated with a tendency (P=0.06) for a reduction in antimicrobial usage, with the difference in usage being +0.94 (SE=0.37) vs -0.04 (SE=0.36) mg/kg live weight for Year 2–Year 1, for the Control vs Veterinary visit herds, respectively. There was no effect of farm location (P=0.14), no Veterinary visit by Island interaction (P=0.11) and no effect of herd size (P=0.23) on change in antimicrobial usage.

Conclusion: We conclude that our program of veterinary intervention focused on promoting good stewardship of antimicrobials reduced their usage on dairy farms.

Testing the susceptibility of bacterial mastitis isolates to host defense peptides: technical challenges and data output for clinical isolates

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Background and objectives: The problem of increasing bacterial resistance to antimicrobial agents with multi-resistant strains, e.g. livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) or ESBL-producing *Escherichia coli* requires alternative treatment strategies. Host defense peptides or cationic antimicrobial peptides (AMPs), as for example the cathelicidins, have recently been discussed as a potential new strategy against bacterial infections. They are key players in the innate immune system as they can directly act against microorganisms or modulate the immune system. The aim of our study was to test *S. aureus* and *E. coli* from bovine mastitis for their susceptibility to the two main bovine cathelicidins, namely BMAP-27 and BMAP-28.

Materials and methods: Susceptibility testing was performed in analogy to the broth microdilution method described by the Clinical and Laboratory Standard Institute (CLSI) to determine minimal inhibitory concentrations (MICs). Based on the repetitive (at least 13 times) testing of two selected clinical *S. aureus* isolates, one clinical *E. coli* isolate and one *S. aureus* laboratory strain, the homogeneity of MIC variances for each peptide was determined. Then susceptibility testing of 50 clinical *S. aureus* and 50 clinical *E. coli* isolates for BMAP-27 and BMAP-28 has been performed.

Results: Statistical analysis revealed strong peptide-specific variances in the technical procedure for the three selected *S. aureus* strains. Therefore, we recommend that besides including a reference strain in each single experiment, MIC assays should be repeated at least three times for each individual isolate to track the technical variances when working with cathelicidins. Using this technique, susceptibility testing of the bacterial field isolates revealed statistically significant peptide-specific differences in the MIC values: While BMAP-27 showed lower MIC values for *E. coli* (mode MIC of 16 µg/mL) compared to *S. aureus* (64 µg/ml), BMAP-28 exhibited lower MIC values for *S. aureus* (mode MIC of 16 µg/mL) compared to *E. coli* (64 µg/ml).

Conclusion: Since selected AMPs e.g. the human cathelicidin LL-37 are already in the clinical phase of development, also bovine cathelicidins might be promising candidates for future treatment strategies against mastitis in dairy cattle. For this, harmonized and standardized analysis of different bacterial species for their susceptibility to AMPs should be conducted in the future.

Longitudinal study of ESBL-carriage on an organic broiler farm: horizontal plasmid transmission

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Background and objectives: Extended-spectrum β-lactamase *E. coli* (ESBL-E) are frequently reported in broilers all around the globe. In the Netherlands, all conventional and organic broiler farms investigated were ESBL-E positive. ESBL-E can potentially be transmitted to humans by direct contact, through the food-chain or via the environment. Most studies are cross-sectional surveys on prevalence and molecular characteristics on different broiler farms. Longitudinal studies, in which transmission dynamics can be studied, are scarce. In a recent longitudinal study on an organic broiler farm (Huijbers et al., 2016) tagged broilers were followed individually from arrival until slaughter age. In this study, the prevalence of ESBL-E increased sharply after arrival of day-old chicks at the farm, but decreased again towards slaughter. ESBL-E isolates were characterised by CTX-M group and phylogenetic group analysis. All isolates carried CTX-M group 1 genes and different phylogenetic groups were found. The objective of the present study was to characterise these ESBL-E further to study transmission dynamics in more detail.

Materials and methods: ESBL-E isolates obtained in the former longitudinal study by selective pre-enrichment of samples taken from one organic broiler-fattening farm at several time points within two consecutive production rounds were typed further by MLST. ESBL-genes were sequenced and plasmids were characterized by transformation, PCR-based replicon typing and subtyped by plasmid MLST. Included isolates originated from 80-tagged broilers, the environment of the broiler house, a sample taker and the transport van.

Results: Analysis of a selection of isolates (158/1166) displayed the presence of $bla_{\text{CTX-M-1}}$. Plasmid typing (36/158 isolates) revealed the presence of an incI1/ST3 plasmid in all of them. MLST analysis (334/1166 isolates) showed that on arrival in round 1, *E. coli* A1/ST88 dominated, while on days 3, 4, 7 and 10 A1/ST10 was most often found and at slaughter age, B1/ST155 and D2/ST1551 predominated. A shift in ST types was also observed in round 2. ST types of isolates from broilers and the environment were partly the same.

Conclusion: The rapid dissemination of ESBL-E on this broiler farm was not due to the spread of one specific *E. coli* clone, but to horizontal transfer of a specific incI1/ST3 plasmid carrying $bla_{\text{CTX-M-1}}$ and/or to a shift in the predominant ESBL-E flora in broilers.

Plasmid and chromosomal location of *bla*$_{CTX-M-15}$ genes detected in *Escherichia coli* from diseased cattle

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Background and objectives: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* have been increasingly detected worldwide. The ESBL *bla*$_{CTX-M-15}$ gene has been commonly detected in human or animal *E. coli* isolates. The aim of this study was to characterize CTX-M-15-producing *E. coli* from diseased cattle and their respective *bla*$_{CTX-M-15}$-carrying plasmids.

Materials and methods: Among 2,961 *E*. *coli* isolated from diseased cattle, collected in the German National Monitoring Program GE*RM*-Vet (2008-2015), the *bla*$_{CTX-M-15}$ gene was detected by PCR and sequencing in 59 isolates. These isolates were characterized by antimicrobial susceptibility testing (AST), XbaI-PFGE, multilocus sequence typing, phylotyping, hybridization experiments and 20 isolates were submitted to whole genome sequencing using Illumina MiSeq. Transformants carrying the *bla*$_{CTX-M-15}$ gene were investigated by AST and conjugation and the respective transferred plasmids were characterized by replicon typing, S1-nuclease PFGE and PCR for the detection of resistance genes and the genetic environment of *bla*$_{CTX-M-15}$ genes.

Results: The 59 bovine CTX-M-15-producing *E*. *coli* were distributed among 17 sequence types (STs), with ST167 (n=16), ST410 (n=6) and ST10 (n=5) most commonly detected. Isolates of ST167, assigned to phylogroup A, were detected in six years (2009, 2011-2015) and most of them showed related XbaI patterns. The *bla*$_{CTX-M-15}$ genes were linked to intact or truncated IS*Ecp1*. Ten of these ESBL genes were not transferable, neither by transformation nor by conjugation, and were likely to be located in the chromosomal DNA. The remaining 49 *bla*$_{CTX-M-15}$ genes were found on plasmids (80-180 kb) of incompatibility groups IncF-type and IncI1 (n=12). The majority of the IncI1 plasmids (11/12) proved to be conjugative and did not carry co-located resistance genes. However, 28/37 IncF-type plasmids were non-conjugative and conferred a multidrug-resistance phenotype. Co-located resistance was commonly detected to tetracycline [*tet*(A) and/or *tet*(B) n=33], sulfonamides (*sul1* and/or *sul2* n=19) and trimethoprim (*dfrA* variants n=18).

Conclusion: This study underlines the risks of ESBL-producing isolates to public health, since such bovine isolates also represented sequence types commonly found in human isolates (e.g. ST167, ST410). Moreover, the chromosomal location of the *bla*$_{CTX-M-15}$ genes may assure their vertical transfer, whereas the plasmid location may favour their horizontal dissemination.

Antimicrobial resistance prevalence in Harbour (*Phoca vitulina*) seal pups stranded in the Netherlands and antibiotic treatment effect in their gut microbiome during rehabilitation

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Background and objectives: The Sealcentre Pieterburen rehabilitates seals stranded in the Netherlands. Every year orphan harbour (*Phoca vitulina*) seal pups with critical health status are admitted during summer season. Since seals share the coastal environment with humans, they may also serve as sentinels for ocean and human health.

Firstly, we aimed to reveal the prevalence of Antimicrobial Resistance (AMR) in bacteria isolated from the rectum of harbor seal pups admitted at the Sealcentre. Secondly, we analyzed the gut microbiome composition of the seals before and during rehabilitation to investigate the influence of antibiotic (AB) therapy and the rehabilitation process on their commensal gut flora.

Materials and methods: During summer 2015, rectal swabs were collected from 100 harbour seal pups at admission, during rehabilitation and before release. If the seal received AB treatment, samples were taken before and after treatment. The swabs collected at admission were streaked onto different selective agar plates to screen for clinically relevant AMR bacteria. Whole genome sequencing (WGS) was performed on positive cultures to determine the antibiotic resistance genes and for comparison with human isolates by a core genome multi-locus sequence typing (cgMLST) approach.

From all swabs collected, DNA was isolated and amplicon sequencing was performed using Illumina Miseq 2x300bp on 450 bp of the 16S V3–V4 region. Reads were analyzed using Mothur. α- and β-diversity were determined using Shannon and Unifrac, respectively. Statistical analyses were performed using Wilcoxon signed rank. Random Forest analysis was performed using the Bioconductor *randomForest* package 4.6-10.

Results: At admission, ESBL-producing *E. coli* were isolated from 4 harbour seal pups (4%). They carried CTX-M-15 and CTX-M-27 and differed only in 40 of 2764 analyzed genes from human *E. coli* isolates. Furthermore, our results indicate that AB treatment has a severe but short-lived effect on the seal microbiome which returns to normal within approximately four days.

Conclusion: We observed low prevalence of ESBL-producing *E. coli* in stranded harbor seal pups. However, they are closely related to those found in humans and contain the same resistance genes. The effect of AB treatment on the seal gut microbiome is extensive, however it is transitory. Analysis of the complete resistome will be undertaken using shotgun metagenomics and qPCR.

Extended spectrum beta-lactamase producing *Enterobacteriaceae* in imported and domestic food products purchased at retail in Canada

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Background and objectives: The Canadian Integrated Program for Antimicrobial Surveillance (CIPARS) tests for resistance in bacteria from major meat commodities including routine screening of *E. coli* and *Salmonella* for extended spectrum beta-lactamase (ESBL) production; it does not routinely test seafood, use selective media, or test other *Enterobacteriaceae*. The aim of this study was to assess, using selective media, the occurrence of ESBL-producing *Enterobacteriaceae* and related bacteria in imported spices and raw seafood, and domestic raw meat and fish sold in Canada.

Materials and methods: Domestic (n=835) and imported (n=1348) products were collected from 2012 to 2016 through CIPARS retail surveillance and targeted studies, and tested using differential chromogenic selective media, ChromAgar™ ESBL. Suspected colonies were tested by double-disk diffusion and the CMV3AGNF Sensititre® plate. Putative isolates were speciated using Vitek2™, and further characterized by multiplex PCR and sequencing.

Results: There was a significant difference (OR=3.8; CI 2.0, 7.7) in the prevalence of ESBL isolates between domestic [1.4% (12/835)] and imported products [5.3% (72/1,348)]. *E. coli* comprised 75% and 46% of ESBL isolates from domestic and imported products respectively, and *Klebsiella pneumoniae,* 1.4% (1 isolate from turkey) and 19% respectively. The remaining ESBL isolates were: 2 *Serratia* (domestic pork); and 10 *Enterobacter cloacae*, 1 *Vibrio cholerae,* 1 *V. parahaemonlyticus* (containing bla_{PER}), and 16 other species from imported products. ESBL *E. coli* contained bla_{SHV} (1 from a domestic product, 1 imported), bla_{TEM} (4, 20), $bla_{CTX\text{-}M}$ (5, 32) and $bla_{OXA\text{-}1}$ (1, 2) alone or in combination, while *K. pneumoniae* isolates harboured bla_{SHV} (1 domestic, 13 imported), bla_{TEM} (0, 7), $bla_{CTX\text{-}M}$ (1, 7), and $bla_{OXA\text{-}1}$ (0, 4). Resistance to six or more antimicrobial classes was found only in *E. coli* [36% (12/33)], *K. pneumoniae* [23% (3/13)] and 1 *Raoultella planticola* isolate from imported products. Sequence type was highly variable but clinically important clones were found: *E. coli* ST38 (1 isolate from domestic chicken; 1, imported shrimp); *E. coli* ST155 (2) and *K. pneumoniae* ST14 (1) from imported seafood.

Conclusion: Although uncommon, ESBL bacteria, including multidrug resistant strains and clones of public health significance, are present in the Canadian food system, more frequently and with greater diversity in imported raw products, primarily seafood.

Abstracts – Poster Presentations

7th ARAE2017 Poster No. 1:

Adaptation of *Salmonella* Typhimurium strains to organic acids and consequences on antibiotic resistance

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Background and objectives: The rapid emergence of antimicrobials resistance worldwide constitutes a major public health concern (WHO). The recent creation by the European Commission of a new functional group of additives for the decontamination of animal feed (regulation (UE) 2015/2294), which can encompass organics acids, has raised new questions about their potential impact (see ANSES 2015-SA-0097). Some works previously reported the potential development of antibiotic resistance following exposure to various susbtances. The potential correlation between the use of organic acids and the emergence of cross-resistance to antibiotics should thus be evaluated.

Materials and methods: A panel of 9 *Salmonella enterica* ser. Typhimurium strains isolated from pig production chains and 1 reference strain (ATCC 13311) displaying different levels of antibiotic resistance were exposed through single 15 min or 24h exposures to various concentrations of 13 organic acid molecules representative of those which may be used in field conditions. Levels of bacterial resistance to these organic acids and to 14 antibiotics were assessed following exposures. The stability of potential increase of resistance was also evaluated by 10 successive subcultures in growth medium without organic acids. The ability of acid organics to generate viable but non cultivable (VNC) bacteria was also evaluated using a propidium monoazide qPCR (PMA-qPCR) method for strain ATCC 13311 after exposition to 5 acids.

Results: Single exposures of *Salmonella* strains to organic acids did not significantly impact their level of resistance to these molecules. Conversely, the development of cross-resistances against 4 antibiotics (ampicillin, tetracycline, sulfamethoxazole and chloramphenicol) was observed in different strains both after 15 min or 24h exposures to 8 of the 13 organic acids tested. Reciprocally, increases of susceptibility to the same antibiotics were also observed for some strain-organic acid combinations. In the vast majority of cases, the high resistance levels remained stable after 10 subcultures in medium without organic acids suggesting that the resistance should be due to inheritable genetic modifications rather than transient phenotypic adaptation. In addition, the quantification of VNC bacteria after exposure to some organic acids clearly showed the presence of large amount of VNC cells with ratio up to 10^4 between viable cells enumerated using PMA-qPCR or standard plating methods.

Conclusion: This study demonstrated the potential ability of organic acids to induce stable antibiotic resistance in *Salmonella* ser. Typhimurium and also to generate high levels of VNC bacteria forms.

7th ARAE2017 Poster No. 3:

Fecal carriage and characterization of extended-spectrum β-lactamase- and AmpC β-lactamase-producing *Escherichia coli* from healthy horses in France

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Background and objectives: Companion animals in close contact with humans should be regarded as a potential reservoir of extended-spectrum cephalosporins (ESC)-resistant bacteria. The objective was to determine the fecal carriage of ESC-resistant *Escherichia coli* isolated from healthy horses in France and to characterize the genetic determinants responsible for ESC resistance.

Materials and methods: Fecal samples from 738 randomly selected healthy adult horses were collected in 41 horse stables during the summer of 2015 in France and screened for the presence of ESC-resistant *E. coli* strains. The ESC resistance genes among non-redundant *E. coli* strains were determined using PCR and sequencing. ESC phenotypes were horizontally transferred by conjugation or transformation. Extended-spectrum-β-lactamase (ESBL)- or AmpC-carrying plasmids were typed by PCR-based replicon typing, restriction fragment length polymorphism, and multilocus sequence typing. The ESC-resistant *E. coli* strains were typed by *Xba*I macrorestriction analysis, phylogroup determination, and virulence genes profile.

Results: In 16/41 (39%) of the stables, at least one horse carrying ESC-resistant *E. coli* isolates was identified. ESC-resistant *E.coli* isolates were found in 26/328 (7.9%) of the horses screened individually. Fifty-one non-duplicate ESC-resistant *E. coli* isolates were included in the molecular resistance analysis. All these isolates showed a great diversity of *Xba*I macrorestriction profiles, belonged mainly to phylogroup B1, and were negative for major *E. coli* virulence genes in animals (*eae*, *stxA*, *stx2A*, *iutA*, *eltB*, *estA*, *estB*) suggesting that they are commensal, non-pathogenic isolates. The ESBL $bla_{CTX\text{-}M}$ genes were dominant ($bla_{CTX\text{-}M\text{-}1}$, n=35; $bla_{CTX\text{-}M\text{-}2}$, n=8; $bla_{CTX\text{-}M\text{-}14}$, n=2). The ESBL/AmpC genes were identified on various conjugative plasmids belonging to the IncHI1, IncI1, IncN, and IncY groups and with different additional non-β-lactam resistance phenotypes. Interestingly, the most prevalent ESBL genes, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}2}$, were mainly located on large conjugative IncH1 plasmids. RFLP and MLST plasmid analysis are underway to assess the relatedness of these ESBL plasmids.

Conclusion: For the first time, a large-scale survey revealed a significant carriage of ESBL-producing *E. coli* and plasmids carrying ESBL genes in faeces from healthy horses in France. Presence of ESBL plasmids in the intestinal microflora of horses may have significant implications for horses and public health in France.

7th ARAE2017 Poster No. 4:

Characterization of ESBL/AmpC-producing *Salmonella enterica* from the Colombian poultry chain using whole genome sequencing.

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Background and objectives: *Salmonella enterica* have been isolated from baseline studies as part of the Colombian integrated program for antimicrobial resistance surveillance (Coipars). Our aim is to investigate the diversity of genes, plasmids and strains associated to the spread of ESBL/AmpC genes along the Colombian poultry chain.

Materials and methods: A total of 578 epidemiologically independent, non-clinical isolates from broiler farms (n=28), slaughterhouses (n=140) and retail (n=410) were analysed for antimicrobial susceptibility using the automated system Phoenix BD™. Isolates resistant to cefotaxime (MIC ≥ 4 mg/L) were screened by PCR for *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CMY}$ and *bla*$_{OXA}$. Based on the distribution of genes a selection of isolates was made and subjected to Whole Genome Sequencing (WGS) with Illumina Miseq and Nextseq. Genomes were assembled with SPAdes. *In silico* characterization of ESBL/AmpC gene variants, plasmid replicon typing and strain Multi Locus Sequence Typing (MLST) was done using ResFinder 2.1, PlasmidFinder 1.3 and MLST 1.8, respectively. Investigation of resistance genes with an identity percentage < 100% was done using Basic Local Alignment Search Tool (BLAST).

Results: In total, 260 isolates were resistant to cefotaxime, of which 168 were carrying *bla*$_{CMY}$, 51 *bla*$_{CTX-M}$, 7 *bla*$_{SHV}$, 5 a combination of *bla*$_{CMY}$-*bla*$_{SHV}$ and 3 a combination of *bla*$_{CMY}$-*bla*$_{CTX-M}$. Except for 1 isolate, *bla*$_{TEM}$ was found in 49 isolates together with *bla*$_{CMY,}$ *bla*$_{CTX-M}$ or *bla*$_{SHV}$. Furthermore, 25 strains were negative for all tested genes. A random selection of *bla*$_{CMY}$-positive isolates (n=13), *bla*$_{CTX-M}$ (7) and negative isolates (5) was made for WGS. In addition, all positive isolates for *bla*$_{SHV}$, *bla*$_{CMY}$-*bla*$_{SHV}$ and *bla*$_{CMY}$-*bla*$_{CTX-M}$ were included. WGS characterization demonstrated 21 strains to carry *bla*$_{CMY-2}$, 11 *bla*$_{CTX-M-165}$, 10 *bla*$_{TEM-1B}$, 7 *bla*$_{SHV-12}$, 5 *bla*$_{SHV-129}$ and 1 *bla*$_{TEM-1A}$. Moreover, plasmids ColRNAI (n=33), IncI1 (26), IncA/C2 (15), IncX1 (14) and p0111 (12) were most frequently found. Finally, 18 strains belonged to ST15, 17 to ST28, 3 to ST11, 1 to ST152 and 1 to ST292.

Conclusion: Resistance to third generation cephalosporins in selected strains of *S. enterica* from Colombian poultry is mainly caused by *bla*$_{CMY-2}$ and *bla*$_{CTX-M-165}$ genes. These genes are mostly contained in strains belonging to ST28 and ST15, respectively.

7th ARAE2017 Poster No. 5:

Vigilance for *Salmonella* in Feedstuffs in Costa Rica: Prevalence and Tetracycline Resistance

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Background and objectives: Animal feed could serve as vehicle to introduce *Salmonella* serovars and antimicrobial resistant bacteria/genes, into the food chain. Tetracyclines, are frequently use in intensive crop and intensive livestock worldwide, especially in developing countries. Our aim is to provide an epidemiological background of *Salmonella* prevalence and resistance to tetracycline in compound feeds and feed ingredients taking into account that the feed industry is at the beginning of the food-chain and a contamination in this stage could affect animals and humans.

Materials and methods: 1724 feedstuffs were collected in Costa Rica as part of a countrywide surveillance program. *Salmonella* was analyzed using a culture method (FDA Bacteriological Analytical Manual). Antibiotic susceptibility testing MIC for TET was determined using Mueller-Hinton media and TETE-tests trips (0.016 to 256 µg/mL).

Results: *Salmonella* prevalence in feedstuff was 6.4% (n=110/1724). MBM samples presented the highest prevalence (n=23/86; 26.7%), followed by other feedstuff (n=8/129; 6.2%), poultry feed (n=76/1420; 5.4%), and pet food (n=3/89; 3.4%). From the different *Salmonella enterica* serovars recovered (n=21), the most common were serovar Give (n=18; 13.8%) and Rissen (n=6; 4.6%) for meat and bone meal (MBM) and serovar Havana (n=14; 10.8%), Rissen, Soerenga, and Schwarzengrund (n=8; 6.2% each) in poultry feed. Recovered strains were regarded to be sensitive or have an intermediate resistance to TET as evidenced by their MIC50 and MIC90 concentrations of 4 and 8 µg/mL for MBM and poultry feed. Compound feed and MBM samples exhibited strains characterized by 86.8 and 88.9% of the isolates classified (according to CLSI) as sensitive, 7.7 and 3.7% as intermediate, and 5.5% (with >256 µg/mL as the highest concentration) and 7.4% (with 64 µg/mL as the highest concentration) as resistant to TET, respectively. *Salmonella* serovars Anatum and Havana exhibited the highest resistance profile >256 and 128 µg/mL.

Conclusion: Poultry feed presented *Salmonella* strains more resistant to TET when compared to MBM-recovered strains; this could be explained by the use of antibiotics in compound feed in Costa Rica (previously reported). The relative high prevalence (26.7%) of *Salmonella* in meat and bone meal is worrying as it is a common ingredient for pet food and monogastric animals feed and is also used as fertilizer; hence feed could serve as contamination source for animals and crops and finally humans, hereafter a stricter surveillance program may be in order.

7th ARAE2017 Poster No. 6:

Characterisation of a colistin resistance plasmid isolated from the gastrointestinal tract of broiler chickens

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Background and objectives: Plasmid-mediated antibiotic resistance (AR) is a major problem affecting human and animal health. Many plasmids have the ability to transfer between different bacterial species. This is a threat to human and animal health if a plasmid carrying a resistance gene is transferred to commensal bacteria or clinical pathogens. This study focused on investigating the presence of colistin resistance on plasmids in the caecal bacteria of broilers, which are raised for meat production. Colistin is used as an antibiotic of last resort for the treatment of multi-drug resistant (MDR) infections.

Materials and methods: DNA was extracted directly from a broiler caecal sample using a modified alkaline lysis method. Sheared genomic DNA was removed using Plasmid-Safe DNase and plasmid DNA amplified with phi29 DNA polymerase. DNA was transformed into *Escherichia coli* DH5α and selected on colistin (16 mg/L). We obtained a plasmid conferring resistance to colistin. Antibiotic susceptibility testing was performed on the transformant using agar dilution and disk diffusion methods. Conjugation was performed between the transformant and a rifampicin resistant *E. coli*. PCR was used to detect for the presence of the *mcr-1* gene. The plasmid was sent for Oxford Nanopore minION sequencing.

Results: We obtained a plasmid capable of conferring resistance to colistin in *E. coli*. A disk diffusion assay revealed no zone of inhibition around a 10µg colistin disk. The transformants have a minimum inhibitory concentration to colistin of >128 mg/L. Conjugation was successful, showing the plasmid is transferrable. PCR was negative for the presence of the *mcr-1* gene. Analysis of the plasmid sequence will allow for the identification of a novel mobile gene responsible for high-level colistin resistance.

Conclusion: We identified a plasmid conferring high-level colistin resistance from the caecum of broilers. This raises concerns over both disease control in animals and food safety, as there is a possibility of the transfer of resistance to humans through food. As colistin is an antibiotic of last resort, the identification of a plasmid conferring high-level resistance is concerning, as it may have the ability to transfer to other pathogenic Enterobacteriaceae. This would further limit the available treatments for MDR infections.

7th ARAE2017 Poster No. 7:

Pig faecal bacteria exhibiting colistin and imipenem resistance

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Background and objectives: The World Health Organisation currently considers antibiotic resistance (AR) one of the greatest threats to animal and human health. It is crucial that all sources of AR are identified and controlled to minimise the transfer of resistance genes and/or bacteria within animals, and between animals and humans. Imipenem (a carbapenem) and colistin (a polymyxin) are two antibiotics that play key roles in the treatment of infections that are not readily treated with other antibiotics. However, resistance to these antibiotics have recently been identified in food animals. The aim of this study was to investigate and characterize antibiotic resistance of bacterial isolates from pigs.

Materials and methods: Selective agars (EMB, Cetrimide, HiChrome, McConkey and XLT-4) with antibiotics (Amikacin, Cefotaxime, Colistin, Imipenem and Kanamycin) were used to isolate AR bacteria from pig faecal samples. The cultured isolates underwent antibiotic susceptibility disk testing (antibiotics same as above). Agar dilution susceptibility testing was performed on all colistin resistant isolates. The presence of metallo-beta-lactamases was identified by the double disk synergy test using Imipenem and Imipenem EDTA. All resistant isolates were speciated by 16S rRNA PCR and sequencing.

Results: Thus far, this work has identified 47 isolates resistant to colistin and 23 resistant to imipenem. Ten of these isolates exhibited resistance to both antibiotics, as well as resistance to aminoglycosides, which demonstrates multi-drug resistance. Furthermore, 91 isolates displayed reduced susceptibility to imipenem. Eight imipenem resistant isolates were positive for metallo-beta lactamases. These resistant isolates were identified as *Enterococcus faecium* and *Citrobacter* spp.

Conclusion: The identification of both colistin and imipenem resistant isolates from pig faecal samples is concerning and highlights the need to identify all potential reservoirs of AR bacteria in the gut microbiomes of animals. The emergence of AR in the environment, the use of antibiotics in veterinary medicine and the possible transfer of resistance through the food chain to humans are issues of high priority at both the national and EU policy levels.

7th ARAE2017 Poster No. 8:

F33: A-: B-, IncI1/ST136, and IncN plasmids accelerate the emergence of the fosfomycin resistance gene *fosA3* in *Escherichia coli* from pigs, chickens and dairy cows in Northeast China

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Background and objectives: The aim of this study was to investigate the occurrence of fosfomycin-resistant *Escherichia coli* isolates from pigs, chickens and dairy cows and to characterize their *fosA3*-positive plasmids.

Materials and methods: A total of 370 *E. coli* isolates, collected from pigs, chickens and dairy cows in three Northeastern provinces of China during 06/2015 to 04/2016, were screened by PCR for fosfomycin resistance genes. Plasmids were further characterized using PCR-based replicon typing, pMLST and restriction fragment length polymorphisms. Representative plasmids were sequenced completely using the next-generation Illumina MiSeq system. Similar plasmids or genetic environments of *fosA3* were analysed by overlapping PCR.

Results: Of the 370 *E. coli* isolates, 39 (10.5%) isolates showed resistance to fosfomycin and contained *fosA3* genes. Thirty-three (85%) of the 39 *fosA3*-positive *E. coli* isolates were clonally unrelated, and all *fosA3* genes were co-located on conjugative plasmids with IncN (n=12), IncN-F33:A-:B- (n=2), IncF33:A-:B- (n=14), IncF14:A-:B- (n=2), and IncI1/ST136 (n=9). Whole nucleotide sequences of three *fosA3*-carrying plasmids pECF12 (IncF33:A-:B-, 77822 bp); pECB11 (IncF33:A-:B-, 92545 bp) and pECM13 (IncI1/ST136, 113006 bp) and a partial 16293-bp *fosA3*-containing fragment from the IncN/ST7 plasmid pECXH3 were obtained. Four different genetic contexts of *fosA3* (types I-IV) were detected in all 39 *fosA3*-producing *E. coli* isolates. The genetic structures, IS*903*-$bla_{CTX-M-65}$-*fipA*-IS*26*-*fosA3*-1760bp-IS*26*-*tetR*/*tet*(A) and IS*26*-$bla_{CTX-M-14}$-*fosA3*-IS*26*-*aac(3)*-III-*tmrB*-IS*Cfr1*-bla_{TEM-1}-*rmtB*-IS*CR1*-*sul1*-*aadA2*-*dfrA12*-*intI1* were most frequently located on IncN and IncI1/ST136 plasmids, respectively. In addition, overlapping PCRs showed that two, two and six FosA3-producers also contained pECF12-like, pECXH3-like and pECB11-like plasmids. While the backbones of these plasmids resembled those of other plasmids of the same replicon type, the multi-resistance regions differed substantially in the arrangements and types of resistance genes or insertion sequences.

Conclusion: Our results showed that the fosfomycin resistance rate of animal *E. coli* isolates in China is higher than previously reported. Three epidemic multiresistance plasmids of different Inc groups play an important role in the rapid spread of the *fosA3* gene. Co-selection of these plasmids by antimicrobial agents other than fosfomycin may be an explanation for their emergence.

7th ARAE2017 Poster No. 9:

Prevalence and anti-microbial resistance (AMR) profile of non-typhoidal *Salmonella* of pigs in Kenya and Malawi

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Background and objectives: Non-typhoidal Salmonella (NTS) causes a higher incidence of disease in humans in developing than developed countries. It is hypothesised that human-to-human transmission may not be the sole route of ST313 spread and that zoonotic transmission may occur. Free-range pig production is common in Kenya and Malawi. Currently the prevalence of NTS in pig populations in sub-Saharan Africa has not been described.

The study objectives were to compare to prevalence, strains and AMR profile of NTS in porcine faecal and mesenteric lymph node samples collected post mortem from slaughterhouse sites in three study areas; Nairobi (urban) and Busia (rural), Kenya and the Chikwawa valley, Malawi.

Materials and methods: Faecal and mesenteric lymph node samples were taken from pigs at post-mortem at slaughterhouses in Busia, western Kenya and in Nairobi. Samples were enriched in buffered peptone water for 24 hours at 37°C followed by 24 hours in Rappaport Vassiliadis medium at 42°C. Selective culture of the samples occurred in brilliant green agar (Oxoid) and Harlequin ABC Salmonella media (Lab M). Salmonella colonies were isolated and cultured for a further 24 hours on nutrient agar prior to antisera testing (Prolab) to confirm the presence of Salmonella. All NTS isolates underwent antimicrobial susceptibility testing (AST) against a panel of 12 antibiotics using the standard disc diffusion method. End-point PCR using the primer tetrathionate was carried out to confirm the presence of Salmonella prior to submission for whole genome sequencing. This method will be repeated on samples yet to be collected from Malawi.

Results: A total of 267 pigs were sampled in Busia and 304 in Nairobi, Kenya. Salmonella was isolated and confirmed from 64 (24%) pigs in Busia (43 mesenteric lymph nodes and 21 faecal samples) and 95 (31%) pigs in Nairobi (43 mesenteric lymph node samples and 52 faecal samples).
AST of these isolates revealed the prevalence of AMR was tetracycline (38.7%), trimethoprim-sulphonamide (18.7%), streptomycin (18%), ampicillin (16.7%), amoxicillin-clavulanate (6.0%), chlor-amphenicol (4.7%), cefoxitin (4.0%), ciprofloxacin (3.3%), cefotaxime (2.7%), gentamicin (1.3%), kana-mycin (1.3%), and ceftazidime (0.6%).

Furthermore there were 8 multidrug resistant (greater then 3 classes of antibiotics) isolates from pigs in Busia, and 17 multidrug resistant isolates from pigs in Nairobi, which were resistant to a range of combinations of antibiotics.
Whole genome sequencing results are currently pending.

Conclusion: Multidrug resistant Salmonella were detected in faecal and mesenteric lymph node samples from pigs at slaughter in Busia and Nairobi.

7th ARAE2017 Poster No. 10:

Antibiotic resistance patterns in bacteria isolated from slaughterhouse worker faeces in western Kenya.

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Background and objectives: In Kenya, antimicrobials are widely available without prescription; farmers can easily acquire them and self-administer to animals. Livestock carry antimicrobial resistant (AMR) bacteria, which could be a risk to public health as they are transmissible to humans via meat consumption. Another route of transmission may be via the preparation of meat in slaughterhouses. This study aimed to identify patterns of AMR in faecal bacteria (*E.* coli) from slaughterhouse workers at 142 randomly selected livestock slaughterhouses in the Victoria Lake Basin, with a 45km radius of Busia town, Western Kenya.

Materials and methods: A census of slaughterhouses was recruited across the study site, covering different livestock species (cattle, sheep, goats and pigs). Up to 12 volunteers were recruited per slaughterhouse. Participants were interviewed (via questionnaire) regarding risk behaviours (e.g. smoking/eating in slaughterhouses), exposure to livestock and personal hygiene practices. Faecal samples were collected from volunteers and cultured on eosin-methylene blue (EMBA) agar. *E. coli* was selected and AMR was detected via antibiotic disc diffusion; extended-spectrum β-lactamase-producing *E. coli* were confirmed by double-disc test.

Results: In total, 441 faecal samples were collected; AMR *E. coli* was detected in 82.5% of samples to tetracycline (82.1%, n=362), trimethoprim (53.1%, n=234), sulfathiazole (50.8%, n=224), ampicillin (33.1%, n=146), chloramphenicol (9.8%, n=43), ciprofloxacin (7.3%, n=32) and gentamicin (3.2%, n=14). ESBL-producing *E. coli* were detected in 41 (9.3%) faecal samples. Multi-drug resistance (to 3 or more classes of antimicrobials) was found in 36.5% samples; two samples yielded *E. coli* which were pan-resistant.

Conclusion: This study demonstrated high levels of AMR, specifically to tetracycline and sulfonamide drugs in slaughterhouse workers' faecal bacteria. Such resistance reflects known high use of such antimicrobials in local farm animals and in the human population. We hypothesise that zoonotic transmission of AMR bacteria during meat processing occurs. ESBL *E. coli* were detected, albeit at low prevalence, perhaps reflecting that cephalosporins are not commonly used in agriculture in Kenya. ESBL production was more likely to be associated with MDR *E. coli*, suggesting co-carriage on the same mobile genetic elements. Further investigation will include full genotyping of isolates and resistance elements, as well as molecular analysis of isolates from livestock as part of ongoing work.

7th ARAE2017 Poster No. 11:

Characterization of extended-spectrum β-lactamase- and AmpC-producing *Escherichia coli* from legally and illegally imported meat

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Background and objectives: Extended-spectrum β-lactamase-(ESBL-) producing *Escherichia* (*E.*) *coli* are commonly resistant to a wide variety of antimicrobial agents. This constitutes a major healthcare concern as therapy options for infections caused by these bacteria are often severely limited. It has been suggested that the transmission through the food chain, and poultry meat in particular, plays an important role in the incidence of such infections in the community. The aim of this study was to examine isolates obtained from meat, which was introduced into the European Union both legally and illegally in order to assess potential risks associated with these products.

Materials and methods: A total of 36 ESBL/AmpC-producing *E. coli* isolated from imported meat were examined in this study. They were recovered from samples of legally and illegally imported pork and poultry meat in the course of a previous study. The isolates were characterized by antimicrobial susceptibility testing, multilocus sequence typing, macrorestriction analysis, microarray analysis and additional PCR assays.

Results: The most prevalent ESBL gene detected among the isolates was $bla_{\text{CTX-M-2}}$ (n=15), followed by the AmpC β-lactamase gene $bla_{\text{CMY-2}}$ (n=7). Other isolates carried genes belonging to CTX-M groups 8, 1 and 9, or $bla_{\text{SHV-12}}$. Most isolates showed additional phenotypic resistances to non-β-lactam antibiotics and a variety of resistance genes could be detected. Most common was *aadA1* (n=27), followed by *sul2* (n=23), and *sul1* (n=21). Quinolone-resistance genes *qnrB* and *qnrS* were detected in 6 and 3 isolates, respectively.

All isolates carried at least one virulence-associated gene. Most prevalent were *hemL* (n=33), *iss* (n=29), *tsh* (n=22) and *lpfA* (n=18). Among the genes coding for toxins, *astA, cma* and *mchF* were most common and present in 16 isolates each. One isolate carried genes typically associated with EPEC*: eae, espB*_O26 and genes connected to type III secretion systems.

Molecular typing results showed a great heterogeneity among the isolates. No more than 3 isolates shared the same sequence type (ST). Most common were ST101 and ST117.

Conclusion: A variety of antimicrobial resistance genes and virulence-associated genes could be detected among the isolates examined in this study. These results highlight the potential risks associated with international trade of meat products as a route for the transmission of ESBL/AmpC-producing *E. coli.*

7th ARAE2017 Poster No. 12:

Extended spectrum β-lactamase producing *Enterobacteriaceae* in local and imported poultry meat in Ghana

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Background and objectives: Antibiotics in animal feed to prevent infections have raised concerns on the emergence and spread of resistant microorganisms. While animal products are traded globally with unprecedented ease, the control of antimicrobial resistance is challenging, in particular in sub-Saharan Africa, where surveillance systems do not exist. This study aims to characterize and compare extended spectrum beta lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* from imported and locally produced poultry products sold in Ghana.

Materials and methods: During an eight months period, local and imported chicken meat is collected from 94 stores and markets throughout the city of Kumasi (Ghana) and cultured on chromogenic ESBL screening agar. Phenotypic ESBL-producing *E. coli* and *K. pneumoniae* isolates are confirmed by combined disc test, further characterized by amplifying the *bla*CTX-M, *bla*TEM and *bla*SHV genes and correlated to their country of origin.

Results: ESBL-producing *E. coli* (n=49) and *K. pneumoniae* (n=36) have been found on 37% of meat samples (n=200) with local chicken (47%) being significantly more contaminated than imported products (31%; p=0.03). However, ESBL rates vary by importing country, ranging between 88% (Belgium) and 23% (USA). Most ESBL-producing isolates belonged to the CTX-M1 group (80 %) and are distributed similarly among countries.

Conclusion: High numbers of ESBL-producing bacteria, particularly on local but also imported poultry meat, represent a potential source for human exposure and spread within the community. Surveillance along the poultry production-food-consumer value chain might be an important tool to identify and target sources of circulating and emerging multidrug resistant pathogens.

7th ARAE2017 Poster No. 13:

NDM-1 producing *Vibrio parahaemolyticus* isolated from imported shrimps

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Background and objectives: *Vibrio parahaemolyticus* is a common seafood foodborne pathogen. This species lives in tropical and warm seas and, with the global warming, this bacterium is an emerging risk. On the other hand, the genus *Vibrio* is able to exchange mobile genetic element carrying antibiotic resistance genes. It can play a role as a transmission vector and/or a reservoir of these genes.

Material and methods: The antimicrobial susceptibility of *V. parahaemolyticus* isolated from seafood was tested by disk diffusion, following the CLSI standards. The presence of known β-lactamase genes was checked by microarray (Check MDR CT101, CheckPoints, NL), in parallel carbapenemase activity was tested by the Carbapenemase Inactivation Method (CIM test). A whole genome sequencing (WGS) was performed to detect other resistance genes and look for their genetic environment.

Results: One strain, *V. parahaemolyticus* 16-B3PA-006, isolated from shrimps imported from Vietnam, displayed growth contact to the disk for cephalothin, cefoxitine, cefotaxime and ceftazidime disks. The CIM test evidenced a carbapenemase activity and bla_{NDM} gene was detected by microarray. WGS revealed the presence of bla_{NDM-1} on a class1 integron previously described in a clinical context and detected other resistance genes: *sul1, sul2, dfrA16, strA, strB, aadA2, floR and tetA*.

Conclusions: The strain *V. parahaemolyticus* 16-B3PA-006 carry genes that can give resistance to important antibiotic families: β-lactams, sulphonamides, phenicols, aminoglycosides and tetracyclines. To the best of our knowledge, bla_{NDM-1} has already been isolated from *V. cholerae* in clinical cases, but this is the first description of a *V. parahaemolyticus* strain producing NDM-1 isolated through food. It is generally assumed that NDM-1 is introduced in Western Europe mostly by travelers returning from endemic part of the world and being carriers of bla_{NDM-1} positive bacteria. However, the introduction of carbapenemase producing bacteria by contaminated imported food should certainly not be underestimated anymore.

7th ARAE2017 Poster No. 14:

Minimum inhibitory concentrations and antibiotic resistant genes in the freshwater cyanobacteria *Microcystis aeruginosa*

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Background and objectives: Native aquatic bacteria have been considered as important players in the emergence/dissemination of antibiotic resistance (AR) in water environments. Conversely, the role of cyanobacteria (CB) on water resistome is unknown. We have been hypothesizing that CB may contribute to the spread of AR in the environment, considering that: i) they are ubiquitous prokaryotes in aquatic habitats; ii) they are exposed to antibiotics and AR bacteria; iii) they maintain biological relations with their bacterial neighbors; iv) they can change genetic material by horizontal gene transfer. *Microcystis aeruginosa* is one of the most common CB in freshwater reservoirs worldwide, often exhibiting long residence time. This work aimed to evaluate the antibiotic susceptibility patterns and resistance mechanisms in *M. aeruginosa* in order to assess their putative contribution to the global pool of resistance determinants in freshwater environments.

Materials and methods: Antibiotic susceptibility of 9 strains of *M. aeruginosa*, isolated from different freshwater reservoirs was evaluated by a microdilution method adapted for cyanobacteria, against beta-lactams (amoxicillin, ceftazidime, ceftriaxone), aminoglycosides (kanamycin, gentamycin), quinolones (norfloxacin, nalidixic acid), trimethoprim and tetracycline. Minimum inhibitory concentrations (MIC) were determined according to CB cell density (DO, 450nm) and microscopic examination of cultures integrity. All strains were subjected to the search of AR-encoding genes and class 1, 2 and 3 integrons by PCR/sequencing.

Results: *M. aeruginosa* is not susceptible to trimethoprim, tetracycline and nalidixic acid within the tested concentration range (0.0015-1.6 mg/L). However, the cell growth is strongly inhibited by norfloxacin for the majority of the strains (0.05 mg/L≤MIC≤0.1 mg/L). The MICs of aminoglycosides and beta-lactams varied between 0.1 - 0.4 mg/L and 0.1 – 1.6 mg/L, respectively. The search for AR-encoding genes revealed a *str*A-*str*B gene in one strain and a *sul*1 gene in four strains. Among these four, two also presented an *int*1-type gene, and one co-harboured a *qac*ΔE gene.

Conclusion: The presence of AR-encoding genes and integrons, as well as the reduced susceptibility to antibiotics, supports the hypothesis that CB play a role on freshwater resistome, contributing, eventually, to the dissemination of AR in freshwater environments.

7th ARAE2017 Poster No. 15:

Wastewater is a reservoir for clinically relevant carbapenemase and 16S rRNA methylase producing *Enterobacteriaceae*

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Background and objectives: The aim of the study was to evaluate wastewater for carbapenemase producing *Enterobacteriaceae* (CPE) and 16S rRNA methylase producing Gram negative bacteria (MPB), and to assess their occurrence following wastewater treatment.

Materials and methods: Wastewater samples were collected between June 2015 and March 2016 in the sewage network of the city of Basel from sites located before and after influx of wastewater from the hospital into the sewage network. Samples were also obtained from the influent and from the effluent of the receiving wastewater treatment plant. Samples were screened for CPE and for MPB using selective media. *E. coli* and *K. pneumoniae* were typed by MLST. Carbapenemase and 16S rRNA methylase genes were identified by PCR and sequencing. Resistance profiles were obtained by the disk diffusion test and Etest.

Results: The occurrence of CPE and MPB was increased downstream of hospital wastewater influx. Of 49 CPE isolates, nine belonged to OXA-48 producing *E. coli* clone D:ST38, seven were OXA-48 producing *C. freundii* and six were KPC-2 or OXA-48 producing *K. pneumoniae* belonging to clonal complex 258. NDM (NDM-1, NDM-5, NDM-9) and VIM (VIM-1) producers were detected sporadically. MPB included ARMA and RMTB producing *E. coli* and *Citrobacter spp.* Isolates corresponding to strains from wastewater were detected in the effluent of the treatment plant.

Conclusion: CPE and MPB, predominantly OXA-48 producing *Enterobacteriaceae*, are readily detected in wastewater, survive wastewater treatment and are released into the aquatic environment. OXA-48 producers may represent an emerging threat to public health and environmental integrity.

7th ARAE2017 Poster No. 16:

Agricultural soils harbor high levels of potentially mobile antibiotic resistance genes

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Background and objectives: Several studies have suggested that stress inducers, just like antibiotics, could contribute to a general mobilization of genetic material in bacterial communities. By studying the genes involved in the mobility of resistance determinants in a pool of agricultural soils, we aimed to specifically target bacterial communities that have been subjected to a variety of selection pressures throughout the years, and that are directly associated to the food-chain.

Materials and methods: Three different agricultural soil samples located within a Portuguese region, representative of the microorganism populations, were pooled in order to minimize spatial variability and specificity of the different practices. DNA was extracted from the samples and amplified using the Illustra GenomiPhi V2 DNA Amplification Kit. DNA was then purified using Illustra GFX PCR DNA and Gel Band Purification Kit and quantified. The DNA sample (10µg each) was high-throughput sequencing using Illumina Hiseq2000 at BGI. Furthermore, quality filtering of data, metagenomic assembly, taxonomic classification and functional analysis, identification of antibiotic resistance genes (ARG) and mobile genetic elements (MGE) were performed with specific tools.

Results: A library consisting of about 180-bp DNA fragment sequences was constructed before DNA sequencing. The strategy "Index 101 PE" (Paired End sequencing, 101-bp reads and 8-bp index sequence) was used for sequencing. About 3% of the reads could be mapped to bacterial sequences using Blast and an E-cut off of 10^{-10}, with proteobacteria (e.g. *Escherichia coli*) as the high represented bacterial phylum (49.7%), followed by bacteroidetes (e.g. *Cytophaga hutchinsonii*) (15.1%), firmicutes (e.g. *Bacillus subtilis*) (12.6%), and actinobacteria (e.g. *Mycobacterium tuberculosis*) (5.9%). Biosynthesis processes (25.0%), translation (10.3%) and tRNA modification and processing (7.7%) amount to >50% of all mappable reads; response to antibiotic represented 0.5%. We identified acquired ARG related to aminoglycosides, fosfomycin, tetracycline, macrolides, glycylcyclines, β-lactams and lincosamides, along with MGE, non-acquired ARGs and compounds associated with resistance to heavy-metals and quaternary ammonium compounds.

Conclusion: These results highlight that agricultural soil microbial communities are potential reservoirs of ARGs; the MGEs found may provide ARGs with the tools for their dissemination in different environments.

7th ARAE2017 Poster No. 17:

An *in vitro* chicken gut model demonstrates transfer of a multidrug resistance plasmid from *Salmonella* to commensal *Escherichia coli*.

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Background and objectives: Chicken caeca are replete with bacteria that fulfil various beneficial roles for the host, including helping to resist colonisation by pathogens, but they can also facilitate transfer of plasmids between bacteria. The potential for dissemination of multidrug resistance (MDR) plasmids via conjugation in the caeca has not been fully defined, but presents a significant public and animal health concern as it may affect our ability to treat bacterial infections. The aim of this work was to model the chicken ceaca microbiota using a chemostat system, simulate colonisation by *Salmonella* and examine the dynamics of transfer of its MDR plasmid harbouring the ESBL gene $bla_{CTX\text{-}M1}$. The impact of cefotaxime administration on plasmid transfer and microbial diversity was also evaluated.

Materials and methods: A chemostat system employing up to six separate vessels in parallel was developed to approximate the chicken caecal microbiota. The microbiota was challenged by inoculation of a *Salmonella* strain harbouring an MDR plasmid and by administration of cefotaxime. Changes in the bacterial populations were monitored using culture-independent 454 sequencing methods and by culture on selective agar plates. 454 data was analysed in Qiime to obtain microbial profiles and to identify significant alterations in bacterial populations. Representative isolates of *E. coli* were recovered from plates containing cefotaxime and examined for the presence of the MDR plasmid by WGS.

Results: Microbial profiles showed *Salmonella* inoculation resulted in no significant changes to alpha- and beta-diversity of the microbiota, whereas administration of cefotaxime caused significant alterations to both measures giving results closely paralleling those reported for *in vivo* studies. Transfer of the MDR plasmid from *Salmonella* to commensal *E. coli* was demonstrated by WGS. Transfer occurred at high rates to seven distinct sequence types of *E. coli* found in the microbiota, even in the absence of cefotaxime, with resistant isolates being recovered within three days.

Conclusion: The chemostat system provided a valuable surrogate of the chicken caecal microbiota and gave insight into the dynamics of plasmid transfer of antibiotic resistance to multiple commensal *E. coli* strains. We suggest the chemostat approach models dissemination of antibiotic resistance which in future can be used to develop interventions that mitigate spread of AMR genes, before employing animal studies.

7th ARAE2017 Poster No. 18:

Multiple drug resistance identified from a soil microbiome using functional metagenomics

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Background and objectives: It is well documented that antibiotic resistance is a clinical concern that affects both human and animal health but antibiotic resistance in the soil environment is not as well understood. As conventional methods only culture a small percentage of soil bacteria, culture-independent methods such as functional metagenomics, are sometimes used to characterise soil bacteria.

Materials and methods: DNA was extracted from the soil. A fosmid library was generated using *Escherichia coli* (*E. coli*) containing a fosmid and DNA insert. Next generation sequencing of non-identical fosmids was completed in order to identify resistance genes or operons and novel genes through assembly and comparisons to existing databases. Proteomics was then used to identify which genes are expressed. The proteins were extracted and analysed using mass spectrometry (Q exactive). Whole genome sequencing was carried out to look for mutations. A gene expression qPCR assay was designed to target two identified novel proteins and analyse up and down regulation of genes.

Results: Four novel proteins were identified which were closest relatives of peptidase, uromethyltransferase, pyrrolo-quinone, and a hypothetical protein. The *E. coli* containing fosmid conferring resistance to nalidixic acid (Nal2) was cultured; the four proteins were cloned and sequenced. Minimum Inhibitory Concentration results showed that the Nal2 fosmid grew on 8 mg/L nalidixic acid compared to 1-4 mg/L for bacteria without plasmid. One uromethyltransferase clone and two peptidase clones were resistant to nalidixic acid. Whole genome sequencing revealed four mutations that resulted in amino acid changes. No mutations were found for GyrA or ParC. Gene expression analysis showed no significant difference between reference genes and resistant strains of peptidase and uromethyltransferase clones.

Conclusion: Novel quinolone resistance genes were identified from soil. Several techniques were employed to establish their mechanism of resistance. Whole genome sequencing and gene expression analysis showed that it was neither amino acid changes, nor up regulation or down regulation of genes that resulted in resistance to nalidixic acid.

7th ARAE2017 Poster No. 19:

Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* in Latvian broiler chickens

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Background and Objectives: Campylobacteriosis is a foodborne disease caused by *Campylobacter* spp. and it the most commonly registered bacterial human intestinal infection in the EU. The prevalence of *Campylobacter* in Latvian broiler faecal samples in 2010 was 92.5%. Various studies demonstrate that the antimicrobial resistance of *Campylobacter* spp. has increased among the clinical isolates. Nowadays, the resistance level of *Campylobacter* isolates is higher than in previous decade.

The aim of the present study was to determine the antimicrobial resistance of *Campylobacter* spp. isolated from broiler chicken cecal samples in Latvia in 2008 and 2014.

Materials and methods: A total of 2870 broiler chicken cecal samples were collected from two poultry slaughterhouses in Latvia at the evisceration stage. Ten samples were combined in one pooled cecal sample. Samples were tested according to ISO 10272 standard. MIC method was used for detection of antimicrobial resistance according to CLSI standard. Multiplex PCR method for confirmation of *Campylobacter* spp. and identification to the species level was applied (Wang et al., 2002).

Results: *Campylobacter jejuni* and *C. coli* from broiler chicken pooled cecal samples were isolated in 158/287 samples. All of the 158 *Campylobacter* isolates were resistant to ciprofloxacin (100%) and nalidixic acid (100%). A total of 2.5% (n=4) of *Campylobacter* spp. isolates were resistant to erythromycin, 6.3% (10/158) to streptomycin, 8.9% (14/158) to gentamicin, and 19.6% (31/158) to tetracycline. The multiresistance was observed in 5.1% (8/158) of samples, the most common antimicrobial multiresistance pattern was the combination of nalidixic acid, ciprofloxacin, tetracycline, gentamicin, and streptomycin. Resistance against streptomycin was significantly higher in *C. coli* than in *C. jejuni* ($p \leq 0.001$). There were no significant ($p > 0.05$) differences in *C. coli* and *C. jejuni* antimicrobial resistance against other antimicrobials.

Conclusion: The present study shows that *Campylobacter* jejuni and coli from broiler chicken cecal samples in Latvia had a high-level of antimicrobial resistance, especially against quinolones.

Acknowledgements: This study was financed by Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia (AgroBioRes 2014-2017) Project No. 5. Resistance of microorganisms and other biological and chemical risks research procedures development and application in the food chain (RISKS).

7th ARAE2017 Poster No. 20:

Effects of different floor designs in fattening turkeys on the development of antibiotic resistance in commensal *E. coli*

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Background and objectives: Currently, there are limited data regarding effects of birds with different exposure to their excreta on development of antimicrobial resistance. The aim of this study was to evaluate the development of enrofloxacin resistance in turkeys housed on different floor designs.

Materials and methods: 240 turkeys (Big 6) reared in large groups for 7 days were randomly assigned to four groups (G 1-4) with three subgroups each: G1 – entire floor pens with litter, G2 - floor pens with litter and heating pad, G3 – partially (50:50) slatted floors including an area that was littered, G4 - fully slatted floors with a sand bath (900 cm^2). Number of animals was reduced by dissection on day 22 to 144 animals in every trial. Enrofloxacin (10 mg/kg BW) was given on day 10-14 via drinking water. Resistance of 207 commensal *E. coli* isolates to enrofloxacin (ENR) and ampicillin (AMP) was evaluated testing 11, resp. 12 different concentrations on Micronaut-Plates (Merlin). *E. coli* was isolated on d2, 21 and 35 from cloacal swabs and on d 9, 15 and 35 from litter samples. T-test was done to investigate significance.

Results: Prior to antibiotic treatment all *E. coli* isolates from cloacal (d2) and litter (d9) samples were sensible. Results of the development of MIC values are shown in table 1.

Table 1: Average MIC-values for enrofloxacin for each group and day of sampling

	Litter/excreta samples			Cloacal samples	
	d9 (n=24)	d15 (n=24)	d35 (n=24)	d21 (n=60)	d35 (n=60)
G1: entire floor + litter	0,03	10.76 ^{b}	21.34 ^{ab}	25,61	17,08
G2: litter + heating pad	0,03	26.67 ^{ab}	26.68 ^{ab}	32,00	19,21
G3: litter + slatted floor	0,03	32.00 ^{a}	32.00 ^{a}	32,00	16,58
G4: fully slatted floor	0,03	32.00 ^{a}	16.02 ^{b}	29,87	14,95

^{a,b} different super-scripts within a column indicate significantly different values ($p < 0.05$)

Interestingly ampicillin resistance (data not shown) revealed very similar MIC`s as shown for enrofloxacin, although no ampicillin was administered in this study.

Conclusion: Idependent of the floor design, 3 weeks after antibiotic treatment for E. coli there was still a higher MIC value for cloaca, but was decreased compared to d21. Further studies (11) have to be evaluated in this ongoing project, before a conclusion concerning the floor design could be given.

Acknowledgement: The project is supported by funds of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV).

7th ARAE2017 Poster No. 21:

Personalized medicine - Fast detection schemes towards antibiotic susceptibility testing

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Background and objectives: Fast and efficient methods for the diagnosis and monitoring of infectious diseases and severe complications such as sepsis are essential in order to allow targeted and optimal therapy and thereby ensure the survival of the patient. Today, infectious diseases are responsible for about 20% of all deaths worldwide, in most cases the cause of death being due to sepsis. The high mortality can be attributed inter alia to the lack of rapid tests for the analysis of the antibiotic resistance of infectious agents. In addition to gram-positive infectious agents, such as methicillin-resistant Staphylococcus aureus (MRSA) and glycoprotein-resistant enterococci (VRE), gram-negative pathogens are increasingly becoming more and more common. Currently established methods of microbiological diagnostics are based on culture and microscopy methods, which are often very time-consuming and to be carried out with trained specialist personnel.

Materials and methods: Within this contribution, we will show that Raman spectroscopy offers the potential to bypass time-consuming cultivation procedures and allows for an identification of microbial pathogens on a single cell level in less than three hours.

Results: By the additional integration of simple sample preparation methods into miniaturized constructions, e.g. selective filters with different pore sizes, functionalized particles, chip surfaces or electrode structures for the utilization of dielectrophoretic forces in a microfluidic chip, the combination with Raman spectroscopy results in a rapid and efficient analysis of infectious agents and antibiotic resistance. Changes in the bacterial Raman spectra due to antibiotic treatment can be identified already after 30 minutes of treatment.

Conclusion: To summarize, the introduced processes form the entire process chain, from sampling to the result, and have a high potential to maximally reduce the critical parameter 'time' of microbiological diagnostics, enabling an efficient diagnosis, whereby an often life-saving therapy can be started in a very short time. Thus, Raman spectroscopy holds great promise as point-of-care-approach for a bedside diagnosis and therapy of infectious diseases.

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7th ARAE2017 Poster No. 22:

Probing novel natural products for antibiotic activity against zoonotic bacteria

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Background and objectives: The last decades have seen the approval of only few new antimicrobial drugs, which are derived from a limited number of classes of antibiotics that had been discovered by the mid-1980s. However, despite the discovery of many new chemical entities (NCEs) in recent years, financial, regulatory and scientific issues frequently impede their evaluation and development to new therapeutics.

Our aim was to preselect a set of nine promising NCEs, including eight natural products, *in vitro* for activity testing in an animal model. Published minimal inhibitory concentrations (MICs), if available, were validated through testing the substances against up to nine bacterial species (*Escherichia coli, Staphylococcus aureus, Mannheimia haemolytica, Pasteurella multocida, Brachyspira hyodysenteriae, Clostridium difficile, Mycobacterium avium* complex*, Klebsiella pneumoniae, Enterobacter cloacae*).

Materials and methods: To assess the antimicrobial activity of the NCEs, MIC values were determined using established CLSI protocols. ATCC quality control strains were tested to ensure conformity with CLSI standards. Most of the bacterial strains tested were field isolates. They were first tested against standard therapeutics such as gentamicin, ampicillin, chloramphenicol and tetracycline and then divided into groups of susceptible and resistant strains. In the next step, five isolates, taken from both groups, were selected to pre-screen the NCEs for activity. Substances with very low MIC values in the pre-test were then tested against up to 50 isolates per species to validate their activity margins.

Results: Our tests identified two NCEs with high activity against *P. multocida* and *M. haemolytica*, including up to 50 field isolates. Other NCEs with good activity against laboratory and quality control strains had higher MIC levels with field isolates.

Conclusion: The study shows that MIC tests should be carried out with relevant clinical isolates in addition to well-established laboratory and quality control strains from the ATCC, or other culture collections. Field isolates frequently show higher MIC values, allowing a more thorough evaluation of NCE activity. This approach identified two promising NCEs with activity against the novel targets *P. multocida* and *M. haemolytica*. They will be selected for further investigation, including *in vivo* studies in murine and bovine animal models.

7th ARAE2017 Poster No. 23:

Use of on-farm bacterial culture and decision support tools to reduce antimicrobial usage in cases of mild to moderate clincial mastitis in cows

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Background and objectives: The aim of the study was to assess antimicrobial usage for clinical mastitis following implementation of a novel on-farm culture system and selective therapy based on culture results.

Materials and methods: Milk samples were collected from 506 quarters with mild to moderate clinical mastitis from 7 farms for on-farm microbiology culture. The culture system was a plate which included 4 quadrants (a 5% blood agar, a Gram positive selective media, a Gram negative selective media, and a yeast/fungi selective media; Check-Up Farm Medix, Auckland, New Zealand). Within sequential pairs of cows, half of these quarters were assigned to be treated without regard to the culture results (Blanket treatment group), while the culture results were used to decide the treatment outcome for the remaining quarters (Selective treatment group), i.e. no treatment for quarters from which no bacteria or gram-negative organisms were isolated, intramammary infusion of cloxacillin for quarters from which *Staphylococcus aureus* was isolated, and intramammary or parenteral narrow-spectrum penicillin for quarters from which *Streptococcus* spp., coagulase-negative *Staphylococcus* spp. or other bacteria were recovered. Milk samples were also submitted for routine laboratory-based microbiology culture. On-farm culture results and mastits treatment records were recovered and the natural log of the sum of antimcrobial doses/cow was compared between treatment groups. The level of agreement between the on-farm and laboratory-based culture techniques was compared with c^2 analysis.

Results: Compliance with the treatment protocols was higher amongst quarters assigned to the Selective (199/233; 85.4%), compared with the Blanket (171/249; 68.7%) treatment group ($p<0.001$). Quarters assigned to the Selective group had a lower mean Ln dose (1.00 (SEM 0.03)) than those assigned to the Blanket (1.22 (SEM 0.03)) group ($p=0.005$). There was no difference between treatment groups in the hazard that cows would be re-diagnosed with clinical mastitis within 60 days of enrolment (hazard ratio (Selective relative to Blanket) = 0.82 (95% CI=0.39-1.69); $p=0.59$). *Streptococcus* spp. (61%) were the most common isolates followed by *S. aureus* (13%) and no growth (12%). The agreement between on-farm culture and laboratory testing was 188/331 (56.8%).

Conclusion: Use of on-farm culture with Selective therapy based on culture results resulted in approximately a 20% reduction in antimicrobial usage.

7th ARAE2017 Poster No. 24:

CXC chemokines exhibit antimicrobial activity against multidrug-resistant Gram-negative pathogens

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Background and objectives: The continued rise and spread of antimicrobial resistance among bacterial pathogens poses a critical threat to global health. Of particular concern is resistance against carbapenems and colistin, antimicrobial agents regarded as last-line defenses for treating infections caused by Gram-negative pathogens. Countering the risks posed by antimicrobial resistant bacterial pathogens will require a multifaceted effort that includes the discovery of novel therapeutic approaches. Here we present the antibacterial capacity of human CXC chemokines to kill multidrug-resistant Gram-negative pathogens.

Materials and methods: Multidrug-resistant *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii* isolates were obtained from Pakistan, Italy, and the United States. Antimicrobial resistance was established by Vitek. Susceptibility to recombinant human CXCL9 and CXCL10 was measured by colony-forming unit determination. New Delhi metalloprotease-1 (NDM-1) and oxacillinase-48 (OXA-48) carbapenemases were identified by PCR. Lipid A chemical modifications conferring colistin resistance were detected using MALDI-TOF mass spectrometry.

Results: Multidrug-resistant bacteria, including NDM-1- and OXA-48-producing isolates, were susceptible to chemokine-mediated antimicrobial activity. Colistin resistance arising from disruptions in chromosomal loci (e.g. *mgrB* and *pmrB*), and consequent modification of lipid A with L-4-aminoarabinose (L-Ara4N), resulted in varying levels of isolate-specific resistance against CXCL10; genetic complementation reversed these phenotypes. Of interest, colistin-resistant *E. coli* harboring the plasmid-borne *mcr*-1 gene were fully susceptible to CXCL10-mediated killing despite the presence of phosphoethanolamine (pEtN)-modified lipid A in the outer membrane of these organisms.

Conclusion: Our observations demonstrate that CXC chemokines are capable of killing multidrug-resistant, carbapenemase-producing Gram-negative bacterial pathogens. In regards to colistin-resistant bacteria, L-Ara4N-modified lipid A limited killing by CXCL10; however, pEtN-modified lipid A did not. This distinction may reflect disparate effects of L-Ara4N and pEtN modification on outer membrane charge neutralization and / or permeability. Collectively, our findings will inform the development of innovative strategies for treating infections caused by antimicrobial-resistant pathogens.

7th ARAE2017 Poster No. 25:

Identification of a novel multiresistance integrative and conjugative element ICE*Pmu2* in a bovine *Pasteurella multocida* isolate from Germany

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Background and objectives: In North America, multiresistant *Pasteurellaceae* harbouring integrative and conjugative elements (ICEs) - such as ICE*Pmu1* carrying twelve resistance genes and conferring resistance to eight classes of antimicrobial agents including the ones licensed for the treatment of respiratory tract infections in cattle - are meanwhile widespread. The aim of the study was to identify the antimicrobial resistance genes in a multiresistant *Pasteurella multocida* isolate and to detect their location on mobile genetic elements.

Materials and methods: *P. multocida* isolates (n=375) originated from the German national resistance monitoring program GE*RM*-Vet (2004-2010) were investigated by antimicrobial susceptibility testing. A single isolate from 2010 was resistant to tilmicosin with a minimal inhibitory concentration (MIC) of ≥256mg/L. It was also resistant to chloramphenicol (MIC 32mg/L) and tetracycline (MIC 16mg/L). The plasmid content was checked by plasmid extraction via alkaline lysis. Whole genome sequencing was performed to identify the resistance genes and to analyse their genetic environment.

Results: Whole genome analysis revealed the presence of an integrative and conjugative element (ICE). No plasmid was detectable. Similarities were seen in comparison to the first described ICE in *P. multocida* ICE*Pmu1*. The integration site and the core genome of the ICE was virtually the same as in ICE*Pmu1*. The novel ICE, designated ICE*Pmu2*, is to the best of our knowledge the first ICE identified in a German isolate. One resistance gene region comprising at least four resistance genes was located in the same positions as seen in ICE*Pmu1*. The genes *sul2* (sulfonamide resistance), *catA3* (chloramphenicol resistance), *strA* and *strB* (streptomycin resistance) were located in the same orientation. The same cluster of resistance genes, *sul2-catA3-strA-strB*, has been detected previously on plasmid pMVSCS1 from *Mannheimia varigena*. No macrolide resistance gene was identified in ICE*Pmu2*. In contrast, ICE*Pmu2* conferred also tetracycline resistance. The gene *tet*(Y) was for the first time identified in *P. multocida* and located downstream of the resistance gene cluster *sul2-catA3-strA-strB*.

Conclusion: The identification of an ICE conferring multiresistance in an isolate from Germany is alarming. While widespread in North America, such multiresistant bovine respiratory tract pathogens have been rarely detected in Europe.

7th ARAE2017 Poster No. 26:

Resistome of multidrug resistant *Klebsiella pneumoniae*

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Background and objectives: Particularly concerning has been the recent acquisition by *Enterobacteriacee* of carpapenemases, i.e. enzymes able to inactivate most beta-lactams, including last resort antibiotics such as carbapenems. Whole-genome sequencing (WGS) can play a significant role in rapid and accurate differentiation of the existing and emerging carbapenemases, which will be essential for surveillance and controlling their spread. We characterized the occurrence of carbapenemase genes and extended-spectrum ß-lactamase (ESBL) genes in 10 multidrug-resistant (MDR) *Klebsiella pneumoniae* isolates from Pakistan.

Materials and methods: The resistance profiles for the isolates were determined using Vitek. WGS data from sequencing on Illumina platforms was used for multilocus sequence typing (MLST), as well as antimicrobial resistance gene and plasmid replicon sequence analyses.

Results: Resistance was observed for 15 of the 25 tested antibiotics in all strains except one. Seven isolates were resistant to colistin, and all were susceptible to tigecycline. The highest number of resistance genes was observed for aminoglycosides (n=12) and beta-lactams (n=11), with at least two genes of each class present in every isolate. The $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ genes were detected in 7 and 5 samples, respectively. In 2 isolates, both genes were present. Several ESBL genes were identified: $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{SHV-11}}$ and $bla_{\text{SHV-28}}$. No plasmid-mediated colistin resistance genes were detected, but disruptions in chromosomal loci (i.e. *mgrB* and *pmrB*) were observed. Six sequence types (STs) were detected: ST11 (n=3 isolates), ST14 (n=3), ST15 (n=1), ST101 (n=2), and ST307 (n=1). The IncL/M (pOXA-48) replicon, indicating the presence of a ~60kb plasmid carrying no other resistance genes, was found in the OXA-48 positive isolates.

Conclusion: The numerous potential transmission routes at the human-animal-environment interface underline the importance of the One Health approach for effective control and prevention. Global movement of people, animals and food is amplifying the geographic distribution of MDR isolates making antibiotic resistance a borders-transcending issue, requiring international cooperation among institutions and multidisciplinary approach for its control. WGS-applying molecular epidemiology studies will provide a better understanding of the worldwide dissemination of MDR isolates and a surveillance tool useful in detecting possible new emerging threats.

7th ARAE2017 Poster No. 27:

First extended-spectrum β-lactamase in *Mannheimia haemolytica*

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Background and objectives: The facultative pathogen *Mannheimia haemolytica* is the major bacterial component of the multifactorial bovine respiratory disease (BRD) complex. Due to the increase of resistant *M. haemolytica* isolates to antimicrobial agents, licensed β-lactams like cefquinome or ceftiofur, are a good alternative option for the treatment. The aim of this study was to identify and analyse the first extended-spectrum β-lactamase (ESBL) from *Mannheimia haemolytica*.

Materials and methods: The *M. haemolytica* isolate 48 showed high cephalosporin minimal inhibitory concentrations (MICs) of 2mg/L and was investigated for the genetic basis of resistance. The plasmid was extracted via alkaline lysis and transferred by electro transformation into *Pasteurella multocida* and *Escherichia coli* recipient cells with selection on ampicillin. The transformants were checked by plasmid extraction with subsequent bla_{ROB} PCR and tested for their susceptibility. Susceptibility testing was done according to CLSI by broth microdilution as well as a double disk test for confirmation of the ESBL phenotype were performed. The sequence was determined by whole genome sequencing and confirmed via Sanger sequencing by primer walking.

Results: A single plasmid, designated pKKM48, with a size of 4,323bp was isolated from the *M. haemolytica* isolate 48. Plasmid pKKM48 harboured a bla_{ROB} gene and was transferred to *P. multocida* B130 and to *Escherichia coli* Jm107. The encoded ROB β-lactamase differed in three amino acid from proteins from the database. In addition to this resistance gene pKKM48 carried the mobilization genes *mobA-mobC-mobB*. PCR assays and susceptibility testing confirmed the presence and activity of the gene bla_{ROB} in the *P. multocida* and in the *E. coli* recipient carrying plasmid pKKM48. The *P. multocida* transformant had distinctly higher MIC values for ceftiofur (4mg/L), cefquinome (8mg/L) compared to the empty recipient with ≤0.03 mg/L and 0.03mg/L, respectively. An ESBL phenotype was seen in the *E. coli* transformant with an increase of the inhibitory zone of 7mm for cefotaxime and of 9mm for ceftazidime in the presence of clavulanic acid.

Conclusion: These findings reveal the first ESBL gene in *Pasteurellaceae*. The transferability to *Enterobacteriaceae* with the functional activity of the gene underlines the possibility of the spread of this gene beyond species or genus restrictions.

7th ARAE2017 Poster No. 28:

Understanding the behavioural influences and perceptions on antimicrobial resistance and antimicrobial use of UK Veterinary Surgeons: A mixed methods study

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Background and objectives: The use of antimicrobials in food-producing animals has been linked with the emergence of antimicrobial resistance in bacterial populations, with consequences for animal and public health. This study explored the underpinning drivers and reasoning behind prescribing decisions made by veterinary surgeons working in the UK pig industry.

Materials and methods: Initially, qualitative interviews were conducted with veterinary surgeons (n=21) selected using a purposive sampling approach to encompass the spectrum of veterinary surgeons working within the UK pig industry. The key themes from these were explored further in a quantitative questionnaire study on a census population of UK veterinary surgeons (n=62) whose clinical caseload included pigs kept as production animals.

Results: Ensuring optimum pig health and welfare was a major driver for antimicrobial use by many veterinary surgeons and was considered a professional and moral obligation. Veterinary surgeons also exhibited a strong sense of social responsibility over the need to ensure that antimicrobial use was responsible.

Antimicrobial susceptibility testing on first presentation of clinical disease was described as being carried out often or always by only 20% of participants however, this figure doubled if testing was being carried out following treatment failure (43%). 49% of respondents indicated that they sometimes encountered treatment failure, however 67% either rarely or never associated this with antimicrobial resistance. Whilst some veterinary surgeons identified that they had encountered resistance in their clinical pig work, many shared the opinion that resistance was an issue faced by other pig practitioners and other species sectors.

A close relationship between management practices, health and economics was evident, with improvements in management commonly identified as being potential routes to reduce antimicrobial usage; however, these were not always considered economically viable.

Conclusion: The drivers behind prescribing decisions by veterinary surgeons were complex and diverse. Any interventions to reduce antimicrobial use at a farm level would rely on the veterinary surgeons' ability to communicate and educate the farmer. Pig health and welfare were deemed highly important hence alternative methods to treat and prevent disease are needed.

7th ARAE2017 Poster No. 29:

Definition of national Defined Daily Doses (DDDch) and Defined Course Doses (DCDch) for antimicrobial preparations for pigs in Switzerland

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Background and objectives: In order to gain better insight into antibiotic use in pig production in Switzerland, national monitoring systems are under development. Based on the proposal by the ESVAC project (EMA), we defined Defined Daily Doses (DDD_{CH}) and Defined Course Doses (DCD_{CH}) for Switzerland with the aim of providing technical units to collect data on antimicrobial consumption. DDD_{CH} and DCD_{CH} were compared to the DDD_{vet} and DCD_{vet} recently published by the EMA. The extent of differences between the values is presented and the impact on estimating antimicrobial consumption in Switzerland is discussed.

Materials and methods: DDD_{CH} and DCD_{CH} were defined for all drugs containing antimicrobial ingredients and approved for pigs in Switzerland. DDD_{CH} were defined by using the highest authorized daily dosage according to the national Summaries of Product Characteristics (SPC). DCD_{CH} were calculated by multiplying the corresponding DDD_{CH} unit with the maximum treatment duration as presented by the SPCs. In cases the SPC did not provide clear information concerning maximum dosage or maximum treatment duration, SPCs of Swiss products containing the same ingredients in equal dosage were used in the first instance. If there still was no plausible information available, values were harmonized with published DDD_{vet}/DCD_{vet} in a pragmatical approach. After definition of DDD_{CH} and DCD_{CH}, all values were compared to the corresponding DDD_{vet} and DCD_{vet} by calculating the percentage difference.

Results: 138 different values for DDD_{CH} and DCD_{CH} were defined for the antimicrobial ingredients of 102 drugs. For 9 drugs and 17 corresponding antimicrobial ingredients no comparison to the DDD_{vet} resp. DCD_{vet} was possible because no values have been published by the EMA yet.

The percentage differences between newly defined DDD_{CH}/DCD_{CH} and DDD_{vet}/DCD_{vet} ranged from 90% to +1500%. 69 DDD_{CH} values and 77 DCD_{CH} values showed a difference of more than 25% to the DDD_{vet} and DCD_{vet} respectively.

Conclusion: The newly defined DDD_{CH} and DCD_{CH} show considerable differences to the published DDD_{vet} and DCD_{vet}. As a consequence the relative use of certain active compounds could be massively overestimated compared to other antimicrobials when using the DDD_{vet}/DCD_{vet} for national monitoring systems. The great value of DDD_{vet} and DCD_{vet} for international comparison is undisputed, but we propose the use of nationally defined units for more accurate national monitoring.

7th ARAE2017 Poster No. 30:

Development of antimicrobial resistance of fecal *Escherichia coli* in growing pigs

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Background and objectives: Antibiotics are used as growth promoters and for therapeutic purposes in pigs including treatment of *Escherichia coli* associated diarrhoea. Misuse, abuse and overuse of these antibiotics has led to development of resistant bacterial strains. This study compared phenotypic and genotypic antibiotic resistance pattern of *E. coli* in piglets treated and not treated with antibiotics to establish the development of antibiotic resistance. This is to provide insight into distribution and pattern of antibiotics resistance in pigs and its potential transfer to humans.

Materials and methods: Two groups of 5 piglets each, at day 0 were kept under routine management practices but without any form of antibiotics use in one. Rectal swabs were collected weekly until day 70, *E.coli* was isolated and used for antibiotics resistance testing through disk diffusion and genomic DNA extracted was amplified by PCR.

Results: Overall [68.04% ($CI_{95\%}$: 61.9, 73.6)] of the isolates showed significant phenotypic resistance to the 7 tested antibiotics (*P = 0.02*). Resistance to oxytetracycline was most common and were significant (*P* = 0.03) in samples of days 10 (*P* = 0.02) and 21 (*P = 0.01*). Significant resistance to amoxicillin on days 56 (*P* = 0.04) and 70 (*P* = 0.01) and trimethoprim on days 5, 10, 21, 56 and 70 (*P<0.05*) were observed. A total of 17 phenotypic antibiotics resistance combinations were observed and 8 were multidrug resistant. Oxytetracycline phenotype was most common[54.8% ($CI_{95\%}$: 44.1, 65.1)] in the antibiotics group and [40.2% ($CI_{95\%}$: 30.3, 51.0)] in the non antibiotics group. Furthermore, [63.9% ($CI_{95\%}$: 57.6, 69.7)] possesed one or more of the 4 tested tetracycline resistance genes. *TetA* was the most common in the antibiotics group [23.3% ($CI_{95\%}$: 16.9,31.1)] while in the non-antibiotics group, *tetB* was most detected [43.5% ($CI_{95\%}$: 34.5, 52.9)].

Conclusion: In this study, Oxytetracycline, Amoxicillin and Trimethoprim have the highest level of resistance. Perhaps environmentally resistant *E. coli* or gene transfer between the two groups might be responsible. We concluded that Tetracycline resistance genes in pigs can be found at any point during the growth period with or without antibiotic usage. Phenotypic resistance to commonly used antibiotics was abundant and diverse throughout the growing period. Therefore, free access to some antibiotics amongst farmers might be a likely factor for the aggravated resistance seen in South Africa.

7th ARAE2017 Poster No. 31:

Quinolone resistant *Escherichia coli* from broiler in Norway - characterization, comparison to human isolates and molecular risk assessment

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Background and objectives: Usage of fluoroquinolones (FQ) in Norwegian livestock production is negligible. Historically the occurrence of quinolone resistant *Escherichia coli* (QREC) in the Norwegian monitoring program for antimicrobial resistance in the veterinary sector (NORM-VET) has been rare. However, in NORM-VET 2014 a selective method was implemented for screening of QREC in broilers and QREC was found in 89.5% of caecal samples and in 70.7% of meat samples. In this study we aim to characterize and compare QREC from broilers and humans in Norway in order to investigate possible links between isolates of the two reservoirs.

Material and methods: NORM-VET 2014 isolates from chicken meat (n=47) and caecal samples (n=53) resistant to nalidixic acid (NAL) and/or ciprofloxacin (CIP) were selected for sequencing. Human QREC isolates were collected via the Norwegian surveillance program for antimicrobial resistance (NORM) from urinary tract infections and bacteraemia (n=77), and from screening healthy carriers for QREC (n=23). Data regarding antimicrobial resistance were extracted from the monitoring programs (NORM-VET and NORM) and compared between isolates. The 200 isolates were sequenced using Nextera XT library prep on HiSeq 2500 with Rapid Run. The sequence data were trimmed using Trimmomatic, and assembled using SPAdes. We are currently analysing the sequence data for presence of resistance- and virulence genes, plasmids, MLST and SNP analysis.

Results: NORM-VET 2014 resistance data showed that the majority of the broiler isolates exhibited high level resistance to NAL (MICs >128 mg/L), but low level tolerance to CIP (MICs 0.12-0.5). Mutations in the *gyrA* gene were identified in most isolates, and a few exhibited the plasmid mediated resistance gene *qnrS1*. Data from the NORM program showed that the human isolates had a different MIC distribution with the largest proportion of isolates exhibiting high level CIP resistance (MICs >0.25), indicative of more mutations in the *gyrA*/*parC* genes. However, a considerable proportion of human isolates exhibited a quinolone resistance MIC profile corresponding to the most common profile found among the broiler isolates.

Conclusions: Preliminary results indicate that the main mechanism behind quinolone resistance in this study is mutations in *gyrA*, but plasmid mediated resistance were also identified. Further, the human isolates exhibit high level CIP resistance compared to low tolerance in the broiler isolates.

7th ARAE2017 Poster No. 32:

Antimicrobial susceptibility to critically important antibiotics of *Enterococcus faecium* and *Enterococcus faecalis* recovered from healthy cattle, pigs and chickens in nine EU countries (EASSA Study)

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Objectives: The European Antimicrobial Susceptibility Surveillance in Animals (EASSA) collects zoonotic and commensal bacteria from food-producing animals at slaughter across the EU and tracks their susceptibility to important human-use antibiotics. Results of *E. faecium* and *E. faecalis* (2013-2014) are presented here.

Methods: Intestinal content from cattle (C), pigs (P) and chickens (Ch) was randomly sampled (5-6 countries/host; ≥4 abattoirs/country; 1 sample/animal/farm) for isolation of enterococci to be identified biochemically and/or by MALDI-ToF. MICs of 9 antibiotics were assessed by agar dilution (CLSI, VET01-A4) in a central laboratory. Except for quinupristin/dalfopristin (Q/D) and tigecycline (EUCAST breakpoints), clinical resistance was interpreted using CLSI breakpoints (M100-S26, 2016), and epidemiological cut-off (ECOFF) values to assess decreased susceptibility (EFSA, 2012), i.e. MICs > ECOFF values but < clinical breakpoint.

Results: In total 960 *E. faecium* (C: 134, P: 328, Ch: 498) and 779 *E. faecalis* (C: 115, P: 176, Ch: 488) strains were recovered. Little or no clinical resistance to ampicillin was observed for *E. faecium* (0[C]-7.6[Ch]%) and almost none for *E. faecalis* (0[C;P]-0.2[Ch]%). For gentamicin 0.0[C]-1.6[Ch]% clinical resistance was observed for *E. faecium* and 0.8[Ch]-5.1[P]% for *E. faecalis.* Clinical resistance of *E. faecium* to Q/D was clearly higher (2.2[C]-12.0[Ch]%) and 59.6[Ch]-80.8[P]% of the strains were classified as intermediate susceptible to Q/D. Of all 1739 strains tested, 7 porcine *E. faecium/faecalis* were resistant to linezolid and 2 avian/porcine *E. faecalis/faecium* strains were non-susceptible (MICs 8 mg/L) to daptomycin (no resistance breakpoint defined). Clinical vancomycin resistance in *E. faecium* and *E. faecalis* was absent; 2 avian *E. faecium* and 1 bovine *E. faecalis* of 1739 strains were intermediate susceptible, all with MICs of 8 mg/L. Extremely high percentages resistance to tetracycline (67.4[P]-78.3[Ch]%) and high resistance to erythromycin (27.1[P]-57.0[Ch]%) were noted for both enterococci species, except for C (5.2-30.4 and 9.0-10.4%, respectively). None of the strains tested were clinically resistant to tigecycline. Decreased susceptibility was only apparent for Q/D (59.6[Ch]-80.8[P]% for *E. faecium*) and negligible to low for the other antibiotics.

Conclusions: This EU survey shows high variability in antibiotic susceptibility of commensal enterococci from healthy food animals. Clinical resistance to critically important antibiotics for human medicine was absent or very low, except for erythromycin.

7th ARAE2017 Poster No. 33:

Quinolone resistance despite low antimicrobial usage: comparison of occurrence in different species in Norway the last ten years

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Background and objectives: The use of fluoroquinolones in Norwegian livestock production is nearly negligible, and quinolone resistance in production animals was rarely seen before the implementation of a selective method in the Norwegian monitoring program for antimicrobial resistance in the veterinary sector (NORM-VET) in 2014. To compare the occurrence of quinolone resistance in different species and detect possible trends, we collected all historical *Escherichia coli* isolates in NORM-VET during the years 2006 to 2016.

Materials and methods: In total, 4712 isolates of *E. coli* have been obtained from fecal or environmental samples collected in NORM-VET (2006 to 2016) divided among different subgroups as follows; broilers (n=1098) including isolates from broilers with septicemia (n=39), layers (n=186), turkey (n=318), pig (n=918), cattle (n=374), horse (n=171), sheep (n=207), dogs (n=339), 430 isolates from fox (n=430), reindeer (n=107), wild birds (n=303) and blue mussels (n=261). Quinolone resistant isolates not previously tested on Sensititre® before 2014 were retested to allow for comparison. MIC - values of ciprofloxacin (CIP) and nalidixic acid (NAL) of >0.06 and >16, respectively, was defined as resistant.

Results: The occurrence of CIP and NAL resistant *E. coli* was higher in isolates originating from all broilers with an occurrence of 3.7% compared to the other subgroups. However, the occurrence of quinolone resistance was 7.7% [95%CI: 1.6-20.9] in broilers with septicaemia in 2011. So far, only isolates from broilers, which have been included in NORM-VET in 2006, 2009, 2011, 2012, 2014 and 2016, could be assessed for possible trends. The occurrence of quinolone resistance among *E. coli* isolates from broilers was significantly ($p < 0.05$) higher in 2009 and 2016 with 8.0% [95%CI: 4.7-14.5], and 6.1% [95%CI: 3.1-10.6], respectively, as compared to the other years, except for the year 2014 where the corresponding results was 3.4% [95%CI: 1.4-6.9]. Resistance to CIP or NAL was not detected in isolates from horses, reindeer or sheep.

Conclusion: The results indicate a difference in the occurrences of quinolone resistance among different species, and also an increasing occurrence in the faecal microbiota of broilers. The higher occurrence in the isolates originating from septicaemia might indicate that factors others than antimicrobial usage, such as for instance stress factors, could play a role in the development of quinolone resistance.

7th ARAE2017 Poster No. 34:

Characterization of quinolone resistance mechanisms in *Enterobacteriaceae* isolated from companion animals in Europe (ComPath II study)

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Background: ComPath is an ongoing monitoring program dedicated to the collection of bacterial pathogens from diseased dogs and cats not treated with antibiotics for 4 weeks pre-sampling across Europe. The purpose is to determine the antibiotic susceptibility of recovered isolates. Objective in this study was to characterize plasmid-mediated quinolone resistance (PMQR) associated with quinolone resistance among *Enterobacteriaceae* recovered in this monitoring program.

Methods: 844 non-duplicate *Enterobacteriaceae* strains from urinary tract, skin/wounds/ear and respiratory tract infections were collected in 9 EU countries and Switzerland from 2013 to 2014. A total of 161 strains (101 *Escherichia coli,* 45 *Proteus mirabilis*, 14 *Klebsiella pneumoniae*, 1 *Enterobacter aerogenes*) with enrofloxacin non-wild type MICs of ≥ 0.25 µg/mL; for *P. mirabilis* ≥ 0.5 µg/mL (agar dilution according to CLSI VET01-A4 standard) were selected and screened for *qnr, oqxAB* and *qepA* genes using real-time PCR methods and *aac(6')-Ib-cr* using a pyrosequencing-based approach.

Results: 19.9% (32/161) enrofloxacin non-wild type strains carried at least one PMQR (18/32 *qnrB, qnrS* or *qnrD,* 10/32 *aac(6')-Ib-cr,* 13/32 *oqxAB*) and 80.1% (129/161) no PMQR. Six strains carried *qnrD1*, 5 strains *qnrS1*, 2 strains *qnrB1,* 1 strain *qnrB4,* 2 strains group *qnrB8 ,*1 strain *qnrS2* and 1 strain carried both *qnrB1* and *qnrS1. qnrB* was detected in 3 *E. coli*, 2 *K. pneumoniae* and 1 *E. aerogenes* strains; *qnrS* in 6 *E. coli* and 1 *P. mirabilis* and *aac(6')-Ib-cr* in 4 *E. coli*, 5 *K. pneumoniae* and in 1 *E. aerogenes* strains. All *qnrD1* were detected in *P. mirabilis*. All *K. pneumoniae* strains except 2, carried *oqxAB* genes. The *E. aerogenes* also carried a *oqxAB* gene not associated to *Tn*6010. No *qepA* genes were found. The PMQR strains originated from 21 urinary tract, 5 ear canal, 4 nasal and 2 skin specimens; 28 specimens were from dogs, 4 from cats. Of the 32 PMQR positive strains, 1 showed enrofloxacin MICs < 2 µg/mL, and 31 were resistant with MICs ≥ 2 µg/mL. For the 129 non-PMQR, non-wild type strains, 35 showed MICs ≤ 2 µg/mL.

Conclusions: Among PMQR-positive strains (3.8%; 32/844), *qnr* was predominant (*qnrB, qnrD, qnrS*). *qnrD* genes were only detected in *P. mirabilis*. Additionally *aac(6')-Ib-cr* and *oqxAB* were frequently detected, but *oqxAB* was limited to *K. pneumoniae* and *E. aerogenes*. Investigation of chromosomal mutations in DNA gyrase and topoisomerase IV genes is required to complete the mechanisms of quinolone resistance.

7th ARAE2017 Poster No. 35:

Antimicrobial susceptibility and genetic relatedness of respiratory tract pathogens before and after antibiotic treatment

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Background and objectives: The cooperative project VASIB aims at reducing the antibiotic consumption in pig production by integrating epidemiologic information from consulting expertise in clinic, hygiene, microbiology and pharmacology. In this VASIB subproject, we investigated the antimicrobial susceptibility of respiratory tract pathogens before and after application of antimicrobial agents.

Materials and methods: *Streptococcus suis* (n=109), *Bordetella bronchiseptica* (n=12) and *Pasteurella multocida* (n=22) were isolated from 30 piglet-producing farms. The farms were sampled at three different time points: (A) initial visit (n=30), (B) day of acute respiratory problems (n=12) and (C) last day of antibiotic treatment (n=12). The minimal inhibitory concentration (MIC) to ampicillin AMP, amoxicillin/clavulanic acid AMC, penicillin PEN, ceftiofur XNL, colistin COL, clindamycin CLI, enrofloxacin ENR, erythromycin ERY, florfenicol FFC, gentamicin GEN, tetracycline TET, tulathromycin TUL and tilmicosin TIL were determined by broth microdilution according to CLSI recommendations. Resistance genes were detected via PCR assays. Macrorestriction analysis was performed for selected isolates.

Results: All *B. bronchiseptica* isolates were AMP resistant, TUL susceptible and FFC intermediate (n=7) or susceptible (n=5). The MICs for AMC were 4/2mg/L, for ENR 0.12-1mg/L, gentamicin 2 or 4mg/L, tetracycline 0.25 or 0.5mg/L, colistin 0.06-0.25mg/L. In contrast SXT MICs ranged from 0.06-4mg/L. All *B. bronchiseptica* isolates from farm 1 showed indistinguishable XbaI-patterns. The 22 *P. multocida* isolates were susceptible to AMP, XNL, ENR, FFC, TUL and TIL. Resistant (n=2) and intermediate (n=4) isolates were solely seen for TET. The SmaI-PFGE-patterns of *P. multocida* from farm 3 showed different DNA fragment patterns at the three time points, suggesting the presence of different isolates at different times. The majority of the *S. suis* isolates was susceptible to FFC (100%), ENR (92%), XNL (84%) and PEN (74%). The isolates were resistant to ERY (85%), CLI (87%) and TET (84%) and harboured *erm*(B) and *tet*(O), respectively.

Conclusion: *B. bronchiseptica*, *P. multocida* and the majority of the *S. suis* isolates showed MIC values similar to those from the national German resistance monitoring program (GE*RM*-VET) or VetPath. *B. bronchiseptica* and *P. multocida* isolates were susceptible and showed overall low MIC values, whereas *S. suis* isolates were frequently ERY/CLI and TET resistant.

7th ARAE2017 Poster No. 36:

Monitoring of antimicrobial susceptibility of major PPDS (MMA) pathogens recovered from acute cases in sows across Europe: VetPath results

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Objectives: VetPath is an ongoing pan-European antimicrobial susceptibility monitoring programme collecting pathogens from diseased cattle, pigs and poultry not recently treated with antibiotics. Results for *Escherichia coli,* staphylococci and streptococci isolated from post-partum dysgalactia syndrome (PPDS; previously known as mastitis metritis agalactia, MMA syndrome) are presented.

Methods: Non-duplicate uterine samples or milk of the infected quarter were collected from sows with acute PPDS in 7 European countries from 2009 to 2012. Bacterial strains were isolated by standardised methods, and identified biochemically and/or by MALDI-ToF. Antimicrobial susceptibility to 16 antibiotics was assessed in a central laboratory by broth microdilution methodology (CLSI, VET01-A4), and interpreted using clinical breakpoints (CLSI, VET01S) where available.

Results: In total, 158 strains (73 *E. coli,* 14 *S. aureus*, 36 coagulase-negative *Staphylococcus* (CNS), 17 *Streptococcus dysgalactiae* and 18 *Streptococcus* spp.) were recovered.

For *E. coli*, resistance to amoxicillin/clavulanic acid (AMC) was low (1.4%) in contrast to tetracycline (64.4%). First generation cephalosporins (CPs; cephalexin, cephapirin, cephalonium) showed an MIC_{90} of 8-32 µg/mL. For 3rd/4th generation CPs (ceftiofur, cefquinome), MIC_{90} varied between 0.12 and 0.5 µg/mL. MIC_{90} of fluoroquinolones (FQs; enrofloxacin, marbofloxacin) was 0.5 µg/mL. The aminoglycosides (AGs) neomycin and kanamycin showed a MIC_{90} of 64 and >128 µg/mL respectively.

For *S. aureus*, resistance was high to erythromycin, penicillin and tetracycline (57.1-71.4%) but lower (14.3%) for AMC. Cloxacillin showed a MIC_{90} of 1 µg/mL and 1-32µg/mL for 1st generation CPs. Cefquinome and ceftiofur showed a MIC_{90} of 2 and 4 µg/mL respectively which is similar to FQs (4 µg/mL). MIC_{90} for tylosin, kanamycin and neomycin was >32, >128 and 8 µg/mL respectively. For CNS, % resistance and $MIC_{50/90}$ was slightly lower than for *S. aureus*.

S. dysgalactiae isolates remained fully susceptible to penicillin and AMC but resistance was 35.3% for erythromycin and 94.1% for tetracycline. MIC_{90} varied from 0.015 to 1 µg/mL for β-lactams, from 1 to 2 µg/mL for FQs and >32 for other macrolides and AGs. Similar results apply to *Streptococcus* spp.

Conclusions: PPDS pathogens exhibit distinct antimicrobial susceptibility patterns, and results may be used to monitor susceptibility over time. Due to lack of clinical breakpoints, interpretation of susceptibility remains difficult for some of these antimicrobials.

7th ARAE2017 Poster No. 37:

Antimicrobial resistance after fluoroquinolone treatment

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Background and objectives: Preservation of the effectiveness of antimicrobials is an important political target. Treatment with an antimicrobial can induce selection of resistance to other antimicrobials (i.e. from different classes). Little is known about possible co-selection through use of fluoroquinolones. Resistance to antimicrobials of different classes was investigated in fecal *Escherichia coli* from pigs after fluoroquinolone treatment or contact to treated animals.

Materials and methods: Two groups of 14 weaners were housed together: TREATED weaners received enrofloxacin at therapeutic dosage using a drencher on study days 1-5. CONTACT weaners received no treatment but were comingled with TREATED weaners. Untreated CONTROL weaners (n=14) were housed separately. Rectal swabs were taken repeatedly before, during and 42 days after treatment.

Swab samples were cultured on MacConkey-agar. Minimum inhibitory concentrations (MIC) to 14 antimicrobials were determined for one *E. coli* isolate per sample using broth microdilution. Panel of substances and evaluation criteria were chosen according to Decision 2013/652/EU).

Prevalence of resistance in the study groups was compared between study days. Probability of resistance to antimicrobial agents in *E. coli* on single study days was compared to the initial value on study day 1 (pre-treatment) using logistic analysis (SAS 9.4, GENMOD Procedure).

Results: During and shortly after enrofloxacin treatment, resistance to ampicillin, sulfamethoxazole and trimethoprim increased with a similar pattern in *E. coli* of TREATED and CONTACT pigs compared to pre-treatment. Resistance to ciprofloxacin was not observed.

In the logistic analysis, the risk of *E. coli* to be resistant to ampicillin was higher on days 5 and 7 compared to day 1 ($p < 0.05$). Thereafter the odds decreased. No significant differences were observed between isolates from study day 42 and day 1.

On study days 5 and 7, the MIC differed by at least 4 dilution steps between susceptible and resistant isolates within treatment groups (e.g. for ampicillin in TREATED) and compared to day 1 within isolates from the same animal that were resistant after treatment.

Conclusion: After enrofloxacin treatment, a transient increase of resistance to other antimicrobials than fluoroquinolones occurred in treated and their contact animals without concurrent resistance to ciprofloxacin.

7th ARAE2017 Poster No. 38:

Monitoring of antimicrobial susceptibility of enteric pathogens isolated from diseased cattle and pigs across Europe: VetPath results

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Objectives: VetPath is an ongoing pan-European resistance monitoring program for veterinary pathogens from diseased cattle, pigs and poultry. Antimicrobial susceptibilities of cattle and pig bacteria from enteric samples are presented.

Methods: Faecal or caecal samples were collected from animals with acute clinical signs, before antibiotic treatment, in 9 countries during 2009-2012. *Escherichia coli* and *Salmonella* spp (one isolate/bacterial species/outbreak) were isolated and identified by standard methods. Susceptibility to 13 antibiotics was determined in one laboratory by broth microdilution (CLSI, VET01-A4). MIC data against target pathogens were interpreted using resistance breakpoints (VET01-S2) where available; susceptibility of *Salmonella* to ciprofloxacin (CIP) was interpreted following CLSI M100-S26.

Results: In total, 543 *Enterobacteriaceae* isolates (cattle: 203; pigs: 340) were recovered. For bovine *E. coli* (n=146) resistance varied between 16.4% (amoxicillin clavulanic acid; AMC) and 67.1% (tetracycline; TC). MIC_{90s} for the fluoroquinolones (FQs) enrofloxacin, danofloxacin and marbofloxacin were 16-32 mg/L. MIC ranges for apramycin and neomycin were 2-≥64 and 1-≥128 mg/L, with MIC_{90}s of 16 and >64 mg/L, respectively. For porcine *E. coli* (n=213), resistance varied between 3.8% (AMC) and 75.8% (TC). MIC_{90}s of FQs were 4 mg/L. Apramycin and neomycin displayed MIC_{90}s of 16 and 64 mg/L, respectively, and the colistin MIC_{90} was 4 mg/L.

Predominant serovars for bovine *Salmonella* were Typhimurium (61.4%) and Montevideo (14.0%); 69.5% of porcine *Salmonella* were Typhimurium. Various Typhimurium phage types were identified. In bovine *Salmonella* (n=57), resistance (1.8-3.5%) was low for AMC, gentamicin and trimethoprim/sulfamethoxazole (TMS) versus 50.9% for TC. Whereas five isolates were intermediate to CIP (MICs 0.12 and 0.25 mg/L), none were clinically resistant. Among porcine *Salmonella* (n=127), TMS and TC resistance were 29.9 and 70.1%, respectively. Eight isolates (6.3%) were intermediate (MICs 0.12-0.5 mg/L) and none resistant to CIP. For all *Salmonella*, apramycin, colistin and neomycin showed MIC_{90} values of 1-8 mg/L.

Conclusions: The results show variable antimicrobial susceptibility among *E. coli* and *Salmonella* isolated from diseased but non-treated cattle and pigs across the EU: from low resistance for some of the compounds to high levels of resistance for TC and its part TMS. Clinical resistance of *Salmonella,* including *S.* Typhimurium, to human FQ-representative CIP was absent.

7th ARAE2017 Poster No. 39:

Cross-sectional study on the prevalence of ESBL-/AmpC-producing *E.coli* in food in Germany

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Background and objectives: Extended Spectrum Beta-Lactamases (ESBL)-producing *E. coli* are a major public health issue. Whereas their frequent presence in healthy animals was described, little is known about their prevalence in foodstuffs in Germany. For assessing the role of the foodborne transmission pathway, detailed data on their major characteristics are necessary. The objective of this study was to describe the prevalence and characteristics of ESBL/AmpC-producing *E. coli* in foods in Germany.

Materials and methods: In four federal states food samples were collected by official staff in food production plants and retail shops during their routine visits in the years 2012 and 2013. Samples were investigated in the regional veterinary investigations centres following a standardized protocol. 25g of each sample were incubated overnight in Luria-Bertani Bouillon, followed by cultivation on Mac Conkey Agar supplemented with 1 µg / ml Cefotaxim. Suspicious *E. coli* isolates were confirmed by biochemical tests or Malditof and *E. coli* isolates were further typed by phylogenetic grouping. Cephalosporin-resistance was confirmed by broth microdilution method and EU-protocol. ESBL-/AmpC-encoding genes were identified by PCR amplification/sequencing. Statistical analysis was performed using R.

Results: Overall, out of 2293 samples tested 409 (17.8%) samples gave a presumptive positive result. The highest prevalence was observed in chicken meat (ca 75%), followed by turkey meat (ca 40%). Compared to the poultry derived samples, prevalence in beef, pork and minced meat was considerably lower (4-15%). Whereas around 18% of the raw milk samples, collected at farm level were positive, only few cheeses and vegetables were positive. ESBL resistance genes of the CTX-M-group were most frequently detected, followed by genes of the SHV-, TEM- families or plasmidic AmpC. Detailed analysis showed that distribution of ESBL/AmpC-encoding resistance genes and phylogroups was significantly different between the chicken related food samples and other food items. Furthermore, additional factors have an impact on the patterns observed which needs detailed analysis and consideration.

Conclusion: Current results show significant differences in the prevalence of ESBL/AmpC producing *E.coli* and associated ESBL-/AmpC genes of isolates depending on their origin. Further detailed analysis may give insights into the factors contributing to their prevalence, distribution and relevance as a foodborne threat.

7th ARAE2017 Poster No. 40:

Quality of veterinary pharmaceuticals and their use by pastoralists in the Far North Region of Cameroon

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Background and objectives: Previous studies conducted in Cameroon highlight the potential for problems with veterinary medication quality but do not describe how these drugs are used by different pastoralists, which is another potential risk to animal and human health. This study evaluates the quality of the veterinary drugs most frequently used in the Far North Region of Cameroon and describes how pastoralists use them to treat their cattle herds.

Materials and methods: We conducted a survey that included questions relating to epidemiology of a common and recognizable disease, treatments used in cases of this disease, and how herders use veterinary pharmaceutical medicines to treat it. In addition to describing medication use by pastoralists, we sampled the most commonly used medications and tested them. High Performance Liquid Chromatography (HPLC) was used to identify and quantify the active ingredients in the drugs (penicillin G, levamisole, oxytetracycline, diminazene diaceturate, vitamin A and vitamin E acetate) and Gas Chromatography Mass Spectrometry (GC/MS) to determine if organic chemical contaminants were present.

Results: The results showed that 69% of surveyed pastoralists used veterinary medicines to treat common illnesses. In addition, the most commonly used medications (procaine penicillin G and oxytetracycline) were used in a manner inconsistent with the recommended dosage, frequency, duration, and withdrawal period by 98% of the pastoralists. However, contrary to previous studies, the quality of the medications available for sale was generally good.

Conclusion: The poor compliance with recommended treatment protocols was much more prevalent than use of poor quality medications and presents a potential for treatment failure, drug resistance of animal pathogens, and harmful drug residues in the human food supply, all of which have potentially negative consequences for animal and human health.

7th ARAE2017 Poster No. 41:

Long term effects of antimicrobial use in feedlot cattle early in the feeding period on *Salmonella enterica* spp. population, distribution and antimicrobial resistance profiles in feces during feeding period and on hide and in lymph nodes at slaughter.

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Background and objectives: Salmonellosis is a leading causes of foodborne disease. Multidrug resistant non-typhoidal *Salmonella enterica* is considered serious public health threat. Beef is one of the causes of salmonellosis. Antimicrobial use in food producing animals is considered a key factor in the occurrence of resistant non-typhoidal *Salmonella*. Ceftiofur and tulathromycin are commonly used antimicrobials to treat and control of bovine respiratory disease in cattle. Our study focused on the effects of these antimicrobials on *Salmonella enterica* population, distribution and antimicrobial resistance profiles in cattle feces, hide and lymph nodes before the treatment, immediately after the treatment and at slaughter.

Materials and methods: 12 pens (134 cattle) were subjected to 3 regimens: 4 pens were treated with ceftiofur, 4 pens received tulathromycin and 4 pens remained as control. Feces were collected on the pre-treatment day (day 0), 7, 14, 28, 56, 99 and slaughter age. Hide swabs and subiliac lymph nodes were collected at slaughter age. Samples were homogenized and spiral-plated on brilliant green agar (BGA) with novobiocin to obtain CFU. BGA plates were used to isolate *Salmonella* colonies after the *Salmonella* specific broth enrichments. O- antigen test and Maldi -TOF analyses were performed to confirm the isolates. Microbroth dilution method was used to determine phenotypical antimicrobial susceptibility profile. Statistical analyses were performed in STATA®.

Results: 898 fecal, 224 lymph node and 132 hide samples were collected and processed. *Salmonella* prevalence estimates were 47% in feces, 75% in lymph nodes and 85% in hide samples. Total of 698 isolates were tested by micro broth dilution. The maximum number of isolates resistant to 3 or more antibiotic classes was in hide isolates (3/111), all lymph nodes isolates were pansusceptible. Prevalence and the CFU counts of *Salmonella* in fecal samples significantly ($P < 0.05$) decreased in all treatment groups by Day 7 and then increased by Day 99; however, there was no significant differences of *Salmonella* in hide samples and lymph nodes among the treatment groups ($P>0.05$).

Conclusion: The vast majority of *Salmonella* isolates were pansusceptible or singly resistant and this was unaffected by treatment. Treatments did not affect the susceptibility profile of *Salmonella* in the lymph nodes. Seasonal changes likely provided the strongest effect on increased prevalence and CFU counts of *Salmonella*.

7th ARAE2017 Poster No. 42:

Monitoring of antibiotic resistance in bacteria in pigs

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Background and objectives: Antibiotic resistance is a major public health concern. Any use of antibiotics can lead to resistance and a transmission of bacteria resistant to antibiotics is possible between humans and animals. Extended-spectrum ß-lactamase (ESBL)-producing *E. coli* strains and livestock-associated *Staphylococcus aureus* (LA-MRSA) are widely distributed among pigs. They seem to play a more important role in conventional farms compared to organic farms.

Materials and methods: A literature search was performed in PubMed and Web of Science. The keywords pig(s), MRSA, ESBL, antibiotic resistance, Germany, Mecklenburg-Western Pomerania, organic farming and livestock were used.

Results: Two cross-sectional studies had investigated the occurrence of MRSA and ESBL in livestock in conventional as well as organic farms in Mecklenburg-Western Pomerania. There were MRSA-positive pig farms in all tested districts, the organic farms tested MRSA-negative. Most conventional pig farms and all organic farms tested positive for ESBL. Other studies sampled pigs of different age and production groups. Mostly fattening pigs as well as suckling and weaned piglets were affected by MRSA. Other studies identified specific risk factors associated with the occurrence of antimicrobial resistance.

Conclusion: The cohort studies conducted in the past did not monitor more than one fattening period. Therefore, five conventional and five organic pig farms will be examined for the presence of resistance marker genes in a longitudinal study over a period of twelve months. The burden of ESBL-producing Enterobacteriaceae as well as methicillin-resistant *S. aureus* in pig farms in Mecklenburg-Western Pomerania will be determined. Risk factors associated with the extend of occurrence of antimicrobial resistance will be identified. Several faecal and dust samples as well as boot swab samples will be collected within several fattening periods. A detailed farm questionnaire will be developed to collect information about farm management and the use of antimicrobials on the farms. In conjunction a mobile PCR based approach for quantification of resistance marker genes will be developed. With the help of this PCR, faeces samples will be analyzed for the presence of resistance marker genes directly on the farm. According to the results, a categorization scheme will be developed to divide the farms into high- and low- resistance farms. An intervention study will be implemented on selected farms modifying crucial factors identified in the longitudinal study.

7th ARAE2017 Poster No. 43:

Extended-spectrum cephalosporin resistance in *Escherichia coli* from Alberta beef cattle

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Background and objectives: Non-selective culturing methods have suggested that there is a low prevalence of resistance towards extended-spectrum cephalosporins (ESCs) in bacteria from Canadian beef cattle. Our recent findings have challenged this, as resistance determinants were found in approximately 90% of fecal samples collected from Alberta beef cattle following the use of an enrichment culture. This suggests ESC resistant bacteria may have been present for some time; however, low levels of these bacteria in combination with non-selective methods used in the past have made them difficult to detect. The objective of this study was to use molecular methods to identify and characterize resistance determinants for ESCs and other antimicrobials from beef cattle.

Materials and methods: Composite fecal samples (n=846) from Alberta beef cattle were collected from four feedlots from May 2015 to April 2016. The samples were enriched using EC broth with cefotaxime (2 mg/L) and underwent DNA extraction and purification. The DNA extracts were screened by real-time PCR custom primer/probe TaqMan assays for the ESC resistance gene families: bla_{CMY}, $bla_{CTX\text{-}M}$, and bla_{SHV}. Aliquots of enriched samples were plated on MacConkey agar with ceftriaxone (1 mg/L) for the isolation of *Escherichia coli*. Those isolates that were lactose-fermenting and tested indole-positive were presumed to be *E. coli*; one isolate from each culture-positive enrichment sample was selected for further screening. These fecal isolates (n=728) were screened for bla_{CMY}, $bla_{CTX\text{-}M}$, and bla_{SHV}, using conventional PCR.

Results: Of the isolates examined 65.8% of isolates tested positive for bla_{CMY}, 28.7% for $bla_{CTX\text{-}M}$, and 1.4% for bla_{SHV}. Further characterization of $bla_{CTX\text{-}M}$ isolated found that 72.7% were members of the $bla_{CTX\text{-}M\text{-}1}$ group and 27.7% were part of the $bla_{CTX\text{-}M\text{-}9}$ group.

Conclusion: These results show that ESC resistance is present in the Alberta beef cattle population and suggests that resistance determinants can be commonly recovered from Alberta beef cattle feces if enrichment techniques are employed. The results warrant further studies on the potential source of these resistance determinants, the potential risk factors associated with acquiring these genes, and their implications for public health.

7th ARAE2017 Poster No. 44:

More than 50% reduction of antibiotic sales in German livestock farming between 2011 and 2015

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Background and objectives: Resistance against antimicrobial agents is an increasing problem in human as well as in veterinary medicine. In June 2015 in Elmau (Germany) the G7 clearly articulated the growing global health threat of antimicrobial resistance (AMR) in their final declaration [1]. In this context it is important to gain a quantitative insight into the use of antibiotics in human as well as in veterinary medicine, to implement risk management measures against the development and spread of AMR.

Materials and methods: In Germany a legal basis for obtaining national sales data from marketing authorisation holders and whole salers who sold veterinary antimicrobial products (VAPs) to veterinarian end users was established in February 2010 (DIMDI-AMV [2]).

Results: By now, results of five years (2011 – 2015) are available. The total sales of antimicrobial active substances decreased by 53% between 2011 and 2015. Apparently this was not caused by a decline in animal population. In 2011, 1,706 t of antimicrobial active substances were sold to veterinarians registered in Germany. In 2012 the amount decreased to 1,619 t, in 2013 to 1,452 t, in 2014 to 1,283 t and in 2015 to 805 t. With regard to antimicrobial classes, tetracyclines and penicillins had the largest shares, followed by sulfonamides, macrolides and polypeptides. Fluoroquinolones and 3rd/4th generation cephalosporins together accounted for less than 1% of the total amounts. Compared to 2011, sales of macrolides, sulphonamides and tetracyclines decreased by more than 60%, penicillins by more than 40%, while sales of 3rd generation cephalosporines and fluoroquinolones increased over the five years by 10% resp. 29 %.

Conclusion: Without any quantitative targets for the reduction of the use of antibiotics the sold quantities in Germany could be reduced by more than 50%. But only setting quantitative targets for the reduction of the use of antibiotics without considering preventive measures (e. g. vaccination, hygiene, structural measures, selection for more robust breeds) will not be sufficient or successful in the long run. The development for requires very careful observation. In regard to AMR it is also important to limit the use of 3rd generation cephalosporines and fluoroquinolones. Since most of the VAPs are licensed for use in several species the data summarize the amounts of antimicrobial agents sold to the veterinarian, but cannot differentiate between amounts used for different animal species.

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7th ARAE2017 Poster No. 45:

Motivations for treatment decisions made by calf care workers on western United States dairies.

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Background and objectives: Cases of dairy calf diarrhea tend to be over treated with antimicrobials. On large United States dairies, identification and treatment of sick calves is in the hands of employees. Understanding the motivation behind why and how calf caretakers make treatment decisions could help dairy advisors create more tailored messages about judicious antimicrobial use. The purpose of this study was to better understand decision making by assessing employee motivation and influences on their learning.

Materials and methods: Western US dairy farms and calf ranches with >300 pre-weaned calves were contacted through their veterinarian to participate. Using employee motivation work by Leonard et al. (1999), we developed a survey tool about motivations for treatment decision-making. We used 5 motivation type–responses for each of 10 questions: Intrinsic- motivated by satisfaction with the tasks; Extrinsic-motivated by financial rewards; Internal-motivated by belief system/values; Goal Internal-motivated to follow the goals of the farm; and External-motivated by recognition from supervisor or coworkers. The survey was administered in interviews on farm in English or Spanish and included questions on information sources, communication and calf health goals. Summary statistics and latent class analysis of motivation type-responses to 10 questions were developed.

Results: Twenty-eight farms were enrolled with 1-9 people interviewed per farm for total of 107 surveys. Calf health goals were not aligned among employees of the same farm. Motivation questions/responses fell into 4 latent classes. External-17% of individuals would identify sick calves and make treatment decisions based on positive social feedback relative to others about their traits, competencies and values; Internal/Intrinsic-41% of individuals would identify sick calves and make treatment decisions on values, beliefs and satisfaction with tasks and are relatively indifferent to social and task feedback; Goal Internal- 17% would likely identify sick calves and make treatment decisions based on farm goals and protocols; and Goal Internal/Internal-about 25% would identify sick calves based on belief systems but make treatment decisions mostly based on farm goals or protocols.

Conclusion: Addressing antimicrobial use reduction would require alignment of worker and farm goals and education addressing employees' beliefs about sick calf identification and reasons for and use of protocols.

7th ARAE2017 Poster No. 46:

Antibacterial activity and mechanism of action of dryofragin against methicillin-resistant *Staphylococcus aureus.*

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Background and objectives: In this study aimed at identifying the antibacterial activity of dryofragin, a phloroglucinol from *Dryopteris fragrans (L.) Schott*. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were treated with dryofragin to determine the differential expression of genes and associated pathways following the drug treatment.

Materials and methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth microdilution and dynamic germicidal efficacy of dryofragin was evaluated and the killing curve of dryofragin was mapped on the basis of the results of MIC and MBC, through which the concentration and time for the optimal efficacy of dryofragin was obtained. In the subsequent *in vivo* experiment, mice lethal and non-lethal systemic MRSA infection model experiments were carried out to comprehensively investigate the in vivo antibacterial activity of dryofragin. To identify the key pathways for anti-MRSA activity of dryofragin, transcriptome analysis was used, in which the transcriptome data of MRSA strains before and after the dryofragin treatment was compared. Furthermore, the transcriptome data was verified by the fluorogenic quantitative PCR method, and the consistent results indicated the authenticity and reliability of the transcriptome data.

Results: The dryofragin possessed significant anti-MRSA activity both *in vivo* (MIC=2μg/mL) and *in vitro*, and reached an antibacterial effect comparable to that of vancomycin. In the lethal septicemic study, a dose of 50 mg/kg of both dryofragin and vancomycin provided a significant protection from mortality. In the non-lethal septicemic study, dryofragin and vancomycin produced a significant reduction in mean bacterial load in murine organs including the spleen, lung and liver. After treated with dryofragin, the expression of genes involved in β-lactam resistance, amino acid synthesis, pathogenicity, ferritin synthesis and ribosome function was significantly reduced.

Conclusion: Our results showed that dryofragin has significant anti-MRSA activity both in vivo and in vitro, and its functional mechanism may be related to the inhibition of the key genes on β-lactam resistance, amino acid synthesis, pathogenicity, ferritin synthesis and ribosome function.

7th ARAE2017 Poster No. 47:

Improved DNA extraction and purification with nano-magnetite beads facilitates highly sensitive detection of MRSA directly from swabs

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Background and objectives: Molecular methods offer fast, safe and cost-efficient detection of pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). These tests depend on a rapid extraction of pure bacterial DNA. The aim of this study was to optimize an extraction and purification protocol for MRSA using nano-magnetite beads.

Materials and methods: A DNA extraction method for MRSA using silica-coated magnetite nanoparticles should be improved by testing an MRSA isolate regarding the influence of proteinase K, lysostaphin and achromopeptidase. In addition, tests with different concentrations of sodium azide were performed. To increase the DNA yield mechanical tools, such as glass beads and chelex, were comparatively tested. Moreover, a time reduction and/or omission of individual reaction steps was also investigated. The purity of the extracted DNA was assessed by photometric measurements using the Nanodrop® and the amount of DNA was detected by a Real-Time PCR with the QuickBlue Real-Time PCR Test MRSA (Q-Bioanalytic GmbH, Germany). The optimized protocol was tested using 11 strains with different SCC*mec* types. Stability testing experiments were performed.

Results: The optimized protocol can save approximately 20 min time and is as follows: The swab is transferred directly into 400 µL of lysis buffer (100 mM Tris-HCl, 10 mM EDTA, 1 M NaCl, pH 8.0). The solution is mixed and subsequently lysed for 5 min at 100°C on a thermal mixing block. The liquid part is transferred to a new sterile tube with 400 µL of binding buffer [20% PEG (Mr 8000) in 4M NaCl] and 60 µL of silica-magnetite nanoparticles (20 mg/mL). After incubation at room temperature for 10 min, the nanoparticles were magnetically immobilized and the supernatant was removed. The supernatant was resuspended in 100 µL elution buffer (distilled, deionized water) and incubated for 5 min at 65°C. The DNA yield obtained with the improved protocol was 53.35 ng/µL compared to 46.4 ng/µL with the previous method. The detection limit if the real-time PCR was 234 cfu/PCR vs. 57 cfu/PCR for the old and the new protocol respectively. The average DNA yield of the 11 MRSA strains was 65.9 ng/µL. The test kit was stable for at least six months.

Conclusion: The results show that the optimized DNA extraction protocol can be successfully used to extract and purify MRSA DNA from swabs. This method offers a quick and easy handling in applications outside highly equipped laboratories.

7th ARAE2017 Poster No. 48:

Staphylococcus aureus from zoo animal and wildlife

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Background and objectives: Antimicrobial resistance of *Staphylococcus aureus* is a major problem in human and veterinary medicine, but also in the context of the One Health principle. The aim of this study was to characterize *S. aureus* isolates from free-living animals, animals from zoos and from captivity.

Materials and methods: In total, 26 *S. aureus* isolates, including 11 from free-living animals and 15 from zoo animals or animals in captivity, were obtained during routine diagnostics. All isolates were subjected to *spa* typing, macrorestriction analysis, antimicrobial susceptibility testing and *S. aureus*-specific DNA-microarray analysis. Resistant isolates were also tested for their respective resistance genes by PCR.

Results: The characterization of the isolates revealed 19 different *spa* types, including the novel types t15467, t15473 and t15865 as well as 16 main macrorestriction patterns with up to one subpattern. Only a few isolates were resistant to selected antimicrobial agents. A single isolate, belonging to CC22-MRSA-IV (UK-15/Barnim), was methicillin-resistant and harbored the gene *mecA*. Another four isolates were resistant to penicillin. The β-lactamase gene *blaZ* was detected in the MRSA isolate and three of the penicillin-resistant isolates. The remaining penicillin resistant isolate harbored the *mecC* and the SCC*mec* XI specific β-lactamase, showing penicillin and oxacillin MICs of 2 mg/L and 1 mg/L, respectively. It was assigned to CC130-XI. Two isolates from free-living animals and one from a zoo animal showed elevated MICs of fluoroquinolones and had amino acid substitutions in GyrA/B and/or GrlA. Tetracycline resistance, mediated via *tet*(K) or *tet*(L), was detected in two isolates. Two isolates from wildlife harbored the acetyltransferase gene cat_{pC221} mediating chloramphenicol resistance. The DNA microarray analysis revealed that two isolates from zoo animals harbored the toxic shock syndrome toxin gene. Moreover, several enterotoxin genes were present in the *S. aureus* collection. All isolates were negative for PVL genes, but the animal-associated leukocidin genes *lukM*/*lukF-P83* were found in four isolates.

Conclusion: The isolates from zoo animals and wildlife showed a high diversity of CCs, *spa* types and PFGE patterns. The antimicrobial susceptibility testing revealed only resistance to four classes of antimicrobial agents and included one isolate each carrying the genes *mecA* or *mecC*. Moreover, major virulence genes were also detected.

7th ARAE2017 Poster No. 49:

MRSA carriage among human volunteers visiting a swine farm

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Background and objectives: Transmission of methicillin-resistant Staphylococcus aureus (MRSA) from animals to humans is of great concern due to the negative implications for the human health and health care system.

We studied the transmission routes of MRSA from pigs to humans in a swine farm. The primary research objectives were to investigate MRSA colonization in human volunteers during a short time exposure in a swine farm and the duration of the MRSA carriage.

Materials and methods: We conducted an experimental study using 32 human volunteers staying one hour in an MRSA-positive swine farm in four trials. In two of the trials the influence of physical contact with pigs was measured using a cross-over design. The quantity of MRSA in nasal swabs, throat swabs, and air samples were measured at different time points and analyzed in relation to relevant covariates.

Results: This investigation has shown that 95% of the volunteers became colonized after one hour stay in the stable. The quantitative level of nasal MRSA carriage was positively correlated to the personal exposure to airborne MRSA and physical contact with pigs and negatively correlated to the time passed since leaving the farm and smoking. No association was observed between MRSA carriage and age, gender, nasal MSSA carriage, and face touching behavior. MRSA was not detected in any of the throat samples. All volunteers exposed to an MRSA air level above 310 CFU/m3 were MRSA contaminated when leaving the stable. After 48 hours, 94% of the volunteers were MRSA-negative.

Conclusion: Short term exposure to airborne MRSA pose a substantial risk of becoming contaminated. The contamination is however typically cleared within hours to few days. The experimental approach made it possible to elucidate the relative importance of transmission of MRSA by air and physical contact during a short time visit to a swine farm.

7th ARAE2017 Poster No. 50:

Methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* from employees and the environment of a small animal clinic

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Background and objectives: Methicillin-resistant staphylococci constitute a major challenge in antimicrobial therapy in human and veterinary medicine. The aim of the present study was to investigate the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) among employees of a small animal clinic and in the clinic environment.

Materials and methods: In total, 169 swabs, including 96 from employees after informed consent (one from the nose and one from the hands of 47 persons, only nasal swabs of two persons) and 73 from the clinic environment, were investigated. The swabs were enriched in Mueller-Hinton broth with 6.5% sodium chloride and then plated on Mueller-Hinton agar supplemented with 2% NaCl and 0.25 mg/L oxacillin. Suspicious colonies were subcultured and the species determined using MALDI-TOF MS. The isolates were subjected to PCR-directed detection of *mecA*, macrorestriction analysis, and antimicrobial susceptibility testing via broth microdilution.

Results: MRSA isolates were detected in samples obtained from five employees and in six environmental samples, whereas MRSP isolates were present in samples obtained from two employees and in three environmental samples. All those isolates harboured the *mecA* gene. Susceptibility testing revealed three resistance patterns each for the MRSA and the MRSP isolates, with all isolates but one being classified as multiresistant (= resistant to at least three classes of antimicrobial agents). All isolates were resistant to β-lactams and fluoroquinolones. One MRSA isolate was intermediate to erythromycin, whereas the remaining isolates were resistant to macrolides and lincosamides. A single MRSA isolate was also resistant to gentamicin. All MRSP isolates were resistant to trimethoprim/sulfamethoxazole, all but two isolates to gentamicin, while the remaining two were classified as gentamicin intermediate. One of the latter two isolates was also resistant to tetracycline. Macrorestriction analysis revealed three SmaI restriction patterns with up to one subpattern for the MRSA isolates and two SmaI restriction patterns with one subpattern each for the MRSP isolates.

Conclusion: The finding of MRSA and MRSP isolates among employees and in the environment of a small animal clinic suggests the possibility of the transfer of these bacteria between humans, animals, and the clinic environment via direct or indirect contact.

7th ARAE2017 Poster No. 51:

Identical genotypes of community-associated MRSA (ST59) and livestock-associated MRSA (ST9) in both humans and pigs in rural China

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Background and objectives: Human and animal health are interlinked and a One Health-approach should be applied to understand the dissemination of MRSA between the human and the animal sectors. The aim of the current study was to investigate the prevalence and epidemiological characteristics of MRSA in households in villages in a rural area of Shandong Province, China.

Materials and methods: Human nasal samples from nostrils and skin samples from behind the ear of pigs were collected during July 2015 in 12 villages in a rural area of Shandong. Presumptive MRSA were isolated from the CHROMagar™ MRSA (CHROMagar Company, Paris, France) after a sample pre-enrichment in 7.5% Sodium Chloride Broth (Land Bridge, Beijing, China) and confirmed as MRSA by PCR assay. MRSA isolates were characterized by MLST-, *spa*-, SCC*mec*-, and *dru*-typing. Antimicrobial susceptibility testing (AST) of isolates was performed using agar dilution in Muller-Hinton agar (Oxoid, Basingstoke, UK). Susceptibility was determined using epidemiological cut-off values (ECOFFs) by the European Committee of Antimicrobial Susceptibility Testing (EUCAST, 2017).

Results: In total, 404 pig ear swab samples and 768 human nasal samples were collected from 245 and 753 households in the 12 villages, respectively, and from which 13 (1.7%) households in seven villages were identified with human MRSA isolates, 7 (2.8%) households in five villages had pigs with MRSA. All isolates belonged to either ST9 (n=12) or ST59 (n=8). ST9 isolates were equally distributed among the human and pig samples, while ST59 isolates were mainly identified among humans except for one isolate which was recovered from a pig sample. In three households, humans and pigs shared the same genotypes; two households with ST9-t899-SCC*mec*IVb-dt12w and one with ST59-t437-SCC*mec*IVa-dt10a. The AST showed the ST9 isolates were resistant to more antibiotics compared to the ST59 isolates.

Conclusion: Our data showed a lower prevalence of MRSA among rural residents and domesticated pigs in China and an even distribution of CA-MRSA and LA-MRSA among humans while a predominance of LA-MRSA among pigs. The household CA-MRSA isolates with shared genotypes could be isolated from both human and pigs, implying that transmission of CA-MRSA from human to pig can take place.

7th ARAE2017 Poster No. 52:

Characterization of two novel small plasmids in *staphylococci sciuri* of animal origin

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Background and objectives: To identify the small plasmids in staphylococci of animal origin containing the *cfr* or *spd* genes, which confer the resistance to five classes of drugs including phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics or spectinomycin, respectively.

Materials and methods: The MICs determination was performed using the two-fold broth dilution method. The resistance genes were detected by PCR. The plasmids in the wild type strains were isolated using the plasmid extraction kit (Qiagen, Germany) and transformed into *staphylococcus aureus* RN4220. S1-PFGE and southern blot using the *cfr* or *spd* genes as the probes were performed for the plasmids from the transformants. The inverse PCR and sanger sequencing were performed to obtain the whole sequence of the plasmids.

Results: Two novel small plasmids SA-1 and SA-2 were identified in staphylococci, which were 8,237 and 6,250bp in length, respectively. Plasmid SA-1 contains *tet*(M) and *cfr* genes, while SA-2 contains *spd* and *erm*(C) genes. Plasmid SA-1 contains a backbone repU-*tet*(M)-pre/mob-*cfr*-△pre/mob, which exhibits the greatest similarity with pBS-03, pSS-03 and pMSA16, except that the *tet*(M) gene was substituted with the *aadY*, *erm*(C) and *erm*(A) genes, respectively. Plasmid SA-2 contains a backbone repN-*spd*-*erm*(C)-rec, which exhibits the greatest similarity with pDJ91S. However, the additional *erm*(C) was inserted into the backbone repN-*spd*-rec in pDJ91S.

Conclusion: The diversity of the resistance genes located on these small plasmids in staphylococci reflects its strong evolving adaptability in confronting with the sophiscated antimicrobial pressure.

7th ARAE2017 Poster No. 53:

Novel pseudo-staphylococcal cassette chromosome *mec* element (ϕSCC*mec*$_{T55}$) in methicillin-resistant *Staphylococcus aureus* ST9

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Background and objectives: The methicillin resistance gene *mecA* is usually transferred by staphylococcal cassette chromosome *mec* (SCC*mec*) elements, which achieve excision from or integration in the staphylococcal chromosomal DNA by cassette chromosome recombinases (Ccr), the genes of which are also part of the SCC*mec* elements. The aim of this study was to gain insights into the structures of SCC*mec* elements of two closely related MRSA ST9 isolates (CD9 and T55), one of which lacks *ccr* genes.

Materials and methods: The MRSA isolates were obtained from nasal swabs of apparently healthy pigs in China. Whole genome sequencing was performed to determine the genetic structure of the SCC*mec* elements. The programs Easyfig and Mauve were employed for genomic comparison.

Results: Genome analysis revealed that these two MRSA isolates contained different but related SCC*mec* elements. In isolate CD9, the SCC*mec* element was similar to the novel type SCC*mec* XII harbouring the *ccrC2* gene and being flanked by a pseudo-SCC element carrying a truncated *ccrA1* gene. In isolate CD9, the type C2 *mec* gene complex was located in opposite orientation compared to the one in SCC*mec* XII. In addition, the SCC*mec* element in isolate CD9 was disrupted by the core chromosome of the strain into two segments of 41.6 kb and 12.2 kb. The 41.6-kb segment contained the key structures of the type XII SCC*mec* element (*ccr* and *mec* gene complexes) and the pseudo-SCC element. The 12.2-kb segment contained the J3 region of SCC*mec* XII but with two additional IS*431* elements and one IS*256* element. In isolate T55, a novel pseudo SCC*mec* (ϕSCC*mec*T55) was identified. The 29.2kb ϕSCC*mec*$_{T55}$ contained a relic of SCC*mec* XII. Compared with CD9, a 19.3-kb segment was missing in T55. This segment encompassed the part from the truncated *ccrA1* gene until immediately upstream of the first IS*431* and included the entire *ccr* gene complex. A 3.2-kb sequence, which contained the type III restriction-modification system methylation subunit and was bracketed by two copies of IS*431* in the same orientation, was also lost in isolate T55 and replaced by a single copy of IS*431*.

Conclusion: This finding proposed an independent excision of the *ccr* gene complex instead of the complete SCC*mec* element, as well as a potential role of IS*431* in forming a novel SCC*mec* element.

7th ARAE2017 Poster No. 54:

Methicillin-resistant *Staphylococcus aureus* in raw cow milk and soft cheese (wara) sold in Abeokuta, Nigeria

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Background and objectives: The emergence of antibiotic-resistant microorganisms in foods of animal origins poses a major public health concern. Methicillin resistant *Staphylococcus aureus* (MRSA) is an important opportunistic pathogen both in humans and in dairy cattle. Milk and milk products can be vehicle for transmission of MRSA. This study was carried out to investigate the presence and resistance profile of MRSA and in raw milk and milk-product (soft-cheese/wara (local name for soft cheese) sold in Abeokuta.

Materials and methods: Two hundred samples including 100 raw cow milk from randomly selected lactating cows in herds and 100 soft cheese/wara from street vendors were collected from Abeokuta, Nigeria. The samples were examined using conventional bacteriological methods for the isolation and phenotypic identification of MRSA. Penicillin-binding protein 2a (PBP2a) latex agglutination test of was used to further confirmed MRSA isolates. The antibiotic susceptibility testing of isolates was determined by agar disk diffusion method.

Results: In all, 52 (26%) of 200 samples yielded *S aureus* out of which 50 (25%) were MRSA. Fifteen (15%) MRSA isolates were from 100 raw milk samples and 35 (35%) were from 100 soft cheese/wara samples. The *Staphylococcus aureus* isolates from raw milk showed high rates of resistance to ceftazidime 17 (100%), ampicillin 16 (94.1%), doxycycline 11 (64.7%), tetracycline 17 (100%), oxacillin 15 (88.2%), augmentin 17 (100%), gentamycin 15 (70.6%), colistin 15 (70.6%), and sulphamethoxazole 16 (88.2%). For isolates from soft cheese/wara, there were resistance to cefoxitin (100%), ceftazidime (100.0%), ampicillin (100%), doxycycline (20.0%), tetracycline (65.7%), oxacillin (100.0%), streptomycin (20.0%), augmentin (94.3%), gentamicin (17.1%), colistin (94.3%), and sulphamethoxazole (40.0%)**.**

Conclusion: The presence of MRSA in the raw cow milk and soft cheese/wara sold in Abeokuta may be due to indiscriminate and/or overdependence on antibiotics use in cattle production. Contamination along processing and marketing chain of soft cheese/wara due to unhygienic practices among milk handlers**,** processors and vendors could also contribute to the presence of MRSA in the product. This constitutes a potential public health risk to consumers of milk and milk products in the study areas.

7th ARAE2017 Poster No. 55:

Airborne colonization of piglets with livestock-associated MRSA

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Background and objectives: Methicillin-resistant-*Staphylococcus aureus* (MRSA) are considered as a major concern in public healthcare systems. Since 2005, the occurrence of livestock-associated MRSA (LA-MRSA) is a common phenomenon in agriculture - especially in pig farming. Particularly the sequence type ST398 was identified to be predominant in these surroundings. In recent years, the number of LA-MRSA isolates in hospital increased. Due to the fact that MRSA was regularly found in air samples, a spread via an airborne route is discussed.

The aim of our study is to identify the target dose for a successful colonization of piglets with MRSA ST398 via the airborne way. Furthermore, we want to define risk factors which could influence a successful MRSA colonization.

Materials and methods: Therefore, we exposed groups of nine MRSA-negative tested piglets, each, in an aerosol chamber to a defined in air MRSA-concentration of 10^2, 10^4 and 10^6 cfu/m^3. The used strain derived from a healthy pig and was characterized as MLST ST398.

During the exposure time of 24 hours, air samples were taken via impingement to verify the desired MRSA concentration. After that, different animal swab samples were taken three times a week for a period of 21 days to monitor the MRSA colonization. Each screening period was finalized by necropsy to examine the presence of MRSA in the internal organs.

Results: The first group was exposed to the lowest MRSA concentration (10^2 cfu/m^3). Therein, only one animal was tested MRSA-positive, directly after staying in the chamber. No other positive sample could be detected during the whole observation time. In the second group (10^4 cfu/m^3) all animals were positive after the exposition. Then the number of positive swabs varied until day 16. After that, the samples remained negative. The last group was exposed to the full load of MRSA (10^6 cfu/m^3). All animals were MRSA-positive for at least one kind of swab sample for the whole screening period.

Conclusion: The data indicated that an airborne route spread of MRSA exists. Whereas group two showed a transient colonization, the animals of group three were permanent colonized. The difference between our target MRSA dose and the mean MRSA concentration in barn air (10^2 cfu/m^3) could be explained with the limited duration of our MRSA exposition. Other factors, like endotoxins or corrosive gases in the air of pig barns could also influence the target dose for a permanent colonization. The effect of immunosuppression is currently examined and these data will be also presented.

7th ARAE2017 Poster No. 56:

ESBL and colistin resistance dynamic during veal calves fattening in France

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Background and objectives: The prevalence of ESBL-producing *E. coli* in veal calves at slaughterhouse has reached 29.4% in 2014 in France. This prevalence was even higher in the 3-weeks-old animals entering the fattening farms. The goal of this study was to investigate the dynamic of ESBL-producing *E. coli* in 3 different veal calves farms.

Materials and methods: Between October 2015 and April 2016, rectal swabs of 45 veal calves from 3 different farms were plated on ChromID ESBL media for the selection of EBSL/AmpC-*Enterobacteriaceae*. In each farm, calves were divided into 3 groups (negative, low carrier, high carrier) depending on their ESBL load at arrival on the farm. Samples were collected every two weeks until animals were slaughtered. One presumptive colony per morphology was identified by Maldi-Tof. Antimicrobial susceptibility was tested by disk diffusion and broth microdilution for colistin. Resistance genes were identified by PCR/sequencing. Plasmid characterization was performed by PBRT, S1-PFGE and Southern blots using adequate probes.

Results: Over the whole study, 84, 15 and 76 ESBL-producing *E. coli* were collected from farm 1, 2 and 3, respectively. In farm 1, all selected animals were ESBL-carriers at arrival and remained positive over two months. ESBLs, mostly of CTX-M-1 group, then disappeared over the 3 following samplings and only re-occurred sporadically. Calves were treated by colistin at arrival, and the *mcr-1* colistin-resistance gene was indeed identified in 53 (63.1%) ESBL isolates. Preliminary results showed that *mcr-1* was carried by IncX4 plasmids, with or without the ESBL gene. In farm 2, ESBL-producing *E. coli*, also mostly producing CTX-M-1 group enzymes, rapidly disappeared and no colistin-resistant isolate was found. In farm 3, ESBLs (both of CTX-M group 1 and 9) presented a bi-modal distribution since they re-appeared in the second part of the fattening period.

Conclusion: The prevalence of ESBL-producing *E. coli* decreased in all farms during the fattening process. However, this dynamic largely differed among farms, most probably in line with different ESBL status of the calves at arrival and variable intercurrent diseases and antibiotic usages. Indeed, the only farm where the *mcr-1* gene was identified was the one where metaphylactic treatment with colistin had been administered to all calves.

7th ARAE2017 Poster No. 57:

Vegastudy: Do vegetarians less often carry ESBL-producing *E. coli/Klebsiella pneumoniae*?

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Background and objectives: ESBL/AmpC-producing *E. coli/Klebsiella pneumoniae* (ESBL-E/K) can be found on meat in the supermarket. In the Netherlands, prevalence rates differ (up to 67% can be contaminated), depending on the animal species from which the meat was derived (MARAN, 2016). As the ESBL/AmpC genes in isolates from farm animals and meat often overlap with the types found in humans, eating meat is generally considered an important transmission route to humans, although direct evidence for this is lacking. The objective of this study was to investigate whether ESBL-E/K prevalence among individuals that do not eat meat (vegans/vegetarians) is lower than among individuals that do.

Materials and methods: Vegetarians, vegans and controls (persons that eat meat or fish more than once a week) were asked to send in a faecal sample and to fill in a questionnaire. Samples were cultured on Brilliance™ E. coli/coliform Selective Agar with (BECSA$^+$) and without 1 mg/L cefotaxime and incubated overnight in Luria Burtani Broth containing 1 mg/L cefotaxime. When direct culture was negative, broth was cultured on BECSA$^+$. Suspected colonies were biochemically confirmed as *E. coli* or *K. pneumoniae*. The questionnaire contained questions about diet and other risk factors for ESBL-E/K carriage, like contact with animals and travel behavior. Up to three isolates per sample were collected and typed by MLST. The ESBL/AmpC genes were typed by sequencing. ESBL-E/K prevalence of the vegetarians/vegan group was compared to the control group using Wilson Score Interval in EpiTools (http://epitools.ausvet.com.au).

Results: In total 1641 persons sent a faecal sample and a questionnaire. 96/1218 vegetarians/vegans (7.88%; 96%CI 6.5 – 9.5) and 23/423 controls (5.43%, 95%CI 3.7 – 8.0), carried ESBL-E/K. Results of the questionnaires are currently analysed. Preliminary sequence data of isolates derived from 50 vegetarians/vegans and 10 controls show the presence of 11 different ESBL/AmpC genes. In 53/60 of those samples analysed, 28 different STs were found. ST69, ST131 and ST648 were most prevalent.

Conclusion: ESBL-E/K prevalence among vegetarians/vegans was not lower than the control group. The hypothesis that eating meat increases the risk of carriage of ESBL-E/K was not confirmed. Analysis of the questionnaires will further reveal which risk factors account for ESBL-E/K carriage among vegetarians/vegans and whether those factors differ from persons that eat meat.

7th ARAE2017 Poster No. 58:

Extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing *Enterobacteriaceae* in water sources in Lebanon

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Background and objectives: The presence of extended-spectrum β-lactamases (ESBLs) has been recurrently reported in both human and veterinary medicine, and carbapenemases have also emerged in these two reservoirs. Such resistance phenotypes are increasingly reported in the environment, which is both receiving and further disseminating multi-drug (MDR) resistant bacteria. This study aimed at estimating the prevalence of ESBL- and carbapenemase-producing *Enterobacteriaceae* in water intended for human consumption (rural wells and spring water) or for animal consumption and agriculture (estuaries) in Lebanon.

Materials and methods: A total of 292 water samples were collected between March 2014 and January 2015, 155 originating from wells, 115 from spring water and 22 from estuaries. A 100µl sample was directly plated on MacConkey agar supplemented with ceftazidime or imipenem. One presumptive *Enterobacteriaceae* per morphology was further identified by Maldi-Tof. Antimicrobial susceptibility was tested by disk diffusion. Resistance genes were identified by PCR/sequencing. Clonality was assessed by PFGE and MLST typing. Plasmid characterization was performed by PBRT, S1-PFGE and Southern blots using adequate probes.

Results: Fifteen (15/22, 68.2%) water samples collected in estuaries were contaminated by MDR bacteria. In total, 33 Enterobacteriaceae were recovered; 21 produced an ESBL (including 14 CTX-M-15) and 4 produced a carbapenemase (2 OXA-48 and 2 OXA-244). ESBL-contamination with CTX-M-15-producing *E. coli* was also identified in 1.9% (3/155) and 6.1% (7/115) of the water samples from rural or spring water, respectively. Among the 33 isolates, resistance genes were mostly carried by IncF-type plasmids (n=27). A high clonal diversity was observed, with only a few sequence types (ST38, ST10 or ST131) found on several occasions.

Conclusion: A massive contamination by ESBL- and carbapenemase-positive bacteria was observed in estuaries, which are the receptacle of hospital, urban and farm effluents. Indeed, the clones identified suggested both animal and human contaminations. Moreover, ESBL-producing *E. coli* were also detected in water from rural wells and springs, which is directly intended for human consumption. Taken together, our data strongly suggest that water may be an important source of human/animal contamination by ESBL-producing *Enterobacteriaceae* in Lebanon.

7th ARAE2017 Poster No. 59:

Extended-spectrum β-lactamase (ESBL) of faecal *Escherichia coli* isolate recovered from European free-tailed bat (*Tadarida teniotis)* in Portugal

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Background and objectives: The aim of this study was to characterize the diversity of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates recovered in faecal products from European free-tailed bat (*Tadarida teniotis*) in Portugal. Bats have a wide variety of ecological behaviors and habitats. *E. coli* is a commensal microorganism of the intestinal flora of bats. The emergence/dissemination of ESBLs in clinical *E. coli* isolates is a public health problem because of its ability to acquire multiple antibiotic resistance.

Materials and methods: We collected 146 faecal samples that were seeded in Levine agar plates supplemented with cefotaxime (2µg/mL). *E. coli* isolates were identified through classical biochemical methods and by the API 20E system. Susceptibility to beta-lactam antimicrobial agents was performed by the disc diffusion method. *E. coli* ATCC 25922 was used as standard quality-control strain. ESBL-phenotypic detection was carried out by double-disc diffusion test. The presence of genes encoding TEM-, OXA-, SHV- and CTX-M-type beta-lactamases was studied by PCR to identify the beta-lactamase gene. Lastly, the phylogenetic groups (*chuA, yjaA*, and *TspE4)* and virulence determinants (*fimA*, *papG* III, *cnf1*, *papC* and *aer*) were also investigated.

Results: 19 cefotaxime-resistant *E. coli* isolates were recovered from 14 free range *T. teniotis*. Beta-lactamase genes detected were: $bla_{\text{CTX-M-3}}$ (5 isolates), $bla_{\text{CTX-M-1}}$ (6 isolates), $bla_{\text{CTX-M-1}}$+$bla_{\text{TEM-type}}$+$bla_{\text{SHV-type}}$ (1 isolate), $bla_{\text{CTX-M-1}}$+$bla_{\text{TEM-type}}$+$bla_{\text{OXA-type}}$ (1 isolate), $bla_{\text{CTX-M-1}}$+$bla_{\text{TEM-type}}$+$bla_{\text{SHV-type}}$+$bla_{\text{OXA-type}}$ (1 isolate), $bla_{\text{CTX-M-1}}$+$bla_{\text{TEM-type}}$ (1 isolate), $bla_{\text{CTX-M-1}}$+$bla_{\text{CTX-M-9}}$ (1 isolate), $bla_{\text{CTX-M-3}}$+$bla_{\text{TEM-type}}$+$bla_{\text{SHV-type}}$ (1 isolate), $bla_{\text{CTX-M-9}}$+$bla_{\text{SHV-type}}$ (1 isolate) and $bla_{\text{SHV-type}}$ (1 isolate). The phylogenetic groups were 7 isolates from group A, 4 from group B2 and 8 from group D. The virulence determinant *fimA* was detected in 13 isolates, the *aer* gene in 3 isolates, *cnf1* in 7 isolates and *papG*III in 2 isolates.

Conclusion: The results indicate that this is the first time that ESBL-producing *E. coli* isolates have been detected in wild populations of *T. teniotis*. Bats could acquire resistant microorganisms from insects and environmental sources. The present study represents an understanding of how *T. teniotis* might contribute to the spread of bacteria into the environment, while also highlighting the need for additional studies.

7th ARAE2017 Poster No. 60:

Spread of CTX-M-9-producing *Enterobacteriaceae* among rescued birds in France

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Background and objectives: Wild birds are not directly exposed to antibiotics, but numerous studies showed that they can be colonized by ESBL- or carbapenemase-producing *Enterobacteriaceae*. Birds can thus disseminate resistance either locally or over thousands of kilometres when they are migrating. They may also be considered as indicators of the environmental ESBL burden. The goal of this study was to estimate the prevalence of ESBL- and carbapenemase-producing *Enterobacteriaceae* in wild birds at their arrival at a wildlife rescue centre in Southern France.

Materials and methods: Between April and November 2015, rectal swabs of 437 birds were plated on ChromID ESBL and CarbSMART selective media for the selection of EBSL/AmpC- and carbapenemase-producing *Enterobacteriaceae*. One presumptive colony per morphology was identified by Maldi-Tof. Antimicrobial susceptibility was tested by disk diffusion and ESBL production was determined by double-disk synergy tests. Resistance genes were identified by PCR/sequencing. Clonality was assessed by PFGE, and MLST for *E. coli*.

Results: Over the 437 samples, 114 (26.1%) presented at least one colony on ESBL/AmpC-selecting plates, but none was positive for the presence of carbapenemases. Three AmpC- and 159 ESBL-producing isolates were identified from 3 (3/437, 0.7%) versus 114 (114/437, 25.4%) different animals, respectively. *E. coli* (n=99) was the most frequently identified species, followed by *Enterobacter cloacae* (n=55) and *Citrobacter* spp (n=5). The ESBL phenotype was due to the $bla_{\text{CTX-M-9}}$ gene in all *E. cloacae* and *Citrobacter* spp isolates, and in 76 *E. coli* (76.8%). The 23 remaining *E. coli* presented the $bla_{\text{CTX-M-1}}$ gene. PFGE performed on *E. cloacae* and *E. coli* revealed the spread of a limited number of major clones, which belonged to ST746, ST1246, ST223 and ST40 for *E. coli*.

Conclusion: The prevalence of ESBL-producing *Enterobacteriaceae* was high (26.1%) and positive birds belonged both to migrating and non-migrating species. Surprisingly, 34.6% of the identified bacteria were *E. cloacae*, and 85.5% of all ESBL phenotypes were due to CTX-M-9 enzymes. These data do not mirror the human or animal epidemiology reported in France. Considering the detection of a limited number of major clones, we hypothesize cross-contamination through human handling of rescued CTX-M-9-positive birds. Further studies and plasmid characterization are needed to understand the origin and routes of transmission of these isolates.

7th ARAE2017 Poster No. 62:

Molecular characterisation of CTX-M-15 producing isolates from food in Germany

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Background and objectives: Spread of extended-spectrum β-lactamases (ESBL) and AmpC producing Enterobacteriaceae is a major public health concern. *bla*$_{CTX-M-15}$ is the most prevalent resistance gene in isolates of human origin, but underrepresented in bacteria from animals. As food may serve as a vehicle for transmission from livestock to the consumers, 3rd generation cephalosporin resistant *E. coli* isolates recovered from food samples between 2011-2013 were screened for the presence of *bla*$_{CTX-M-15}$ genes.

Materials and methods: 437 3rd generation cephalosporin resistant *E. coli* isolates from poultry, swine, cattle meat and raw milk were screened for ESBL-/AmpC-encoding genes by PCR amplification/sequencing and further typed by phylogenetic grouping. Antimicrobial resistance of CTX-M-15 producing isolates was determined by broth microdilution. Additionally, isolates were characterized by MLST and XbaI-PFGE. Plasmid analyses were carried out by transformation experiments and subsequent Inc-typing and S1-PFGE. In addition, whole genome sequencing (NGS) was carried out.

Results: 21 out of 437 food-isolates harboured a *bla*$_{CTX-M-15}$ gene. Majority of isolates (n=17) were assigned to phylogenetic group A followed by B1, D and B2. XbaI-PFGE revealed diverse restriction patterns but two clusters (P1, P2) could be identified. Cluster P1 includes six strains, one originated from beef and five were isolated from raw milk over a period of six month within the same geographical region.

Plasmids encoding CTX-M-15 enzymes belonged to IncF multi-replicon plasmids (FII/FIA/FIB) (n=10), IncI1 (n=5), IncI2 (n=1) and IncN (n=1). In four isolates a chromosomal localisation of the gene was assumed. IncF plasmids are mostly associated with class 1 integrons containing *aadA5*/*dfrA17* gene cassette as well as with further resistance genes (e.g. *aac(6)-1b-cr*). MLST revealed diverse sequence types with ST167 (n=8) and ST410 (n=4) as most abundant once. NGS revealed a close phylogenetic relationship for isolates of ST167 as well as for ST410 isolates.

Conclusion: In conclusion, CTX-M-15 producing *E. coli* in Germany can be found in different food matrices with low prevalence. Comparison with isolates obtained from livestock samples revealed a number of similarities and show the spread of some prominent clones and a probable transmission from animals to food. The distribution of CTX-M-types should be closely monitored to notice actual trends in the dissemination of the β-lactamases.

7th ARAE2017 Poster No. 63:

Characterization of clinical bovine and human CTX-M-15-producing *Escherichia coli* isolates by biocide susceptibility testing

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Background and objectives: Biocides have been widely used in veterinary clinics and hospitals, as well as in animal husbandry and food animal production. In Germany, CTX-M-15 is one of the most commonly reported extended spectrum-β-lactamases (ESBLs) among *Escherichia coli* isolates from different sources. This study aimed at investigating the tolerance to biocides of clinical CTX-M-15-positive *E. coli* from cattle and humans.

Materials and methods: A total of 105 clinical CTX-M-15-positive *E. coli* isolates, from cattle (faeces n=32, intestinal tissue n=10, milk n=12, udder n=2; sampling period 2008-2015) and from humans (faeces n=8, rectal swabs n=12; anal swabs n=5; urine n=24; sampling period 2009-2015), were subjected to biocide susceptibility testing by broth macrodilution. The biocides tested were: glutaraldehyde (312.5-10000 mg/L), benzalkonium chloride (BAC) (5-80 mg/L), chlorhexidine (0.25-32 mg/L) (2-fold dilution series) and isopropanol (1-8 %) (2 % steps). The *E. coli* reference strains ATCC© 10536 and ATCC© 25922 were used for comparative reasons.

Results: The distribution of the minimal inhibitory concentrations (MICs) detected for BAC, isopropanol and glutaraldehyde was usually within two or three dilution steps. The MICs for BAC were 20-40 mg/L (0.002-0.004 %) and 20-80 mg/L (0.002-0.008 %), and for isopropanol 4-6 % and 4-8 % for bovine and human isolates, respectively. The same glutaraldehyde MIC distribution (625-2500 mg/L; 0.0625-0.25 %) was seen among isolates of bovine and human origin. In comparison, a MIC distribution of six dilution steps [0.5-16 mg/L (0.00005-0.0016 %)] was seen for chlorhexidine with bovine isolates originating from faeces, intestinal tissue or milk. The human isolates displayed chlorhexidine MICs of 1-8 mg/L (0.0001-0.0008 %). Most of the *E. coli* isolates from the different sources showed MICs similar to the *E. coli* reference strains.

Conclusion: In this study, the distribution of the MICs does not suggest the presence of acquired tolerance to glutaraldehyde, BAC and isopropanol in the bovine and human CTX-M-15-producing isolates. Further studies with larger numbers of isolates and including genotypic investigations of isolates with elevated MICs (if available) may allow a better evaluation of the distribution of biocide MICs and the identification of acquired tolerance mechanisms to biocides in the isolates investigated.

7th ARAE2017 Poster No. 64:

Diversity of VIM-1 producing *E. coli* from German livestock

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Background and objectives: Carbapenemase-producing *Enterobactericeae* (CPE) are of major concern in human medicine. CPEs are resistant to the majority of β-lactam antibiotics and are often associated with a number of further resistance genes. There is a world-wide distribution of CPEs and a growing number of carbapenemase variants from human infections. In comparison CPEs in companion animals and livestock are reported only sporadically. Nevertheless, a close monitoring of CPEs in livestock and food is recommended by the European Food Safety Authority. In this study, isolates phenotypically resistant to carbapenems were screened for the most abundant carbapenemase genes. The occurrence of bla_{VIM-1} positive *E. coli* in a pig fattening farm in 2016 was investigated to determine its genetic relationship to previously characterized isolates from the same farm in 2015.

Materials and methods: Four VIM-1 producing isolates recovered from faecal samples of three different barns of one farm were isolated using a slightly modified version of the EFSA protocol. To characterise the genetic background of the isolates and the location of the bla_{VIM-1} resistance gene further molecular analyses were performed by PCR, PFGE, Hybridisation and whole genome sequencing.

Results: XbaI PFGE revealed a clonal spread of VIM-1 producing *E. coli* on the farm since 2015. However, S1 PFGE displayed certain variability in the modular organisation of the plasmid. Resistance to carbapenems was persistent on the farm without selective pressure for at least three months. This may be based on several identified chromosomal and plasmidal persistence factors. Dissection of the whole genome sequences disclosed variability in the composition of the VIM-associated sequences, while the genetic background of the chromosome of the host bacteria was almost equal. Genetic analysis outlined the mechanism of plasmid evolution of VIM-1 producing *E. coli*.

Conclusion: VIM-1 producing *E. coli* were still present in German pig farming. Persistence in one farm over a period of at least five months and presence in separate barns of the farm was observed. As some of the bacteria were isolated from subsequent herds housed in the same barn and the piglets originated from the same breeding herd, failure of cleaning procedures to eliminate the bacteria from the environment, as well as repeated introduction of colonized pigs from the breeding herd could be involved in the transmission of the VIM-1 producing *E. coli*.

7th ARAE2017 Poster No. 65:

Detection of plasmid-mediated AmpC beta-lactamase CMY-2 in *Escherichia coli* isolated from diseased food-producing animals

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Background and objectives: CMY-2 has been reported as the globally most widespread plasmid-mediated AmpC β-lactamase (pAmpC) in *Escherichia coli*. This study aimed at the characterization of AmpC genes in *E. coli* from diseased food-producing animals.

Materials and methods: A total of 7,616 *E. coli* from diseased cattle, pigs or poultry was investigated by antimicrobial susceptibility testing (AST) in the GE*RM*-Vet program (2008-2015). Cefoxitin-resistant isolates were tested by an AmpC β-lactamase detection kit (Mast D68C), and PCR and sequencing of pAmpC genes. The pAmpC-positive isolates were characterized by XbaI-macrorestriction and PFGE and multilocus sequence typing (MLST). Transformation and conjugation experiments were performed. Transformants, pAmpC gene-carrying plasmids and the genetic environment of pAmpC genes were analyzed using AST, S1-nuclease PFGE, EcoRV/PstI restriction and PCR/sequencing. Five pAmpC-positive isolates were submitted to whole genome sequencing using the Illumina MiSeq platform.

Results: The presence of pAmpC genes was detected in seven *E. coli* isolates and all of them harboured the bla_{CMY-2} gene. These isolates showed unrelated XbaI-patterns and belonged to the sequence types (STs) ST10 (n=3), ST88, ST117, ST429 and ST3778. The bla_{CMY-2} genes were linked to intact or truncated IS*Ecp1*. Five isolates carried conjugative plasmids with transfer efficiencies of $6.0x10^{-5}$-$1.8x10^{-3}$. The non-conjugative IncK plasmid (115 kb), from an avian *E. coli* isolate ST429, carried a class 1 integron (*aadA1* gene cassette, streptomycin/spectinomycin resistance) including the *sul1* gene (sulfonamide resistance). In contrast, the remaining six plasmids belonged to IncI1 and carried bla_{CMY-2} as the only resistance gene. Two IncI1 plasmids (100 kb) of plasmid ST (pST) pST12, showed indistinguishable EcoRV- and PstI-restriction patterns, but originated from unrelated avian isolates (ST117; ST3778). The three other 85-95 kb IncI1-pST2 plasmids (two of them were conjugative), originating from unrelated bovine, porcine or avian isolates (all ST10), displayed similar EcoRV- and PstI-restriction patterns. Moreover, an IncI1-pST55 plasmid (95 kb) was found in a bovine isolate (ST88).

Conclusion: This study suggests that plasmid transfer, especially IncI1-pST2 plasmids, may play an important role in the dissemination of the bla_{CMY-2} gene among *E. coli* isolates from diseased food-producing animals in Germany.

7th ARAE2017 Poster No. 66:

Molecular analysis of plasmids coding for cephalosporin resistance in *E. coli* from broilers

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Background and objectives: Resistance to extended spectrum cephalosporin (ESC) is a major health concern. This ESC resistance is mostly borne by conjugative plasmids. In 2010-2011, this resistance could be detected in Enterobacteriaceae of poultry flocks and the impact of ceftiofur administration in hatchery was established. The aim of the present study was to evaluate the characteristics and the diversity of ESC resistance plasmids from different free-range broiler flocks, and their persistence in flocks during the breeding period.

Materials and methods: Two hatcheries were selected. Faecal samples were collected from 30 flocks from before arrival on the production farm to the end of the breeding period (Baron et al, 2014). Nineteen plasmids from *E. coli* isolates obtained at different times and from different flocks and harbouring an ESC resistance gene were selected and sequenced using Mi-seq Illumina technology or Ion Proton System (Ion Torrent).

We cleaned reads with trimmomatic, aligned with bwa to *E. coli* DH5 alpha strain to remove chromosomal reads. Samples reads were downsampled to obtain an estimated coverage depth of 80 for assembly. Then we performed spades assemblies on cleaned reads and mira assemblies on related raw reads. The ESC resistance genes, the replicons and the sequence types of the plasmids were determined (https://cge.cbs.dtu.dk), and the sequences of the plasmids were compared using Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results: Nine IncI1 ST12 plasmids had the bla_{CMY-2} gene and seven of them had no other resistance gene. All nine were obtained from four flocks produced by the same hatchery, placed in three different farms and sampled from the 1st to the 7th day of life. For five of these plasmids for which the resulting assembly gave one or two contigs, the comparison showed that they shared more than 99.99% identity. Nine IncI1 ST3 plasmids were obtained from 2 to 77-day-old broilers from seven flocks on six farms. These plasmids harbored the $bla_{CTX-M-1}$ gene, and eight had also the *tetA* and *sul2* genes. For three of these IncI1, ST3 plasmids obtained from three flocks originating from two hatcheries, for which the resulting assembly gave a unique contig, the comparison showed that they shared more than 99.98% identity.

Conclusion: The molecular characterization of these plasmids showed that most of them shared a high percentage of identity and could persist during several months in a flock.

7th ARAE2017 Poster No. 67:

Occurrence and characterization of ESBL-encoding plasmids among *Escherichia coli* isolates from fresh vegetables

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Background and objectives: Fresh vegetables contaminated with extended-spectrum β-lactamase (ESBL)-producing bacteria may pose risks to human health. The objectives of this study were the detection and characterization of ESBL-producing *Escherichia coli* from vegetables.

Materials and methods: Among 245 samples of vegetables investigated (2011-2013), seven ESBL-producing *E. coli* isolates were found in salad (2/202) and sprouts (5/43). The ESBL-producing isolates were analysed by antimicrobial susceptibility testing (AST), XbaI-PFGE, multilocus sequence typing and phylotyping. ESBL genes were detected by PCR and sequenced. Transformants carrying ESBL genes were characterised by AST, S1-nuclease PFGE, replicon typing, conjugation and were tested for co-located antimicrobial resistance genes. Plasmid sequencing of one $bla_{\text{CTX-M-15}}$- and $bla_{\text{CTX-M-125}}$-carrying plasmids was performed using a HiSeq 2500 system.

Results: XbaI-patterns revealed no clonal relationship among the seven ESBL-producing *E. coli*. They were associated to phylogenetic groups A (n=4), B1 (n=2) or D (n=1) and to unique sequence types (STs). Two plasmids carrying $bla_{\text{CTX-M-14}}$ genes (incompatibility group IncK, 90 kb; IncHI2, 245 kb) were seen in isolates from salad (D/ST973) or sprouts (B1/ST527). Three $bla_{\text{CTX-M-15}}$-carrying plasmids (IncFIA-FIB, 170 kb; IncFIB, 125 kb; non-typable, 80 kb) were found in isolates from sprouts (A/410; B1/ST847) or salad (A/ST120). The IncN $bla_{\text{CTX-M-65}}$- (225 kb) and IncHI2 $bla_{\text{CTX-M-125}}$-carrying plasmids (70 kb) were found in isolates from sprouts (A/ST10; A/ST542). Multidrug-resistance (resistance to antimicrobial agents of at least three classes) was seen for 6/7 plasmids. Sulfonamide (*sul1*, *sul2*) and tetracycline [*tet*(A)] resistance were found on four of them. Sequence analysis of two plasmids revealed the ESBL gene in close location to other resistance genes: the plasmid-mediated quinolone resistance gene *qnrS1* and $bla_{\text{TEM-1}}$ (*qnrS1*-IS*2*-Δ*tnpA*-$bla_{\text{CTX-M-15}}$-IS*Ecp1*-Δ*tnpA*-*tnpR*-$bla_{\text{TEM-1}}$) or the fosfomycin resistance gene *fosA3* ($bla_{\text{CTX-M-125}}$-ΔIS*903*-*fosA3*-*orf1*-*orf2)*. All plasmids were conjugative, except the IncFIA-FIB plasmid. The IncN plasmids displayed higher conjugation efficiency ($1.2\text{x}10^{-2}$-$1.2\text{x}10^{-1}$) than the remaining plasmids ($2.9\text{x}10^{-7}$-$1.8\text{x}10^{-6}$).

Conclusion: *E. coli* isolates from vegetables carrying multidrug-resistance ESBL gene-carrying plasmids may increase risks to human health and the co-located resistance genes may contribute to the persistence of ESBL genes.

7th ARAE2017 Poster No. 68:

OXA-23 and OXA-58 carbapenemase-genes in *Acinetobacter indicus* isolates from cattle in Germany

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Background and objectives: *Acinetobacter* (*A.*) *indicus* was described as a novel environmental species in 2012. Two years later, OXA-23 mediated carbapenem-resistance (CPR) was determined in an *A. indicus* isolate from a patient in France. In the same year, we observed two clinical CPR *A. indicus* isolates from calves. Therefore, *A. indicus* was one targeted species in a field study on CPR *Acinetobacter* spp. in cattle in Germany.

Materials and methods: 422 cattle (nasal/rectal swabs (NS, RS) and collective fecal samples (CS)), obtained from 353 Hessian farms in 2015 were screened for *Acinetobacter* spp. Screening for CPR was done with Müller-Hinton Agar with 2 and 4 µg/ml meropenem (MHM). Species were identified by MALDI-TOF MS and 16S rRNA sequence analysis. Main Spectrum Profiles (MSPs) were created with Biotyper 3 software. $Bla_{OXA-23/40/58}$ genes were identified by PCR, phenotypic resistance by using the VITEK2 system. Whole genome sequences were used to determine virulence associated genes (VAGs) and resistance genes (http://www.genomicepidemiology.org/). Virulence was evaluated in the *Galleria* (*G.*) *mellonella* infection model.

Results: 30% of the farms were positive for *A. indicus*. The strains were mostly found in CS (n=76) followed by NS (n=34) and RS (n=23). Among 36 archived isolates 7 carried bla_{OXA-23}-like and 6 bla_{OXA-58}-like genes, respectively. One isolate was resistant to imipenem (MIC=8 µg/ml). Comparative genomics of 16 bovine (11 field, 5 clinical), one human and two environmental strains revealed separated clusters for bovine and non-bovine isolates and a clear grouping of carbapenem-resistant strains. Mean survival rate for 27 *A. indicus* strains tested in the *G. mellonella* model was higher than for *A. baumannii* ATCC17978 (81% vs. 19% 4 d p.inf.). *A. indicus* genomes contained 10 to 14 VAGs in comparison to 82 genes detected in ATCC17978, among them genes involved in motility (*pilT*/*W*) and adherence and apoptosis (*ompA*) (79.7%- 85% identity). Additional resistance genes including *strA*, *strB*, *tet*(X), *tet*(Y), bla_{OXA-51}, and *sul2* were identified among the isolates.

Conclusion: The frequent possession of carbapenemase genes in bovine *A. indicus* isolates indicates a role in the spread of resistance. Initial genomic and phenotypic analysis suggests a rather low virulence of this species. Separate clustering of bovine isolates in the genomic context of human and environmental strains should be verified by including additional strains from other sources.

7th ARAE2017 Poster No. 69:

Crystal structure of the multidrug resistance regulator RamR complexed with bile acids

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Background and objectives: *Salmonella enterica* serovar Typhimurium has at least nine multidrug efflux systems. Among them, the AcrAB-TolC system is particularly effective in generating bile acid resistance. Bile induces the expression of *acrAB*, and this induction is mediated by the transcriptional regulators RamA and RamR. From biochemical and structural studies, we previously found that RamR can recognize multiple drugs including berberine, crystal violet, dequalinium, ethidium bromide and rhodamine 6G. Binding of these compounds to RamR resulted in increased expression of *ramA*. All compounds recognized by RamR are also known substrates of AcrAB; however, these five compounds are not usually present in environments that *Salmonella* inhabit, such as the intestine. Because bile induces *ramA* expression, we hypothesized that RamR recognizes some components of bile acids.

Materials and methods: Purified RamR protein was prepared, then co-crystals of RamR with cholic and chenodeoxycholic acids were grown from hanging drops at 25°C using the vapour diffusion method.Crystals were picked with LithoLoops (Protein Wave) and subjected to flash cooling in cold nitrogen gas stream (100 K) from a cryostat (Oxford Cryosystems). All data sets were collected on beamline BL44XU at SPring-8 with a CCD detector MX225-HE (Rayonix). All data sets were collected at a cryogenic temperature of 100 K.

Results: Here, we describe the crystal structures of RamR in complexes with bile components, including cholic acid and chenodeoxycholic acid, determined at resolutions of 2.0 and 1.8 Å, respectively. Both cholic and chenodeoxycholic acids form four hydrogen bonds with Tyr59, Thr85, Ser137 and Asp152 of RamR instead of π–π interactions with Phe155, a residue important for the recognition of multiple drugs including berberine, crystal violet, dequalinium, ethidium bromide and rhodamine 6G. Binding of these compounds to RamR reduced its DNA-binding affinity, resulting in the increased transcription of *ramA* and *acrAB-tolC*.

Conclusion: We have extended our knowledge of recognition of bile acids by RamR, a regulator of multidrug resistance in several enterobacterial pathogens. Both cholic and chenodeoxycholic acids form four hydrogen bounds with RamR instead of the π–π interaction that is important for recognition of other drugs. These different recognition mechanisms highlight the wide substrate specificity of RamR, whereby the substrate-binding pocket accommodates diverse ligands.

7th ARAE2017 Poster No. 70:

Antibiotic resistance profile of bacteria isolated from mobile phones in Yaba College of Technology, Lagos, Nigeria

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Background and objectives: The constant handling and heat generated by mobile phones increase the incidence of bacteria on them. The public health significance is thus, the risk of transmission of antibiotic resistant bacteria within communities. The aim of the study was to determine the antibiotic resistance pattern of bacteria isolated from mobile phones from lecturers, students, food handlers and shop owners in Yaba College of Technology.

Materials and methods: 80 mobile phones were swabbed and the swabs cultured for bacterial isolation and identification using standard conventional microbiological procedures. Multiple isolates per sample were tested for susceptibility to antibiotics including amoxicillin, ofloxacin, gentamicin, streptomycin, ceftriaxone, pefloxacin, ciprofloxacin, cefuroxime, augumentin, streptomycin, sulfamethoxazole-trimethoprim, erythromycin, ampliclox and chloramphenicol using the Kirby Bauer disk diffusion method.

Results: Two hundred and seventy-five bacterial isolates including *Staphylococcus aureus* 94 (34.2%), *Pseudomonas aeruginosa* 89 (32.4%), *Klebsiella pneumoniae* 43 (15.6%), *Staphylococcus epidermidis* 40 (14.4%), *Staphylococcus saprophyticus* 5 (1.8%) and *Escherichia coli* 4 (1.5%) were obtained from the mobile phones. One hundred and nineteen (43.3%) of the isolates were resistant to all the antibiotics tested. Overall antibiogram showed high resistance of Gram positive bacteria to ceftriaxone 113 (81.3%), amoxicillin 112 (80.6%) and perfloxacin 111 (79.9%) while Gram negative bacteria demonstrated high resistance to sulfamethoxazole-trimethoprim 128 (94.1%), streptomycin 121 (89%) and gentamicin 116 (85.3%).

Conclusion: The results suggest that mobile phones can serve as vehicles of transmission of antibiotic resistant bacteria within communities. Good personal hygiene, strict adherence to infection control such as hand washing and safe antibiotic use should be emphasized.

7th ARAE2017 Poster No. 71:

Antimicrobial usage and risk of extended-spectrum β-lactamase producing *Escherichia coli* in animal-rearing households of selected rural and peri-urban communities, Nigeria

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Background and objectives: Antimicrobial usage promotes the emergence of antimicrobial resistance. Poor environmental sanitation, unhygienic household practices and close interaction between humans and animals facilitate the dissemination and zoonotic transmission of resistant bacteria. This study investigated antimicrobial usage, hygiene practices and occurrence of extended-spectrum β-lactamase producing *Escherichia coli* in animal-keeping households of rural and peri-urban communities.

Materials and methods: In-depth interviews, focus group discussions and observational studies were employed to assess knowledge, practices and attitude regarding household antimicrobial usage and hygiene in 320 households from 16 communities. Four hundred and fifty-seven biological samples were examined for the detection of ESBL-producing *E. coli*. Extended spectrum β-lactamase producing *E. coli* strains were isolated on MacConkey agar supplemented with ampicillin (100 mg/L; Amp_{100}) and/or cefotaxime supplements (1 mg/L; CTX_1) and confirmed phenotypically using the cefpodoxime/cefpodoxime-clavulanic acid combination discs test.

Results: Many households used antimicrobials in humans (69.4%) and animals (60.6%) without prescription. Antimicrobials used in humans included ampicillin (38.4%), ampicillin/cloxacillin (37.5%), tetracycline (28.1%), penicillin (26.9%), amoxicillin (26.6%), trimethoprim/sulphamethoxazole (22.2%), chloramphenicol (10.9%) and ciprofloxacin (9.1%) while long acting oxytetracycline (10.0%), penicillin/streptomycin (7.8%), tetracycline (8.4%), ampicillin (6.9%) and ampicillin/cloxacillin (5.3%) were used in animals. Animals were reared predominantly (60.2% - 100%) under the extensive system with unrestricted access to human space, cooking utensils and foods. Waste disposal methods and toilet facilities were often inadequate. Phenotypic ESBL-producing *E. coli* were detected in the faeces of goats (n=20), dogs (n=15), sheep (n=4), chickens (n=3), ducks (n=2), human (n=1) and turkey (n=1) as well as from environmental (n=6) and food (n=1) sources within and around human dwellings.

Conclusion: Non-prescription antimicrobial usage was common in both humans and animals. Poor regulation of antimicrobial marketing and inadequate access to veterinary care contributed to non-prescriptional use of antimicrobials in humans and animals. Household animals served as vehicles for the dispersal of ESBL-producing *E. coli* in the environment.

7th ARAE2017 Poster No. 72:

Mastitis and antimicrobial resistance in Austrian dairy cows.

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Background and objectives: Bovine mastitis is a worldwide problem which leads to significant economic loss and high use of antibiotics in dairy industries. Precise identification and demonstration of resistance to antibiotics of mastitis isolates are important steps for prevention and treatment of this infectious disease. In this study, we focussed on antibiotic resistance of major bovine mastitis pathogens.

Materials and methods: 1016 mastitis isolates from quarter milk samples of Austrian dairy cows were collected over one year in five different Austrian veterinarian laboratories. Species-specific polymerase chain reaction methods were used for *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli*. Amplification and sequencing of the 16S *rRNA* gene was done for all other species. Antibiotic resistance to eight antibiotic agents was determined for all strains of the genus *Staphylococcus* using disk diffusion method according to EUCAST 2015/2016. Additionally, genotyping based on the *spa*-gene sequence was done for all *S. aureus* strains.

Results: Half of isolates (50 %) belonged to the genus *Staphylococcus* with 26 % coagulase-negative staphylococci (CNS) and 24 % *Staphylococcus aureus* strains, followed by *Streptococcus uberis* (19 %) and Enterobacteriaceae (14 %). Other bacterial species or yeasts (0.7 %) were rare. Antibiotic resistance of coagulase-negative staphylococci was mainly found against tetracycline, clindamycin and erythromycin (9 % - 7 % - 4 % resistant isolates), whereas *S. aureus* had highest resistance rate against benzylpenicillin (14 % resistant isolates). There was no resistance against fluoroquinolone norfloxacin and trimethoprim-sulfamethoxazole. Multi-resistance including resistance against three or more antibiotic classes was present in 3 % of CNS and 4 % of *S. aureus* strains.

Conclusion: *Staphylococcus* was found to be the major genus in mastitis isolates from Austrian dairy cows. Whereas antibiotic resistance was mainly detected against benzylpenicillin, tetracycline and clindamycin, susceptibility to other antibiotic agents was high. Also, the occurrence of multi-resistance was low, which underlines a still existing high antibiotic susceptibility of bovine staphylococci isolates. Therefore, a prudent use of antibiotics should be pointed out clearly to save the high antibiotic susceptibility in Austrian dairy cows and to prevent the spreading of antimicrobial resistance.

7th ARAE2017 Poster No. 73:

Antimicrobial resistance: a sheep mastitis treatment problem or a sheep in wolf's clothing?

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Background and objectives: Intramammary infections (IMI) are a serious problem for sheep farms. To inform correct use of antimicrobials for mastitis treatment, AMR profiles of mastitis pathogens must be determined. Because many mastitis pathogens are commensals of other body sites, e.g. nares, oropharynx or gut, such profiling also gives an indication of the prevalence of AMR in sheep-associated bacteria.

Materials and methods: A preliminary AMR study was performed on sheep mastitis pathogens from a cross-sectional study on three farms in Scotland and a longitudinal study of a single sheep flock in Norway in 2016. Using standard culture methods for milk samples, isolates were obtained from subclinical and clinical infections and from udder abscesses. The susceptibility of isolates to different antimicrobial agents, many of which are commonly used in mastitis therapy, was tested by disk diffusion method. As an alternative to clinical breakpoints, AMR can be described using epidemiological cut-off values (ECOFF). ECOFFs are based on the distribution curve for inhibition zone diameters and represent the **lower** limit of the curve for the majority of the population. Isolates with values **below** this breakpoint are considered resistant even if this value is **above** the clinical breakpoint, which predicts clinical success.

Results: The most common species were *Staphylococcus aureus* and Coagulase-Negative Staphylococci (CNS), which are considered major and minor pathogens, respectively. Other major pathogens included Escherichia coli, *Mannheimia* spp., *Streptococcus dysgalactiae* and *Streptococcus uberis*. AMR was low, particularly among staphylococci. Using clinical breakpoints, only 4% of 55 *S. aureus* isolates and 14% of 35 CNS were considered resistant to penicillin, which is very low compared to levels reported from bovine mastitis or humans. In our data, EUCAST-recommended ECOFF values often bisected normal distributions of observed inhibition zone diameters, e.g. for cephalosporin or carbapenem resistance in *E. coli*, which could lead to erroneous reporting of high resistance levels.

Conclusion: Our preliminary results suggest that AMR will not be the main reason for treatment failure of IMI in sheep. Moreover, they indicate that the industry should establish and seek recognition for bespoke cut-off values for AMR monitoring in bacterial isolates derived from sheep to avoid inappropriate use of cut-offs from human medicine. The latter could lead to misclassification and high apparent resistance rates, suggesting an AMR problem that may not actually exist.

7th ARAE2017 Poster No. 74:

Diffusion of antimicrobial resistance across management niches on dairy farms

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Background and objectives: A major challenge to develop strategies that mitigate the global impact of farm origin antibiotic resistance is our failure to describe dissemination patterns of resistance within the farm via animals or environmental flow. This study focuses on the presence and diversity of phenotypic antibiotic resistance within niches on the whole dairy enterprise. The goals are: identify niches where diversity is generated and maintained, identify niches connected by phenotype similarity suggesting dissemination, and identify niches that narrow diversity. The premise is: diversity is generated in niches with high antibiotic use and disseminate through the dairy system with animal movement or environmental flow.

Materials and methods: We sampled from commercial dairy herds that maintain a milking herd and rear replacement animals. Farm niches are defined by housing and function. Housing niches were areas with pre-weaned heifers, weaned and bred heifers, early lactation cows, lactating cows, pregnant non-lactating cows, antibiotic treated cows, and cows leaving the herd. Function niches included milk, waste, water, and feeds. For each sampling, 72 animal fecal samples, 8 water, 8 feed samples, 1 milk filter, samples from the waste stream, and soil samples were collected. We used *Escherichia coli (EC)* as our model bacterium and antimicrobial susceptibility tested 4 isolates from each sample against 13 antimicrobials. Latent class analysis (LCA) was used to organize isolate-based resistance patterns.

Results: Twelve farms were enrolled and each visited 3 times. More than 13,000 EC isolates were tested for antimicrobial susceptibility patterns. Based on LCA, 24 different susceptible patterns were observed. The most common pattern was pan-susceptible (68%) followed by a pattern with tetracycline resistance only (7%). The greatest resistance diversity was observed in pre-weaned and weaned calves followed by treated animals. EC from adult cows were pan-susceptible (approaching 90% of isolates). EC from soil samples were pan-susceptible except for samples in pre-weaned housing areas. There were farm-specific patterns and between farm differences in diversity.

Conclusion: There are niche specific patterns of resistance that suggest there is little to no dissemination across niches. The between-farm patterns also suggest that resistance traits are farm-specific and driven more by local features rather than shared across spatially distinct farms.

7th ARAE2017 Poster No. 75:

Prevalence and distribution of ESBL and AmpC β-lactamases producing *Escherichia coli* in food-producing animals and meat in Latvia

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Background and objectives: The aim of the present study was to determine the prevalence and distribution of extended-spectrum-βeta lactamases (ESBL) and AmpC β-lactamases producing *Escherichia coli* in food-producing animals (calves and pigs) as well as in beef and pork.

Materials and methods: A total 630 samples, including caecal contents of pigs (n=150), rectal swabs of calves (n=180) and raw meats (n=300) were investigated in 2015. Samples were collected as a part of national official control program and within VPP AgroBiores project. Samples for isolation of ESBL and AmpC producing *Escherichia coli* were investigated in accordance to DTU National Food Institute EURL-AR laboratory protocols (DTU, 2014). The classifications of phenotype of β-lactamases producing *Escherichia coli* are based on EFSA recommendations (EFSA, 2012). In total, 112 strains of ESBL un AmpC β-lactamases producing *Escherichia coli* were obtained during this period. Multiplex PCR was used to detect the family type of β-lactamases. In addition, nine PCR negative isolates were screened for the presence of specific family types of plasmid-mediated AmpC (pAmpC) β-lactamases.

Results and conclusion: The highest prevalence of β-lactamases producing *Escherichia coli* (48.7%) was identified in pigs. The prevalence of β-lactamases producing *Escherichia coli* in calves was 11.1 % (20/180), in pork meat 8.6 % (13/150) and beef meat 8.0 % (12/150), respectively. The ESBL was the most common *Escherichia coli* phenotype in food producing animals (calves, pigs), pork and beef in Latvia. Predominant family types of β-lactamases were TEM, CTX M I and CMY II.

7th ARAE2017 Poster No. 76:

Characterization of antimicrobial resistant *Escherichia coli* from wild reindeers in Norway and Svalbard

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Background and objectives: Resistance against antimicrobials is an expanding problem, and it is clear that it is spreading and emerging globally. Antimicrobial resistance (AMR) is usually studied in bacteria from humans and domestic animals, but for further insight into the dissemination and occurrence of AMR, wild animals could be a good source of information. In Norway, wild reindeer (*Rangifer tarandus tarandus*) live in National Parks and areas that are only occasionally visited by humans. On Svalbard, the reindeers (*Rangifer tarandus platyrhynchus*) live in remote environments with almost no contact with human infrastructure. This makes Norwegian and Svalbard reindeer a good source for studying AMR and AMR dissemination in the wilderness. Whole Genome Sequencing (WGS) is an important tool for in-depth characterization of resistant isolates that can give indication on the origin of the resistance determinants. The aim of this study was to characterize antibiotic resistant *Escherichia coli* from wild reindeer in Norway and Svalbard.

Material and Methods: From previous field studies 27 resistant *E. coli* were available. The isolates originated from 27 different healthy, wild reindeers in three national parks (including Svalbard) and one wild reindeer area. The bacteria were isolated by selective culturing and/or by susceptibility testing of randomly chosen *E. coli*. Disc diffusion and minimum inhibitory concentration determination were conducted to assess phenotypic resistance. WGS was used to obtain sequence data for in silico typing and analysis on genetic relatedness. Transferability of resistance plasmids were investigated by conjugation.

Results: Fifteen isolates were resistant to more than one antimicrobial agent. Resistance against streptomycin (n=19) was most common, followed by tetracycline (n=12), ampicillin (n=12) and sulfamethoxazole (n=11). The strains were genetically diverse. Some strains had several resistance genes closely arranged in their genome and some had resistance genes arranged in conjunction with replicons that indicated plasmid location. Resistance regions with high homology to plasmid regions previously described in bacteria from humans and/or animals were identified.

Conclusion: This study demonstrated the presence of AMR *E. coli* in wild reindeer in Norway and Svalbard. Furthermore, resistance regions discovered in the isolates have previously been identified in isolates obtained from human, bear and bird. This supports the fact that resistance genes are widespread and a global problem.

7th ARAE2017 Poster No. 77:

High prevalence of cephalosporin-resistant commensal *E. coli* in calves in Latvia

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Background and objectives: Application of antibiotics in animal husbandry may lead to the development of antimicrobial resistance in commensal and pathogenic microflora. Commensal *E. coli* may serve as an indicator of resistance and reservoir for resistance genes for pathogenic microflora, therefore the monitoring of antibiotic resistance in commensal *E. coli* isolates is needed. The aim of the present study was to detect the antimicrobial resistance in commensal *E. coli* isolates from calves in Latvia.

Materials and methods: Altogether, 110 commensal *E. coli* isolates from calves from 18 farms in Latvia were tested for antimicrobial susceptibility according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) requirements. All the calves were sampled at the farm aged up to 1 year and were without clinical signs of disease. The information on veterinary care and antibiotic usage pattern in the farm were collected.

Results: Resistance at least to one antibiotic was found in 74 out of 110 isolates were tested (74%). Multiresistance was found in 45% of isolates with the maximum number of resistances up to eight different classes of antibiotics. Resistance to sulfamethoxazole (52%), ampicillin (51%) and tetracycline (48%) was the most frequently observed. Resistance to cephalosporins (cefotaxime, ceftazidime) was identified in 22% of isolates. All *E. coli* isolates were susceptible to meropenem, azithromycin, tigecycline and colistin. Calves with *E. coli* cephalosporin-resistant isolates were housed at farms, which have a history of the application of cephalosporins for treatment of cows.

Conclusion: Pattern of the antimicrobial resistance of commensal E. coli isolates was comparable with other studies in Europe. Despite, the resistance to cephalosporins in commensal *E. coli* isolates in calves in Latvia was higher than in the previously reported. Application of cephalosporins for cow treatment at the farm could contribute the development of resistance to cephalosporins in commensal *E. coli* isolates.

7th ARAE2017 Poster No. 78:

WGS and plasmidome-analysis of related broiler and human cephalosporin-resistant *Escherichia coli* isolates to study possible transmission events

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Background and objectives: ESBL/pAmpC-producing *E. coli* (ESBL-E) are frequently found in livestock in the Netherlands, especially in broilers. Previous research, performed with classical methods that have limited resolution, suggested transmission between broilers and humans at multiple farms. Here, ESBL-E from broilers and persons working and/or living at broiler farms were studied by using Whole Genome Sequencing (WGS) in order to investigate transmission events with higher resolution.

Materials and methods: Twenty ESBL-E isolates were selected from two previous studies. The isolates originated from eight farms and included nine isolates coming from broilers, four from farmers, and seven from family members that lived on the broiler farm who had no/limited contact with the animals. The selection included isolates that were expected to be 'transmission pairs', based on identical MLST, ESBL/pAmpC gene and/or plasmid replicon type. DNA was isolated and sequenced using Hiseq (Illumina) and PacBio sequencing.

A core genome MLST (cgMLST) based on 3059 genes was performed for genome comparison, using SeqSphere 3.1.0. (Ridom GmbH). ESBL/AmpC containing plasmids were compared using the Basic Local Alignment Search Tool (BLAST) by performing a bidirectional blast analysis.

Results: The cgMLST showed that five ESBL-E pairs had a maximum allelic difference of six. The distance between a pair of isolates and unrelated isolates ranged from 89 to 2130 alleles. One of the five pairs was from a broiler and a farmer, two pairs were from a broiler and a family member, and two pairs were from a farmer and a family member. The other pairs did not cluster with each other and the minimum distance between them was more than 250 alleles.

The plasmid comparison of two non-clustering pairs did show low variance. One pair originated from a broiler and its farmer and another from a broiler and a family member. The plasmid variance of the other pairs was similar to variance between unrelated isolates.

Conclusion: The classical methods suggested clonal transmission in seven ESBL-E pairs and three horizontal transmission events. The WGS approach removed false positives, confirming clonal and horizontal transmission for five and two ESBL-E pairs, respectively. Moreover, WGS gives valuable information regarding mutation events, which will contribute to the understanding of ESBL-E transmission.

7th ARAE2017 Poster No. 79:

Antimicrobial susceptibility of *Avibacterium paragallinarum* isolates from outbreaks of infectious coryza in Dutch commercial poultry

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Background and objectives: *Avibacterium paragallinarum* (AVP) is the causative agent of infectious coryza, a disease of the upper respiratory tract in chickens. The disease occurs worldwide and causes economic losses due to an increased numbers of culls, a marked egg production loss and reduced hatch. The disease has no zoonotic relevance. Since 2008, there is an increase in the number of outbreaks of infectious coryza in commercial poultry in the Netherlands. To allow more prudent use of antimicrobial drugs, information on the susceptibility profile of AVP is important. As publications on the antimicrobial susceptibility of this pathogen are scarce, a study was performed to examine the antimicrobial susceptibility of AVP isolates obtained from infectious coryza outbreaks in Dutch commercial poultry.

Materials and methods: The collection comprised isolates from chickens with clinical symptoms of coryza, submitted to GD Animal Health for post-mortem examination between 2008 and 2017. Isolates were cultured from affected sinuses and confirmed as AVP by PCR. Minimal inhibitory concentrations (MICs) were assessed by broth microdilution using commercially available MIC plates. The broth used was CAMHB plus NADH and sterile filtered heat-inactivated chicken serum, a new medium, recently described for MIC testing of *Haemophilus parasuis*. For each antimicrobial agent, the range of MIC results, the MIC50, and MIC90 values were calculated.

Results: Preliminary results obtained for antimicrobial agents approved for the treatment of infectious coryza in the Netherlands (or belonging to the same chemical class) show lowest MIC values for trim/sulfa (MIC50 and MIC90 of 0.0625/1.1875 and 0.5/9.5 µg/ml, respectively), followed by ampicillin (0.125 and 0.25 µg/ml), penicillin (0.25 and 1 µg/ml) and erythromycin (1 and 2 µg/ml), with highest MIC values for tetracycline (16 and >16 µg/ml). Applying the criteria of Blackall et al. (1989) to define isolates as susceptible, intermediate susceptible, and resistant results in 100% of the isolates being classified as susceptible for ampicillin, 96% for both penicillin and erythromycin, and 36% for tetracycline.

Conclusion: Preliminary results of this first study on antimicrobial susceptibility of AVP isolates obtained from outbreaks of infectious coryza in Dutch commercial poultry show relatively good susceptibility to antimicrobial agents that are recommended for the treatment of infectious coryza in the Netherlands, except for tetracycline.

7th ARAE2017 Poster No. 80:

Distribution of the *pco* operon among swine *Escherichia coli* from a controlled feeding trial

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Background and objectives: As one component of a study on alternatives to antibiotics, copper, zinc, and oregano oil (with negative controls and low- and high-dose tetracycline positive controls) were administered in feed to 400 pigs over a 49-day period. *Escherichia coli* were isolated from fecal samples on Days 0 and 28. The *pco* operon, a copper transport system, has been previously shown to increase tolerance to copper by active efflux.

Materials and methods: The prevalence of the operon was determined by PCR among 403 *E. coli* isolates, as well as the extended spectrum beta-lactamase genes bla_{CMY} and bla_{CTX-M}, and tetracycline resistance genes *tet*(A) and *tet*(B).

Results and conclusion: Thirty-four isolates (8%) were *pco*-positive, while 15% carried bla_{CMY} and 3% carried bla_{CTX-M}. Isolates with and without the *pco* operon had virtually identical susceptibility to copper in the form of copper(II) sulfate, with MICs around 20mM; these MICs were unaffected by induction with lower concentrations of copper (1mM and 5mM). The expression of the *pco* operon was compared between several isolates, induced and uninduced, using reverse transcription of total RNA, and no significant differences were observed. There was no statistically significant association between the presence of the *pco* operon and any other treatment factor, including addition of 125 ppm copper in feed, though *pco* was negatively associated with the presence of bla_{CMY}. Targeted sequencing revealed the operon to be closely associated with a Tn*7*-like transposon, and was flanked by both chromosomal and plasmid-like DNA in different isolates. The *pco* operon also appears to be randomly distributed among various multi-locus sequence types, independently of genetic relationships. Whole genome analysis using next-generation sequencing of a subset of isolates was performed to further characterize the local genetic environment of the operon. In the absence of an associated copper resistance phenotype and of any selective effect of copper in feed, the significance of *pco* for co-selection of other antimicrobial resistance factors may be questionable. However, the association with a transposable element, as well as documented co-location with other antimicrobial resistance genes warrants further study.

7th ARAE2017 Poster No. 81:

First report of *bla*$_{OXA-58}$ positive *Acinetobacter pittii* isolates from pet animals

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Background and objectives: Non-baumannii *Acinetobacter* strains such as *A. pittii* are increasingly reported in human clinical samples. In addition, carbapenem resistance is increasing among these species, and *A. pittii* producing OXA-58-like carbapenemases have emerged worldwide. We describe the first detection of *bla*$_{OXA-58}$-positive *A. pittii* isolates from animals.

Materials and methods: Five *A. pittii* isolates were recovered in the context of a routine in-house screening, where clinical *Acinetobacter* spp. isolates are tested for carbapenem non-susceptibility by using Mueller-Hinton agar with 2 and 4 mg/L meropenem. The presence of *bla*$_{OXA-23/OXA-40/OXA-58}$ genes was tested by PCR. Antimicrobial susceptibility was determined with the VITEK2 system. Whole genome sequences were analysed using services provided at the Center of Genomic Epidemiology to identify resistance genes and multi locus sequence types (STs). The isolates were further tested by PFGE and comparative genome analysis. PCR-based plasmid mapping was performed based on a ca. 53 kb contig spanning the entire OXA-58 plasmid available from one the isolates.

Results: Sequence analysis confirmed the presence of *bla*$_{OXA-58}$ in the five *A. pittii* isolates, which were all carbapenem-susceptible. Isolates were obtained from the nose, skin and bronchi of five different animals that had been treated in two veterinary clinics (clinic A: two dogs; clinic B: two cats and one rabbit) at different time points between 2014 and 2016. All isolates were assigned to ST93, a sequence type that has been previously observed among two human *A. pittii* isolates carrying OXA-23- and Gim-1-carbapenemases, respectively, from Germany. Based on the alignment of the maximum common genome the animal isolates clustered according to their origin from clinic A and B. The *bla*$_{OXA-58}$ genes were located on identical 53.8 kb plasmids with only partial similarity to published plasmid sequences. Resistance genes s*trA*, *strB*, *aacC2*, *sul2*, and *tet39* were co-localized on the OXA-58 plasmid. The genetic context of *bla*$_{OXA-58}$, i.e. IS*Aba3*-like element upstream and IS*Aba3*, *araC1* and *lysE* downstream of the carbapenemase gene has recently been described for OXA-58 plasmids of various *Acinetobacter* spp., including *A. baumannii*.

Conclusion: The presence of *bla*$_{OXA-58}$ genes in carbapenem-susceptible *A. pittii* isolates highlights the threat of hidden reservoirs of carbapenemase-encoding genes, since laboratory detection usually targets phenotypic resistance.

7th ARAE2017 Poster No. 82:

Detection of *mcr-1* using enrichment media and real-time PCR for chicken cecal and porcine fecal samples from Ontario, Canada

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Background and objectives: The newly described *mcr-1* plasmid-mediated colistin resistance gene has been found in more than 30 countries around the world, and from numerous animal and human sources. Although other forms of colistin resistance have been well documented, this is the first transmissible determinant to be characterized. Its potential spread throughout the world into various bacterial populations is of great concern to public health, as colistin is one of the last resort antimicrobials for some infections. The objective of this study was to develop a method for screening animal fecal samples for the *mcr-1* gene, using broth enrichment and real-time PCR detection. This highly sensitive but non-quantitative approach would allow us to detect the presence, if any, of *mcr-1* in samples using a high throughput method.

Materials and methods: Thirty swine fecal samples and 242 chicken cecal samples were collected from farms in Ontario between 2015 and 2017. Chicken samples were collected at slaughter through the Canadian Integrated Program for Antimicrobial Resistance Surveillance and pooled in sets of five to minimize workload. Additional porcine samples are continuing to be collected. Initial testing was performed with known concentrations of *mcr-1*-positive *Escherichia coli* added to fecal samples to determine the effect of possible inhibitors, and to determine the minimum detection limit of the real-time PCR assay. Approximately 1g of sample in 9mL of EC broth (supplemented with 1µg/mL colistin) was incubated overnight with shaking. A boiled lysate of each resulting culture was used as template for the PCR.

Results: The assay's detection limit after a 16 hour incubation of the enrichment culture was determined to be between 10-15 CFU per sample when using 1uL of lysate. None of the initial samples appeared to have an inhibitory effect on the assay. All swine and chicken samples were PCR-negative for *mcr-1*.

Conclusion: Although no *mcr-1*-positive samples were found in this collection, this very sensitive method could be used for monitoring this highly important resistance gene in the future.

7th ARAE2017 Poster No. 83:

Monitoring of Antimicrobial Susceptibility of Poultry Pathogens in The Netherlands, 2014-2016

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Background and objectives: GD Animal Health, a leading organization in animal health in the Netherlands, monitors antimicrobial susceptibility (AMS) of pathogens originating from different animal species. Isolates were obtained from diagnostic submissions and actively sent in by field veterinarians for the purpose of monitoring.

Materials and methods: The objective of the present study was to analyze the in vitro antimicrobial susceptibility of *Escherichia coli*, *Enterococcus* species and *Staphylococcus aureus* isolates from meat-type chickens, in the period from October 2014 to December 2016. Minimal inhibitory concentrations of 18 and 12 antimicrobial agents for *E. coli* and both types of cocci, respectively, were assessed, and MIC_{50} and MIC_{90} values were determined. MICs were tested using the broth microdilution method with commercially manufactured plates. Clinical & Laboratory Standards Institute (CLSI) veterinary breakpoints (when available) were used to indicate whether isolates were susceptible, intermediate or resistant.

Results: Results are provided for *E. coli* (n=350 and n=488), *Enterococcus cecorum* (n=106 and n=95) and *Enterococcus* faecalis (only 2016, n=57), and *S. aureus* (n=46 and n=75) isolates from 2015 and 2016 respectively. Susceptibility of *E. coli* isolates from 2015 for tetracycline and trimethoprim-sulfamethoxazole was 65.8 and 68.5%, respectively. Five percent of the *E. coli* isolates were resistant to cefotaxime. The *E. cecorum* isolates from 2015 were all found to be susceptible for ampicillin and amoxicillin-clavulanic acid. Ninety percent of the *E. cecorum* isolates from 2015 were found to be susceptible for clindamycin. Different susceptibility patterns were obtained for *E. cecorum* and other *Enterococcus* species. Susceptibility of *S. aureus* isolates from 2015 for tetracycline, ampicillin, amoxicillin-clavulanic acid and the combination of trimethoprim-sulfamethoxazole was 91.3%, 100% of the last three. Results of 2016 and MIC_{50} and MIC_{90} data will be presented.

Conclusion: In some drug/pathogen combinations interpretation is hampered by the lack of CLSI-defined clinical veterinary (and specifically poultry) breakpoints. Consequently, correct interpretation of clinical relevant drug/pathogen combinations is not always possible. In order to overcome these difficulties more veterinary breakpoints are urgently needed.

7th ARAE2017 Poster No. 84:

Characteristics profiles of cefotaxime-resistant *E. coli* from German livestock farms and potential association with farm factors

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Background and objectives: Resistance to third-generation cephalosporins and other beta-lactam antibiotics is of major concern for animal and human health. To better understand the epidemiology of ESBL/AmpC-producing *E. coli* we investigated the association of management factors of livestock farms in Germany with the characteristics of cefotaxime-resistant *E. coli* strains from these farms.

Materials and methods: In a cross-sectional investigation on the prevalence of cefotaxime-resistant *E. coli* in 2010-2011 samples from 194 livestock farms in Germany were collected. During farm visits, data on farm management were recorded by questionnaires developed for each production type.

Cefotaxime-resistant *E. coli* were isolated from samples of 150 farms, and characterised further. These farms comprise 34 broiler farms, 38 fattening pig farms and 78 cattle farms. For 469 isolate,s the ESBL-genes and the phylogroup were determined. Additionally, the phenotypic antimicrobial resistance was tested. This information was used to define different profiles characterising the isolates. Multivariate analyses using the distance-based permutation test were performed to investigate dependencies between characteristics profiles and conditions observed in the farms (e.g. farm size, hygiene factors or antimicrobial use).

Results and conclusion: First results show, that the characteristics profiles of isolates from broiler farms differ substantially from the characteristics profiles of isolates from fattening pig and cattle farms. Results on characteristics profiles and association analyses will be presented.

7th ARAE2017 Poster No. 85:

Detection of OXA-181-carbapenemase, colistin resistance determinant MCR-1 and AmpC β-lactamase CMY-2 genes in an *E. coli* strain from swine

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Background and objectives: Plasmid-mediated resistance in *Enterobacteriaceae* against carbapenems and colistin represents an emerging threat for public health. Although animals have been identified as a relevant source of multidrug-resistant (MDR) bacteria, there are still only few reports about the presence of carbapenemases, and even less regarding the co-existence of carbapenemase and colistin resistance genes in animal isolates. The present study intended to investigate the occurrence and molecular characteristics of carbapenemase genes among fecal *E. coli* isolates from swine.

Materials and methods: Using Mueller-Hinton agar with 0.5 mg/L meropenem (MHM), 7,850 fecal *E. coli* isolates obtained from 2,253 pigs, predominantly from Germany (91.8%) and also from five other European countries, were screened for carbapenem resistance from May 2015 to August 2016. Strains that grew on MHM agar were tested for $bla_{\text{KPC-like}}$, $bla_{\text{NDM-like}}$, $bla_{\text{VIM-like}}$, and $bla_{\text{OXA-48-like}}$ genes by PCR. Antimicrobial susceptibility was determined with the VITEK2 system. Whole genome sequences (WGS) were analysed using services provided at the Center of Genomic Epidemiology to identify resistance genes, plasmid incompatibility groups and multi locus sequence types (STs).

Results: Eleven isolates showed growth on the MHM agar but only two proved positive for a carbapenemase gene, namely $bla_{\text{OXA-48-like}}$, by PCR. These isolates were obtained from different pigs housed at the same farm in Italy and were genetically unrelated (ST359 and ST641). WGS revealed the presence of $bla_{\text{OXA-181}}$ in both isolates and in addition of colistin resistance gene *mcr-1*, AmpC β-lactamase gene $bla_{\text{CMY-2}}$, and 16S rRNA methyltransferase gene *armA* in one of them. OXA-181 was encoded on a 51.5 kbp non-conjugative IncX3 plasmid that co-harbored *qnrS1* and revealed 100% identity to pOXA181_14828 (*E. coli*, human, China). The *mcr-1* plasmid (33.3 kbp, IncX4) was conjugative and almost identical (99.9%) to pESTMCR (*K. pneumoniae*, peritoneal fluid, human, China).

Conclusion: Although the overall prevalence of carbapenemases in porcine *E. coli* seems to be low, our finding of a strain that encodes for MCR-1, OXA-181, CMY-2, QnrS1, and ArmA and harbours human-like plasmids suggests that livestock may become a relevant reservoir of strains conferring resistance to a number of (last-line) antibiotics, including carbapenems, polymyxins, third-generation cephalosporins, fluoroquinolones and aminoglycosides.

7th ARAE2017 Poster No. 86:

Investigation of potential risk factors for the occurrence of *Escherichia coli* isolates from German fattening pig farms harbouring the *mcr-1* colistin resistance gene

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Background and objectives: The ongoing discussion about an increase of untreatable bacterial infections was fuelled by the detection of the plasmid-mediated colistin resistance gene *mcr-1*, reported in November 2015. Soon after this finding, working groups all over the world confirmed the presence of the *mcr-1* gene in their strain collections. However, to date no analysis of factors associated with the occurrence of the *mcr-1* gene in livestock has been published. The analyses described here are based on existing data and samples collected in 2011 and 2012. The obtained bacterial samples as well as the particular farm information were re-analysed in this study with the aim to investigate factors associated with the occurrence of *mcr-1*.

Materials and methods: Within the scope of a cross-sectional investigation on fattening pig farms conducted in 2011 and 2012, 48 fattening farms in different agricultural regions of Germany were investigated. Primary cultures of boot swabs and collective faecal samples were stored at -80°C and currently screened for the presence of the *mcr-1* colistin resistance gene. The laboratory results were linked to farm related data collected via questionnaire. We used logistic regression models to investigate the association between occurrence of *mcr-1* and farm information.

Results and conclusion: In 26 (12.0%) out of 216 mixed bacterial cultures originating from 12 out of 48 farms (25.0%), *Escherichia coli* carrying the *mcr-1* gene have been isolated. Results of the logistic regression analyses indicate that the transmission between pigs or their direct environment is crucial for the occurrence of resistant bacteria. However, we found no statistically significant association between antimicrobial use and the occurrence of the *mcr-1* gene.

7th ARAE2017 Poster No. 87:

Early detection of Polymyxin-resistant Gram-negative bacteria using chromID® Colistin R agar, a new chromogenic medium

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Objectives: To preliminary assess chromID® Colistin R agar performance for the screening and pre-identification of colistin-resistant *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from human and veterinary specimens.

Methods: The first part of the study was performed using 46 colistin-resistant strains and 20 colistin-sensitive strains from several species, and with various colistin MICs and resistance mechanisms. They were inoculated onto chromID Colistin R from a standardized inoculum (10^4 or 10^6 CFU per plate). In a second part, study was performed with 30 stool samples and 31 veterinary samples. Stool specimens were analyzed either directly or after being contrived (n=20) with a colistin-resistant strain such as *E. coli, Salmonella, Klebsiella* or *Pseudomonas* species to get around 10^4 CFU per specimen. All the assays with human stools and veterinary samples were firstly introduced into a Brain Heart Infusion broth (BHI) containing 2 colistin disks (2x10 µg) added extemporaneously. After a 4-5 hours enrichment at 35°C, 10 µl of the broth were inoculated onto chromID Colistin R agar. All plates were incubated under ambient atmosphere at 35°C and were read after 18-24 hours.

Results: Among the 46 colistin-resistant strains, 44 of them (95%) were able to grow onto chromID Colistin R agar with the expected color and all the 20 strains susceptible to colistin were correctly inhibited (specificity, 100%).Human stools and caecal samples from swine and chicken were also tested. A preliminary study had shown that an enrichment step in a selective broth prior to inoculation onto chromID Colistin R agar is required to prevent false positive results. By applying this protocol, which includes a 4-5 hours enrichment step, chromID Colistin R agar was able to recover all the 20 stools artificially contrived with colistin-resistant strains. For natural human stools and chicken samples, no growth was observed on chromID Colistin R agar inoculated from the BHI broth. In contrast, 20 different colonies were recovered from 14 out of 16 swine samples. Among these, three different colors were observed: colorless (13), blue-green (4), and red (3). Colorless colonies were identified as *Aeromonas hydrophila*, blue-green colonies as *Serratia liquefaciens* and red as *E. coli*. The two first species are naturally resistant to colistin. *E. coli* isolates recovered in three samples were confirmed sensitive to colistin by BMD. Taking account all the results with natural specimens, specificity for chromID Colistin R agar is 92% (38/41).

Conclusion: The present study shows that chromID Colistin R agar, a new chromogenic medium currently in development, when used in conjunction with a short selective enrichment protocol in a BHI broth, is a promising solution for easy and sensitive recovery of Gram-negative isolates having an acquired resistance to colistin. Main advantages are the high selectivity and specificity combined to the possibility to get a pre-identification, at the species or group level, thanks to different colors of the colonies. Use of a chromo-genic medium, compared to a conventional one, could improve reading in case of mixed cultures, and also could make possible distinction of species known to carry transmissible *mcr* genes (*E. coli* and *Salmonella*).

7th ARAE2017 Poster No. 88:

Insights in the genetic diversity of *E. coli* from livestock and food harboring *mcr*-1

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Background and objectives: In 2015 the plasmid-encoded colistin resistance gene *mcr*-1 was detected in China. Subsequently studies revealed worldwide distribution of this gene in different Enterobacteriaceae-genera from the environment, food, livestock, and humans (infected patients and asymptomatic carriers). As colistin is considered a last choice antimicrobial in human medicine, spreading of the *mcr*-1 mediated resistance might become a problem for therapeutic issues. Here we provide an overview on the genomic composition of different *mcr*-1 harboring *E. coli* from livestock and food samples, collected in Germany (2010- 2015).

Materials and methods: Isolates analyzed in this study originated from commensal *E. coli* isolated from animals and food samples of animal origin in the course of the German national zoonoses monitoring. Out of 10,600 investigated *E. coli*, 505 isolates from poultry, pigs and cattle food chains had a MIC for colistin of >2 mg/l. In 78% of these isolates the *mcr*-1 gene was detected using real-time PCR. Whole genome sequencing on 24 isolates, reflecting the different sources considered, was done on an Illumina MiSeq benchtop sequencer.

Results: Bioinformatical analysis revealed a broad spectrum of *E. coli* comprising different MLST-, phylo-, and serotypes. All strains exhibited distinct patterns of virulence, resistance genes and/or mobile genetic elements. Interestingly, only three different *mcr*-1 carrying plasmid variants were identified. The distribution of these variants could not be attributed to a specific time period, source, or serotype. The spread of these plasmids does not seem to be associated with different MLST-, phylo- or serotypes, but is rather driven by the susceptibility of strains for the self-transmissible plasmid and antibiotic selection pressure. The evolutionary events behind the appearance of the different *mcr*-1 encoding plasmid variants need further investigation.

Conclusion: The *mcr*-1 resistance gene is widely distributed among commensal *E. coli* from different sources at least since 2010. However, in the sequenced isolates only three different plasmid variants were found. The likelihood of plasmid transfer might be associated with the susceptibility of the strains for *mcr*-1 encoding plasmids and selection pressure. In future, *mcr*-1 prevalence should be monitored and epidemiological data from other countries are urgently needed to determine the predominant plasmid variants, their evolution and their transferability.

7th ARAE2017 Poster No. 89:

Active screening for *mcr*-1 in faecal samples from livestock in the Netherlands by non-selective enrichment and PCR

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Background and objectives: Previously performed retrospective studies revealed low prevalence of *mcr-1* in a selection of colistin resistant *E. coli* and *Salmonella* isolates from livestock faecal samples and meat from 2010 to 2015. This information was insufficient to assess the risks of this transferable resistance gene from farm animals through food to humans. To get more accurate data on the prevalence and the genetic characteristics of *mcr-1* in livestock faecal samples, active monitoring for the presence of *mcr-1* was performed followed by molecular analysis of collected isolates.

Materials and methods: Faecal samples collected in 2016 during the national monitoring program on antimicrobial resistance (AMR) were included in the study. From each sample 1 gram of faecal material was inoculated in 9ml buffered peptone water (BPW). After overnight incubation at 37^0C BPW cultures were screened for the presence of *mcr-1* with PCR according to the EURL-AR protocol. In case of positive PCR, 10 µl of O/N enrichment was streaked on MacConkey agar with 4 mg/L colistine. From each plate at least five *E. coli* colonies were screened by PCR for the presence of *mcr-1*. Complete DNA sequence of the strains was identified by Next-Generation Sequencing (NGS) and the genetic environment of *mcr-1* and the genetic characteristics of the bacterial strains were determined.

Results: In total, 1800 faecal samples of livestock (600 broilers, 200 layers, 100 ducks, 300 pigs, 300 calves and 300 dairy cows) were screened for the presence of *mcr-1*. We detected *mcr-1*-positive *E. coli* at a low frequency in broilers (n=2), veal calves (n=4), slaughter pigs (n=1) and dairy cows (n=1). The first analysis of NGS data revealed the presence of chromosomally located in *E. coli* with different genotypes (ST34, ST93, ST1429, ST1434 and ST1508) or on IncX4 or IncF plasmids.

Conclusion: Active screening in faecal samples demonstrated the presence of *mcr*-1 at a low prevalence in different livestock species. In all PCR-positive samples *mcr*-1 was identified in *E. coli* on different plasmid types or on the chromosome. Within the AMR monitoring program, *mcr*-1 was not detected in non-selectively collected *E. coli* from the same faecal samples (data not shown). Therefore, the current results demonstrate an increased sensitivity to detect *mcr*-1 in livestock faecal samples by using non-selective enrichment and PCR.

7th ARAE2017 Poster No. 90:

Emergence of plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* isolates from patients and poultry products in Germany

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Background and objectives: In November 2015 a high prevalence of the plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* from livestock and several human cases was reported from China. Thus, intensive screening of strain collections started worldwide to assess the extent of *mcr-1* distribution. In January 2016 laboratories throughout Germany were asked to send colistin-resistant isolates to the Robert Koch Institute (RKI) for *mcr-1* screening. Here we report the characterization of these colistin-resistant isolates.

Materials and methods: The retrospective *mcr-1*-screening by PCR included AmpC- or extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates from poultry products (n=211) and 819 ESBL-*E. coli* isolates from human patients. Moreover, we screened 70 colistin-resistant clinical isolates that were sent to the RKI since January 2016. Antimicrobial susceptibility testing was performed, and presence of various resistance genes were analysed by PCR and sequencing. Bacterial strain typing was performed by enzymatic macrorestriction and subsequent pulsed field gel electrophoresis (PFGE). Resistance gene transfer was tested in conjugation assays.

Results: Colistin resistance and presence of *mcr-1* was confirmed in 16 ESBL/AmpC-producing *E. coli* isolates from poultry products collected in 2011 and 2014. These 16 isolates additionally carried CMY-2, CTX-M-1 or SHV-12 enzymes. PFGE-typing confirmed the presence of ten different *E. coli* clones. Among the 70 colistin-resistant Enterobacteriaceae from 2016/17, we detected *mcr-1* in 15 *E. coli* that were mainly originating from one clinical laboratory. These were isolated from urine (n=11), blood culture (n=2), wound swab and pharyngeal swab. Using PFGE-typing we differentiated 13 *E. coli* clones - not related to the isolates from poultry - with additional resistance to ciprofloxacin and ampicillin due to production of QnrS1 and TEM-1 beta-lactamase, respectively. The majority of *mcr-1*-positive *E. coli* harboured an IncX1 plasmid of 30-35kb size. In contrast, none of the colistin-resistant *Klebsiella pneumoniae* (n=25) produced Mcr-1 but sequence analyses of the intrinsic *mgrB* gene of these isolates showed various mutations resulting in loss of functionality of this gene.

Conclusion: Although our data indicate a low prevalence of *mcr-1* in *E. coli* isolates from humans, intensified surveillance is needed to assess the spread of this resistance gene in the next years.

7th ARAE2017 Poster No. 91:

Occurrence of MCR-1-producing Enterobacteriaceae in pigs, Portugal

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Background and objectives. The *mcr-1* gene encoding a phosphoethanolamine transferase has been recently identified as a source of acquired resistance to polymyxins in *Escherichia coli*. It has been occasionally identified in other species such as *Enterobacter cloacae* and *Klebsiella pneumoniae*. It is now admitted that the *mcr-1* gene has very likely emerged first in animals due to a high selective pressure with colistin in veterinary medicine since the mid 1960's. Our study aimed to prospectively analyze the prevalence and the occurrence of the *mcr-1* gene among Enterobacteriaceae recovered from two pig farms in Portugal.

Materials and Methods. One-hundred fecal samples from two different Portuguese pig farms were screened for polymyxin-resistant Enterobacteriaceae using the Superpolymyxin® selective plates. Isolates were confirmed to be resistant to colistin by using the biochemical Rapid Polymyxin NP test. Susceptibility testing was evaluated by broth microdilution for colistin and by disk diffusion for other antibiotics.

Screening of the *mcr-1* gene and other resistant determinants was performed by PCR amplification followed by sequencing. PCR-based replicon typing (PBRT) was realized with the PBRT kit (Diatheva®). Clonality and phylogeny assays were determined by PFGE analysis, by MLST, and by the Clermont method identifying *E. coli* phylogroups.

Results. Ninety-four isolates (18 *K. pneumoniae* and 76 *E. coli*) being resistant to colistin were recovered. Noteworthy, they all carried the *mcr-1* gene. All the isolates presented an MIC to colistin ranged from 4 to 64 µg/ml. Among the *E. coli mcr-1* positive strains, 7 co-produced an extended-spectrum ß-lactamase. All *K. pneumoniae* isolates belonged to Sequence Type ST45 and all *E. coli* belonged to the B1, A and F phylogenetic groups. Twenty-nine different *E. coli* clones were identified belonging to twenty-six different ST mostly detected as new STs. PBRT showed that the *mcr-1* gene was carried on a diversity of plasmid including IncP, IncHI2, IncX4 and IncY plasmids. Insertion sequence IS*Apl1* was identified upstream and/or downstream of the *mcr-1* gene in IncHI2-carrying *mcr-1* isolates.

Conclusion. This study showed a wide dissemination of MCR-1 in two different pig farms in Portugal, further highlighting the wide diffusion of that colistin resistance determinant in veterinary medicine. Furthermore, we showed here the worrying dissemination of *mcr-1* not only in *E. coli* but also significantly in *K. pneumoniae*

7th ARAE2017 Poster No. 92:

Characteristics of *mcr-1* harboring plasmids isolated from *Escherichia coli* at the human-animal-environment interface

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Background and objectives: The recent identification of Enterobacteriaceae harbouring the plasmid-mediated transferable colistin resistance *mcr-1* gene is of great concern to public health. Here, we report on *mcr-1* harbouring plasmids from Enterobacteriaceae isolated from patients with diarrhea in Switzerland, river water in Switzerland, imported poultry meat and from ready-to-eat imported vegetables.

Materials and methods: In total, fifteen non-intrinsic colistin resistant enterobacterial strains isolated from over 300 non-duplicate stool samples or fecal swabs of patients with diarrhea, seventyfour ESBL-producing Enterobacteriaceae isolated from twenty-one rivers and lakes sampled in 2012 in Switzerland, thirty-three non-intrinsic colistin resistant isolates from imported poultry meat and sixty ESBL-producing Enterobacteriaceae isolated from forty-two imported vegetable samples from the Dominican Republic, India, Thailand, and Vietnam were screened for the presence of the of the *mcr-1* gene. From a selection of *mcr-1*-positive strains the plasmids were transferred by transformation experiments into *E. coli* DH5α, and colistin-resistant transformants were selected on LB agar supplemented with 2 mg/liter colistin. The *mcr-1*-harboring plasmids were sequenced on a PacBio RS2 device.

Results: The *mcr-1*-carrying plasmids circulating at the human-animal-environment interface belong to the incompatibility types I2, X4, HI1 and HI2 and their size vary between 33 kb and over 247 bp. The *mcr-1* genes were found to be located within various genetic contexts in the sequenced plasmids. In all plasmids, a 735-bp ORF encoding a hypothetical protein was detected immediately downstream of the *mcr-1* gene.

Conclusion: The results prove that *mcr-1*-encoding plasmids have arrived in Switzerland as in other countries. This is worrisome, because polymyxins are currently being chemically modified to become less toxic in order to replace currently used antimicrobials that are increasingly corrupted by multiple resistance.

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