RESET & MedVet-Staph - Final Scientific Symposium

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Imprint

BfR Abstracts

RESET & MedVet-Staph - Final Scientific Symposium

All authors are responsible for the content of their respective abstracts.

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Dear Ladies and Gentlemen,

It is my pleasure to welcome you to the final scientific symposium of the two research consortia RESET and MedVet-Staph funded by the Federal Ministry of Education and Research (BMBF).

These two consortia addressed highly relevant questions related to antimicrobial resistance with a special focus on extended-spectrum beta-lactamase producing *E. coli* (ESBLs) and *Staphylococcus (S.) aureus* including methicillin-resistant *S. aureus* (MRSA). By funding these two interdisciplinary research consortia, the BMBF has facilitated important progress in our understanding of the underlying mechanisms of the development and spread of antimicrobial resistance and in the assessment of risks associated with antimicrobial resistance development in animal husbandry.

Antimicrobial resistance has gained substantial public attention. Risks related to resistant, in particular multi-resistant bacteria are considered severe threats to human health. In the public, particular attention is paid to ESBLs and MRSA due to their relevance for public health and their high prevalence in livestock and along the farm-to-fork food chain. However, the extent of the real risks for consumers related to MRSA and ESBL needs to be assessed separately as their epidemiology differs significantly.

Research in the consortium RESET focused on resistance to cephalosporins in enterobacteria. These antimicrobials are classified as “prioritised critically important antimicrobials” for treatment in humans by WHO. The results obtained by this research consortium improved our understanding of the spread of this resistance and its underlying mechanisms. They shed light on the role of risk factors and the relevance of different transmission pathways. In the long term, these research results contribute to an improved assessment of the risks for humans related to foodborne exposure to such bacteria.

In the consortium MedVet-Staph, particular attention was paid to zoonotic pathogenic *S. aureus* and MRSA with livestock origin. As a result of the research conducted within the consortium, the extent to which livestock-associated MRSA, but also MRSA from pets, contributes to colonisation and (treatment-related) infections in humans and animals in Germany was elucidated. The results make a significant contribution to the identification and development of suitable intervention strategies allowing for a reduction of human exposure to this kind of antimicrobial resistance.

In both consortia, scientists working at the BfR collaborated intensively with partners from other academic and public research institutions in the fields of medicine, public health and veterinary public health. The aim of the BfR is to contribute to a science-based discussion on possible links between antimicrobial use and development of resistance in livestock populations and problems with antimicrobial resistance in the medical field. BfR achieves this by means of collaborative research projects such as RESET and MedVet-Staph, but also by conducting independent investigations within its mandate. Knowledge exchange and scientific discussion is fostered in various formats of scientific events and by presenting scientific results to government bodies, the public and various stakeholders. At the same time, BfR intends to identify and quantify the real risks, to recommend appropriate control measures and to adequately communicate these risks to the public.

The two consortia contributed to the implementation of the German Antibiotic Resistance Strategy (DART2020). This strategy emphasises the particular relevance of antimicrobial resistance and the urgent need for the development and implementation of control measures to reduce antimicrobial resistance in both human and veterinary medicine. At this symposium, the consortia present their results to you to enable a scientifically sound discussion on the implications of the findings.

I wish you and all of us plenty of interesting presentations and fruitful discussions on antimicrobial resistance to improve our understanding of the complexity of the burden of antimicrobial resistance from a One Health perspective.

Professor Dr Dr Andreas Hensel

Dear Sir or Madam,

On 22 March 2006, a press release announced that the Federal Cabinet of the Federal Republic of Germany had submitted a research agreement on zoonoses between the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV), the Federal Ministry for Education and Research (BMBF) and the Federal Ministry of Health (BMG). The main objectives of this agreement were to clarify interdisciplinary questions concerning the transmission of pathogens from animals to humans or vice versa, to improve cooperation between veterinary and human medical scientists, and to create a dense network of "One Health" experts in Germany.

This decision led to an extensive research programme, from which a large number of research networks emerged as of 2007. In a second funding phase, the topic of antibiotic resistance was also included, which is of particular relevance for the health of humans and animals. Even Alexander Fleming already warned against the selection of penicillin-resistant bacteria through the use of antibiotics. The World Health Organization (WHO) reported the risk of resistance as early as 1963. The promotion of research on the spread of antimicrobial resistance and its prevention are also essential components of the "Deutsche Antibiotika-Resistenzstrategie" DART and of the "Global Action Plan on Antimicrobial Resistance" of the WHO. Within the scope of these initiatives, two interdisciplinary research groups, RESET and MedVet-Staph, were founded in 2010/2011 and funded by the BMBF.

Within the RESET research consortium, findings on epidemiology, molecular genetics and pharmacology of resistant bacteria were obtained and incorporated into a concept for risk assessment (www.reset-verbund.de). The activities of the compound concentrated on Escherichia (E.) coli-producing extended spectrum beta-lactamases (ESBLs) and plasmid-coded cephamycinases (pAmpCs). In contrast, the consortium MedVet-Staph (www.medvetstaph.net) focused on assessing the molecular epidemiology, zoonotic transmission, virulence and pathogenicity of antimicrobial-resistant Gram-positive microorganisms including methicillin-resistant Staphylococcus aureus (MRSA).

The work of the RESET and MedVetStaph consortia has now come to a formal end, after more than six years of collaboration between a large number of research groups and researchers from various disciplines in which more than 200 publications were published, numerous lectures and posters presented at scientific conferences, as well as SOPs and press contributions issued. We know that the work has not only resulted in a great deal of

knowledge and qualifications (for example, over 20 young colleagues have successfully finalised their theses), but that new networks of scientific cooperation have emerged. We also hope that the scientific findings of our work will be of benefit to the society which has funded them.

Therefore, it is our wish that all the participants will be able to take a lot of insights from the two research groups, which will be gathered in the coming days, and we hope that the event goes well and can provide important impetuses for the future.

Lothar Kreienbrock, Hannover & Robin Köck, Münster / Oldenburg

1 Programme

Wednesday, 26 April 2017

11:00–11:30 a.m.
Opening & Welcome
Prof. Dr Dr Andreas Hensel, Federal Institute for Risk Assessment, Berlin
Bärbel Brumme-Bothe, Federal Ministry of Education and Research, Berlin

Tasks and objectives of the consortia

11:30 a.m.–12:00 p.m.
MedVet-Staph
PD Dr Robin Köck, University Hospital Münster

12:00–12:30 p.m.
RESET
Prof. Dr Lothar Kreienbrock, University of Veterinary Medicine Hannover, Foundation

12:30–1:30 p.m. lunch

Main results of the consortia

1:30–2:30 p.m.
MedVet-Staph
The MedVet-Staph team

2:30–3:30 p.m.
RESET
The RESET team

3:30–4:00 p.m. coffee break

Consequences from the research results

4:00–4:30 p.m.
Statement: Consequences – BMEL point of view
Dr Karin Schwabenbauer, Federal Ministry of Food and Agriculture, Bonn
Statement: Consequences – BMG point of view
Dr Antina Ziegelmann, Federal Ministry of Health, Berlin
Statement: Consequences – Health authority point of view
Dr Matthias Pulz, Health office of Lower Saxony, Hannover

4:30–5:30 p.m.
Discussion

Thursday, 27 April 2017

8:30–8:45 a.m.
Welcome
Prof. Dr Lothar Kreienbrock, University of Veterinary Medicine Hannover, Foundation

8:45–9:30 a.m.
Invited Talk: Antimicrobial resistance as a central One Health task
Prof. Dr Lothar Wieler, Robert Koch Institute, Berlin

Research results from RESET

9:30–9:45 a.m.
Molecular epidemiology of ESBL-/AmpC-producing enterobacteria in the broiler production chain
Michaela Projahn, Freie Universität Berlin

9:45–10:00 a.m.
Occurrence of carbapenem-resistant *Escherichia coli* and *Salmonella enterica* isolates within German chicken fattening farms
Dr Nicole Roschanski, Freie Universität Berlin

10:00–10:30 a.m.
Analyses of associations of *E. coli* isolate characteristics and epidemiological information
Katja Hille, University of Veterinary Medicine Hannover, Foundation

10:30–11:00 a.m. coffee break

Research results from MedVet-Staph

11:00–11:25 a.m.
Multidrug-resistant bacteria entering a horse clinic
Dr Birgit Walther, Freie Universität Berlin

11:25–11:50 a.m.
***Staphylococcus aureus* CC398: Factors promoting host adhesion and immune evasion**
Dr Phillip Jung, University Saarland, Homburg/Saar

11:50 a.m.–12:15 p.m.
How big is the risk? Update on MRSA in the food chain
Dr Alexandra Fetsch, Federal Institute for Risk Assessment, Berlin

12:15–12:40 p.m.
Living in livestock-dense regions: Impact of livestock-associated MRSA on human infection and colonisation
PD Dr Robin Köck, University Hospital Münster

12:40–01:00 p.m.
Development of diagnostic kits for selected markers of resistance, virulence and zoonotic transmission among methicillin-resistant *Staphylococcus aureus*
Dr Boris Oberheitmann, Q-Bioanalytic, Bremerhaven

1:00–2:00 p.m. lunch

Research results from RESET

2:00–2:30 p.m.
From ESBL colonisation to infection: rates and risk factors within the hospital setting
Dr Rasmus Leistner, Charité, Berlin

2:30–3:00 p.m.
NGS-based Analysis of AmpC-beta-lactamase CMY-2-producing *Escherichia coli* from humans, livestock and food in Germany
Dr Michael Pietsch, Robert Koch Institute, Wernigerode

3:00–3:15 p.m.
Resistance transmission dynamics and circulation of ESBL-encoding *E. coli* in the One Health context
Linda Falgenhauer, Justus Liebig University, Gießen

3:15–3:30 p.m.
Novel insights into the phylogenomics of ESBL-*E. coli* in the One Health Concept
Judith Schmiedel, Justus Liebig University, Gießen

3:30–4:00 p.m. coffee break

Research results from MedVet-Staph

4:00–4:20 p.m.
Social networks in the pig barn – Implications for the infection dynamics of MRSA
Tobias Kaufholz, Federal Institute for Risk Assessment, Berlin

4:20–4:50 p.m.
Novel antimicrobial resistance genes among staphylococci in livestock environments
Prof. Dr Stefan Schwarz, Freie Universität Berlin

4:50–5:10 p.m.
Antibiotic resistance profiles of coagulase-negative staphylococci in livestock environments
Sonja Schoenfelder, University of Würzburg

5:10–5:30 p.m.
Colonisation with MRSA CC398 among a cohort of veterinarians in Germany
Dr Jan Walter, Robert Koch Institute, Berlin

5:30–6:30 p.m.
Poster

6:30 p.m. evening meal

Friday, 28 April 2017
Research results from RESET

9:00–9:30 a.m.
Significance of environmental contaminations on the development of bacterial resistance to antibacterial agents in indicator animals
Jessica Meißner, University of Veterinary Medicine Hannover, Foundation

9:30–10:00 a.m.
New occurrence of VIM-1 producing *E. coli* in German pig production
Dr Alexandra Irrgang, Federal Institute for Risk Assessment, Berlin

10:00–10:30 a.m.
Investigation of extended-spectrum β-lactamase (ESBL) and plasmid-mediated quinolone resistance (PMQR) gene-carrying plasmids in *Escherichia coli* and *Salmonella enterica* from diseased animals: their role in antimicrobial resistance, biocide tolerance and virulence of the isolates
Dr Geovanna Brenner Michael, Freie Universität Berlin

10:30–10:45 a.m.
Possibilities and limits of logistic regression for the study of the transmission dynamics of ESBL/AmpC-producing *E. coli* between broiler flocks
Dr Guido Correia Carreira, Federal Institute for Risk Assessment, Berlin

10:45–11:00 a.m.
Assessment of the foodborne transmission pathway for ESBLs
Prof. Dr Annemarie Käsbohrer, Federal Institute for Risk Assessment, Berlin

11:00–11:30 a.m. coffee break

Research results from MedVet-Staph

11:30 a.m.–12:00 p.m.
Breaking the species barrier: Epidemiological dynamics and genetic changes in livestock-associated MRSA
Sarah van Alen, University Hospital Münster
and Dr Britta Ballhausen, Federal Institute for Risk Assessment, Berlin

12:00–12:15 p.m.
MRSA in equine clinics and the impact for humans
Dr Christine Cuny, Robert Koch Institute, Wernigerode

12:15–12:30 p.m.
Occurrence of cfr-encoded linezolid resistance in coagulase-negative staphylococci from livestock and exposed humans
Prof. Dr Wolfgang Witte, Robert Koch Institute, Wernigerode

12:30–1:15 p.m.
Invited Talk: Intercultural differences in the prevention of antimicrobial resistance – the Dutch experience
Prof. Dr Alexander W. Friedrich, University Medical Center Groningen, Netherlands

1:15–1:30 p.m.
Farewell

2 Abstracts

2.1 Molecular epidemiology of ESBL-/AmpC-producing enterobacteria in the broiler production chain

Michaela Projahn[1], Katrin Dähre[1], Philine von Tippelskirch[2], Greta Gölz[2], Stefanie Orquera[2], Thomas Alter[2], Sebastian Guenther[1], Torsten Semmler[3], Anika Friese[1], Uwe Rösler[1]

[1] Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health
[2] Freie Universität Berlin, Institute for Food Safety and Food Hygiene
[2] Robert Koch-Institute, Microbial Genomics, Berlin

Extended-spectrum beta-lactamase (ESBL-) and AmpC beta-lactamase enzymes reduce the effectiveness of specific antibiotics. Therefore, ESBL-/AmpC-producing enterobacteria represent an increasing problem in human and veterinary medicine. The occurrence of these resistant bacteria in broiler fattening farms was shown in many studies. In previous studies, the detection of ESBL-/AmpC-producing enterobacteria was already possible in cloacal swabs of one-day old chicken. This leads to the hypothesis of an early entry or emergence of these resistant bacteria in the broiler production chain but possible transmission routes, vertical and/or horizontal, could not yet be clarified.

In our study, seven broiler fattening flocks, preselected by positive initial testing of the parent flocks, were tracked along the entire production chain. The hatchery, as suspected bottleneck for bacteria transmission, was sampled intensively and in the farms the chicken and their environment were investigated from arrival until departure. At the slaughterhouse, samples from the respective animals and the environment were investigated as well.

ESBL-/AmpC-producing enterobacteria were isolated and examined for their species, phylogroup and resistance genes using MALDI-TOF, Disk Diffusions Tests, (real-time) multiplex PCR and sequencing approaches. To identify possible transmission routes and epidemiological relationships, further investigations such as pulsed-field gel-electrophoresis (PFGE) and whole genome analyses (WGA) were performed.

In total, we analysed 36 samples from the parent flocks of which 24 were positive for ESBL-/AmpC producers of different species (*E. coli, E. fergusonii*) and *bla* resistance genes (bla_{TEM}, bla_{CMY}, $bla_{CTX\text{-}M}$). In the hatchery, only 0.6% of the samples (n=1,571; eggs and environment) tested positive for ESBL-/AmpC-producing enterobacteria. At the first sampling at the fattening farm, these bacteria were detected in 0.7% (n=280) of the investigated individual animals only. Nevertheless, positive samples were found in the middle and at the end of the fattening period in all investigated flocks, with widely varying prevalence, although only one of the seven flocks was treated with a macrolide antibiotic. The prevalence of the animal samples ranged from 0 to 87.5% and of the environmental samples from 7.7 to 66.7% at the end of the fattening period. The detection frequencies for ESBL-/AmpC-producing enterobacteria in

caecum samples collected at slaughterhouse varied between 0 and 88%. Similar differences were also shown for positive environmental samples and swabs (0 -75%).
As isolates from six of the parent flocks differ from the corresponding fattening flocks, a direct vertical transmission could not be proven. Interestingly, we determined isolates with comparable genotypes (species, phylogroup, resistance gene) at different production steps for some broiler flocks, which indicated possible transmission events. Using PFGE analyses we could verify a pseudo-vertical transfer of ESBL-/AmpC-producing enterobacteria from the parent flock to the hatchery via contaminated outer egg surfaces. Early contamination of the recently hatched chicks in the hatchery and/or during the transportation to the respective farms could also be shown during our study. Additionally, horizontal transmission routes occurred in the investigated farms, as comparable isolates were found in flock E and F, fattened consecutively in the same barn. Using WGA, we determined indications of an entry of the resistant bacteria due to a contaminated farm environment. Therefore, the cleaning and disinfection procedures also have an impact on the occurrence of ESBL-/AmpC-producing enterobacteria. At the slaughterhouse, we found ESBL-/AmpC producers in the scalding water even before the animals of the respective flocks were slaughtered. Investigations of both skin and fillet samples revealed isolates with equal molecular characteristics which is a strong indication of cross-contamination on slaughterhouse level.
Taken together, we showed the occurrence of ESBL-/AmpC-producing enterobacteria in the different levels of the broiler production chain and due to analyses of the molecular background we identified different transmission routes of these bacteria along the broiler production chain. These findings have to be taken into consideration for the application of successful intervention strategies against ESBL-/AmpC-producing enterobacteria.

Contact: uwe.roesler@fu-berlin.de

2.2 Occurrence of carbapenem-resistant *Escherichia coli* and *Salmonella enterica* isolates within German chicken fattening farms

Nicole Roschanski[1], Jennie Fischer[2], Linda Falgenhauer[3], Sebastian Günther[1], Michael Pietsch[4], Lothar Kreienbrock[5], Trinad Chakraborty[3], Yvonne Pfeifer[4], Beatriz Guerra[2,§], Uwe Rösler[1]

[1] Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health

[2] Federal Institute for Risk Assessment, Department Biological Safety, Berlin

[3] Justus Liebig University Giessen, Institute of Medical Microbiology and German Center for Infection Research Partner Site Giessen-Marburg-Langen, Giessen

[4] Robert Koch Institute, Nosocomial Pathogens and Antibiotic Resistance, Wernigerode

[5] University of Veterinary Medicine Hannover, Institute for Biometry, Epidemiology and Information Processing, WHO Collaborating Center for Research and Training for Health at the Human-Animal-Environment Interface

[§] Present address: European Food Safety Authority, Biological Hazards and Contaminants, Parma, Italy

Carbapenems belong to the group of last resort antibiotics in human medicine. Therefore, the increasing number of reports describing carbapenemase-producing Enterobacteriaceae is worrying. Within the last couple of years, it was shown that the occurrence of carbapenemase-producing bacteria is no longer limited to clinical settings. Increasing numbers of carbapenemase-producing bacteria have been isolated from environmental surroundings, wild birds, companion- and food-producing animals all over the world. This represents an important issue for the public health sector.

In 2011, the first VIM-1 producing *Salmonella* Infantis (R3) was isolated from a German chicken-fattening farm. Due to this observation, we retrospectively investigated more than 500 stored bacterial cultures, isolated from 45 chicken-fattening farms during the years 2011-2012. After a non-selective overnight incubation, the bacteria were transferred to selective agar plates. Growing *E. coli* as well as *Salmonella* isolates were investigated for the presence of carbapenemase genes by real-time PCR. Aside from the already described *Salmonella* Infantis isolate R3, one additional *Salmonella* subspecies I (rough phenotype, G-336-1a) as well as two *E. coli* isolates (G-336-2, G-268-2) were isolated from the selective agar plates. The real-time PCR-based screening indicated the presence of the bla_{VIM-1} gene within the isolates G-336-1a and G-336-2. For the second *E. coli* isolate (G-268-2), the reason for the carbapenem resistance remained unknown. Whole genome sequencing using MiSeq (Illumina) was performed for all isolates and the data were analysed using the CGE platform (http://www.genomicepidemiology.org/). The phylogenetic likelihood of the two *Salmonella enterica* isolates was determined by a core genome comparison using parsnp analysis (http://harvest.readthedocs.io/en/latest/content/parsnp.html). In addition, the carbapenemase-containing plasmids were compared using BRIG (http://brig.sourceforge.net/). Both *Salmonella* isolates (R3 and G-336-1) were closely related: They belonged to ST32, and both PFGE and SNP analysis showed only small differences of 2 bands and 36 SNPs,

respectively. The *bla*$_{VIM-1}$-encoding plasmids (IncHI2; ~ 300 kb) derived from the two *Salmonella* isolates as well as the *E. coli* (G-336-2) were 100% identical. Therein, the *bla*$_{VIM-1}$ gene was part of a class 1 integron, accompanied by *aacA4* and *aadA1* in its variable region. On the same plasmid, the AmpC gene *bla*$_{ACC-1}$ was detected. Aside from the IncHI2 plasmid, G-336-2 (ST131) contained an IncI1α plasmid, carrying the AmpC gene *bla*$_{CMY-2}$. In the course of *in vitro* cultivation and transformation experiments, one *E. coli* isolate was received, which contained the *bla*$_{VIM-1}$-encoding class1 integron integrated into the *pilU* gene of the *bla*$_{CMY-2}$-encoding IncI1α plasmid (mediated by insertion sequence IS1). These data showed that - at least *in vitro* - this class 1 integron is highly mobile and self-transmissive. In case of the second *E. coli* isolate G-268-2 (ST-354), the whole genome data did not indicate the presence of a known carbapenemase gene. However, a plasmid isolation and electroporation showed that the carbapenem resistance is transferable to an *E. coli* recipient strain. Hence, G-268-2 has to be investigated in more detail in the future.

Taken together, our data showed that carbapenem-resistant and carbapenemase-producing Enterobacteriaceae have been found in three different chicken-fattening flocks and, in addition, the VIM-1 plasmids distributed therein were closely related. This finding is alarming and emphasises the importance of obligatory monitoring programmes within the food production chain as well as intervention strategies to contain the environmental spread of resistant bacteria in animals and humans.

Contact: nicole.roschanski@fu-berlin.de

2.3 Analyses of associations of *E. coli* isolate characteristics and epidemiological information

Katja Hille[1], Mayala Felski[1], Inga Ruddat[1], Johanna Woyd[1], Annette Schmid[2], Anika Friese[3], Anika Friese[3], Jennie Fischer[4], Linda Falgenhauer[5], Stefan Hörmansdorfer[2], Can Imirzalioglu[5], Trinad Chakraborty[5], Annemarie Käsbohrer[4,6], Uwe Rösler[3], Lothar Kreienbrock[1]

[1] University of Veterinary Medicine Hannover, Institute for Biometry, Epidemiology and Information Processing, WHO Collaborating Center for Research and Training for Health at the Human-Animal-Environment Interface, Hannover

[2] Bavarian Health and Food Safety Authority, Oberschleissheim

[3] Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin

[4] Federal Institute for Risk Assessment, Department: Biological Safety, Berlin

[5] Justus Liebig University Giessen, Institute of Medical Microbiology and German Center for Infection Research (DZIF), Partner Site Giessen-Marburg-Langen, Giessen

[6] University of Veterinary Medicine, Institute for Veterinary Public Health, Vienna, Austria

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.

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 animal-specific questionnaires.

From samples of 160 farms, cefotaxime-resistant *E. coli* were isolated and further characterised. These farms comprised 34 broiler farms, 41 fattening pig farms and 39 cattle farms. For 587 isolates, the ESBL genes and the phylogroup were determined. Additionally, the phenotypic antimicrobial resistance was tested. This information was used to define a profile characterising each isolate. A multivariate analysis using the distance-based permutation test was then performed to investigate dependencies between detected profiles and conditions observed in the farms (e.g. farm size, hygiene factors or antimicrobial use etc.).

Contact: Katja.hille@tiho-hannover.de

2.4 Multidrug-resistant bacteria entering a horse clinic

Birgit Walther[1], Katja S. Klein[1], Antina Lübke-Becker[1], Heidrun Gehlen[1]
[1] Freie Universität Berlin, Berlin

The introduction rate of multi-drug resistant (MDR) pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and extended-spectrum beta-lactamase (ESBL)-producing Enterobactericeae is worth knowing, since colonised horses provide a continuing source of MDR pathogens for themselves, other equine patients and, not least, personnel (1-3).

Thus, a total of 341 equine patients were screened for MDR pathogen carriage within two six-month study periods in 2014 and 2015, respectively. Horses suffering from either colic (n=233) or (open) wounds (n=108) were selected for microbiological examination of nostril swabs and faecal samples at hospital admission.

The detected introduction rates of MDR pathogens via colonised equine patients were alarming, since 10.7% (34 of 318) of the valid faecal specimens were positive for ESBL-producing Enterobactericeae (94%: *E. coli*).
Considering the two distinct groups, an enteral colonisation of 10.5% was detected in horses with “colic” (23/220), while a comparable rate of 11% (11/98) showed a positive result in the “open wound” group.
MRSA (0.6%) and *A. baumannii* (0.9%) were rarely proven, while *Clostridium difficile* and Salmonella were not detected.

Screening results for the equine nostrils were 3.5% for MRSA (12/340 valid specimens), with 4.3% in the “colic” group (10/232) and 1.9% (2/108) in the “open wound” group. Likewise, the ESBL-producing Enterobactericeae rate was 3.4% among colic patients and 0.9% in the injury group, with an average rate of 2.6% (9/340) for both indications.
These results demonstrated a massive introduction of MDR pathogens in equine clinics via colonised equine patients, depicting a constant threat to any hygiene management system (4).

Literature

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2.5 *Staphylococcus aureus* CC398: Factors promoting host adhesion and immune evasion

Philipp Jung[1], Britta Ballhausen[2], Mohamed M. Abdelbary[3], Karsten Becker[4], Wilma Ziebuhr[5], Robin Köck,[4] Alexandra Fetsch, [2], Wolfgang Witte[3], Christiane Cuny[3], Andrea T. Feßler[6], Stefan [6], Nicolas Thewes[1], Christian Spengler[1], Karin Jacobs[1], Markus Bischoff[1]

[1] University of Saarland
[2] Federal Institute for Risk Assessment, Berlin
[3] Robert Koch-Institute, Wernigerode
[4] University Hospital Münster, Münster
[5] Universty of Würzburg, Würzburg
[6] Freie Universität Berlin, Berlin

Staphylococcus aureus of the Clonal Complex (CC) 398 is able to overcome the species barrier between livestock and humans. Especially in regions with a high density of pig farming, methicillin-resistant *S. aureus* (MRSA) CC398 strains are increasingly found in clinical settings, causing severe infections in humans. The bacterial potential to adhere to host structures and to evade the host immune response are of major importance for the initial pathogenicity of *S. aureus* and are focuses of our research.

In an initial approach, we demonstrated a decreased adhesive potential to human epithelial and endothelial cells of MRSA CC398 when compared to widespread human-adapted MRSA lineages. However, an epidemiological CC398 sublineage (*spa* type t108) exhibited an adhesion capacity which was on the same level as that of human-adapted, community-acquired MRSA and hospital-acquired MRSA lineages, and exceeded the adhesion of other CC398 sublineages, such as *spa* type t011 and t034. These variations might be due to an altered adhesive potential to the host protein fibronectin, attributed to massive transcriptional changes of the genes of both cell wall-anchored fibronectin binding proteins A and B (*fnb*A/B) and to the frequency of mutations in the gene *fnb*B. Our results underline the previously described pronounced heterogeneity of virulence properties within the CC398 pool.

In a second approach, we studied the impact of the bacteriophage Sa*int*3-coded immune evasion cluster (IEC) on the capacity of phagocytic protection of *S. aureus* CC398 isolates. The IEC, targeting the host complement system, is believed to be strictly human-specific. However, we demonstrated that the IEC mediates a protective effect against phagocytosis by PMN in human blood and equine blood alike, while no protection against phagocytosis by porcine PMN is provided. Our findings indicate that the host specificity of the IEC is broader than currently assumed and might partly explain the distribution pattern of IEC-positive *S. aureus* isolates described in recent prevalence studies.

2.6 How big is the risk? Update on MRSA in the food chain

Alexandra Fetsch[1], Britta Ballhausen[1], Britta Kraushaar[1], Jens Hammerl[1], Petra Hiller[1], Ylanna Kelner-Burgos[1], Kaesbohrer Annemarie[1], Tenhagen Bernd-Alois[1]

[1] Federal Institute for Risk Assessment, Berlin

Methicillin-resistant *Staphylococcus aureus* (MRSA) found in livestock and along the food chains have attracted increased attention for over a decade. These livestock-associated (LA) MRSA, mainly belonging to clonal complex (CC) 398, have also emerged among humans indicating a zoonotic transmission from animals to humans. Livestock professionals, such as farmers, veterinarians and slaughterhouse workers handling live animals, are at increased risk [1]. However, some cases of LA-MRSA carriage in humans cannot be explained by livestock contact. Thus, one could speculate that humans might have acquired such MRSA by human-to-human transmission or via contaminated food[2]. In Germany, previous investigations embedded in a national monitoring programme for zoonotic agents and resistant bacteria in the food chain have estimated a high MRSA prevalence in meat, particularly poultry meat, in retail. While the risk of acquiring infections via contaminated meat is estimated to be low, the exact extent to which food of animal origin contributes to the occurrence of human infections with MRSA cannot be quantified at present.

Our research projects conducted in the course of the BMBF-funded consortium MedVet-Staph help to fill data gaps and, in return, allow for more reliable risk assessment. We focused on open questions related to the level of MRSA cross-contamination in the kitchen environment by mimicking common practices of the consumer in private households during food preparation. Our results show that transfer of MRSA via hands and equipment and from there to food intended to be eaten raw occurs in household kitchens once contaminated broiler meat is introduced, and that consumers may be at risk during handling and/or ingestion[3]. In a second study, we addressed the risk related to the spread of MRSA in commercial kitchens after introduction via contaminated raw poultry meat. It was shown that MRSA spread seems to be limited to positions which are directly linked to the processing and preparation of a meal. Nevertheless, our results underline the necessity of strict separation of working areas and equipment used in commercial kitchens[4].

In a third study, the high prevalence of MRSA along the poultry food chains and the emergence of a novel hybrid LA-MRSA CC9/CC398 genotype were further elucidated. This CC9/CC398 genotype has been described in human clinical cases in Denmark[5]; moreover, poultry meat was considered to serve as a vehicle of possible livestock-to-human transmission of CC9/CC398 MRSA. By applying phylogenetic single nucleotide polymorphism tree analysis, a high identity of CC9/CC398 strains from human cases and of poultry meat (mainly

turkey) origin was confirmed by our group, providing further evidence that specific LA-MRSA lineages with unique genetic characteristics are adapting to humans[6].

Finally, our group characterised and compared MRSA of turkey and broiler origin sampled on different production levels and in different years. It was shown that isolates of the same clonal complex clustered together according to their common virulence and resistance profiles. Moreover, other poultry-associated clones of MRSA (mainly CC9 and CC5) besides the predominant CC398 were found in both poultry species[7].

In summary, our results underline the continuing need for comprehensive monitoring and intervention strategies from farm to fork to prevent further spread of pathogenic MRSA of livestock origin to humans via the global food chains.

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2.7 Living in livestock-dense regions: Impact of livestock-associated MRSA on human infection and colonisation

Robin Köck[1], Stefan Bletz[1], Christian Deiters[1], Julia Fischer[1], Karsten Becker[1], Sarah van Alen[1], Britta Ballhausen[2], Ursula Kaspar[1], Alexander Mellmann[1]
[1] University Hospital Münster, Münster
[2] Federal Institute for Risk Assessment, Berlin

After the emergence of MRSA among livestock, transmission to humans was a major concern, especially in regions with a high density of pig, cattle, and poultry farming. Here, we summarise the results of epidemiological studies performed within MedVet-Staph to elucidate how livestock-associated (LA) MRSA clonal lineages (CC398 and others) emerged among humans in the rural area around the city of Münster in Westphalia.

To assess transmission between livestock and directly exposed humans, we investigated the prevalence of nasal MRSA and rectal ESBL-*E. coli* colonisation among 85 pig farmers working on 51 regional farms. Overall, MRSA was found on all except two farms (96%) and ESBL-producing *E. coli* was detected on 61% of the farms. 85% of the farmers carried MRSA (all MRSA CC398) and 6% carried ESBL-producing *E. coli*. In a pilot study, we confirmed that most farmers did not spontaneously clear MRSA carriage during summer breaks, indicating rather persistent carriage. In a review of the literature, we summarised case reports documenting various types of occupation-related MRSA CC398 infections among exposed persons including superficial skin or wound infections, as well as lethal cases of endocarditis and pneumonia.

Besides farmers, other persons are also affected by LA-MRSA. However, in a prospective cohort study, we demonstrated that the overall prevalence of MRSA carriage in the regional non-hospitalised population remained relatively low. We found that among 1878 volunteers, only 0.7% carried MRSA. However, MRSA CC398 caused 31% of these cases. Indeed, the risk factors contact with livestock (OR 31), taking antidepressant/neuroleptic drugs (OR 5) and chronic renal insufficiency (OR 41) were associated with MRSA carriage in the community.

Hospital inpatients are usually more often MRSA carriers than persons in the community. In a regional investigation, we found that, on admission to 39 hospitals and 11 rehabilitation care centres, 1.2-1.6% of the patients carried MRSA. In 2006, 17% of these MRSA carriers were colonised by MRSA CC398. When analysing data for >14000 MRSA isolates derived from various specimens collected between 2008 and 2012 in the same regional hospitals and practices, 18.6% of all isolates were associated with *spa* types indicative of MRSA

CC398, while other typical LA-MRSA clonal lineages were rarely found (0.14% CC9 (t1430), 0.01% CC97 (t3992), 1% CC5 (t002), 0.04% CC30 (t007)).
At the University Hospital Münster, where general admission screening is performed and all MRSA isolates have been typed since about 2006, this proportion has increased over time. The analysis of data over fifteen years showed that MRSA CC398 were first isolated from local patients in 2000. Until 2013/2014, the proportion of MRSA CC398 among all MRSA increased to 29-34%. Considering absolute numbers demonstrated that the number of patients affected by non-MRSA CC398 strains (e.g. classic healthcare associated MRSA) remained relatively stable. Thus, MRSA CC398 represented an additional regional MRSA burden. Investigations in non-outbreak situations revealed that regional nurses carried MRSA in 5.6% and physicians in 1.2% of cases, which demonstrates that close contact to hospital settings seems to increase carriage rates compared to the general population. However, none of the healthcare workers participating in this study carried MRSA CC398 or other LA-MRSA clonal lineages.

The increase of MRSA CC398 in the region led to the question as to whether this clonal lineage remained LA-MRSA or whether it disseminated via other routes. In a case-control study among 384 MRSA-positive patients, direct livestock contact (odds ratio 46) and living directly on a farm (OR 13) were independent risk factors for MRSA CC398. However, we found that 21/55 patients (38%) carrying MRSA CC398 reported no direct contact to livestock, indicating other indirect transmission routes. This was confirmed in a study in rehabilitation centres where 31% (5/16) of patients carrying MRSA CC398 had no livestock contact.
The burden of severe human infections was assessed in a state-wide survey characterising MRSA 1952 isolates from blood cultures in the federal state of North Rhine-Westphalia (NRW). Among all isolates collected, MRSA CC398 accounted for 1.7% of the bacteraemia cases. However, in some livestock-dense districts of NRW, this clonal lineage represented >11% of all bacteraemia isolates. We confirmed this in another regional study where LA-MRSA-associated *spa* types accounted for a proportion of 8% among MRSA isolates from blood cultures and 14% from deep respiratory fluids.

Finally, the regional, “Münsteranian” data assessed in MedVet-Staph allowed us to estimate the burden of LA-MRSA in German livestock-dense regions. We estimated that the incidences of MRSA CC398 cases (colonisation and infection), MRSA CC398 infections and MRSA CC398-bacteraemia were 61, 6.7 and 0.3 per 100000 inhabitants, respectively.

2.8 Development of diagnostic kits for selected markers of resistance, virulence and zoonotic transmission among methicillin-resistant *Staphylococcus aureus*

Boris Oberheitmann[1], Constanze Seidel[1], Sonja Peters[1], Erik Eschbach[1], Andrea T. Feßler[2], Stefan Schwarz[2]
[1] Q-Bioanalytic GmbH, Bremerhaven
[2] Freie Universität Berlin, Berlin

The aim of the study was to develop real-time PCR and PCR NALFIA tests to detect resistance and virulence genes of *Staphylococcus aureus* (Seidel et al., 2016). In addition, a real-time PCR test for the zoonotic strain CC398 was developed. Tests were evaluated for performance characteristics, such as limit of detection, sensitivity, specificity, positive/negative predictive value (PPV, NPV).

A magnetic nanoparticle-based DNA purification method was optimised for application in nasal swabs for routine screening.

Literature

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2.9 From ESBL colonisation to infection: rates and risk factors within the hospital setting

Luisa A. Denkel[1,2], Petra Gastmeier[1,2], Rasmus Leistner[1,2]

[1] Charité – Universitätsmedizin Berlin, Institute of Hygiene and Environmental Medicine
[2] National Reference Centre for the Surveillance of nosocomial infections

Background: The number of patients colonised with ESBL-positive Enterobacteriaceae (ESBL-E) is growing worldwide. Subsequent infections with these organisms are associated with limited treatment options and elevated mortality and morbidity. Hospitals are trying to prevent these infections with additional and costly prevention measures. But how often do these patients develop an infection with ESBL-E compared to infections with other bacteria and what are the risk factors? Within a hospital setting, we aimed to determine the incidence of these ESBL-E infections as well as the risk factors associated with these infections after rectal ESBL-E colonisation.

Material/methods: The setting of this study was a German university hospital with more than 3,000 beds. The study period was two years (2014 and 2015). We included all patients that were found to be rectally colonised with ESBL-positive *Escherichia* (*E.*) coli or *Klebsiella (K.) pneumoniae* and subsequently stayed at least 3 days in our hospital. The patients were prospectively tracked, looking for microbiological examination followed by adequate antimicrobial treatment indicating a possible infection. Cases were manually reviewed by infection control professionals to identify a new infection with either the colonising organism or any other bacterium. In order to analyse risk factors for a bacterial infection, we conducted a nested case-case-control study. Cases were either ESBL-E infections or infections with any other bacteria. Controls were patients without bacterial infection with an onset between ESBL-E colonisation and hospital discharge. Data were analysed using univariate and multivariable regression models.

Results: Within the study period, 3,036 patients fulfilled the inclusion criteria. The patients were found to be colonised with the following ESBL-E: *E. coli* (78.2%), *K. pneumoniae* (19.0%) or both (2.8%). Of these patients, 3.8% (n=117) developed a bacterial infection with the colonising ESBL-E, 5.9% (n=180) with any other bacteria and 0.5% (n=14) with both. The overall infection rate on intensive care units was 21.8% (7.5% associated with the colonising ESBL-E and 14.2% with any other bacteria). The overall infection rate on normal wards was 6.6% (2.9% with the colonising ESBL-E and 3.6% with any other bacteria). The most common infection in the ESBL-E infection group was urinary tract infection, whereas it was bloodstream infection in the other infection group. All cases (n=117+180) and 230 controls were included in the nested case-case-control study. Independent risk factors for an ESBL-E infection after colonisation were: Intestinal surgery (OR 2.6, 95%CI 1.24 – 5.55), steroid intake (OR 2.3, 95% CI 1.29 – 4.19), central venous catheter (OR 2.1, 95%CI 1.25 – 3.68),

urinary catheter (OR 1.9, 95%CI 1.08 – 3.04). Carbapenem intake between colonisation and discharge served as a protective factor (OR 0.3, 95%CI 0.15 – 0.66). On the other side, risk factors for a bacterial infection with any other organism were: Intestinal surgery (OR 2.7, 95%CI 1.38 – 5.14), steroid intake (OR 2.3, 95%CI 1.32 – 3.93), central venous catheter (OR 1.8, 1.08 – 2.95).

Conclusions: The incidence of a nosocomial infection in rectal ESBL-E carriers was high with overall 10.2%. ICU patients had an increased incidence of nosocomial infections, especially with bacteria other than the colonising ESBL-E. Risk factors for subsequent infections with the colonising ESBL-E were associated with typical colonisation sites for the Gram-negative organisms.

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

Michael Pietsch[1], Alexandra Irrgang[2], Nicole Roschanski[3], Geovana Brenner Michael[4], Heime Rieber[5], Annemarie Käsbohrer[2,7], Stefan Schwarz[4,6], Uwe Rösler[3], Yvonne Pfeifer[1], Guido Werner[1]

[1] Robert Koch-Institute, Nosocomial Pathogens and Antibiotic Resistance, Wernigerode

[2] Federal Institute for Risk Assessment, Department of Biological Safety, Berlin

[3] Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health

[4] Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Neustadt-Mariensee

[5] Medizinisches Versorgungszentrum Dr. Stein, Division of Microbiology, Mönchengladbach

[6] Freie Universität Berlin, Institute of Microbiology and Epizootics

[7] Veterinary University Vienna, Austria

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 approx. 1% and approx. 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".

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 included and sequenced using the Illumina MiSeq platform. The sequences were analysed for resistance genes and phylogenetic markers, such as multi-locus sequence type and plasmid replicon types, were identified.

The 170 sequenced isolates showed a highly diverse distribution of sequence types (STs) and replicon types. Fifty-nine different STs were identified; the 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 was 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; and 12 *E. coli* from broilers and retail chicken meat carried the colistin resistance gene *mcr-1*.

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 ST57, ST429 and ST38. In contrast, IncI1 was associated with all seven ST58 isolates. 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.

Contact: pietschm@rki.de

2.11 Resistance transmission dynamics and circulation of ESBL-encoding *E. coli* in the One Health context

Linda Falgenhauer[1], Can Imirzalioglu[1], Hiren Ghosh[1], Konrad Gwozdzinski[1], Judith Schmiedel[1], Katrin Gentil[1], Rolf Bauerfeind[2], Jennie Fischer[3], Alexandra Irrgang[3], Harald Seifert[4], Yvonne Pfeifer[5], Michael Pietsch[5], Geovana Brenner Michael[5], Stefan Schwarz[6], Annemarie Käsbohrer[3,7], Uwe Roesler[8], Nicole Roschanski[8], Peter Kämpfer[9], Lothar Kreienbrock[10], Alexander Goesmann[11], Trinad Chakraborty[1]

[1] Justus Liebig University Giessen, Institute of Medical Microbiology and German Center for Infection Research, Partner site Giessen-Marburg-Langen
[2] Justus Liebig University Giessen, Institute of Hygiene and Infectious Diseases of Animals
[3] Federal Institute for Risk Assessment, Department of Biological Safety, Berlin
[4] University of Cologne, Institute for Medical Microbiology, Immunology, and Hygiene and German Center for Infection Research, Partner site Köln-Bonn
[5] Robert Koch Institute, Division of Nosocomial Pathogens and Antibiotic Resistances, Wernigerode
[6] Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Neustadt-Mariensee
[7] Veterinary University Vienna, Institute for Veterinary Public Health, Austria
[8] Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health
[9] Justus Liebig University Giessen, Institut für Angewandte Mikrobiologie
[10] University of Veterinary Medicine Hannover, Foundation, Institute for Biometry, Epidemiology, and Information Processing
[11] Justus Liebig University Giessen, Institute of Bioinformatics and Systems Biology

Multidrug-resistant *Escherichia coli* frequently harbour extended-spectrum beta-lactamase (ESBL) genes, thereby impairing the treatment options in case of an infection with bacteria carrying these genes. In particular, isolates coding for CTX-M-type ESBLs are often isolated from humans as well as from companion animals, livestock, wild animals and the environment, raising concerns regarding the exchange and spread of clonal isolates and/or plasmids between and among these host populations and habitats, respectively. To address this question, we performed a detailed, whole genome-based, molecular epidemiological analysis of CTX-M-15-producing *E. coli* isolated from various sources in Germany.

Whole genome sequencing using Illumina technology was performed for 397 CTX-M-producing *E. coli* isolates (CTX-M-1, n= 214, CTX-M-14, n=70, CTX-M-15, n= 113) from humans, companion animals, livestock, food and the farm environment. Based on the whole genome sequences, the multi-locus sequence type (ST) of these isolates and phylogeny based on single nucleotide polymorphisms (SNPs) was assessed. Additionally, genomes were scanned for virulence genes.

CTX-M-15-producing *E. coli* isolates were the most homogenous group of isolates reflected by the low number of different STs. Among these, ST410 was the most frequent ST (31/121) and was detected in isolates from humans (n=9), companion animals (n=4), livestock (n=8), food (n=4) and farm environments (n=6). Based on SNP analysis, five clades (A-E) were identified within the ST410 isolates. Isolates of clade B were present in all four populations and their core genomes differed by fewer than 75 SNPs. In addition, isolates of clade B and C were clonally marked by chromosomal insertion of the $bla_{\text{CTX-M-15}}$ gene either in the *rhs*E locus (clade B) or in a defective lambdoid prophage (clade C). Virulence genes found in

these isolates were mostly related to iron acquisition and adhesion to eukaryotic cells. All ST410 isolates displayed a fluoroquinolone resistance.

Our data provides strong evidence for circulation of fluoroquinolone-resistant CTX-M-15-producing *E. coli* ST410 clones encompassing different populations. These isolates are closely related to each other and display a broad-host-range including humans. As the isolates originated from diseased humans and animals, pathogenic potential of these isolates can be assumed. Further studies will address the presence of these clones in other European countries by investigating a larger set of isolates.

Contact: linda.falgenhauer@mikrobio.med.uni-giessen.de

2.12 Novel insights into the phylogenomics of ESBL-*E. coli* in the One Health Concept

Judith Schmiedel[1], Anita Windhorst[2], Hiren Ghosh[1], Swapnil Doijad[1], Moritz Fritzenwanker[1], Linda Falgenhauer[1], Can Imirzalioglu[1], Rolf Bauerfeind[3], Trinad Chakraborty[1]

[1] Justus Liebig University Giessen, Institute of Medical Microbiology and German Center for Infection Research, Partner site Giessen-Marburg-Langen
[2] Justus Liebig University Giessen, Institute of Medical computer science
[3] Justus Liebig University Giessen, Institute of Hygiene and Infectious Diseases of Animals

Extended-spectrum β-lactamase (ESBL)-producing multidrug-resistant *Escherichia (E.) coli* have become a significant problem in human and veterinary medicine. While many studies address the antimicrobial resistance characteristics of these bacteria, few studies have investigated their virulence properties. This study combines whole genome sequencing, phylogenomic analyses, a phenotypic virulence assay and statistical modelling to assess the pathogenic potential of ESBL-producing *E. coli* strains from human and animal sources (horses, dogs and cats).

101 *E. coli* isolates were submitted to whole genome sequencing. Assembled contigs were queried using in-silico methods. This included screening for selected virulence and resistance genes as well as multi-locus sequence typing. Concatenated multi-locus sequence typing (ConMLST) was used to uncover phylogenetic relationships between the isolates. Subsequently, 40 isolates (20 human and 20 animal isolates) were randomly chosen and tested for virulence in the *Galleria (G.) mellonella* model. To identify differences in larvicidal effects, Cox regression analysis was conducted and a stepwise multinominal logistic regression was used to identify over- or underabundant virulence factors. To evaluate the observed larvicidal effect in *G. mellonella*, growth in human serum was tested subsequently.

The majority of the tested 101 *E. coli* isolates carried genes or operons, respectively, encoding products that have been associated with bacterial iron metabolism, serum resistance and adhesion. More than half of the 40 in-depth investigated isolates were found to be clearly larvicidal as defined by killing larvae of *G. mellonella* within 24 hours after inoculation of the respective *E. coli* isolate. Cox regression identified the ConMLST phylogenetic group (PG) C as the most larvicidal, exhibiting a higher risk of larvicidal effects than the least larvicidal ConMLST PG A. The clonal cluster (CC) 23 was the most larvicidal CC. Occurrence of *iss* and *ompT* increased the hazard, while *usp, sitA* and *kpsMT II* gene cluster decreased the hazard for larvicidal effects in *G. mellonella*. Strains encoding only aerobactin show an increase in larvicidal effects compared to strains without any siderophore.

The identified genes and gene combinations are promising candidates to establish a genome-based scoring system for predicting virulence of ESBL-producing *E. coli* strains in ex-

traintestinal infection. Future studies will expand on these findings and serve to evaluate and refine the preliminary scoring system.

Contact: Judith.schmiedel@mikrobio.med.uni-giessen.de

2.13 Social networks in the pig barn – Implications for the infection dynamics of MRSA

Tobias Kaufholz[1], Maike Will[2], Thomas Selhorst[1]

[1] Federal Institute for Risk Assessment, Berlin
[2] University of Kiel, Kiel

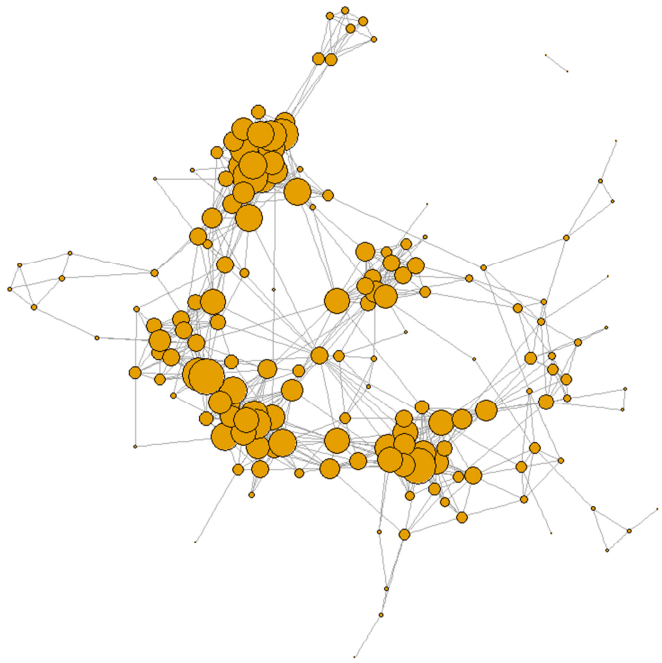
The spread of antibiotic resistant pathogens like MRSA are a major concern for public health, and the threat that livestock-associated variants pose is an emerging new challenge.

We can better understand the spread and dynamics of an infectious disease in a population by looking at the underlying contact network. Many studies focusing on the trade networks generated between livestock handling facilities have already been conducted. However, contact networks on the farm level, e.g. between livestock in a barn, have rarely been investigated since such investigations can only be carried out with considerable effort.

In the BMBF research project MedVetStaph, it is possible for us to conduct such a study. On a farm for the production of fattening pigs[1], roughly 200 sows were equipped with transponders. This allowed us to localise each individual animal inside a barn during ongoing production. The transponders have a frequency of 1Hz, which results in roughly 13-15m position measurements for all pigs every day.

Here, we want to present initial results which encompass the data collection and methods that we used to build contact networks out of the underlying movement data.
We show the preliminary insights of the pig contact networks in a barn. The characteristics and dynamics of the pig network and the possible implications on the spread of infectious diseases on animal contact networks will be presented in the talk.

[1] Lehr- und Versuchszentrum Futterkamp der Landwirtschaftskammer Schleswig-Holstein



A static snapshot of a possible realisation of a pig contact network, accumulated over one hour. Each node represents an individual pig, the size scaled with the node degree, and the edges between them show whether they had contact during the respective time frame.

2.14 Novel antimicrobial resistance genes among staphylococci in livestock environments

Stefan Schwarz[1], Kristina Kadlec[2], Andrea T. Feßler[1], Sarah Wendlandt[2], Wang Yang[3], Jianzhong Shen[2], Cong-Ming Wu[3]
[1] Freie Universität Berlin, Berlin
[2] Friedrich-Loeffler-Institut, Neustadt-Mariensee
[3] China Agricultural University, Beijing

Antimicrobial resistance is a major threat in human, but also in veterinary medicine. During recent years, a number of novel antimicrobial resistance genes have been detected among staphylococci of human and animal origin, which will be summarised in the following section. In 2012, two research groups detected a novel gene mediating methicillin resistance in staphylococci. This gene, first designated as *mecA*$_{LGA251}$, was later renamed as ***mecC***. After the initial finding of this gene in *Staphylococcus aureus* of human and bovine origin, it has also been detected in *S. aureus* of pet and companion animals as well as in coagulase-negative staphylococci (CoNS) from different sources including wildlife. Later on, two novel allotypes of *mecC* were seen, one in a *Staphylococcus xylosus* isolate from bovine mastitis (*mecC1*) and a methicillin-resistant *Staphylococcus saprophyticus* from a common shrew (*mecC2*). In addition, two novel spectinomycin resistance genes, both encoding spectinomycin adenyltransferases, were detected. The gene ***spw*** was found in methicillin-resistant *S. aureus* (MRSA) from pigs and chickens and the gene ***spd*** was detected not only in MRSA ST398 from humans and various animal species, but also in porcine MSSA ST433. Further studies detected the gene among *Staphylococcus hyicus* and CoNS from animals. The gene ***apmA*** is the first and, so far, only apramycin resistance gene in staphylococci coding for an acetyltransferase that confers resistance to apramycin and decreased susceptibility to gentamicin. To date, it has been detected in MRSA CC398 from pigs, cattle and food of poultry origin. During recent years, four genes mediating resistance to macrolides, lincosamides and streptogramin B were detected in staphylococci. The gene ***erm*(T)** was first described in staphylococci in 2010. It was located on a large multiresistance plasmid in a porcine MRSA CC398 isolate. In 2012, the gene ***erm*(43)** was detected in *Staphylococcus lentus* isolates of human, dog and chicken origin where it was located in the chromosomal DNA. The gene ***erm*(44)** was detected in the chromosomal DNA of an *S. xylosus* isolate from bovine mastitis. A novel *erm*(44) variant was detected in *S. saprophyticus* from river water samples. The gene ***erm*(45)** was detected in a genomic island in a *Staphylococcus fleurettii* isolate from bovine milk. Among staphylococci resistance to pleuromutilins, lincosamides and streptogramin A, antibiotics can be mediated by ABC transporter genes, some of which were first described among MRSA from pigs. The gene ***vga*(C)** was located on a multiresistance plasmid, whereas the gene ***vga*(E)** was located on the novel transposon Tn*6133.* Moreover, a variant of the *vga*(E) gene was detected in porcine *Staphylococcus cohnii* and *Staphylococ-*

cus simulans. The ***lsa*(E)** gene was found first in MRSA from pigs and poultry, but later also detected in various CoNS from dairy cattle. The gene ***sal*(A)**, originally described as a lincosamide-streptogramin A resistance gene, has meanwhile been shown to also confer pleuromutilin resistance. This gene was originally considered to be indigenous to *Staphylococcus sciuri* but has recently also been found in a feline *Staphylococcus haemolyticus* and in canine *Staphylococcus epidermidis* and *S. xylosus* isolates. Last but not least, the ***optrA*** gene, which mediates resistance to oxazolidinones (including tedizolid) and phenicoles, was first described in *Enterococcus faecalis* and *Enterococcus faecium* isolates of human and animal origin in China. The *optrA* gene was either located on plasmids and/or in the chromosomal DNA and has meanwhile been detected on a multiresistance plasmid of a porcine *S. sciuri* isolate. In addition to the *optrA* gene, the plasmid harboured the genes *cfr*, *fexA*, *aadD*, *ble* and *aacA-aphD*. The trimethoprim resistance gene ***dfrK*** was initially found on multiresistance plasmids from porcine MRSA ST398 where it was linked to the tetracycline resistance gene *tet*(L). It was also detected to be part of the small non-conjugative transposon Tn*558*.
The detection of novel resistance genes among staphylococci, their localisation on mobile genetic elements, and their co-localisation with other resistance genes increases the risk of co-transfer and co-selection of different resistance genes via a single horizontal gene transfer event under the selection of single antimicrobial agents.

2.15 Antibiotic resistance profiles of coagulase-negative staphylococci in livestock environments

S.M.K. Schoenfelder[1], Ying Dong[1], Andrea T. Feßler[2], Stefan Schwarz[2], Christoph Schoen[1], Robin Köck[3], Wilma Ziebuhr[1]
[1] University of Würzburg, Würzburg
[2] Freie Universität Berlin, Berlin
[3] University Hospital Münster, Münster

In light of the global emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), the question of resistance development needs to be re-addressed. One aspect is the dark matter of a potential resistance gene pool residing in other species co-existing in the livestock environment.
LA-MRSA can carry various novel and uncommon resistance genes against different antimicrobial classes, many of which are found on mobile genetic elements (MGE), e.g. transposons, plasmids, SCCs. The possibility of horizontal gene transfer (HGT) between strains and even species allows for very rapid development of resistant populations.
Coagulase-negative staphylococci (CoNS), often neglected and poorly understood regarding their resistance profile, can carry the same resistance genes as *S. aureus*, suggesting an ongoing genetic exchange between the species. CoNS might therefore function as reservoirs for the evolution and transfer of known or novel antibiotic resistance genes into other bacteria, such as LA-MRSA. In this study, we focused on CoNS samples taken from dust/manure in pig farms and analysed their antibiotic resistance profiles to assess their potential as putative resistance reservoirs.

We obtained samples from 41 pig farms (36/41 with a proven LA-MRSA/MSSA history) and found 18 different species among 344 isolates analysed. *S. sciuri* showed by far the greatest numbers (46%) and was detected in more than 80% of the farms.
Regarding the resistance profile, we found high resistance rates for tetracycline (71%), penicillin (65%) and oxacillin (64%), as well as fusidic acid (50%), due to a reduced susceptibility among the *S. sciuri* isolates. Six isolates showed linezolid resistance, with two carrying the multiresistance gene *cfr*, conferring simultaneous resistance to linezolid, phenicols, streptogramin A and pleuromutilin.
Among the *S. sciuri* isolates, all were resistant to at least 2/ 21 antibiotics tested, and most isolates were multiresistant: 151/158 showed phenotypic resistance to 3 or more and 40/158 even to 6 or more classes of antibiotics. Also, several isolates carried a number of uncommon (multi)resistance genes (*e.g*. *ampA*, *fexA* and *cfr*).
Most alarming was the low susceptibility of *S. sciuri* to one of the last resort antibiotics, daptomycin, with MICs ranging from 2 to 16 mg/L. In addition, we could identify two isolates with

clear daptomycin resistance (MIC of 64 and 128 mg/L, respectively). Ongoing work is now focusing on the potentially novel resistance mechanism of those two isolates.
Taken together, the data obtained suggest that *S. sciuri* harbours a significant resistance gene pool that requires further attention. This species might contribute to the spread of resistance genes in the livestock environment including, possibly, LA-MRSA.

2.16 Colonisation with MRSA CC398 among a cohort of veterinarians in Germany

Jan Walter[1], Werner Espelage[1], Christiane Cuny[1], Wolfgang Witte[1], Tim Eckmanns[1], Julia Hermes[1]

[1] Robert Koch-Institute, Berlin/Wernigerode

As part of the MetVetStaph Project IP9, a cohort of veterinarians and other persons involved in animal health care was recruited to participate in a study on risk factors, prevalence and persistence of nasal colonisation with MRSA attributed to clonal complex (CC) 398, as the most frequent livestock-associated MRSA in Central Europe. The original cohort included 1453 participants, recruited at three meetings of the German Federal Association for Veterinary Practitioners in 2008/2009. The baseline results of MRSA CC398 colonisation were published for the subgroup of 695 veterinarians with involvement in herd health management. MRSA CC398 was present in 9% of the participants. Risk factors for colonisation included working with swine (1).

Of the original cohort, 45 CC398-positive and 180 CC398-negative participants were recruited into the longitudinal part of the study with probing for nasal colonisation with CC398 in 2011, 2012 and 2014. The study also included the probing of household members of the participants for colonisation with MRSA CC398. We found consistent colonisation with CC398 in only 8 (26%) of 31 continuously tested and initially CC398-positive participants. Only 4 (13%) of them were colonised with the same *spa* type at all points in time, suggesting that colonisation with CC398 is largely transient. One hundred and eighty-five participants provided one or more samples of household contacts. Of these, household contacts in 21 (11%) different households were CC398-positive at least at one point in time. The odds for a CC398-positive household contact were 12 times higher (confidence interval 4-37) if the conference participant had been CC398-positive in 2008/2009 (2).

In summary, this prospective cohort study demonstrated the high risk for nasal colonisation with MRSA CC398 for veterinarians and the household members of colonised persons. With declining trends in healthcare-associated MRSA (3, 4), the relative proportion of LA MRSA among all MRSA infections is likely to increase in Germany in the future (3).

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2.17 Significance of environmental contaminations on the development of bacterial resistance to antibacterial agents in indicator animals

Jessica Meißner[1], Stefanie Mielke-Kuschow[1], Gesine Scherz[1,2], Georg Langenkämper[2], Husam Ibrahem Aroud[1,2], Dorle Link[1,2], Christine Schwake-Anduschus[2], Manfred Grote[3], Manfred Kietzmann[1]

[1] University of Veterinary Medicine Hannover, Foundation, Department of Pharmacology, Toxicology and Pharmacy
[2] Max Rubner-Institut, Department of Safety and Quality of Cereals, Detmold
[3] University of Paderborn, Department of Chemistry

The general aim of this project was to investigate which factors select for resistant bacteria in humans and farm animals, respectively. The results obtained so far in RESET I have demonstrated that animals exposed to antimicrobials in subtherapeutic concentrations pose an enhanced risk for the development of bacterial resistances. It was demonstrated that, during animal treatment (especially oral administration), antibiotic contaminations can be found in the direct environment of the animals, as well as in manure.

Therefore, the hypothesis that a low-level intake of drugs resulting from the ingestion of contaminated feed takes place and may result in the development of bacterial resistance was confirmed by our studies.

Based on the observations of RESET I that antibiotic residues in manured soil can be incorporated into crop plants, in particular enrofloxacin, the ingestion of contaminated plants by farm animals or humans constitutes a potential risk for the development of bacterial resistance.

Therefore, white cabbage plants were grown in nutrient solution and exposed to different concentrations of enrofloxacin. The plants were placed in a climate controlled growth cabinet for several days until the plant material was collected and freeze-dried. An *in vitro* setup was used to characterise the antibacterial effect and potential risk of bacterial resistance development of plant material enriched with antibiotic traces.

Furthermore, the freeze-dried plants were used for the *in vivo* study in poultry to gain information about the impact of antibiotic-containing plant material on the MIC of commensal E. coli during digestion. Furthermore, faecal samples of the animals were used and analysed for their content of enrofloxacin after cabbage feeding to obtain knowledge on enrofloxacin bioavailability after ingestion of plants.

Contact: jessica.meissner@tiho-hannover.de

2.18 New occurrence of VIM-1 producing *E. coli* in German pig production

Alexandra Irrgang[1], Jennie Fischer[1], Silvia Schmoger[1], Bernd-Alois Tenhagen[1], Mirjam Grobbel[1], Jens Hammerl[1], Annemarie Käsbohrer[1]

[1] Federal Institute for Risk Assessment, Department for Biological Safety, Berlin

Carbapenemase-producing Enterobactericeae (CPE) are of major concern in human medicine. CPEs are resistant to the majority of beta-lactam antibiotics and are often associated with a number of other resistance genes. There is worldwide 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. In fact, the first VIM-1 beta-lactamase-producing *E. coli* isolates from samples originating from livestock were detected within RESET 1. Subsequently, close monitoring of CPEs in livestock and food was recommended. In Germany, the national antimicrobial monitoring programmes on commensal *E. coli*, ESBL-producing *E. coli* and CPEs are established in the national reference laboratory for antibiotic resistance at the German Federal Institute for Risk Assessment. Within the RESET 2 project, isolates phenotypically resistant to carbapenemases were tested by real-time PCR for the presence of the five most abundant carbapenemase genes. One of the isolates (R1176) obtained from the colon content of a fattening pig taken in December 2015 was positive for the bla_{VIM} gene and sequencing revealed VIM-1 metallo-beta-lactamase. Xba 1 PFGE analysis indicated a close phylogenetic relationship of this isolate to isolates detected in the years 2011/2012. In contrast to those in isolate R1176, the VIM-encoding integron was located on the chromosome. After these findings, another slaughter batch from the same fattening farm was examined in April 2016 and further bla_{VIM-1} positive *E. coli* could be isolated (R1177-1180). Again, a close phylogenetic relationship was observed but these isolates harboured the bla_{VIM-1} gene on typical 180-200 kb IncHI2 plasmids, comparable to the isolates detected in 2011/2012.

As a consequence of these findings, composite faecal samples from all pens and barns of the fattening farm were taken and screened for the presence of VIM-1 encoding *E. coli*. It was possible to detect VIM-1-producing isolates from four samples of three different barns. Isolates were further characterised by PCR, PFGE, Southern Blot Hybridisation and whole genome sequencing.

XbaI PFGE revealed a clonal spread of VIM-1-producing *E. coli* on the farm. S1 PFGE displayed certain variability in the modular organisation of the plasmid. Resistance to carbapenems was proved to be stable in vitro without antibiotic pressure for at least three months, which may be based on several identified chromosomal and plasmidal persistence factors. Whole genome sequencing analysis revealed a close genetic relationship between

the strains but high variability in the composition of the VIM-associated sequences. Genetic analysis outlines the mechanism of evolution and dissemination of VIM-1 producing *E. coli*. In conclusion, VIM-1 producing *E. coli* are still present in German pig farming. Persistence in one farm over a period of at least five months and transmission between different barns of the farm was observed. As the bacteria were isolated from subsequent herds housed in the same barn and originating from the same breeding herd, failure of cleaning procedures to eliminate the bacteria from the environment as well as repeated import of colonised pigs from the breeding herd could be involved.

Contact: alexandra.irrgang@bfr.bund.de

2.19 Investigation of extended-spectrum β-lactamase (ESBL) and plasmid-mediated quinolone resistance (PMQR) gene-carrying plasmids in *Escherichia coli* and *Salmonella enterica* from diseased animals: their role in antimicrobial resistance, biocide tolerance and virulence of the isolates

Geovana Brenner Michael[1,2], Heike Kaspar[3], Amanda Keller Siqueira[2,4], Kristina Kadlec[2], Stefan Schwarz[1,2]

[1] Freie Universität Berlin, Institute of Microbiology and Epizootics
[2] Friedrich-Loeffler-Institut, Institute of Farm Animal Genetics, Neustadt-Mariensee
[3] Federal Office of Consumer Protection and Food Safety, Berlin
[4] Universidade Federal da Fronteira Sul, Laranjeiras do Sul, Brazil

Food-producing animals have been reported as significant reservoirs of antimicrobial-resistant bacteria. In Germany, β-lactam antibiotics are the most frequently sold antimicrobial agents in veterinary medicine, followed by tetracyclines and sulfonamides. Extended-spectrum β-lactamase (ESBL)-producing isolates have gained increased attention due to the risks they represent to human and animal health. Since plasmids play an important role in the dissemination of ESBL genes and plasmid-mediated quinolone resistance (PMQR) genes, the main objective of this individual project of the RESET consortium was the investigation of ESBL and PMQR gene-carrying plasmids in *Escherichia coli* and *Salmonella enterica* from diseased food-producing animals, with particular reference to their role in (a) antimicrobial resistance, (b) biocide tolerance and (c) virulence. In contrast to *E. coli* isolates, no putative ESBL-producing *S. enterica* isolates were identified by our cooperation partners in diseased food-producing animals. Instead, a total of 7,616 *E. coli* isolates, collected from diseased cattle, pigs and poultry in the German national monitoring programme GE*RM*-Vet (2008-2015), were characterised by antimicrobial susceptibility testing and screened for the ESBL phenotype during RESET I and II. ESBL genes were identified by PCR and sequencing. All ESBL-producing isolates were further characterised by PCR-based phylotyping, and 210 isolates and their respective ESBL gene-carrying plasmids were further characterised by phenotypic and genotypic methods. The 513/7,616 ESBL-producers identified included 395/3,062 isolates from cattle, 95/1,833 from pigs and 23/2,721 from poultry. Most of the ESBL-producing isolates were from animals suffering from gastrointestinal infections. The ESBL genes most commonly detected were: $bla_{\text{CTX-M-1}}$ (68.4%), $bla_{\text{CTX-M-15}}$ (14.9%) and $bla_{\text{CTX-M-14}}$ (11.9%). The $bla_{\text{CTX-M-1}}$ gene was detected most frequently over time and in *E. coli* from all animal origins, whereas the $bla_{\text{CTX-M-14}}$ gene was exclusively found in bovine isolates. The phylogroup A was the dominant group among the isolates of all animal origins. In-depth characterisation of the 210 ESBL-producing isolates revealed that all ESBL genes were located on plasmids, except one $bla_{\text{CTX-M-1}}$ and eleven $bla_{\text{CTX-M-15}}$ genes which were located in the chromosomal DNA. In 486/513 ESBL-positive isolates and in 126/198 of the characterised ESBL gene-carrying plasmids, additional resistance to non-β-lactam antibiotics (espe-

cially to tetracyclines, sulfonamides and trimethoprim) was seen. The PMQR genes *qnrS1* (n=5) and *aac(6')-Ib-cr* (n=9) were detected on ESBL gene-carrying plasmids.
Special attention was given to the CTX-M-14-producing *E. coli* isolates from diseased cattle. They were compared to isolates of healthy cattle, which were sampled and initially characterised by groups of the RESET consortium (TiHo-Epi and BfR-Resi). Clonal relatedness (sequence type ST744) was found among the isolates of healthy animals harbouring similar $bla_{CTX\text{-}M\text{-}14}$-carrying plasmids (incompatibility group IncF, ca. 70 kb), suggesting a clonal expansion of isolates among healthy animals. The finding of such similar plasmids in unrelated isolates (e.g. ST362) of diseased cattle indicates a horizontal spread of plasmids among bovine isolates. None of the $bla_{CTX\text{-}M\text{-}14}$-carrying plasmids, from both healthy and diseased animals, carried co-located virulence genes.
Interestingly, a comparison between CTX-M-15-producing *E. coli* isolates from diseased animals and human clinical isolates [provided by the RESET groups of the JLU Gießen and RKI-Wernigerode] showed similar minimal inhibitory concentrations (MICs) to the biocides benzalkonium chloride (MIC 20-80 mg/L), chlorhexidine (MIC 0.5-16 mg/L), isopropanol (MIC 4-8 mg/L) and glutaraldehyde (MIC 625-2500 mg/L). For these investigations, a protocol for biocide susceptibility testing of bacteria by broth macrodilution was developed in cooperation with partner IP6 of the MedVet-Staph consortium and validated via an interlaboratory trial.
The main conclusions of this study are as follows: (a) The presence of additional antimicrobial resistance on ESBL-carrying plasmids may compromise the therapeutic options and underlines the risks of co-selection, persistence and dissemination of ESBL genes even in the absence of direct selective pressure imposed by the use of β-lactam antibiotics, and (b) clonal expansion of ESBL-producing isolates and the horizontal spread of ESBL gene-carrying plasmids seem to play an important role in the dissemination of ESBL genes in Germany.

Contact: stefan.schwarz@fu-berlin.de, g.brenner.michael@fu-berlin.de

2.20 Possibilities and limits of logistic regression for the study of the transmission dynamics of ESBL/AmpC-producing *E. coli* between broiler flocks

Guido Correia Carreira[1], Carolina Plaza-Rodriguez[1], Annemarie Käsbohrer[1]
[1] Federal Institute for Risk Assessment, Department Biological Safety, Berlin

This contribution presents how the technique of logistic regression was used in the development of an epidemiological model of the transmission dynamics of ESBL/AmpC-producing *E. coli* in broiler flocks along the broiler production chain.

The developed model considered the broiler production chain from the parent generation through the hatchery and broiler fattening farm up to the transport of broilers to the slaughterhouse. Among other things, the model keeps track of the prevalence of colonisation with ESBL/AmpC-producing *E. coli* among flocks. The flock prevalence is changed at each stage of the modelled production chain, for example by simulating the effect of horizontal transmission of bacteria between flocks using a logistic regression model.

Logistic regression has previously been used by other authors in order to describe the horizontal transmission of Campylobacter between broiler flocks on broiler fattening farms. This approach was adapted to the transmission model for ESBL/AmpC-producing *E. coli*. However, to parameterise the latter, one could not simply adopt the regression parameters from the original Campylobacter case. Therefore, we investigated how the adopted logistic regression model (and its regression parameters) reacted to systematically fabricated test data in order to obtain a better understanding of how the regression parameters change under different theoretical scenarios. This understanding is important for interpreting the results of the model, since the parameters of a logistic regression model generally have no direct real world interpretation. Therefore, the investigation of the reaction of the model (parameters) to given theoretical data is used to get a reasonable idea about what constitute useful parameter values for different scenarios and where the limitations lie.

Our work shows how the regression model is useful in predicting transmission dynamics on the basis of a relatively low information input and giving indications of where further investigation for understanding the bacterial transmission dynamics might be promising. On the other hand, it is the low information input that limits the established regression model in its ability to help us understand the workings of the inner biological processes of the transmission process of bacteria as well as to assess the impact of specific on-farm interventions.

Contact: correia-carreira@bfr.bund.de

2.21 Assessment of the foodborne transmission pathway for ESBLs

Annemarie Käsbohrer[1,2], Carolina Plaza-Rodriguez[1], Hannah Sharp[1], Lars Valentin[1], Alexandra Irrgang[1], Jennie Fischer[1], Alexandra Fetsch[1], Guido Correia Carreira[1]

[1] Federal Institute for Risk Assessment, Department for Biological Safety, Berlin
[2] University of Veterinary Medicine, Institute for Veterinary Public Health, Vienna, Austria

Extended-spectrum beta-lactamases (ESBL)-producing *E. coli* are found frequently in healthy animals, foodstuffs, humans and the environment. This rapid global distribution of the ESBL-producing *E. coli* has attracted significant attention. More and more, the effectiveness of antibiotics is being limited due to the increasing availability of resistance genes, becoming more critical due to horizontal gene transfer of resistance genes to different bacterial clones and species. Furthermore, horizontal gene transfer complicates the assessment of the relevance of individual pathways.

Up to now, there is little agreement in the scientific community on the role of livestock animals and food derived from them as a source of ESBL-producing *E. coli* for human colonisation or infection. Depending on the approach taken, different perceptions are published. Based on molecular typing, the same resistance genes, the same plasmid families and *E. coli* of the same MLST type are described in livestock, food of animal origin and humans. This is seen as evidence of their spread via the food chain.

Within our research, we followed two approaches to assess the role of the foodborne pathway: (1) the assessment of transfer of ESBLs along the broiler meat production chain and (2) the assessment of the contribution of different animal species by a source attribution approach.

The study of the interplay between different broiler production stages is important to understand the transmission mechanisms along the food chain. In this context, mathematical models can help to analyse these relationships. Modelling the transmission of ESBL-producing *E. coli* in the broiler production chain allowed assessment of the possible impact of vertical and horizontal transmission.[1,2] Furthermore, assessment of the next processing steps confirmed that the magnitude of transfer from livestock to poultry meat might reach a sufficient level of contamination for colonisation or even infection of consumers.[3]

Experimental work documented further that during handling in household kitchens, transfer of ESBLs via hands and equipment and from there to food intended to be eaten raw occurs once contaminated broiler meat is introduced. Thus, consumers may be at risk during handling and/or ingestion. [4]

In an additional study, we addressed the risk related to the spread of cephalosporin- or fluoroquinolone-resistant *E. coli* in commercial kitchens after introduction via contaminated raw poultry meat. It was shown that these resistant Enterobacteriaceae are frequently introduced

into the kitchen, but their spread seems to be limited to positions which are directly linked to the processing and preparation of a meal. Nevertheless, our results underline the necessity of strict separation of working areas and equipment used in commercial kitchens.[5]

To further assess the impact of livestock on human exposure to ESBL-producing bacteria, typing data were used for source attribution approaches. Comparison of isolates from livestock populations with those from food of animal origin reflected that similar resistance gene patterns can be observed in both populations. Analysis of data showed that *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{SHV-12}}$ and AmpC *bla*$_{\text{CMY-2}}$ were the most abundant beta-lactamase genes conferring cefotaxime resistance. Swine, cattle and products thereof were mainly contaminated with *bla*$_{\text{CTX-M-1}}$carrying *E. coli* and to a lesser extent with *bla*$_{\text{CTX-M-15}}$ harbouring isolates. In broilers and poultry meat, besides CTX-M-1, CMY-2 was the most frequently detected enzyme, followed by SHV-12.[6]

Results of a comparative analysis showed that most ESBL genes were found in both human and non-human populations but quantitative differences for distinct ESBL types were detectable. More than 70% of the animal isolates and more than 50% of the human isolates contained the broadly distributed ESBL genes. While the majority of animal isolates carried *bla*$_{\text{CTX-M-1}}$, this was the case for only a limited number of the human isolates. In contrast, *bla*$_{\text{CTX-M-15}}$ was more frequent in human isolates. When *E. coli* subtype definition included ESBL types, phylogenetic grouping and antimicrobial susceptibility data, the proportion of isolates allocated to common clusters was markedly reduced. Nevertheless, relevant proportions of the same subtypes were detected in isolates from the human, livestock and companion animal populations included in this study, suggesting exchange of bacteria or bacterial genes between these populations or a common reservoir.[7]

High detection rates of *bla*$_{\text{CTX-M-1}}$ genes in all livestock and food sources allow no clear conclusion on their individual pathways. Occurrence of ESBL- and AmpC-producing isolates in livestock and food of animal origin may present a public health risk. Further in-depth molecular analysis is currently being carried out to obtain evidence of the extent to which food-producing animals represent a source of ESBL- and AmpC-producing *E. coli* isolates in foodstuffs in Germany. Current knowledge indicates a limited contribution of the food chain to colonisation of humans with ESBL-/AmpC-producing *E. coli,* but quantification of this is still required.

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2.22 Breaking the species barrier: Epidemiological dynamics and genetic changes in livestock-associated MRSA

Sarah van Alen[1], Britta Ballhausen[2], Ursula Kaspar[1], Evgeny A. Idelevich [1], Andreas Schlattmann[1], Georg Peters[1], Robin Köck[1], Karsten Becker[1]

[1] University Hospital Münster, Münster
[2] Federal Institute for Risk Assessment, Berlin

During the last decade, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) of the clonal complex CC398 have emerged in areas with high livestock density and have been introduced into the human healthcare system (Köck et al., 2013) . A prospective study showed that LA-MRSA increased significantly in Germany originating from the north-western regions (Schaumburg et al., 2012). The University Hospital of Münster is located in the pig-dense Dutch-German border region, which is a hotspot for the occurrence of MRSA CC398. Within the past 15 years, the proportion of MRSA isolates from patients who belong to the clonal complex 398 (comprising 45 different *spa*-types) rose to 30% (van Alen et al., 2016).

Little is known about factors enabling the (re-)adaptation and the epidemiological success of this zoonotic MRSA lineage. Therefore, we investigated the influence of different factors such as the acquisition of mobile genetic elements (e.g. bacteriophages) or resistance and virulence genes on the success of LA-MRSA CC398. Our investigations on the virulence characteristics revealed a high cytotoxic potential of LA-MRSA CC398 isolates as compared to that of community-associated (CA)-MRSA strains (Ballhausen et al., 2016). Moreover, it was observed that LA-MRSA CC398 has the capability to cause a similarly broad spectrum of infections as other MSSA and MRSA clonal lineages such as bacteraemia, endocarditis, pneumonia, thoracic empyema, osteomyelitis and prosthetic joint infections, urinary tract infections and various skin infections including abscesses and necrotising fasciitis (Becker et al., 2017).

The virulence and host adaptation of LA-MRSA CC398 might be influenced by the acquisition and incorporation of mobile genetic elements such as bacteriophages in the genome of MRSA CC398 strains. The bacteriophage *phi3* encodes the human-specific immune-evasion cluster (IEC) associated genes *sak*, *chp* and *scn,* which counteract with the innate immune response of the human host. Integration of this phage might thus influence the adaptation of LA-MRSA CC398 back to the human host. During epidemiological screening over 15 years and including the very first CC398 isolates, 572 MRSA CC398 belonging to different *spa*-types were screened for a truncated *hlb*-gene indicating the presence of bacteriophage *phi3*. Overall, the percentage of *phi3*-carrying isolates was low (2.1%), indicating that bacteriophage phi3 is not the key driver for re-adaptation.

Another selective advantage could be decreased heavy-metal susceptibility in MRSA CC398 caused by the extensive use of these compounds in livestock. MIC determination showed a decreased susceptibility of MRSA CC398 to zinc chloride compared to other clonal lineages, and no differences regarding the phenotypic resistance were found for copper sulphate and nickel chloride. Over the past 15 years, an increasing number of zinc-resistant LA-MRSA CC398 has been detected with all isolates found to carry the *czrC* gene, which is responsible for zinc and cadmium resistance in *S. aureus*.

Apart from CC398 strains, *S. aureus* isolates recovered from livestock were found to carry a further beta-lactam resistance determinant named *mecC*. For these *mecC*-MRSA, the underlying genetic basis leading to beta-lactam resistance was studied. By knockout and complementation experiments, we could show that the *mecA* homolog *mecC* confers resistance to beta-lactams irrespective of the genetic strain background. (Ballhausen et al., 2014).

Overall, *S. aureus* arose as a notable zoonotic pathogen. LA-MRSA CC398 and *mecC*-MRSA emerged within the past 15 years resulting in a substantial medical and economic burden. The changing genetic equipment seems to be closely connected to the adaptation processes to new hosts and environments, which promote the epidemiological spread.

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2.23 MRSA in equine clinics and the impact for humans

Christine Cuny[1]
[1] Robert Koch Institute, Wernigerode

The abstract was not present at the time of the editorial.

2.24 Occurrence of *cfr*-encoded linezolid resistance in coagulase-negative staphylococci from livestock and exposed humans

Wolfgang Witte[1], Andrea T. Feßler[2], Stefan Schwarz[2], Franziska Layer[1], Jaishri Mehraj[3], Julia Hermes[1], Christiane Cuny[1]

[1] Robert Koch-Institute, Berlin/Wernigerode
[2] Freie Universität Berlin, Berlin
[3] Helmholtz Centre for Infection Research, Braunschweig

Oxazolidinones (linezolid, tedizolid) are antibiotics of last resort for the treatment of complicated soft tissue infections and pneumonia caused by multiresistant *S. aureus*. There are several mechanisms mediating oxazolidinone resistance. Of particular significance are those coded by transferable resistance genes such as *cfr* and *optr*A.

Here we report the emergence of linezolid-resistant coagulase-negative staphylococci (CoNS) containing the multiresistance gene *cfr* in veal calves and pigs, as well as in humans exposed to these animals.

CoNS (*S. auricularis, S. cohnii, S. lentus, S. kloosii, S. sciuri, S. simulans*), but not *S. aureus*, carrying the gene *cfr* but not *optr*A, were detected in samples of 12 out of 52 calves at three farms which had a history of florfenicol use. Nasal swabs from 10 humans living on these farms were negative for *cfr*-carrying staphylococci. Nasal swabs taken from 42 calves at 16 farms in the same area that did not use florfenicol were also negative for *cfr*-carrying staphylococci.

The *cfr*-carrying CoNS (*S. kloosii, S. saprophyticus, S. simulans*) were also detected in three of eight conventional pig farms investigated. One of 12 humans living on these farms harboured a *cfr*-carrying *S. cohnii.*

Among the nasal swabs taken from 169 veterinarians from all over Germany, four (2.3%) were positive for *cfr*-carrying CoNS (three *S. epidermidis,* one *S. saprophyticus*), and three (1.1%) of 263 contact persons of this group also harboured *cfr*-carrying CoNS (one *S. epidermidis,* two *S. saprophyticus*). These isolates were also negative for *optr*A.

As to be expected, all of the *cfr*-containing isolates were resistant to retapamulin, 56% of them also to fusidic acid.

In vitro conjugation of *cfr* by filter mating to *S. aureus* 8325-4 was possible for 10 of 34 CoNS and the *cfr* gene was associated with plasmids of 38 – 40 kb.

In order to get an idea of the probable spread of *cfr* among staphylococci as nasal colonisers in the community, a total of 363 nasal swabs from humans of a German municipal community were investigated, all of them revealed as negative.

3 List of Poster Presentations

3.1 RESET Poster

Comparative analysis of CTX-M-1 producing *E. coli* and associated plasmids originating from German livestock farms

Jennie Fischer[1], Noemi Alonso[2], Silvia Schmoger[1], Beatrice Baumann[1], Lothar Kreienbrock[3], Bernd Appel[1], Annemarie Kaesbohrer[1], Uwe Roesler[4], Reiner Helmuth[1], Beatriz Guerra[1]

[1] Federal Institute for Risk Assessment, BfR, Germany
[2] Hospital de la Santa Creu i Santa Pau, Barcelona, Spain
[3] Department of Biometry, Epidemiology and Information Processing WHO-Collaborating Center for Research and Training for Health at the Human-Animal-Environment Interface
University of Veterinary Medicine Hannover, Hannover, Germany
[4] Institute for Animal Hygiene and Environmental Health, Freie Universitaet Berlin

Occurrence of ESBL-, AmpC- and carbapenemase-encoding genes in *E. coli* isolates from animal-derived food products in Germany

Jennie Fischer[1], Mateo San José[1,2], Elisabeth Guiral[1,3], Alexandra Irrgang[1], Silvia Schmoger[1], Beatriz Guerra[1], Annemarie Kaesbohrer[1]

[1] Federal Institute for Risk Assessment, BfR, Germany
[2] University of Reading, Reading (Berkshire), United Kingdom
[3] CRESIB, Hospital Clínic-Universitat de Barcelona, Spain

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

Alexandra Irrgang[1], Jennie Fischer[1], Silvia Schmoger[1], Beatriz Guerra[1], Annemarie Käsbohrer[1]

[1] Federal Institute for Risk Assessment, BfR, Germany

Distribution of ESBL-/AmpC-producing *Enterobacteriaceae* along the broiler production chain

Katrin Dähre[1], Michaela Projahn[1], Philine von Tippelskirch[2], Greta Gölz[2], Stefanie Orquera[2], Thomas Alter[2], Anika Friese[1], Uwe Rösler[1]

[1] Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin
[2] Institute for Food Safety and Food Hygiene, Freie Universität Berlin

Prevalence and quantitative analysis of ESBL and AmpC β-lactamase producing *Enterobacteriaceae* in poultry during slaughter

Philine von Tippelskirch[1], Greta Gölz[1], Stefanie Orquera[1], Michaela Projahn[2], Katrin Dähre[2], Anika Friese[2], Uwe Rösler[2], Thomas Alter[1]

[1] Institute of Food Safety and Food Hygiene, Freie Universitaet Berlin
[2] Institute for Animal Hygiene and Environmental Health, Freie Universitaet Berlin

Detection of extended-spectrum β-lactamases (ESBLs)-producing Escherichia coli isolates in diseased food-producing animals in GERM-Vet 2008-2015.

Geovana Brenner Michael[1,2], Amanda Keller Siqueira[2,4], Heike Kaspar[3], Kristina Kadlec[2] and Stefan Schwarz[1,2]

[1] Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany
[2] Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany
[3] Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany
[4] Universidade Federal da Fronteira Sul, Laranjeiras do Sul, Brazil

Characterisation of plasmids encoding CTX-M-14 extended-spectrum β-lactamase (ESBL) detected in *Escherichia coli* isolates from healthy and diseased cattle.

Geovana Brenner Michael[1,2], Heike Kaspar[3], Kristina Kadlec[2] and Stefan Schwarz[1,2]

[1] Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany
[2] Institute of Farm Animal Genetics, Friedrich-Loeffler Institute (FLI), Neustadt-Mariensee, Germany
[3] Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

Location of *bla*$_{CTX-M-1}$ and *qnrS1* genes on IncN plasmids from clinical *Escherichia coli* isolates collected from diseased animals.

Geovana Brenner Michael[1,2], Heike Kaspar[3], Kristina Kadlec[2] and Stefan Schwarz[1,2]

[1] Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany
[2] Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany
[3] Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

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

Yvonne Pfeifer[1], Michael Pietsch[1], Andre Frühauf[1], Nicole Roschanski[2], Uwe Roesler[2], Klaus-Peter Hunfeld[3], Norman Lippmann[4], Sebastian Wendt[4], Corinna Woelke[5], Amelie Eisele[6], Axel Kola[6], Guido Werner[1]

[1] Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany
[2] Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany
[3] Krankenhaus Nordwest, Central Institute of Laboratory Medicine, Microbiology & Hospital Hygiene, Frankfurt (Main), Germany
[4] Universitätsklinikum Leipzig, Institute of Medical Microbiology and Infection Epidemiology, Leipzig, Germany
[5] MVZ Diamedes GmbH, Bielefeld, Germany
[6] Charité Universitätsmedizin Berlin, Institute of Hygiene and Environmental Medicine, Berlin, Germany

Investigations on bacterial fitness of *Escherichia coli* with plasmid-mediated beta-lactam resistance

Michael Pietsch[1], Yvonne Pfeifer[1], Stephan Fuchs[1], Guido Werner[1]

[1] Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany

The RESET research database - Comprehensive microbiological and epidemiological information on ESBL-producing *Escherichia col*i from Germany

Katja Hille[1], Uwe Seibt[2], Walther Honscha[2], Lothar Kreienbrock[1] for the RESET consortium www.reset-verbund.de

[1] Department of Biometry, Epidemiology and Information Processing, WHO-Collaborating Center for Research and Training for Health at the Human-Animal-Environment Interface, University of Veterinary Medicine Hannover, Hannover, Germany
[2] Faculty of Veterinary Medicine, Institute of Pharmacology, Pharmacy and Toxicology, University Leipzig, Leipzig, Germany

The antibiotic ENROFLOXACIN incorporated in white cabbage: high uptake potential and inhibitory effect on *E. coli*

Georg Langenkämper[1], Husam Ibrahem Aroud[1,2], Dorothea Link[1,2], Christine Schwake-Anduschus[1], Gesine Scherz[2], Jessica Meißner[2], Stefanie Mielke-Kuschow[2], Manfred Kietzmann[2], Manfred Grote[3]

[1] Department of Safety and Quality of Cereals, Max Rubner Institute, Detmold, Germany
[2] Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Foundation
[3] Department of Chemistry, University of Paderborn, Paderborn, Germany

Molecular characterisation of extended-spectrum beta-lactamase producing *E. coli* sequence Type 131 (ST131) of human and companion animal origin in Germany.

Hiren Ghosh[1], Swapnil Doijad[1], Boyke Bunk[2], Linda Falgenhauer[1], Yancheng Yao[1], Cathrin Sproer[2], Katrin Gentil[1], Judith Schmiedel[1], Alexander Goesmann,[3] Can Imirzalioglu[1], Jörg Overmann[2], Trinad Chakraborty[1]

[1] Institute of Medical Microbiology, Justus Liebig University Giessen, and German Center for Infection Research, Partner Site Giessen-Marburg-Langen, Campus Giessen, Giessen, Germany
[2] Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, and German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig, Braunschweig, Germany
[3] Institute of Bioinformatics and Systems Biology, Justus Liebig University Giessen, Giessen, Germany

Comparative analysis of extended-spectrum beta-lactamase and carbapenemase-encoding plasmids from humans, companion animals and livestock using next generation sequencing

Linda Falgenhauer, Judith Schmiedel, Hiren Ghosh, Yancheng Yao, Swapnil Doijad, Moritz Fritzenwanker, Can Imirzalioglu, Trinad Chakraborty

[1] Institute of Medical Microbiology, Justus Liebig University Giessen, and German Center for Infection Research, Partner Site Giessen-Marburg-Langen, Campus Giessen, Giessen, Germany

Detection of translocatable units in a blaCTX−M−15 extended-spectrum beta-lactamase-producing ST131 Escherichia coli dog isolate

Hiren Ghosh[1], Swapnil Doijad[1], Boyke Bunk[2], Linda Falgenhauer[1], Yancheng Yao[1], Cathrin Sproer[2], Katrin Gentil[1], Judith Schmiedel[1], Can Imirzalioglu[1], Jörg Overmann[2], Trinad Chakraborty[1]

[1] Institute of Medical Microbiology, Justus Liebig University Giessen, and German Center for Infection Research, Partner Site Giessen-Marburg-Langen, Campus Giessen, Giessen, Germany
[2] Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, and German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig, Braunschweig, Germany

Characterisation of *mcr-1* and ESBL-producing isolates from humans, companion animals and food

Linda Falgenhauer, Judith Schmiedel, Hiren Ghosh, Yancheng Yao, Swapnil Doijad, Moritz Fritzenwanker, Can Imirzalioglu, Trinad Chakraborty for the RESET consortium

[1] Institute of Medical Microbiology, Justus Liebig University Giessen, and German Center for Infection Research, Partner Site Giessen-Marburg-Langen, Campus Giessen, Giessen, Germany

3.2 MedVet-Staph Poster

Analysis of bacteriophage *phi3* in MRSA CC398

S. van Alen[1], B. Ballhausen[2], R. Köck[3], G. Peters[1], K. Becker[1]

[1] Institute of Medical Microbiology, University Hospital Münster, Münster, Germany
[2] Federal Institute for Risk Assessment (BfR), Department Biological Safety, Unit Microbial Toxins, Berlin, Germany
[3] Institute of Hospital Hygiene, University of Oldenburg, European Medical School Oldenburg-Groningen, Oldenburg, Germany

Analysis of heavy metal compounds triggering the success of MRSA CC398 in healthcare settings

S. van Alen[1], R. Köck[1,2], E.A. Idelevich[1], U. Kaspar[1] , G. Peters[1], K. Becker[1]

[1] Institute of Medical Microbiology, University Hospital Münster, Münster, Germany
[2] Institute of Hospital Hygiene, University of Oldenburg, European Medical School Oldenburg-Groningen, Oldenburg, Germany

Staphylococcus aureus adhesion capacity: A biophysical approach

Philipp Jung[1], Henrik Peisker[1], Janina Eisenbeis[1], Nicolas Thewes[2], Peter Loskill[2], Christian Spengler[2], Mathias Herrmann[1], Karin Jacobs[2], Markus Bischoff[1]

[1] Institute for Medical Microbiology and Hygiene, University of Saarland, Homburg/Saar, Germany
[2] Experimental Physics, University of Saarland, Saarbrücken, Germany

Characterisation of opportunistic pathogens of the nasal cultural microbiome of dog and cat owners vs. other participants of a German general population cohort

R. Köck[1*], F. Schaumburg[1], C. Fegeler[2], A. W. Friedrich[3], K. Becker[1] and Prevalence of Multiresistant Microorganisms (PMM) Study Group[4]

[1] University Hospital Münster, Institute of Medical Microbiology, Münster
[2] Heilbronn University, GECKO Institute of Medicine, Informatics and Economy, Heilbronn
[3] University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen
[4] Prevalence of Multiresistant Microorganisms (PMM) Study Group: O. Bindewald, T. T. Bui, C. Eckhoff, R. Epping, L. Kähmann, M. Meurer, P. Werner, J. Steger, L. von Auenmüller, Institute of Medical Microbiology, Münster, and P. Werner, Heilbronn University, GECKO Institute of Medicine, Informatics and Economy, Heilbronn
[*] present address: Institute of Hospital Hygiene, University of Oldenburg, European Medical School Oldenburg-Groningen, Oldenburg

Comparative analysis of canine and feline methicillin-resistant Staphylococcus pseudintermedius (MRSP) from Thailand

Kadlec K1, Weiß S1, Wendlandt S1, Schwarz S1,2, Tonpitak W3

[1] Institute of Farm Animal Genetics, Friedrich Loeffler Institute (FLI), Neustadt-Mariensee, Germany
[2] Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin Germany
[3] Department of Microbiology, Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand

Rifampicin resistance in multi-resistant porcine livestock-associated methicillin-resistant *Staphylococcus aureus* from China

Li J[1,2], Feßler AT[2,3], Jiang N[1], Fan R[1], Wang Y[1], Wu C[1], Shen J[1], Schwarz S[2,3].

[1] Beijing Key Laboratory of Detection Technology for Animal-Derived Food Safety, College of Veterinary Medicine, China Agricultural University, Beijing, P. R. China;
[2] Institute of Farm Animal Genetics, Friedrich Loeffler Institute (FLI), Neustadt-Mariensee, Germany
[3] Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin Germany

Development of a biocide susceptibility testing method for bacteria

Feßler AT[1,2], Geber F[3], Meurer M[1], Hensel V[1], Speck S[3], Reinhardt M[3], Michael GB[1,2], Truyen U[3], Schwarz S[1,2]

[1] Institute of Farm Animal Genetics, Friedrich Loeffler Institute (FLI), Neustadt-Mariensee, Germany
[2] Institute of Microbiology and Epizootics, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
[3] Institute of Animal Hygiene and Veterinary Public Health, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

Targeted hygiene management system in a horse clinic: Rapid beneficial effects

Katja-Sophia Klein[1], Birgit Walther[2], Antina Lübke-Becker[2], Heidrun Gehlen[1]
[1] Equine Clinic and
[2] Institute of Microbiology and Epizootics, Freie Universität Berlin

MRSA in equine clinics and the impact for humans

C. Cuny[1], Scheidemann W[2], Layer F[1]

[1] Robert Koch-Institut, Wernigerode
[2] Tierärztliche Klinik Domäne Karthaus, Dülmen

4 Index of Authors