Final Program and Abstracts

ASM CONFERENCES

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

UNIVERSITY OF COPENHAGEN

3rd ASM-ESCMID Conference on

Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications

November 4 – 7, 2013
Copenhagen, Denmark

1752 N Street, N.W.
Washington, DC 20036-2904
Phone: 202-737-3600
World Wide Web: www.asm.org

ASM CONFERENCES

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ASM Conferences Committee

ASM Conferences Mission

To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 500 people.

To encourage student and postdoctoral participation.

To recruit individuals in disciplines not already involved in ASM to ASM membership.

To foster interdisciplinary and international exchange and collaboration with other scientific organizations.

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Acknowledgments

The American Society for Microbiology and the European Society of Clinical Microbiology and Infectious Diseases gratefully acknowledge the following sponsors of the 3rd ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications. On behalf both organizations, our leadership and members, we thank them for their financial support:

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General Information

REGISTRATION AND NAME BADGES

ASM Staff will be available at the registration desk in the Ceremonial Hall during session hours. Participants may collect name badges and program materials at the registration desk. A name badge is required for entry into all sessions, meals, and social events.

GENERAL SESSIONS

All general sessions will be held in the Ceremonial Hall.

POSTER SESSIONS

Poster boards are located in the main Foyer of the Ceremonial Hall.

Posters 1 – 52 will be displayed in Poster Session A on Tuesday.

Posters 53 – 102 will be displayed in Poster Session B on Wednesday.

Please check your assigned number in the abstract index. The same number is used for the presentation and board number.

"A" session posters may be mounted on the assigned board starting Monday afternoon and must be mounted by no later than the morning coffee break on Tuesday. Posters in session "A" should be removed at the end of the day on Tuesday.

"B" session posters must be mounted by no later than the morning coffee break on Wednesday. Posters in session "B" should be removed at the end of the conference on Thursday.

The posters are grouped by topic.

A Session Posters:
- Epidemiology of MRSA (1-21)
- Diagnostics and Typing (22-52)

B Session Posters:
- Epidemiology of MRSP (53-61)
- Genomics, Virulence, Host-Specificity and Evolution (62-79)
- What is New (80-88)
- Treatment and Control (89-102)

CERTIFICATE OF ATTENDANCE

Certificates of Attendance can be found in the registration packet received at the registration desk.

Note: Certificates of Attendance do not list session information.

CAMERAS AND RECORDINGS POLICY

Digital recorders, cameras (including camera phones) and video cameras (including video phones) are prohibited in the poster hall and session room. Anyone found photographing, videotaping or recording in the prohibited areas will be asked to surrender their badge immediately and leave the conference. No refund will be provided. This rule is strictly enforced.

CHILD POLICY

Children are not permitted in session rooms, poster sessions, conference meals or social events.

CONFERENCE MEALS AND SOCIAL EVENTS

Registration includes the Welcome Reception on Monday, November 4, lunches on Tuesday, November 5 and Wednesday, November 6, and coffee breaks throughout the meeting. All events take place in the Ceremonial Hall.

GUEST REGISTRATION

Registered participants may also register an accompanying guest (age 16 and older) to attend the Welcome Reception for an additional fee of $100. Guests are not permitted in the lunches, coffee breaks, general sessions or poster sessions. Guests must present their guest badge for entrance to the Welcome Reception. Non-registered guests are not permitted to attend any part of the conference, including social events.

Travel Grants

STUDENT TRAVEL GRANTS

To encourage the participation of graduate students and new postdoctoral fellows at this conference, ASM and ESCMID have awarded travel grants of $500 to each of the following individuals:

Mohamed Abdelbary
Raghavendra Amachawadi
Britta Ballhausen
Michelle Chen
Meghan Davis
Alejandro Dorado-Garcia
Thomas Groenthal
Ewan Harrison
Joost Hordijk
Aneta Mroczkowska
Maya Nadimpalli
Alim Nazarali
Matthew Riley
Joana Rolo
Matthew Saab
Jisun Sun
Joany Van Balen
Gianpiero Ventrella
Min Tao Wan

Scientific Program

Monday, November 4, 2013

5:00 – 6:00 pm **Opening Session**

Welcome Remarks
***Luca Guardabassi**, University of Copenhagen*
***Ulla Wewer**, Dean of Faculty of Health and Medical Sciences, University of Copenhagen*

Opening Keynote Lecture
Staphylococci at the Human-Animal Interface
***Ross Fitzgerald**, The Roslin Institute, University of Edinburgh, Edinburgh, UNITED KINGDOM*

6:00 – 8:00 pm **Welcome Reception at the Ceremonial Hall of the University of Copenhagen**

Tuesday, November 5, 2013

9:00 – 11:00 am **SESSION 1: Epidemiology of MRSA**
Chairman: Engeline van Dujikeren

9:00 – 9:30 am LA-MRSA: What Have We Learned and What Are We Still Missing?
***Robert Skov**, Statens Serum Institut, Copenhagen, DENMARK*

9:30 – 10:00 am Role of Plasmids in Antimicrobial (Multi)Resistance of LA-MRSA
***Stefan Schwarz**, Institute of Farm Animal Genetics (FLI), Neustadt-Mariensee, GERMANY*

10:00 – 10:15 am Strong Association Between MRSA Air Exposure and MRSA Carriage in Veal Calf and Pig Farmers
***Marian Bos**, IRAS - Utrecht University, Utrecht, NETHERLANDS*

10:15 – 10:30 am MRSA Contamination in the Vicinity of Poultry and Pig Farms in Germany
***Anika Friese**, Freie Universität Berlin, Inst. for Animal Hygiene and Environmental Health, Berlin, GERMANY*

10:30 – 10:45 am	Residential Proximity to Large Swine CAFOs is Associated with Increased Risk of MRSA Carriage at Time of Hospital Admission in Rural Iowa Veterans ***Margaret Carrel**, University of Iowa, Iowa City, IA*
10:45 – 11:00 am	Environmental Contamination and Pet Colonization in the Households of People Diagnosed with a Community-acquired MRSA Infection ***Meghan Davis**, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD*
11:00 – 11:30 am	**Coffee Break and Poster Viewing**
11:30 am – 1:00 pm	**SESSION 2: Epidemiology of MRSP** Chairman: Scott Weese
11:30 am – 12:00 pm	Evolution and Spread of Multidrug-resistant *Staphylococcus pseudintermedius* Strains ***Lothar Wieler**, Institute of Microbiology and Epizootics, Berlin, GERMANY*
12:00 – 12:30 pm	Emergence of New MRSP Clones ***Stephen Kania**, University of Tennessee, Knoxville, TN*
12:30 – 12:45 pm	Subtle Strain Variation of *Staphylococcus pseudintermedius* Complicates Correct Diagnostics in Infected Dogs ***Joost Hordijk**, Utrecht University, Utrecht, NETHERLANDS*
12:45 – 1:00 pm	The Role of Methicillin Resistant *Staphylococcus pseudintermedius* Colonization as a Risk Factor for Development of Surgical Site Infections in Dogs Undergoing Tibial Plateau Leveling Osteotomy ***Alim Nazarali**, University of Guelph, Guelph, ON, CANADA*
1:00 – 2:00 pm	**Lunch**
2:00 – 3:00 pm	**Poster Session A** *Posters 1-52 will be presented.*

3:00 – 4:30 pm **SESSION 3: Diagnostics and Typing**
Chairman: Dave Bemis

3:00 – 3:30 pm Untangling the Transmission Dynamics of MRSA Using Whole Genome Sequencing
Matthew Holden, *The Wellcome Trust Sanger Institute, Cambridge, UNITED KINGDOM*

3:30 – 4:00 pm New MLST Scheme for *S. pseudintermedius*
Vincent Perreten, *Institute of Veterinary Bacteriology, University of Berne, Berne, SWITZERLAND*

4:00 – 4:15 pm Prevalence of *mecA*-positive Methicillin-resistant *Staphylococcus aureus* in Pigs Exhibits Dose-response to Zinc Supplementation
Raghavendra Amachawadi, *Kansas State University, Manhattan, KS*

4:15 – 4:30 pm Molecular Characterization of Methicillin-resistant and Methicillin-susceptible *S. aureus* (MRSA, MSSA) Clonal Complex 97 Isolates from Pigs and Cattle in Italy
Antonio Battisti, *IZSLT, Rome, ITALY*

4:30 – 5:00 pm **Coffee Break**

5:00 – 6:00 pm **Plenary Session on LA-MRSA**
Part 1: Definition of LA-MRSA
Lothar Wieler and Lance Price
Part 2: Control of MRSA
Jaap Wagenaar and Andreas Voss

Wednesday, November 6, 2013

9:00 – 11:00 am **SESSION 4: Genomics, Virulence, Host Specificity and Evolution**
Chairman: Lothar Wieler

9:00 – 9:30 am
Host Adaptation of LA-MRSA
Jodi Lindsay, *St. George's, University of London, London, UNITED KINGDOM*

9:30 – 9:45 am
The Genome of MRSP ST71
Arshnee Moodley, *University of Copenhagen, Frederiksberg, DENMARK*

9:45 – 10:00 am
New LA-MRSA Clones Emerging in Pigs: An Insight into ST433
Yvonne Agersø, *National Food Institute, Technical University of Denmark, Lyngby, DENMARK*

10:00 – 10:15 am
Phylogenetics and Molecular Epidemiology of *mecC*-MRSA Isolates in Europe
Ewan Harrison, *University of Cambridge, Cambridge, UNITED KINGDOM*

10:15 – 10:30 am
Staphylococcus aureus ST398 Gene Expression Profiling During ex vivo Colonization of Porcine Nasal Epithelium
Birgitta Duim, *Utrecht University, Utrecht, NETHERLANDS*

10:30 – 11:00 am **Coffee Break and Poster Viewing**

11:00 am – 1:30 pm **SESSION 5: What's New?**
Chairman: Birgit Strommenger

11:00 – 11:30 am
The Population Structure of CC398 and the Emergence of Horse-associated Sub-clone
Mohamed M. H. Abdelbary, *Robert Koch Institute, Wernigerode, GERMANY*

11:30 am – 12:00 pm
Staphylococcus stepanovicii: The potential Origin of the Methicillin-resistance Encoding Gene *mecC*
Birgit Walther, *Institute of Microbiology and Epizootics, FU Berlin, Berlin, GERMANY*

12:00 – 12:15 pm	Methicillin-resistant *Staphylococcus aureus* in Urban Rats ***Scott Weese**, University of Guelph, Guelph, ON, CANADA*
12:15 – 12:30 pm	The *mecA* Homolog *mecC* Confers Resistance Against beta-lactams in *Staphylococcus aureus* Irrespective of the Genetic Strain Background ***Britta Ballhausen**, Institute of Medical Microbiology - University Hospital Muenster, Muenster, GERMANY*
12:30 – 1:30 pm	**Lunch**
1:30 – 2:30 pm	**Poster Session B**
2:30 – 4:00 pm	**SESSION 6: Stakeholder's Points of View** Chairman: Luca Guardabassi
2:30 – 3:00 pm	Multi-drug Resistant Staphylococci and Increasing Antimicrobial Resistance: What Are We Currently Learning Using Novel in vitro Measurements ***Joseph Blondeau**, Royal University Hospital, Saskatoon, SK, CANADA*
3:00 – 3:30 pm	Epidemiology and Management of MRSA in Dairy Herds (Moredun Research Institute) ***Ruth Zadoks**, University of Glasgow, Glasgow, UNITED KINGDOM*
3:30 – 3:45 pm	The View on LA-MRSA by the Danish Pig Industry (Pig Research Center) ***Poul Bækbo**, Pig Research Centre, Danish Agriculture & Food Council, Kjellerup, DENMARK*
3:45 – 4:00 pm	Risk Management Initiatives on MRSA CC398 in the Danish Pig Production in a One Health Perspective (Danish Veterinary and Food Administration) ***Charlotte Thrane**, Danish Veterinary and Food Administration, Glostrup, DENMARK*
4:00 – 4:30 pm	**Coffee Break**
4:30 – 5:30 pm	**Plenary Session on MRSP** Use of Critically Important Antimicrobials (CIAs) in Companion Animals: What Should be Done? *Scott Weese, Engeline van Duijkeren, Ulrika Grönlund-Andersson and Luca Guardabassi*

Thursday, November 7, 2013

9:00 – 11:00 am	**SESSION 7: Treatment and Control** Chairman: Andreas Voss
9:00 – 9:30 am	Towards Harmonised Monitoring of MRSA in Animals and Food in the EU ***Pierre Alexandre Beloeil**, European Food Safety Authority (EFSA), Parma, ITALY*
9:30 – 10:00 am	Understanding *Staphylococcus aureus* Colonization in Pigs: Can Selective Breeding Be Used to Produce MRSA-Free Pigs? ***Luca Guardabassi**, University of Copenhagen, Copenhagen, Denmark*
10:00 – 10:15 am	Intervention Measures Reducing Livestock-associated MRSA on Pig Farms in the Netherlands: a Longitudinal Study ***Alejandro Dorado-Garcia**, Institute for Risk Assessment Sciences, Utrecht, NETHERLANDS*
10:15 – 10:30 am	Methicillin-resistant *Staphylococcus aureus* (MRSA) Screening at a Tertiary Veterinary Hospital: is Testing Cost-beneficial from an Integrated Human-animal Perspective? ***Jorge Pinto-Ferreira**, SAFOSO, Bern, SWITZERLAND*
10:30 am – 11:00 am	**Coffee Break**
11:00 – 11:45 am	**Closing Keynote Lecture** Phage Lysins May be Used to Treat and Prevent Infections in Both Human and Veterinary Applications ***Vincent Fischetti**, Rockefeller Univ., New York, NY*
11:45 am – 12:00 pm	**Concluding Remarks** ***Robert Skov***

Speaker Abstracts

OS:1

STAPHYLOCOCCI AT THE HUMAN-ANIMAL INTERFACE

R. Fitzgerald;
The Roslin Institute, University of Edinburgh, Edinburgh, UNITED KINGDOM.

The genus Staphylococcus is a diverse group of bacterial species associated with a wide array of host species. For example, *Staphylococcus aureus* is both a global human pathogen and a major cause of infectious disease in livestock animal species. In this presentation, I will summarise recent studies which have highlighted the origin and evolution of livestock strains of *S. aureus*, revealing the capacity of some clones to switch host species, undergo adaptation, and then transmit widely in new host populations. Such host switching events may have important consequences for veterinary and public health, and food security. *Staphylococcus pseudintermedius* is another major pathogen from the genus, responsible for the common skin infection pyoderma and notable for its increasing multi-drug resistance. Recent population and genomic studies have opened the door towards a better understanding of how *S. pseudintermedius* causes disease. In particular, we have identified the family of encoded cell wall-associated (CWA) proteins which are involved in key host-pathogen interactions. I will summarise how we are investigating the function of the CWA proteins and their potential application as targets for controlling this important canine disease.

S1:1

LA-MRSA: WHAT HAVE WE LEARNED AND WHAT ARE WE STILL MISSING?

R. Skov;
Statens Serum Institut, Copenhagen, DENMARK.

Since LA-MRSA CC398 was first recognized in The Netherlands in 2004 in pig farmers and their families, it has been reported in most European countries, the Americas, Australia and Asia. Although pigs are the dominating reservoir, CC398 has been reported in a wide range of other animal species. LA-MRSA is not confined to CC398 but also includes CC9 (especially in Asia) and ST5 (especially in the US and Canada). In addition, a new variant of methicillin resistance determinant (*mecC*) has been described in specific LA-MRA lineages that are primarily associated with cows and sheep but again display a surprisingly broad host range. Altogether the LA-MRSA phenomenon seems to be much more complex than anticipated at the beginning.
Through eight years of intensive research we have learned a lot about the evolution and epidemiology of LA-MRSA but several pieces of the puzzle are still missing. Today we assume that CC398 originated as a human MSSA which has subsequently adapted to pigs. In the new host it has acquired methicillin resistance and changed phage content by losing phage Φ3 and acquiring phages Φ2 and Φ6. LA-MRSA displays diverse resistance patterns and SCC*mec* types, suggesting that strain evolution is driven by a variety of selective pressures in the agricultural sector. Despite the considerable knowledge gained over the last years, various key aspects regarding the dynamics of transmission within and between herds are still not fully elucidated. How many of the pigs and humans that we score as positive are truly colonized and how many are just contaminated due to the high density of LA-MRSA in farm

environment? Trade has certainly played and is playing a very important role for the dissemination of LA-MRSA but is this the only factor? To what extent do veterinarians and workers moving from farm to farm act as vehicles? LA-MRSA CC398 is increasingly found in people without direct contact to pigs and farm workers, typically in areas with intense farming. As LA-MRSA can be detected in exhaust air from pig barns and even in soil samples at least 300 m from the barn, to what extent does carriage in these people reflect person-to-person transmission and to what extent is this due to environmental contamination? What is the risk that further human adaptation will result in increased human-to-human transmission? And will the risk of foodborne transmission remain negligible? These and other topics of present interest will be addressed by the talk.

S1:2

THE ROLE OF PLASMIDS IN ANTIMICROBIAL (MULTI)RESISTANCE OF LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

K. Kadlec[1], S. Wendlandt[1], A. T. Feßler[1], ***S. Schwarz[2];***
[1]Institute of Farm Animal Genetics (FLI), Neustadt-Mariensee, GERMANY, [2]Molecular Microbiology and Antibiotic Resistance, Institute of Farm Animal Genetics (FLI), Neustadt-Mariensee, GERMANY.

During the last decade research has focused on livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) with particular reference to isolates of the clonal complex 398 and their dissemination between various human and animal hosts. Studies on the resistome of these isolates identified a number of novel resistance genes. Many of the novel resistance genes were located on mobile genetic elements among which plasmids play the most important role.
The novel resistance genes included the trimethoprim resistance gene *dfrK*, the apramycin resistance gene *apmA*, the macrolide-lincosamide-streptogramin B (MLS_B) resistance gene *erm*(T), the pleuromutilin-lincosamide-streptogramin A resistance genes *vga*(C), *vga*(E) and *lsa*(E), as well as the spectinomycin resistance gene *spw*. Usually, multi-resistance plasmids, which carried several resistance genes, were detected in LA-MRSA. Most of these plasmids harbored the *dfrK* gene linked to the tetracycline resistance gene *tet*(L) in addition to one or more other resistance genes, including *apmA*, *aadD* (kanamycin, neomycin, tobramycin resistance), *vga*(A), or MLS_B resistance genes *erm*(B), *erm*(C), or *erm*(T). The co-location of several resistance genes on the same plasmid supports co-selection and persistence of this plasmid under the selective pressure imposed by any of the respective antimicrobial agents. Structural analysis of these multiresistance plasmids showed that they carried a variety of insertion sequences, such as IS*257*, IS*431* and/or IS*Sau10*, all of which are widely disseminated in *S. aureus* and have been postulated to play an important role in the generation of mosaic plasmids by integrating small plasmids into larger plasmids or mediating interplasmid recombination processes. Most recently, a large multiresistance gene cluster of ca. 17 kb, which is flanked by insertion sequences and most likely originated from enterococcal plasmids, was detected in MRSA CC398 and MSSA ST9 isolates from humans, but also in MRSA ST9 isolates from pigs. A 41-kb plasmid, which carried this multiresistance gene cluster, was sequenced completely and harbored - besides the novel resistance genes *lsa*(E) and *spw* - another five resistance genes, including *tet*(L), *erm*(B), *aadE* (streptomycin resistance), *aacA-aphD* (gentamicin, kanamycin, tobramycin resistance), and *lnu*(B) (lincosamide resistance).
In addition to small *erm*(C)-carrying plasmids of < 4 kb, plasmids of < 6 kb from LA-MRSA and other porcine staphylococci have been detected, which carried only the genes *vga*(A), *vga*(C), *dfrK* or *apmA* and - in part - differed distinctly in their plasmid replication and mobilization genes from the corresponding genes usually found on staphylococcal plasmids.

These observations suggest that plasmids from other bacteria can be acquired by and stably maintained in LA-MRSA and other staphylococci from food animal sources.

S1:3

STRONG ASSOCIATION BETWEEN MRSA AIR EXPOSURE AND MRSA CARRIAGE IN VEAL CALF AND PIG FARMERS

M. Bos[1], *W. Dohmen*[1], *A. Dorado-Garcia*[1], *B. Van Cleef*[2], *H. Graveland*[1], *B. Duim*[3], *K. Verstappen*[3], *J. Kluytmans*[4], *J. Wagenaar*[3], *D. Heederik*[1];
[1]IRAS - Utrecht University, Utrecht, NETHERLANDS, [2]St. Elisabeth Hospital, Tilburg, NETHERLANDS, [3]Dept. Infectious Diseases and Immunology - Utrecht University, Utrecht, NETHERLANDS, [4]Amphia Hospital, Breda, NETHERLANDS.

Around 30% of the Dutch veal calf farmers are MRSA carrier with a lower percentage being persistent carrier. Prevalence in pig farmers is even higher. Previous studies showed a strong association with carriage in animals and the number of hours worked in the stables (as proxy for direct animal contact). Furthermore, MRSA was found in air samples obtained outside farms. However, airborne exposure and transmission are poorly studied so far. Therefore, we determined MRSA air levels in stables of pig and veal calf farms, and studied the exposure-response relationship with nasal MRSA carriage in farmers. We analysed samples from three independent populations; population A consisted of 38 assumed frontrunner pig farms, population B of 50 randomly selected pig farms, and population C of 49 randomly selected veal calf farms. Farmers were defined as participants working on the farm at least 20 hours per week. Per farm 1-6 electrostatic dust collectors (EDCs) were analysed by qPCR to determine an equivalent to the number of colony forming units (CFUeq). Furthermore, we used culturing to analyse nasal swabs collected from the participants. The three human populations were comparable regarding length of work week, age, sex, and smoking habits. Prevalence for farmers in population A was 30/78 (53%), for population B 48/67 (72%), and for farmers from population C prevalence was 32/104 (31%). Mean log(CFUeq) MRSA on EDCs per farm for population A was 1.84 (95%CI: 1.62-2.07), for population B it was 2.06 (95%CI: 1.92-2.20), and for population C it was 0.97 (95%CI: 0.75-1.19). We applied a multivariate, pooled analysis and a meta-analysis on the combined datasets. The results showed a consistent strong association between the mean log MRSA exposure on a farm and nasal MRSA carriage in farmers, even after adjusting for working hours, smoking, sex, and age (pooled-OR: 1.98 (95% CI: 1.34-2.93), meta-OR: 1.81 (95% CI:1.10-2.96)). A model with only MRSA exposure provided a better fit than one with only working hours. Model fit was not improved by including an interaction term of exposure level and working hours. The findings suggest that a role for MRSA transmission through air exists, and give new insights into the importance of air exposure. This may have a large impact on predictive models for MRSA transmission and possible intervention measures.

S1:4

MRSA CONTAMINATION IN THE VICINITY OF POULTRY AND PIG FARMS IN GERMANY

A. Friese[1], *J. Schulz*[2], *B. Tenhagen*[3], *A. Fetsch*[3], *J. Hartung*[2], *U. Roesler*[1];
[1]Freie Universität Berlin, Inst. for Animal Hygiene and Environmental Health, Berlin, GERMANY, [2]Institute for Animal Hygiene, Animal Welfare and Farm Animal Behaviour, University of Veterinary Medicine Hannover, Foundation, Hannover, GERMANY, [3]Federal Institute for Risk Assessment, Department Biological Safety, Berlin, GERMANY.

Although the occurrence of methicillin-resistant Staphylococcus aureus (MRSA) is known in different healthy livestock species, there are only little data on the emission amounts and dispersion distances of these resistant bacteria

from farm buildings via the aerial route. Therefore, this study determined the presence and concentration of MRSA in the ambient air and on surrounding ground surfaces of different animal farms in order to give an estimate of the environmental load. Six pig farms were investigated four times within one year and five turkey as well as two broiler farms were analyzed four and three times, respectively within one fattening period. Different samples were collected inside (samples of animals and their direct surrounding including air) and outside (air, ground surfaces). All samples were analyzed for the presence of MRSA. Selected isolates were spa typed and grouped as Livestock associated (LA)-MRSA according to their association to the clonal complex (CC)398. MRSA was found regularly on ground surfaces downwind of the pig (73% of 67 samples) and poultry barns (44.4% of 81 samples), up to the planned investigated distance of 500 m. MRSA was detected in exhaust air samples from three pig farms and two turkey farms, however, with very low concentrations between 7 and 93 cfu/m3. Inside the barn MRSA occurred in samples of animals in high prevalences but also in barn air with higher concentrations than outside as well as regularly in dust and in some fecal samples. Isolates originating from inside and outside the farms were of the same spa types. The relevance of the emission of MRSA from livestock holdings to the environment has to be discussed critically. Neighbouring residents, livestock and wild animals might be exposed and even contaminated via the air and also via contaminated ground surfaces. However, MRSA concentration in the exhaust air was relatively low. This makes a direct airborne colonization of animals and people housed or living in close vicinity of livestock farms rather unlikely. The role of deposited MRSA and thus a potential contamination of crops used for food and feed are not yet well understood. This needs to be studied in more detail, in particular in respect to the survival times of deposited MRSA. We conclude that there seems to be no acute health risk for neighbouring farms or residents due to MRSA emissions from animal husbandries. However, further studies need to verify this presumption.

S1:5

RESIDENTIAL PROXIMITY TO LARGE SWINE CAFOS IS ASSOCIATED WITH INCREASED RISK OF MRSA CARRIAGE AT TIME OF HOSPITAL ADMISSION IN RURAL IOWA VETERANS.

M. Carrel[1], *M. L. Schweizer*[2], *M. Vaughan Sarrazin*[2], *T. C. Smith*[3], *E. N. Perencevich*[2]; *[1]Department of Geography, University of Iowa, Iowa City, IA, [2]Center for Comprehensive Access & Delivery Research and Evaluation (CADRE), Iowa City VA Health Care System, Iowa City, IA, [3]Department of Epidemiology, University of Iowa College of Public Health, Iowa City, IA.*

Background: Methicillin-resistant S. aureus (MRSA) colonization has been documented in livestock, livestock workers and individuals living in areas of high livestock density in the US and Europe. Recently, the US Veterans Administration (VA) implemented procedures that screened all patients at time of admission for MRSA colonization via nasal swabs. We aimed to determine whether residential proximity to swine Confined Animal Feeding Operations (CAFOs) was associated with increased MRSA colonization in patients admitted to the Iowa City VA Health Care System (IC-VAHCS). **Methods:** Nasal swabs were taken from patients on admission to the IC-VAHCS from 12/1/2009 -12/31/2011 and MRSA status was assessed. Patient addresses were geocoded and assigned to either rural or urban residential status based on Census designations. The number of swine within 1 mile of the patient household was calculated using data provided by the Iowa Department of Natural Resources. Relative risk was estimated for rural residents based upon residential proximity to any swine or to large numbers of swine. Generalized estimating equations were used to determine the impact of residential proximity to any or large numbers of swine on MRSA colonization

at time of admission, controlling for age and number of prior admissions to the IC-VAHCS. **Results:** Overall MRSA colonization on hospital admission among 1036 rural IC-VAHCS patients was 7%. Residential proximity within 1 mile of large swine populations was associated with nearly double the risk of MRSA colonization at time of admission (RR=1.8786, 95% CI=1.0928, 3.2289, p=0.0239). After adjusting for age and number of prior hospital admissions, residential proximity to large swine populations was associated with nearly triple the odds of MRSA colonization (OR=2.76, 95% CI=1.2728, 5.9875, p=.0101). The relationship between MRSA and no residential proximity to swine was not statistically significant. **Conclusions:** Residential proximity to large swine CAFO populations in rural Iowans was associated with increased risk of MRSA colonization at time of hospital admission, while proximity to smaller swine populations was not associated with MRSA colonization. Healthcare facilities serving rural populations need to respond to increased risk MRSA introduction into healthcare settings.

S1:6

ENVIRONMENTAL CONTAMINATION AND PET COLONIZATION IN THE HOUSEHOLDS OF PEOPLE DIAGNOSED WITH A COMMUNITY-ACQUIRED MRSA INFECTION

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Background: Households may serve as a point of transmission for methicillin-resistant Staphylococcus aureus (MRSA) among people and pets. Environmental survival of MRSA and related staphylococci enhances opportunities for indirect transmission. **Goals:** (1) To evaluate risk factors for pet acquisition of MRSA given exposure to an infected person. (2) To evaluate the roles of household contamination and pet positivity as sources of continued MRSA exposure for people undergoing decolonization treatment. (3) To characterize staphylococci of veterinary significance in these homes. **Methods:** We sampled environmental surfaces and all pets of any species present in 95 homes of patients diagnosed with a community-acquired MRSA infection. We repeated sampling in 65 homes, three months after randomized administration of a decolonization therapy to 65% of human households. We cultured samples for methicillin-resistant and methicillin-susceptible S. aureus (MRSA/MSSA), S. pseudintermedius (MRSP/MSSP) and S. schleiferi (MRSS/MSSS) using a broth enrichment, media-based protocol. We confirmed staphylococcal species and mecA/mecC presence via multiplex PCR. **Results:** At baseline, MRSA prevalence was 63% of homes (one or more sites contaminated) and 8% of 184 pets (including a fish tank). At follow-up, MRSA prevalence was 50% of homes and 5% of 130 pets, with 3% of pets persistently positive. At baseline, MSSP prevalence was 4% of homes and 18% of pets. At follow-up, MSSP prevalence was 5% of homes and 15% of pets, with 9% of pets persistently positive. At baseline, one dog each was positive for MRSP and MSSS. No reptiles or birds were positive for MRSA, but an aquatic turtle was positive for MSSA at baseline. Pets with a history of antimicrobial use were 6.4 times more likely to be positive for MRSA ($p<0.05$). Dogs were 20 times more likely than cats to be positive for MSSP ($p<0.05$). Home MRSA contamination, MRSA prevalence in people, and household randomization to a treatment group were positively correlated with pet carriage of MRSA. **Conclusions:** The patient's home was commonly contaminated with MRSA and may serve as a reservoir for continued exposure to both people and pets. While household decolonization reduced MRSA carriage in the treated

people, it did not reduce MRSA carriage in pets (pets were not treated). S. pseudintermedius was frequently cultured from pets, particularly dogs. Further work to characterize veterinary staphylococci from people enrolled in this study is planned.

S2:1

EVOLUTION AND SPREAD OF MULTIDRUG-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* STRAINS

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Over the last decade, an increasing prevalence of colonization, infections and infectious diseases caused by multidrug-resistant bacteria particularly in companion animals like cats, dogs and horses, has been observed. Multidrug-resistant methicillin-resistant *S. pseudintermedius* (MRSP) cause infections and infectious diseases often associated with clinical settings and involve mostly wound, skin or ear infections. *S. pseudintermedius* is a typical cause of canine skin infections and until recently regarded as being host-adapted. However, the epidemic spread of MRSP together with the changing socio-cultural interaction between companion animals and humans has even resulted in human cases of MRSP infections.
Methicillin-resistant variants of *S. pseudintermedius* (MRSP) were first reported in the late 1990s sporadically, followed by a sudden rise in incidence only a few years later. Meanwhile diseases caused by MRSP are a huge therapeutic challenge due to their frequently exhibited multi-drug resistant phenotype. Although knowledge on the global infection epidemiology of MRSP is still scarce, spread of a limited number of MRSP clones is envisioned. Currently it is believed that the genetic background of MRSP is associated with its geographic origin, i.e. certain dominating MRSP-lineages spread in Europe or the North American continent. Conventional typing methods like pulsed-field gel electrophoresis (PFGE) are able to compare strains in a restricted geographical area over a limited time period only, while Multi-locus sequence typing (MLST) enables unequivocal comparison of Sequence types (STs) of globally isolated strains. An initial MLST scheme stated the European-wide spread of a clone belonging to ST71.
To get a deeper insight into the population structure, we comparatively analyzed 100 MRSP and 100 MSSP from a convenience sample of strains obtained from various geographic origins in Germany. Representative strains were analyzed by whole genome sequence (WGS) analysis to unravel the population structure and genetic makeup of these clones. DNA sequences encoding the methicillin resistance determinant (*mec*A) seem to be highly conserved among MRSP strains. Despite clearly distinct PFGE patterns, most of the MRSP strains isolated in Germany belong to ST71 by applying both, the old and the updated MLST scheme while MSSP generally display more heterogeneous ST profiles. In contrast to high relatedness of PFGE pattern displayed by epidemic MRSA strains, results of WGS analysis contributes to the understanding of the limited PFGE-clonality of epidemic STs like ST71. In conclusion, MRSP is yet another example highlighting the influence of multidrug-resistance speeding up microbial spread.

S2:2

EMERGENCE OF NEW METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* CLONES

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Studies of clonal populations of *Staphylococcus pseudintermedius* provide important information about the epidemiology of the

organism as well as insight into the mechanisms by which antibiotic resistance genes and genes associated with virulence are spread. An expanding database of genomic information has facilitated molecular characterization of bacterial populations. These molecular studies provide information about the spatial and temporal distribution and host specificity of the bacterium. Correlation of multilocus sequence types with antibiotic resistance phenotypes and genotypes, SCC*mec* types, spa types, optical mapping and other methods of population characterization provide a broad picture of the distribution of various strains of *S.pseudintermedius* and their role in the pathology of this organism. Two clonal populations of methicillin resistant *S.pseudintermedius* represented by ST68 in North American and ST71 in Europe were originally described as predominant strains within their regions. Recent studies, however, have identified additional strains, some of which are genetically closely related, others that appear to represent additional clonal complexes and others that may have more recently acquired methicillin resistance. In the United States several new clonal complexes of methicillin resistant *S.pseudintermedius* (MRSP) have been identified suggesting independent *mecA* acquisition events, clonal expansion and the evolution of new sequence types. In addition, studies on other continents have revealed both the apparent spread of ST71 and ST68 and the presence of independent clonal populations of resistant organisms with diverse cassette types and antibiotic resistance profiles. Recent data suggest that MRSP clonal populations continuously arise and spread, however, they remain primarily concentrated within distinct geographical regions.

S2:3

SUBTLE STRAIN VARIATION OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS* COMPLICATES CORRECT DIAGNOSTICS IN INFECTED DOGS

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Background: S. pseudintermedius is an opportunistic pathogen that colonizes the skin and mucosal membranes of dogs and is the major cause of canine pyoderma. The worldwide spread of multi resistant methicillin resistant S. pseudintermedius (MRSP) has complicated treatment considerably. Genetic diversity among strains from the same dog has been described but many questions concerning the temporal genetic diversity in time and consequences for diagnosis and treatment remain unanswered. The index case for this study was a dog suffering from chronic otitis externa. Differences in antimicrobial susceptibility patterns of strains isolated from the index dog during one year were noted and questioned was if the ongoing infection was caused by multiple strains or one strain that was quickly evolving. Additionally, it was questioned how often strain diversity would be observed in a single sample from other dogs. **Methods:** At the Veterinary Microbiological Diagnostic Center (VMDC), during one year swabs were repeatedly obtained from the index dog. In addition, 5 epidemiologically unrelated dogs suffering from an S. pseudintermedius infection were sampled prospectively. Of each swab ten colonies were subcultured and confirmed with pta PCR-RFLP, mecA, and mecALGA251 PCR as MRSP. All strains were analyzed with PFGE and the MICs were determined. A selected amount of isolates was further characterized by SCCmec typing and spa typing. **Results:** From the 10 swabs of the index case 5 contained multiple strains that showed 9 different antibiotic resistance patterns. PFGE analyses showed that all strains

were highly related. Remarkable was the finding of strains that were genetically related based on PFGE and spa-typing, but were not all positive for mecA. In the prospective study two dogs carried a single MSSP strain, one dog carried two unrelated MSSP strains, and two dogs carried an unrelated MSSP and MRSP strain that were both multi resistant with different β-lactam resistances. **Conclusion:** The finding of genetically related strains expressing a different β-lactam phenotype in one dog is suggestive for excision of SCCmec and needs further confirmation. The observation of multiple strains in a sample with different antibiotic resistances is a risk for misidentification and treatment failures.

S2:4

THE ROLE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* COLONIZATION AS A RISK FACTOR FOR DEVELOPMENT OF SURGICAL SITE INFECTIONS IN DOGS UNDERGOING TIBIAL PLATEAU LEVELING OSTEOTOMY.

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Tibial plateau leveling osteotomy (TPLO) is one of the most commonly performed surgical techniques to stabilize a cranial cruciate insufficient stifle in dogs. Numerous studies have reported high surgical site infection (SSI) rates for TPLO, and methicillin-resistant S. pseudintermedius has emerged as a leading cause of these infections. In humans, pre-operative colonization with methicillin resistant Staphylococcus aureus (MRSA) is a risk factor for subsequent MRSA SSI, but the relationship between MRSP carriage and TPLO infection risk are unknown. The objective of this study was to evaluate the impact of pre-operative MRSP colonization on TPLO SSIs. A prospective, multi-institutional study of dogs undergoing TPLO at 6 surgical facilities in Canada and the Unites States was undertaken. Within 24 hours of admission, samples of the nares, pharynx, rectum and skin at the surgical site were obtained for MRSP screening using enrichment culture and mannitol salt with oxacillin agar. Isolates were typed by dry typing. Active surveillance of all patients was performed 30 days post-operatively and SSIs were documented according to standard definitions. In cases of SSI, samples from the wound were obtained. All patients at 2 institutions were swabbed at time of radiographic re-evaluation to determine MRSP colonization status. The overall SSI rate was 21/338 (6.2%), with 9/21 (43%) caused by MRSP. Ten of 338 (3%) dogs were colonized with MRSP preoperatively. Two of ten (20%) MRSP colonized dogs developed MRSP SSI, compared to 7/323 (2.2%) dogs that were not colonized (P=0.026). 7/138 (5%) of dogs were colonized with MRSP at time of post-operative recheck, between 4 and 8 weeks after surgery. 4/7 (57%) of these dogs were colonized at time of initial admission to the hospital. Isolates consisted of dt9a (n=6, 46%), dt10h (n=3 - 23%), dt10f (n=1, 7.7%), dt11a (n=1, 7.7%) and dt11af (n=2, 15.4%). All dogs with pre-operative MRSP colonization carried the same type at either time of post-operative recheck or diagnosis with MRSP SSI. The pre-operative MRSP colonization rate was consistent with studies of similar populations, as was the SSI rate and commonness of MRSP SSI. Pre-operative colonization was associated with development of MRSP SSI, something that indicates a need to consider pre-operative screening and/or peri-operative prophylaxis measures. However, while there was a significant association, most MRSP infections developed in dogs that were not identified as colonized pre-operatively, so further study of the epidemiology and pathophysiology of MRSP TPLO SSI is needed.

S3:1

UNTANGLING THE TRANSMISSION DYNAMICS OF MRSA USING WHOLE GENOME SEQUENCING

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Next-generation sequencing (NGS) has changed the face of bacterial genomics, allowing larger numbers of samples to be sequenced more rapidly with decreasing costs. This has opened up new opportunities for the application of whole genome sequencing beyond basic research, towards more applied areas such as clinical microbiology diagnostics. The high-resolution genotyping that NGS provides can be used to distinguish closely related isolates, and also place them within a population structure based on data from previously sequenced isolates. Phylogenetic analysis of single nucleotide polymorphisms (SNPs) identified in the genomes of bacterial isolates allows us to reconstruct their evolutionary history, and consequently make inferences about their origins and genetic relationships. Applying these approaches to methicillin-resistant *Staphylococcus aureus* (MRSA), we have used NGS to investigate two MRSA outbreaks in a large teaching hospital. In the first study focusing on a Neonatal Intensive Care Unit (NICU), we were able to demonstrate that whole genome sequencing was able to distinguish outbreak isolates from unlinked MRSA isolates in the hospital. In addition, we used genotype information generated from NGS to characterise the antibiotic resistance gene content of the isolates, and demonstrated congruence with the antibiotic resistance profile generated by standard phenotypic testing. In a second study we investigated a prolonged MRSA outbreak on a Special Care Baby Unit (SCBU). Genome sequencing identified that there had been multiple transmissions on the ward, but also that there had been a series of secondary transmissions that lead to the MRSA infection spreading beyond the hospital into the community. Combining the genomic data with epidemiological data we were able to hypothesise that a member of staff was linked to the transmissions on the ward. Subsequent screening identified a single member of staff who was MRSA positive and colonised with a strain belonging to the SCBU outbreak. The staff member was decolonised, and since then there has been no reoccurrence of the outbreak. These results highlight the potential of whole-genome sequencing to identify outbreaks in a healthcare setting and provide clinically relevant data that can influence patient care and guide initial therapy choices. The challenge for the future is transitioning NGS into the clinical setting, and integrating the results into everyday practice.

S3:2

NEW MLST SCHEME FOR *S. PSEUDINTERMEDIUS*

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Staphylococcus pseudintermedius belongs primarily to the normal flora of dogs and is now established as one of the most common causes of canine dermatitis, as well as hospital-acquired infections, in companion animals. *S. pseudintermedius* has also been reported to be a cause of infection in horses and mastitis in dairy cows. Additionally, *S. pseudintermedius* occasionally causes severe infections in humans. To determine the phylogenetic diversity between *S. pseudintermedius* strains, in 2007 Bannoehr et al. developed a Multilocus Sequence Typing (MLST) method based on 4 housekeeping genes (*cpn60*, *pta*, *tuf*, *agrD*) and the partial 16S rRNA gene (Bannoehr et al. 2007. J. Bacteriol. 189:8685-8692). This MLST scheme permitted the identification of predominant clones, such as e.g. ST71 in Europe and ST68 in North America. In 2013, a new MLST scheme using three of the previously described alleles (*cpn60*, *pta*, *tuf*) as well

as 4 new alleles (*ack*, *fdh*, *purA*, *sar*,) has been published for a better discrimination between isolates of a related clonal lineage (Solyman et al. 2013. J Clin. Microbiol. 51:306-310). It is however still recommended to sequence the *agrD* locus which is useful additional marker for the further discrimination of clones belonging to the same ST.

A website developed and sited at the University of Oxford (Jolley & Maiden. 2010. BMC Bioinformatics 11:595) has been adapted to the MLST scheme of *S. pseudintermedius* and is now available at http://pubmlst.org/spseudintermedius/.

The site contains useful information including methodology, submission form, publication list of the corresponding ST, as well as two databases. The first database consists of the locus/sequence definition database which contains allele sequences, allele profiles and MLST profiles. This database may be used, for example, for sequence, allele and ST queries. The second database contains the isolates database which includes epidemiological information on the different S. pseudintermedius, such as e.g. methicillin-susceptible *S. pseudintermedius* (MSSP) and methicillin-resistant *S. pseudintermedius* (MRSP), country and source of the isolates, health status and type of disease. In September 2013, the databases contained 169 sequences, 280 STs, and 305 isolates. Among them, 69 were MRSP (22.62%) and 236 were MSSP (77.38%), which came from 21 different countries. All users are welcome to submit, using the online submission form, their new allele sequences and the STs, if new for their countries, to the database for *S. pseudintermedius*, as well as the publication in which they have been released. The *S. pseudintermedius* MLST database will provide valuable epidemiological information about this rapidly spreading zoonotic bacteria.

S3:3

PREVALENCE OF *MECA*-POSITIVE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN PIGS EXHIBITS DOSE-RESPONSE TO ZINC SUPPLEMENTATION

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Zinc (Zn) is often supplemented at elevated concentrations in swine diets to promote growth and to prevent enteric infections. It is hypothesized that the occurrence of methicillin-resistant Staphylococcus aureus (MRSA) in pigs is associated with the use of zinc in the diet. In Europe, swine have been identified as an emerging reservoir of livestock-associated MRSA (LA-MRSA). Previous studies from Denmark have suggested a genetic linkage and a phenotypic association between Zn resistance, encoded by czrC, and methicillin-resistance, encoded by mecA, in S. aureus. Such an association has not been reported in the U.S. swine population. We conducted a study to determine the effects of various concentrations of Zn, supplemented as zinc oxide (ZnO), on the prevalence of MRSA in nursery (n=40) and finisher pigs (n=40). The basal diet consisted of zinc as zinc sulfate at 110 and 55 mg/kg of feed in nursery and finisher pigs, respectively. The nursery pigs included control (n= 20; no supplemental ZnO) and zinc groups (n= 20; 1,800 mg/kg of supplemental ZnO). The finisher pigs included four groups, each with ten animals that received 0 (no supplemental ZnO), 75, 150, or 225 mg/kg of ZnO. Nasal swabs were collected from both nursery and finisher pigs. The swab samples were inoculated on to MRSA CHROMagar and presumptive MRSA colonies (mauve or magenta color) were tested for mecA and czrC genes by PCR. Zinc susceptibility [minimum inhibitory concentration (MIC)] was determined by the agar gel dilution method. Statistical analyses

for binary endpoints were carried out using STATA MP (v.12.1) via the multivariable exact logistic regression procedure, considering categorical dose of zinc and controlling for the fixed effect of production age. Zinc MIC susceptibility data also were analyzed using non-parametric survival analysis. The prevalence of mecA-positive MRSA was 0% (0/20) in the nursery control group and 20% (4/20) in piglets fed an elevated concentration of ZnO (P = 0.05). In finisher pigs, the prevalence of mecA-positive MRSA was 0% (0/10), 10% (1/10), 20% (2/10), and 50% (5/10) in groups that received 0, 75, 150, and 225 ppm of supplemental Zn, respectively (P = 0.05). Of the 12 mecA-positive S. aureus isolates, 7 had the czrC gene (58.3%) compared to none that were positive for czrC among the 68 mecA-negative isolates. The presence of both mecA (P = 0.002) and czrC (P = 0.006) genes was strongly associated with higher levels of Zn supplementation. The median MICs of Zn for MRSA and MSSA were 8 and 4 mM, respectively (P = 0.0001). The possible genetic link between czrC and mecA genes suggests the importance of elevated Zn supplementation in co-selection and propagation of antibiotic resistance. Further studies are underway to better understand the molecular epidemiology of MRSA via genetic typing (spa typing) and the effects of feeding zinc versus other antimicrobials on the prevalence of LA-MRSA in pigs.

S3:4

MOLECULAR CHARACTERIZATION OF METHICILLIN-RESISTANT AND METHICILLIN-SUSCEPTIBLE *S. AUREUS* (MRSA, MSSA) CLONAL COMPLEX 97 ISOLATES FROM PIGS AND CATTLE, ITALY.

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AIMS: The aim of this study was to provide molecular characterization of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) Clonal Complex (CC)97, one of the most prevalent lineages in Italian primary productions (Battisti et al., 2010), detected in holdings of pigs and dairy cattle in Italy in the last five years. Recently (Spoor et al., 2013) a livestock origin for human pandemic CC97 MRSA has been demonstrated. METHODS: A total 42 CC97 *S. aureus* were studied: 35 MRSA of swine and bovine origin and 7 MSSA, with one human MSSA from Spain for comparison purposes. Genotyping was performed using multilocus sequence typing (MLST), spa-typing, SCC*mec* multiplex-PCR characterization. Virulence, pathogenicity and resistance genes were investigated by PCR and microarray. RESULTS: The CC97 isolates belonged to ST97 (n=37; spa-types t4795, t1730, t1236, t2112, t267), ST71 (n=3; spa-type 524), ST352 (n=2; spa-types t359). All MRSA carried SCC*mec* cassette type V (5C2), and were all PVL negative. Conversely, all isolates (MRSA and MSSA) were positive for genes of other leukotoxin families: LukF-LukS-HlgA, LukD-LukE and LukX-LukY. One MSSA (ST97) from dairy cattle carried the LukF-PV(P83) gene typical of *S. aureus* from ruminants. One MSSA and one MRSA from cattle, one MRSA from pigs and the human isolate were positive for *sak* and *scn*, within the immune evasion cluster (IEC). All isolates were positive for several superantiges/enterotoxin-like genes, with few isolates from cattle only carrying *seg* (n=1), *sed* (n=1 MRSA), *sed*, *sej*, *ser* (n=1 MSSA), or ORF CM14 (n=3 MSSA). None was positive for *tst*1. As regards antimicrobial resistance, all MRSA carried the *tet*(M) gene, and 27/35 (77%) also the *tet*(K) gene. Aminoglycoside (GEN-KAN) resistance gene *aacA-aphD* was present in 6/42 isolates often in co-presence with *aadD*. Macrolide-lincosamide resistance was mediated by *erm*(B) (n=13), *erm*(C) (n=2) or both genes (n=1). The vast majority (32/35, 91%) MRSA showed pleuromutilin resistance (tiamulin MIC=8 mg/L), attributed to the *vga*(A) gene, also contributing to resistance to streptogramins A and lincosamides. All isolates

carried *sdrM*, a chromosomally-encoded multidrug efflux pump, and two porcine isolates the plasmid borne *qac*C. All MRSA isolates had the same pattern of capsule and biofilm-associated genes, with 4 MRSA (2 bovine, 2 swine) and 1 MSSA isolates (bovine) which carried also the *bap* gene. All isolates carried several genes encoding MSCRAMMs, *bbp*, *clfA*, *clfB*, *ebh*, *ebpS*, *eno*, *fib*, *fnbA*, *fnbB*, *map*, *sasG*, *sdrC*, *sdrD*, *vwb*. The two spa-types t267 had the same genetic "core profile" (including *sak* and *scn*), except for acquired resistance genes/elements (e. g. SCC*mec*, *blaZ*, *aacA-aphD, fus*C). CONCLUSIONS: Very few host- or ST-associated differences were found among MSSA and MRSA CC97 isolates studied. A minority of isolates harbour genes associated with human adaptation. MRSA CC97 from pigs and cattle possesses several virulence, pathogenicity and resistance genes towards other major classes of antimicrobials, and may represent a serious therapeutic challenge in case of invasive infections in humans.

S4:1

HOST ADAPTATION OF LA-MRSA

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CC398 isolates from pigs and humans in contact with pigs have conserved genomes but vary in their carriage of mobile genetic elements (MGE). Variation is partly due to host and partly to geography. More rarely, certain CC398 isolates appear to transmit between humans without animal contact. These isolates have substantially different MGE profiles. MGEs are important as they often encode resistance genes as well as genes involved in virulence and host-adaptation. Snapshots of bacterial DNA content provide important clues as to which genes and MGEs may be selected for in populations of bacteria in different environments. But it does not tell us which genes are selected under controlled conditions during competition and survival on different hosts, nor how mobile the elements are, nor the potential prospects for future evolution. We co-inoculated a pig associated isolate (S0385) and a human adapted isolate (H278) onto gnotobiotic piglets in isolation chambers to establish if the pig adapted strain was fitter and a more successful coloniser. Our data surprisingly revealed both isolates could grow equally well, but this involved extensive gene transfer and exchange of MGE between the isolates. Whole genome sequencing suggests MGE alone are responsible for host-adaptation and survival, and the prospects for the evolution of strains with broader host range is high. Extensive MGE movement also has implications for our understanding of genome stability and bacterial growth and physiology.

S4:2

THE GENOME OF MRSP ST71

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Methicillin-resistant Staphylococcus pseudintermedius (MRSP) E140 was the first fully sequenced strain belonging to the widespread MRSP clone ST71, which has been associated with infections in different animal species and humans. E140 was isolated from a canine bite wound infection in Copenhagen, Denmark in 2008. The draft genome was determined to be 2,77Mb, with a GC content of 38%, and predicted to have 2678 coding sequences. Methicillin resistance was attributed to the presence of SCCmec type II-III, which is typically associated with this genetic lineage. Excluding the presence of the SCCmec cassette, we observed that E140 had a larger genome compared to the two published methicillin susceptible S. pseudintermedius genomes of ED99 and HKU10-03. Using PHAST, an online prophage finder programme, E140 contains prophage related DNA of up to 300Kb, including three intact prophages of ~222Kb, collectively represent-

ing 8% of the total genome. Currently, efforts are underway to annotate the phage related DNA for identification of novel virulence factors, as well as whole genome sequencing of additional MRSP ST71 genomes isolated from humans and different animal species to identify common genetic traits that may explain the rapid emergence and global spread of this clonal lineage.

S4:3

NEW LA-MRSA CLONES EMERGING IN PIGS: AN INSIGHT INTO ST433

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Livestock associated methicillin resistant Staphylococcus aureus (MRSA) in pigs mainly belong to CC398, which is also the most common clonal complex among methicillin susceptible S. aureus (MSSA) in pigs in Denmark. I 2009 MRSA ST433 (CC30) (spa type t1333) was detected among 4% of MRSA in pigs at slaughter. ST433 is the second most common MSSA type among pigs in Denmark.
From 2010 to 2011 pigs and farm workers were sampled for MRSA in 39 farms in Denmark. MRSA ST433 (spa type 1333) was detected in a pig and two farm workers from the same farm. The MRSA isolates were compared to three human MSSA t1333 isolates identified retrospectively in the national collection of S. aureus bacteraemia isolates from 2007-2011. Pulsed-field gel electrophoresis (PFGE) analysis of 14 MSSA and five MRSA from pigs, the two MRSA from farm workers and the three MSSA from bacteraemia revealed two different pulsotypes. Pulsotype A included two MSSA bacteraemia isolates that belonged to ST30 and carried the scn gene (encoding a human-specific complement inhibitor). Pulsotype B included all isolates from pigs and farm workers, as well as one MSSA bacteraemia isolate; these isolates belonged to ST433 and in one case its single-locus variant ST2082 (both CC30) and lacked scn. These findings suggest that in addition to an ancestral population of scn-positive S. aureus CC30 in humans there is a newly emerged scn-negative subpopulation in pigs, which can spread to pig farmers and be a source of S. aureus bacteraemia in humans.
Both pulsotype A and B isolates lacked lukF-lukS encoding PVL, and most (20/22) isolates were tetracycline susceptible; only two porcine MSSA ST433 isolates were tetracycline resistant and carried tet(K). Tetracycline is the most commonly used antimicrobial agent in the Danish pig production. The low rate of tetracycline resistance (9%) in the pig-associated S. aureus ST433/ST2082 isolates is in sharp contrast to the high rate of tetracycline resistance in livestock-associated S. aureus CC398 (~100%) and may partly explain the high occurrence of CC398 in pigs.
Sequence analysis of SCCmec from ST433 and CC398 isolates, respectively showed similar SCCmec cassettes. Isolates of CC30 including ST30 and ST433 were analyzed for mutations in the sau1 restriction-modification system and the result revealed an amino acid change in position 45 of hsdM1 in MRSA ST433 isolates and an amino acid change in position 187 of ST433 isolates when compared to residual CC30 isolates. These amino acid changes could have made MRSA ST433 isolates more capable of taken up foreign DNA and support that SCCmec cassette was exchanged horizontally.
In conclusion, pigs constitute a zoonotic reservoir of S. aureus (MRSA and MSSA) ST433/ST2082 and suggests a link between pigs and MSSA ST433 bacteraemia in humans. Moreover, mutations in the sauI restriction-modification system may have caused up take of the SCCmec cassette in MRSA ST433.

S4:4

PHYLOGENETICS AND MOLECULAR EPIDEMIOLOGY OF *MECC*-MRSA ISOLATES IN EUROPE

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A novel mecA homologue (mecALGA251: now designated mecC) present on an SCCmec type XI element has been identified in methicillin-resistant Staphylococcus aureus (MRSA) isolates from humans and animals throughout Europe. MRSA with mecC present a potential public health problem as they can be missed by current laboratory diagnostics and have been associated with animals. More recently, transmission of mecC-MRSA between livestock and humans has been demonstrated in Denmark by epidemiological follow up and whole genome sequencing. mecC has also been identified in a number of coagulase-negative staphylococci species. To further understand the epidemiology and evolutionary history of mecC-MRSA, isolates from a wide range of sources and geographical locations in Europe were whole genome sequenced. mecC-MRSA isolates and closely related sequence types were subjected to SNP analysis of the core genome, and high resolution phylogenetic trees constructed in order to understand the evolutionary history, transmission and spread of mecC-MRSA. Phylogenetic trees from the core genome and SCCmec elements were compared to gain insight into the evolutionary history of the mecC carrying SCCmec type XI element. Analysis of SCCmec type XI in comparison to orfX regions of mecC-positive coagulase-negative staphylococci (CoNS) was also carried out. Finally, comparison of the content of mobile genetic elements within isolates was made to further understand the evolutionary history of isolates and to identify potential determinates of host specificity.

S4:5

STAPHYLOCOCCUS AUREUS ST398 GENE EXPRESSION PROFILING DURING EX VIVO COLONIZATION OF PORCINE NASAL EPITHELIUM

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Background: Staphylococcus aureus is a common human and animal opportunistic pathogen. In humans nasal carriage of S. aureus is a risk factor for various infections. Methicillin-resistant S. aureus ST398 is highly prevalent in pigs in Europe and North America. The mechanism of successful pig colonization by MRSA ST398 is poorly understood. Here we present the analysis of the transcriptome of MRSA ST398 strain S0462 during maintenance of colonization on ex vivo nasal epithelium. **Methods:** Previously, we developed a nasal colonization model of porcine nasal mucosa explants to identify molecular traits involved in nasal MRSA colonization of pigs. From explants colonized with MRSA strain S0462 bacteria were isolated for RNA isolation in four repetitions. RNA was purified using the NucleoSpin RNA II total RNA isolation kit. The microarray was specifically developed for multiple S. aureus strains and contained 121,901 probes (Nimblegen, Roche). Process-

ing of the data was performed using R (version 2.14.1) and the Bioconductor MAANOVA package. **Results:** Major regulated genes were encoding metabolic processes and regulation of these genes represents metabolic adaptation to nasal mucosa explants. Maintenance of colonization was not accompanied by significant changes in transcripts of main virulence associated genes or known human colonization factors. We studied the regulation of two genes which have potential influence on S. aureus colonization; cysteine extracellular proteinase (scpA) and von Willebrand factor-binding protein (vwbp, located on SaPIbov5). Colonization with isogenic-deletion strains (ΔvwbpandΔscpA) did not alter the nasal S. aureus colonization compared to wild type. **Conclusion:** Our results suggest that maintenance of nasal colonization with MRSA ST398 is a complex event that is accompanied with changes in bacterial gene expression regulation and metabolic adaptation.

S5:1

THE POPULATION STRUCTURE OF CC398 AND THE EMERGENCE OF HORSE-ASSOCIATED SUB-CLONE

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant nosocomial and community acquired pathogen worldwide. The clonal complex 398 (CC398) was described as livestock associated MRSA. Nevertheless, various studies have reported colonisation and infection of humans and various companion animals with CC398. We investigated the population structure of 195 CC398 isolates through mutation discovery. Isolates were collected from various hosts (human, pigs, poultry, horses, and cattle) and countries (Germany, the Netherlands, USA, UK, Canada, Belgium, Italy, Denmark, Austria and Thailand). *Spa*- and SCC*mec*-types were determined for all isolates. The denaturing high-performance liquid chromatography was used for the single nucleotide polymorphism (SNP) discovery at 97 loci (≈ 40 kb of the CC398 genome). The polymorphic PCR products were sequenced and a minimum spanning tree (MST) was constructed by using Bionumerics 6.5. The Bayesian tip-association significance test revealed that *spa* types (t034, t011, t571, t108, t1457, and t899) and SCC*mec* types (IV, and V) were significantly associated with phylogeny. We detected the φAvβ prophage-related sequences only in MSSA turkey meat isolates. In addition, immune evasion genes carried on the β- converting φSa3 prophage (*sak, chp* and *scn*) were found in 17 CC398 isolates, which had been from human and animal origin. Interestingly, we revealed the spread of specific MRSA-CC398 sub-clone within equine settings, which causes infection in horses and humans nasal colonisation. The spread of this CC398 sub-clone may be due to insufficient hygiene practices in veterinary settings or unknown host specificity factors.

S5:2

Staphylococcus stepanovicii: The potential origin of the methicillin-resistance encoding gene *mecC*

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Introduction: Methicillin resistance in coagulase-positive (CPN) staphylococci is a major threat to both, human and veterinary medicine. In recent years, the origin of the methicillin-resistance encoding gene mecA has been identified among coagulase-negative staphylococci (CNS) like Staphylococcus fleurettii, Staphylococcus vitulinus and further CNS. In 2011, a novel mecA homologue (mecC; EMBL FR821779) harbored by SCCmecXI was described for MRSA from human and bovine origin, and later also from companion animals. We suspected that mecC, similar to mecA, originated potentially from CNS. **Material and methods:** The Staphylococcus stepanovicii strain IMT27065 (ODD4) was isolated in August 2011 from a fecal sample of a bank vole (Myodes glareolus) as part of a screening study focusing pathogens from wild rodents in Germany (Network "Rodent-Borne Pathogens"). Rectal swabs were enriched for staphylococcal growth in order to prevent overgrowing gram-negative bacteria. The staphylococcal species of isolate IMT27065 was verified by 16S rRNA sequence analysis (LGC Genomics, Berlin). A positive PCR-result for mecC was the reason for sequencing the whole genome of the strain on a HiSeq (Illumina, USA). The reads were assembled using CLC Genomics Workbench 6.5 (CLC bio, Denmark) and genomic comparative analyses were performed using Geneious 6.1.5 (Biomatters, New Zealand). **Results:** The mecC gene of IMT27065 (EMBL: KC110686.1) shares 99.2% nucleotide and 98.5% amino acid sequence identity with the mecCLGA251 of the recently described SCCmecXI in Staphylococcus aureus. Furthermore, the surrounding region revealed the presence of genomic elements similar to those of the class E mec of SCCmecXI (blaZ, mecC, mecR1, mecI) and the presence of the arsenic resistance operon. **Discussion:** Here we present S. stepanovicii as potential origin of the mecC gene and the class E mec in SCCmecXI. Further comparative genomic analysis including more regions surrounding mecC genes harbored by CNS are needed to reconstruct the phylogeny of SCCmecXI in S. aureus. Our data highlights the role of the environmental resistome as an important source of antibiotic resistance in opportunistic bacteria such as S. aureus.

S5:3

Methicillin-resistant *Staphylococcus aureus* in urban rats

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The Norway and black rat (Rattus norvegicus and Rattus rattus) are among the most common urban pest species and sources of various zoonotic pathogens. Little is known about MRSA in rats, although it has been identified in rats on farms in Europe. This study evaluated MRSA colonization in rats in an impoverished inner city region. Rats were trapped within the core of the Downtown Eastside (DTES) neighborhood of Vancouver, Canada, between Sept 2011 and May 2012 as part of the Vancouver Rat Project. A swab of the oropharynx and nares was collected under general anesthesia. Selective, enrichment culture was performed. Isolates were characterized by spa typing, PVL PCR and whole genome sequencing (WGS). MRSA was isolated from 22/637 (3.5%) rats, with a block prevalence ranging from 0-50%.

Spa type t008/ST8 was most common (n=7, 32%), followed by t034/ST3982 (n=5, 23%), t267/ST97 (n=5, 23%) and t002/ST105 (n=2, 9.1%). 3 other ST8 isolates (2 spa types) were also identified. WGS identified 4 clusters of isolates, corresponding to MLST and spa types. Within 3 clusters, samples were diverse, with 44-107 variant positions. Rat t008 isolates were indistinguishable by WGS from human t008 isolates from the DTES, although there were distinct differences between t008 from rats and humans from other regions. Rats caught in the spring and winter had higher odds of being MRSA positive compared to those in the fall, as did rats with greater body weight and internal fat stores. Two distinct clusters of high-than-expected MRSA prevalence were identified, each corresponding to distinct WGS clusters. The main clone found here, the PVL-positive t008 (which corresponds to USA300) is an important cause of MRSA infection in Canada, and is frequently identified in humans from the DTES. The predominance of this common human epidemic clone, along with the inability of WGS to differentiate rat isolates from those from humans in the same area supports interspecies transmission, although the route(s) and direction(s) are unknown. ST398/t034 was unexpected in this urban population given its association with livestock. Most of the other isolates were recognized human types, including the common t002 (USA100/Canadian epidemic MRSA2). Typing data and spatial clustering suggest that there are independent incidents of MRSA introduction into rats in different blocks over time. The seasonal association might reflect differences in human-rat interactions or the type of indirect contacts between humans and rats. The association of MRSA with higher body fat could suggest that MRSA exposure is influenced by rat social hierarchy, with fatter (dominant) rats at greater risk, as has been reported for Leptospira shedding. The public health consequences of MRSA shedding in rats are unclear but this evidence of interspecies transmission indicates that further study is required.

S5:4

THE *MECA* HOMOLOG *MECC* CONFERS RESISTANCE AGAINST BETA-LACTAMS IN *STAPHYLOCOCCUS AUREUS* IRRESPECTIVE OF THE GENETIC STRAIN BACKGROUND

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Background: In contrast to hitherto known SCCmec elements carrying mecA gene, SCCmec type XI harbors a mecA homolog, designated mecC, along with the regulatory genes mecRI/mecI most probably conferring resistance to beta-lactam antibiotics. Since mecC shares only 70% identity on DNA level to the mecA gene of other SCCmec elements, the impact of mecC on beta-lactam resistance in Staphylococcus aureus was elucidated by generation of a defined mecC knock-out mutant in this study. To compare mecC and mecA directly in its ability to confer resistance to beta-lactams, we analyzed the MICs of oxacillin and cefoxitin in S. aureus strains with different genetic backgrounds by expressing mecA or mecC in trans regulated by its respective wild-type promoter or by a standardized promoter. **Material and Methods:** The mecC gene was knocked out in the MRSA isolate w44646 (spa type t843) by inserting an erythromycin resistance cassette via homologues recombination. For homologous expression, mecC and mecA were cloned into the vectors pCN47.2 and pCN68.2. The resulting plasmids were introduced into methicillin-susceptible S. aureus strains RN4220, ME131 and NE1868 as well as into the w44646ΔmecC mutant. Antimicrobial susceptibility testing with oxacillin and cefoxitin was performed by microdilution according to CLSI guidelines. The expression of mecC, mecRI and mecI of SCCmec XI in presence and absence of oxacillin was comparatively analyzed by real time-PCR. **Results:** The mecC knock-out strain showed considerably reduced oxacillin and cefoxitin MICs compared to its wild type stain (oxacillin

and cefoxitin MICs decreased from 8 to 0.25 mg/L and from 16 to 4 mg/L, respectively). Complementing w44646ΔmecC in trans using both mecC and mecA restored the resistance to oxacillin and cefoxitin. Homologues expression of mecC in S. aureus RN4220, ME131, NE1868 and w44646ΔmecC led to an increase in cefoxitin and oxacillin MICs to 32, 256, 32-64, 64mg/L and to 32, 128, 32-64, 64mg/L, respectively. As described for the mec operon, oxacillin was likewise found to induce the expression of mecC as well as mecRI and mecI. Sub-inhibitory concentrations of oxacillin (2 mg/L) led to a 100-, 9- and 10-fold increase of transcription of mecC, mecRI and mecI, respectively. **Conclusion:** This study proofs for the first time genetically that mecC mediates ß-lactam resistance in MRSA carrying the SCCmec type XI. We demonstrated that mecC alone mediates resistance to oxacillin and cefoxitin irrespective of the underlying genetic background. Comparing the oxacillin/cefoxitin MIC values of strains expressing mecC and mecA in trans, we found only slight differences between mecA and mecC in their ability to confer ß-lactam resistance. Furthermore, this study provides evidence that the mecRI/mecI regulatory system of SCCmec type XI is functional and that it can be activated by ß-lactam antibiotics.

S6:1

MULTI-DRUG RESISTANT STAPHYLOCOCCI AND INCREASING ANTIMICROBIAL RESISTANCE: WHAT ARE WE CURRENTLY LEARNING USING NOVEL IN VITRO MEASUREMENTS?

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Antimicrobial resistance has compromised every antimicrobial agent currently licensed for therapeutic use. Bacterial resistance or susceptibility is based on an in vitro measurement utilizing 10^5 organisms per milliliter standardized for inoculum, media, incubation temperature/duration and atmosphere. In vitro pharmacokinetic and pharmacodynamic (PK/PD) modelling is often based on the minimum inhibitory concentration (MIC) values. Literature on bacterial densities during infection show low to high (10^2 colony forming units per milliliter –CFU/ml) to 10^9 CFU/ml) bacterial densities in meningitis and high density (10^7 ->10^{10} CFUs) burdens in pneumonia. As such, a single bacterial density used for susceptibility testing is misleading and clearly does not represent bug-drug interactions when low/high density burdens are present. The mutant prevention concentration (MPC) defines the antimicrobial drug concentration necessary to block growth of the least susceptible cells present in high density ($\geq 10^9$ CFUs) bacterial populations. MPC measurements are conducted on susceptible strains. MPC measurements with methicillin susceptible *Staphylococcus aureus* (MSSA) and fluoroquinolones showed differences in the ability of each compound to restrict mutant growth despite the fact the compounds are clinically equivalent. MPC testing of quinolone susceptible, methicillin resistant *S. aureus* (MRSA), also showed clear differences in the ability to restrict mutant growth between the quinolones tested. MPC testing of *Staphylococcus pseudintermedius* against quinolones, aminoglycosides, beta lactams and macrolides also showed clear differences between compounds in the amount of drug necessary to block growth of the least susceptible cell in high density inocula. Low MIC values were often followed by MPC values above therapeutic drug concentrations for some compouns. With fluoroquinolones and *S. pseudintermedius*, pradofloxacin had the lowest MPC values of all agents tested. For MRSA and vancomycin (MIC 1ug/ml) MPC values of 16ug/ml were seen which upon retesting by MIC yielded a parental MIC value. The explanation of this observation remains unknown; pulsed field gel electrophoresis analysis showed identical profiles between the parental strain and that with the high MPC value. In vitro kill measurements based on a range of bacterial densities (10^6-10^9 CFU/ml) showed

striking differences between MIC, MPC, maximum serum and tissue drug concentrations and killing of bacteria. How this relates to resistance selection and clinical recovery is currently debated. Novel in vitro measurements are adding considerable discussion around our understanding of the emergence and spread of antimicrobial resistance and how this impacts clinically. How such information should be incorporated in PK/PD modelling is an intriguing but necessary question.

S6:2

EPIDEMIOLOGY AND MANAGEMENT OF MRSA IN DAIRY HERDS

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From the farm perspective, there is no such thing as the management of MRSA in dairy herds because farmers or herd managers would rarely be aware that they have MRSA in their herds. Mastitis, as it presents to farmers, is the presence of clots, flakes or other abnormalities in a cow's milk, possibly accompanied by the classical signs of "-itis", i.e. rubor, tumor, dolor and calor (redness, swelling, pain, increased temperature). In developed countries, such milk is not considered fit for human consumption. As a result, the affected milk is discarded or fed to calves or other animals. Meanwhile, to protect animal welfare and to return the cow to production of saleable milk as quickly as possible, animals may or may not be treated with antimicrobials, anti-inflammatory drugs or supportive therapy. At this stage, it will generally not be known that the disease is caused by *Staphylococcus aureus*, let alone MRSA, so clinical case management will not be targeted to MRSA. Availability and opinions on the usefulness of diagnostics, including identification of bacterial species and, possibly, antimicrobial resistance profiles, differ between laboratories and countries. In some countries, detection of *S. aureus* from milk is automatically followed by screening for penicillin susceptibility whereas this is considered uninformative or economically not justified elsewhere. Screening for methicillin resistance, whether caused by *mecA* or *mecC*, does not normally take place as part of routine mastitis diagnostics. In addition to mastitis with visible signs, *S. aureus* may cause subclinical mastitis or asymptomatic intramammary infection in dairy cattle. This condition affects milk quantity and milk quality, providing an economic rather than a welfare incentive for its control. Depending on herd management, *S. aureus* may spread between cows in a milking herd in a contagious manner, or even between milking animals and non-milking animals. Thus, control of infection in one animal may lead to prevention of infection in other animals. Whether or not the use of antimicrobials is justified under those circumstances depends on the balance of economic and societal factors, e.g. the expected economic gain or the potential risk of selecting for antimicrobial resistance. Information on prevalence and epidemiology of MRSA in dairy herds stems from research rather than diagnostic investigations. Molecular epidemiology studies suggest that MRSA in dairy herds may be a human- or pig-derived problem or a primary cattle problem, with differences between countries and strains. In this presentation, an overview will be given of mastitis management on dairy farms, with specific attention to the management of *S. aureus* mastitis, potential beneficial or harmful consequences of the use of antimicrobials and the feasibility or need for MRSA specific management strategies from the perspective of farms and society.

S6:3

THE VIEW ON LA-MRSA BY THE DANISH PIG INDUSTRY (PIG RESEARCH CENTRE)

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Denmark has a significant pig production of 30 million pigs per year. Denmark is one of

the major players on the world pork marked, exporting nearly 2 mill tons of pork per year. The Danish pig producers are organised in a NGO, The Pig Research Centre (PRC) that is an integrated part of The Danish Agriculture & Food Council (DAFC). DAFC brings together the largest industry grouping in Denmark, representing the Danish food and farming industry. More than 50% of the pig herds are run as SPF-herds being free from some of the major pig diseases and all herds have a Salmonella status according to the Danish Salmonella control program initiated in 1995.

LA-MRSA CC398 was isolated from pigs in Denmark in 2006 for the first time. Surveys performed by the authorities in 2010 and 2011 shows that 16% of the Danish pig herds are infected (www.danmap.org). In humans CC398 constituted 12.5% of the MRSA cases in 2011 (164 persons), of which the majority are seen among persons with direct contact to pigs. From 2012 contact with live pigs is regarded as a general risk factor in the MRSA guideline for the human health sector.

MRSA CC398 is expected to be introduced to the Danish pig production from other countries, presumable from human contacts as the import of live pigs is very limited. MRSA is transmitted between herds by transfer of live pigs, whereas the significance of transmission by other routes is unknown.

The PRC regards LA-MRSA as a problem for the work environment (people working with live pigs) and with no food safety aspects. PRC advices all people working with live pigs to give this information whenever they are in contact with human health system, especially in any case of surgery.

The strategy of the PRC on MRSA CC398 can be put into the following 6 statements:

1. Takes the problem seriously, but we have to live with it
2. Seeks full transparency and high level of information for people working in the pig industry
3. The herd owner of infected herds must inform their workers, visitors and byers of live pigs of the MRSA status
4. Recommend a high level of personal hygiene in all pig herds, whenever people are leaving the farm pig units
5. Seeks a high degree of dialog and cooperation with the human and veterinary health authorities
6. Supports activities to reduce the overall consumption of antibiotics in the pig production (already the amount of antibiotics used per kg produces pork is one of the lowest in the world of modern pig production)

It has been discussed if the spread of infection between herds could be controlled by an infection declaration of all pig herds (positive/negative herds). Based on the present knowledge PRC do not see declaration as a relevant option, because:

- Too many herds already infected
- Reliable free-testing is a challenge and presumably very costly
- False confidence by the stockmen
- No proven way of elimination of MRSA on herd level
- Potential conflicts with other infections of interest (The SPF-pig diseases & Salmonella)

S6:4

RISK MANAGEMENT INITIATIVES OF LA-MRSA CC398 IN THE DANISH PIG PRODUCTION IN A "ONE HEALTH" PERSPECTIVE

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Risk management initiatives of LA-MRSA CC398 in the Danish pig production in a "One Health" perspective

LA-MRSA is present in the Danish pig production. During the last years there has been an increase in the impact of LA-MRSA on the human health. Consequently, we are currently reconsidering how to manage the human risk of LA-MRSA.

The Danish Ministers of Food, Fisheries, and Agriculture respectively of Human Health decided that risk management of LA-MRSA

was best dealt with if handled in a "one health perspective". Thus, at the national level we established a Danish action group with experts from different disciplines across veterinary, food safety, and working environment from Universities, authorities and private stakeholders. This action group has been working together to ensure that managing the LA-MRSA risk is based on "one health" principles, and that resources to combat LA-MRSA are leveling the known risk.

The first task of the action group was to point out short term initiatives, that were supposed to help to decrease the human exposure to LA-MRSA. The prioritized short term initiatives to fight the human impact of LA-MRSA will be presented.

We still have some gaps about the physiology and epidemiology of the LA-MRSA, which is a basis for effectively combatting and controlling the microbe. In Denmark, it was decided that we cannot wait for more knowledge – we need to improve measures starting in the short term.

S7:1

TOWARDS HARMONISED MONITORING OF MRSA IN ANIMALS AND FOOD IN THE EU

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MRSA colonisation, in production animals, detected in recent years, has, in several cases, resulted in infection in humans, and infections with a livestock-associated strain of MRSA may be considered a zoonosis. An assessment of the public health significance of MRSA in animals and in food was issued by EFSA in 2009. In addition, EFSA has also recently issued proposals, as a result of a mandate from the European Commission, to improve the harmonisation of monitoring of prevalence, genetic diversity and antimicrobial susceptibility in MRSA from food-producing animals and food derived thereof, by the EU Member States.

The primary route of zoonotic transmission of MRSA has been considered to be direct or indirect contact of livestock professionals with colonised animals, while the role of food as a source of human colonisation or infection has been deemed of minor importance. Therefore, monitoring the occurrence and diversity of MRSA in primary production, including at slaughter, seems pivotal, while monitoring in food may also help with the assessment of consumers' exposure via this route. MRSA has been shown to evolve continuously, and changes in characteristics, such as virulence and transmissibility, may occur in the future and favour exchanges between animal and human populations, in which the organism may adapt, diffuse, multiply and evolve further. Regular monitoring of MRSA is beneficial in directly providing information about the emergence of strains of potential public health significance, but can also provide important epidemiological data on the spread of particular strains between animal and human populations. Monitoring in animals should be performed in parallel with consistent monitoring in humans.

Monitoring recommendations have proposed a harmonised definition of MRSA and prioritised several different food-producing animal populations previously described as MRSA reservoirs and, to a lesser extent, food produced by these animals. Regular monitoring of broiler flocks, fattening pigs and dairy cattle, as well as in veal calves under 1 year of age, and fattening turkey flocks, is recommended every third year on a rotating basis. It is proposed that breeding poultry flocks and breeding pigs, as well as meat and raw milk products, are monitored on a voluntary basis. Representative sampling should be made within the framework of the national Salmonella control programmes for the poultry populations targeted, at the slaughterhouse for calves and either on the farm or at the slaughterhouse for fattening pigs. Harmonised analytical methodologies for identification, typing and further characterisation of MRSA are also proposed. The use of the microdilution method applied to a harmonised set of antimicrobials through harmonised

concentration ranges, and interpreted using EUCAST epidemiological cut-off values, for antimicrobial susceptibility testing of MRSA, is recommended.

S7:2

UNDERSTANDING *STAPHYLOCOCCUS AUREUS* COLONIZATION IN PIGS: CAN SELECTIVE BREEDING BE USED TO PRODUCE MRSA-FREE PIGS?

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Introduction: *Staphylococcus aureus* colonization is a complex biological phenomenon resulting from interaction of bacterial, host and environmental factors. *S. aureus* colonization rates in the human population are known but virtually nothing is known about frequency and stability of colonization in pigs. This is because most studies to date focussed on MRSA only, had cross-sectional design and assessed MRSA carriage by use of enrichment procedures, which enhance detection but do not provide any quantitative data. **Objective:** This lecture is based on the results of a large longitudinal study where *S. aureus* nasal colonization rates were assessed quantitatively in 480 adult pigs over a period of three weeks. The objective of the study was to understand frequency and stability of colonization in adult pigs.

Material and methods: A longitudinal quantitative study was performed in 20 randomly selected Danish production farms. Within each farm, 24 individual pigs in the finishing sections were ear-tagged and nasal swabs were collected from each individual three times at weekly frequency. *S. aureus* was quantified by the most probable number (MPN) method following enrichment of swab serial dilutions in physiological saline and subsequent plating onto SaSelect (Biorad, USA) and blood agar. Pigs were defined as permanent carriers if positive in all three sampling times, transient carriers if positive in one or two sampling times, and non-carriers if negative in all sampling times. Data were analysed statistically using a logistic regression model for either multinomial or binary data (proc glimmix, SAS Inst.), with sampling round, swab cleanliness and MRSA-positivity for herd as fixed effects when appropriate, and farm, room and pig as random effects, when appropriate.
Results: All farms were *S. aureus*-positive and in-herd prevalence of permanent carriers was on average 23% (range 0 to 75%). Among the 480 pigs tested, 23% were permanent carriers, 54% were transient carriers and 23% were non-carriers. There was a strong effect by farm ($p<0.0001$) and pen ($p\leq0.02$) on in-herd prevalence and load of *S. aureus* in nasal swabs. Within farms, some pigs had a significantly higher load than others ($p=0.004$), and the bacterial load was significantly higher in permanent carriers than in transient carriers, when the sample was positive ($p=0.002$). There was a tendency of negative animals to remain negative during the study period.

Conclusions: This study shows that, even if farm- and pen-related effects are taken into consideration, certain pigs are more predisposed than others to *S. aureus* colonization. The genomes of 50 permanent carriers and 50 non-carriers identified in this study are presently analysed to investigate whether colonization is enhanced by specific host genetic traits. If this hypothesis will be confirmed, selective breeding could be used to produce pigs with reduced susceptibility to *S. aureus*. Potential use of this approach for control of MRSA in pig farming will be discussed taking into consideration sustainability and risks associated changes in the current breeding programmes.

S7:3

INTERVENTION MEASURES REDUCING LIVESTOCK-ASSOCIATED MRSA ON PIG FARMS IN THE NETHERLANDS: A LONGITUDINAL STUDY.

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Livestock-associated (LA) MRSA is widely spread in pig farms in the Netherlands with approximately 70% of them being positive. The aim of this study is to determine the quantitative effect of implementing intervention measures on pig farms in order to reduce MRSA transmission to humans. A longitudinal study in 40 randomly selected pig farms in the Netherlands was done for a period of 18 months. On each farm 10 pools of 6 nasal swabs were obtained from pigs every 6 months. A tailor made intervention protocol was developed by the farmer and the main veterinarian targeting 3 key points: reduction of the use of antimicrobials, improving hygiene and changing the animal contact structure. An extensive questionnaire was used to assess the course of all specific interventions. There was a non-significant decrease in the overall MRSA pool prevalence from the beginning (42.6%) to the end of the study (36.3%). For the purpose of variable reduction, potential determinants, registered by the questionnaire, were evaluated cross-sectionally in the 4 sampling moments by a simple linear regression for pool prevalence. Those with $p<0.20$ in at least 2 sampling moments were further evaluated longitudinally. The selected determinants were included with sampling moment as a factor in a bivariate random intercept model to adjust for trend over time. The difference in mean prevalence between the 2 categories of a variable during the study, is given by the β estimates from the mixed models. Univariate results, adjusted for time trend, showed that farms with finishing pigs and farms without external supply of animals had significantly ($p<0.05$) lower prevalence during the study (β=-17.9 and -24.4 respectively). MRSA prevalence was significantly higher in farms cleaning with soaking agents (β=15.6), farms vaccinating piglets and/or fatteners (β=17.0) and farms injecting antibiotics in piglets during the first week of life (β=15.8). Housing sows in stable groups led to significantly lower prevalence (β=-13.5). Other downward trends in prevalence (borderline significant or $p<0.2$) were shown in farms where showering was mandatory (β=-7.4), delivery platform was cleaned right after animal movements (β=-9.1), no teeth clipping in piglets was done (β=-10.4), and external biosecurity score was high (β=-9.8). Additionally, data on prescription of antimicrobials during the study are being processed . Preliminary results show a positive and significant linear relationship between the Defined Daily Doses (DDD) animal/year and pool prevalence in at least two sampling moments. However, longitudinal evaluation still needs to be done. A final multivariate mixed model by which we attempt to explain change in prevalence over time, as a result of the described interventions, is under development. The longitudinal nature of our data will enable us to give indications of the most effective strategies to decrease MRSA prevalence on pig farms.

S7:4

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) SCREENING AT A TERTIARY VETERINARY HOSPITAL: IS TESTING COST-BENEFICIAL FROM AN INTEGRATED HUMAN-ANIMAL PERSPECTIVE?

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Methicillin-resistant Staphylococcus aureus (MRSA) is an antimicrobial resistant organism of international significance to human and veterinary medicine. In human medicine, policies proposed and tested for MRSA control include variations of universal patient screening and surveillance programs. In veterinary medicine information on MRSA control is scarce. Objective: To develop a cost-benefit (CB) analysis from an integrated human-animal that could be used for policy development and including all animals admitted for hospitalization to NCSU CVM Veterinary Teaching Hospital. Materials and methods: A model was developed to estimate the costs of MRSA of all animals admitted to the hospital taking into consideration the projected economic benefits of the prevention of human infections. Results: The baseline model used the most plausible inputs and different scenarios were considered in the sensitivity analysis. The cost of the screening policy was estimated at $320,104 and exceeded the savings of about $183,409. Variations in the input assumptions, most notably the additional cost for treating a human case, rendered a variety of possible outcomes. Conclusions: Our study suggests that it is not beneficial to implement MRSA screening programs in veterinary hospitals from an integrated economic human-animal perspective.

CS:1

PHAGE LYSINS MAY BE USED TO TREAT AND PREVENT INFECTIONS IN BOTH HUMAN AND VETERINARY APPLICATIONS

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Bacteriophage lytic enzymes (lysins) are highly evolved molecules used by the phage to quickly destroy the bacterial cell wall to release phage progeny. We have exploited the rapid and lethal action of these enzymes to destroy pathogenic bacteria on mucous membranes, in blood and infected tissues. In general lysins are specific for the species or bacterial group for which they were produced, thus avoiding destruction of the surrounding normal commensal organisms found on mucosal surfaces. Lysins have been developed that are specific for *S. equi, S. suis, S. uberis*, *S. pyogenes, S. pneumoniae, S. aureus, B. anthracis, E. faecalis, E. faecium* and group B streptococci. Our results show that in vitro 10^7 bacteria can be reduced to sterility minutes after enzyme contact. In animal model experiments, we were able to colonize mice with either streptococcal or pneumococcal species (orally or nasally) and remove them to undetectable with lysins delivered to these sites using a single lysin dose. In a septicemia model with *S. pneumoniae,* bacteria are reduced by >3-logs from the blood of infected animals with a single intravenous dose of enzyme resulting in survival of nearly all animals. A model of murine pneumococcal pneumonia was developed demonstrating widespread infiltration of the lung and physiologic evidence of systemic infection within 24h. When mice were treated intraperitoneally with either the anti-pneumococcal lysin Cpl-1 or amoxicillin every 12 hours for 3 days, bacteremia was

eliminated from both groups and lung function and histological findings returned to normal, whereas all animals treated with buffer died. Similar results were obtained in endocarditis and meningitis models of pneumococcal infection. Resistance to lysins has not been found nor do antibodies completely neutralize their activity, allowing for repeated use in the same individuals. Furthermore, a combination of antibiotic and lysin has been shown to work synergistically resulting in significant lethal activity in cases of antibiotic resistant bacteria. We also found that lysins have a prominent effect on MRSA biofilms compared to the limited or no effect when antibiotics were used. When we tested the *S. uberis* lysin for its activity in the presence of raw milk, we found that it was not inhibited, suggesting its use in controlling mastitis in combination with the staphylococcal lysin PlySs2. Thus, phage lytic enzymes are a safe new reagent that may be used to control both antibiotic resistant and sensitive pathogenic bacteria in both human and veterinary applications and offer a capability previously unavailable.

Poster Abstracts

1A

PRESENCE, DISTRIBUTION AND TRANSMISSION OF METHICILLIN-RESISTANT *STAPHYLOCOCCI* IN A SMALL ANIMAL CLINIC

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Objective: The aim of this study was to identify and to characterize methicillin-resistant staphylococci (MRS) isolated from samples collected in a small animal clinic and to investigate their distribution, focusing on a possible transmission and on resistance properties. **Materials and Methods:** In total, 72 swabs were taken from animals (n=10), the environment (n=58) and employees (n=4) and screened for the presence of MRS. The staphylococcal species was confirmed biochemically and by 16S rDNA sequencing. Methicillin resistance was confirmed by MIC determination for oxacillin according to the recommendations given by the Clinical and Laboratory Standards Institute and by PCR detection of *mecA*. Further characterization followed standard procedures. **Results:** The 34 identified MRS isolates were *Staphylococcus (S.) aureus* (n=5), *S. epidermidis* (n=21), *S. haemolyticus* (n=6) or *S. pettenkoferi* (n=2). They originated from cats (n=6), the environment (n=24) or humans (n=4). Neither the feline patients nor the employees showed clinical signs of staphylococcal infections. The isolates had SCC*mec* types IV (n=22) or V (n=8) or were non-typeable (n=4) by the multiplex PCR assays used and *dru* typing showed five novel *dru* types (dt5i, dt9bb, dt9bc, dt10cb, dt12w) in addition to five known *dru* types (dt10a, dt10g, dt10r, dt11a, dt11c). Four of the five MRSA isolates belonged to the clonal complex 398, the remaining MRSA had multi locus sequence type (MLST) ST217. The *S. epidermidis* isolates belonged to five different MLST types, ST2, ST5, ST10, ST48, and ST66, with ST5 being predominant. All isolates were multidrug-resistant with resistance to at least three classes of antimicrobial agents. In addition to isolates that varied in their molecular characteristics, a single multidrug-resistant *S. epidermidis* clone was detected in nine samples from cats, clinic environment and an employee. Members of this clone shared SCC*mec* type IV, *dru* type dt10a and MLST type ST5. Moreover, these nine MRSE isolates showed indistinguishable plasmid profiles and SmaI macrorestriction patterns. **Conclusion:** Multiresistant MRS isolates belonging to the same species and showing the same characteristics were isolated from patients without clinical infections, the clinic environment as well as from healthy employees, suggesting a possible transmission. Moreover, MRS were mainly found in the stationary area of the clinic.

2A

MULTIDRUG- AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ARE PRESENT IN SURFACE WATERS NEAR INDUSTRIAL HOG OPERATIONS IN NORTH CAROLINA

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Industrial animal production using subtherapeutic levels of antibiotics for growth promotion and disease prevention has raised concerns about antibiotic resistance in clinically relevant bacteria such as *Staphylococcus aureus*. Although nasal carriage of antibiotic-resistant *S. aureus* in industrial animal workers

has been documented, transmission of *S. aureus* to the water environment in areas where waste products are sprayed has not been characterized. We investigated whether *S. aureus* is present in surface waters near industrial animal operations that manage liquid waste with lagoons and spray fields. Surface water samples (n=183) were collected over the course of approximately one year from nine sites in southeastern NC and analyzed for the presence of *S. aureus*. A total of 698 presumptive *Staphylococcus* isolates exhibiting phenotypic antibiotic resistance were recovered and archived. Culture-based, biochemical, and molecular tests were used to confirm the presence of *S. aureus* and methicillin-resistant *S. aureus* (MRSA). Confirmed *S. aureus* isolates were subject to additional characterization, including antibiotic susceptibility testing using Kirby-Bauer disk diffusion, *spa* typing, and a duplex PCR to detect the *tet*(M) and *scn* genes. Of the 698 isolates, 16 were *S. aureus* and 5 were MRSA. *Staphylococcus aureus* isolates were recovered from 16 distinct samples, 9 sampling events, and 7 sites, with an overall percent detection of 0.09 (16/183 samples). By site, *S. aureus* and MRSA detection ranged from 0-30% and 0-15%, respectively. Eleven isolates were resistant to at least one antibiotic and four were multidrug-resistant (defined as complete resistance to ≥3 antibiotic classes). The most common *spa* types represented in this study were t008 (5/16) and t021 (7/16), which have been associated with clonal complexes 8 and 30, respectively. Other *spa* types represented include t216, t338, and t267. No *spa* types detected in this study have previously been associated with livestock, but phenotypic tetracycline resistance and lack of the *scn* gene--both of which have been considered markers of livestock association--were observed in four and six isolates, respectively. This study demonstrated that multidrug- and methicillin-resistant *S. aureus* were present in surface waters adjacent to industrial hog operation waste spray fields in southeastern NC. To our knowledge, this is the first documentation of waterborne *S. aureus* and MRSA collected from surface waters near industrial hog operations.

3A

EFFECT OF PIG POPULATION DENSITY ON METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* PREVALENCE IN BULK TANK MILK OF ITALIAN DAIRY FARMS

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MRSA contamination of pig holdings in Europe with ST398 and other lineages is a fact. Dairy cows can also be colonized and suffer mastitis by MRSA. Professional categories linked to farming resulted colonized at higher rates than general population due to exposure along all the production chains. The proximity to livestock itself is at risk. A Dutch recent study showed that people without direct contact with pigs or cows and living in a rural area were more likely to be LA-MRSA positive than people in urban settings. The mutual risk between different species in livestock has been not completely investigated yet. We used bulk tank milk (BTM) as a screening sample to detect MRSA in dairy herds located within a high-density pig farming area. Applying Geographic Information Systems (GIS) to the area, the association between density of finishing pigs and MRSA status of dairy herds was evaluated. Bulk tank milk (BTM) samples were collected in March 2011 from 225 dairy farms resulted positive for *taphylococcus aureus* in the bulk tank at a previous control. Each milk sample underwent a double enrich-

ment, firstly in Mueller Hinton broth with 6.5 % NaCl and then in Tryptic Soy broth with 3.5 mg/l cefoxitin and 75 mg/l aztreonam. Each enrichment step required an incubation for 18-20 h at 37°C. Finally, 100 µl were plated onto MRSA Chromogenic Agar (Pronadisa, Spain). After incubation of 48 h at 37°C, putative colonies were confirmed MRSA by a duplex PCR targeting *nuc* and *mec*A genes. Dairy herds with at least a MRSA isolate were classified MRSA-positive. Isolates were typed by Multi Locus Sequence Typing and *spa*-typing. Each milk sample underwent also DNA extraction. Dairy herds in whose milk the detection of *mec*A by PCR occurred were classified *mec*A-positive. All the other dairy herds were classified as negative. Geographic Information System ArcVIEW (ArcGis, 9.3.1, Esri, Redlands, CA) computed number of finishing pig holdings and pig heads within 3 km range around each dairy farm. Statistical analyses by SPSS 19.0 (IBM, SPSS Inc., Chicago IL, USA) and test U Mann-Whitney were applied to compare pigs consistency based on the MRSA status of dairy herds. Statistical significance was accepted when $P<0.05$. A total of 53 herds (23.6%) were *mec*A-positive and 9 herds (4%) were effectively MRSA-positive. Two STs were isolated (ST398 and ST97) and five *spa*-types (t899, t001, t108, t4795 and t9305). The number of finishing pig holdings was significantly higher around both *mec*A-positive ($P=0.004$) and MRSA-positive herds ($P=0.000$) than those surrounding negative herds. Also the number of finishing pigs was significantly higher around *mec*A-positive and MRSA-positive ($P=0.019$ and $P=0.001$, respectively). The present work give evidence to the significant effect of pig population density on dairy herds status regarding MRSA. STs and spa-types confirmed this link too, as they are most common in Italian finishing pigs.

4A

FINDINGS OF MRSA OF THE SAME *SPA*-TYPE IN FARMER AND DAIRY COWS IN SWEDEN

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Background: Methicillin resistant *Staphylococcus aureus* (MRSA) has only been detected sporadically in dairy cows in Sweden. Here we describe the findings of MRSA of the same *spa*-type from both farmer and several of the cows in a dairy herd. The farmer was confirmed positive with *pvl*-positive MRSA of *spa*-type t002, which is one of the most common *spa*-types in humans in Sweden.
Materials and methods: In order to investigate if also cattle in the herd were infected with or carriers of MRSA, all 68 animals in the herd were sampled. Composite milk samples were taken from all 25 lactating cows and one sample from bulk tank milk. Nasal swabs and samples from groin skin were taken from non-lactating cattle, including one dry cow and 42 young cattle and calves. In addition, wounds were sampled, if present. All samples were selectively enriched over night in 37°C in Trypton Soy Broth with mannitol and phenol red, 4% NaCl, 3.5 mg/L cefoxitin and 50 mg/L aztreonam. Composite milk samples and 10 mL of the bulk tank milk were centrifuged and the supernatant discarded before enrichment. Nasal swabs and swabs from groins were pooled five and five in enrichment broth. After incubation, enrichment broth was spread on MRSA Brilliance agar (Oxoid) and bovine

blood agar and incubated over night in 37°C. Suspected colonies were confirmed with PCR for the *nuc*-, *mecA*-, *mecC*- and *pvl*-genes. Confirmed isolates were *spa*-typed. **Results:** MRSA was detected in samples from 12 cows and in bulk tank milk. From 10 of the cows only the milk sample was positive, from one cow both the milk sample and the sample from groin and from one dry cow only the nasal swab. All isolates were positive for the *nuc*-, *mecA*- and *pvl*-genes and were of *spa*-type t002. MRSA was not detected in samples from young cattle and calves. One month later, all previously MRSA-positive cows, except three that had been culled, were sampled again by means of milk samples and nasal swabs. At the second sampling, MRSA was detected in milk samples from seven cows. **Conclusion:** Since MRSA-isolates both from the farmer and from dairy cows belonged to *spa*-type t002 which is one of the most common *spa*-type in humans but has never before been detected in farm animals in Sweden, it was suspected that transmission occurred from humans to dairy cows. All lactating MRSA-positive cows that remained in the herd after one month were still positive at the second sampling, indicating that MRSA of a common human *spa*-type was established in lactating cows in the herd.

5A

THE EFFECT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CARRIAGE AND DIET ON THE NASAL MICROBIOTA IN SLAUGHTER-AGE PIGS

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The nasal microbiota may play a role in determining the fate of bacteria to which it becomes exposed, including MRSA. The objective of this study was to describe the nasal microbiome of slaughter-age pigs and to evaluate the influence of the microbiome on MRSA colonization. Nasal swabs were collected from 16 age-matched pigs (8 MRSA positive, 8 negative) approaching slaughter age on one conventional swine farm. The nasal microbiome was assessed using next generation sequencing following V3-V5 16s rRNA gene PCR. Species richness was high, a mean of 120 species-level OTUs (range 25-277, median 98) and no difference between MRSA carriers and non-carriers (P=0.94). In conventionally fed pigs, the Proteobacteria Phylum was most abundant, followed by Firmicutes (largely based on high abundances of Staphylococcus, Bacillus and Paenibacillus spp). Bacteroidetes, Spirochaetes, Actinobacteria, Cyanobacteria, Thermotogae, Tenericutes, Synergistes, Fibrobacteres, Fusobacteria, Elusimicrobia, Lentisphaerae and Deferribacteres were present at lower abundances. There was no relationship between microbial population structure and MRSA carriage (P>0.05), nor was there any apparent clustering by principal component analysis. There were a few statistically significant differences at lower taxonomical orders, including Sphingobacteriales (Bacteroidetes Phylum, P=0.005), Burkholderiales order (P=0.037) and Comanadaceae family (P=0.036)(Proteobacteria Phylum) and Microbacterium Genus (P=0.021, Actinobacteria Phylum), the biological relevance of which is unclear. There was abundant staphylococcal diversity, with a total of 12 different species identified. ; S. aureus, S. epidermidis, S. equorum, S. fleuretti, S. hominis, S. pseudintermedius, S. kloosii, .S lentus, S. lugdunensis, S. pasteuri, S. saprophyticus and S. schleiferi, although care should be taken with this result because of the limited species resolution with 16s rRNA gene-based studies. There was a significant impact of diet. Liquid-fed pigs had significantly fewer Bacteroidetes (P=0.042) and Proteobacteria (P=0.027), and more Firmicutes (P=0.004). There were numerous differences between groups at lower taxonomic levels, including Paenibacillaceae (P=0.007), Staphylococcaceae (P=0.043), Planococcaceae (P=0.025) and Enterococcaceae (P=0.038). While there was limited influence of microbial population on MRSA colonization status, this study indicates that management factors can influence the nasal microbiome.

6A

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RETAIL MEAT IN THE CANADIAN MARITIME PROVINCES.

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Food animals and their products have been suggested to be an important source of antimicrobial resistant organisms, including methicillin-resistant Staphylococcus aureus (MRSA). While MRSA has been detected in livestock and meat products in various regions of Canada and the world, there are no estimates of MRSA in livestock or retail meat in Atlantic Canada. Overall, this region has lower estimates of methicillin-resistant staphylococci in animals. The objective of this study was to determine the prevalence and molecular types of MRSA in beef, pork and poultry in raw retail samples collected in New Brunswick, Nova Scotia and Prince Edward Island. It was hypothesized that the prevalence of MRSA in retail meats in the Canadian Maritimes would be lower than reported in other Canadian regions. Samples and epidemiologic data were collected as part of the Canadian Integrated Program for Antimicrobial Resistance Surveillance, which focusses on antimicrobial resistance among enteric bacteria. Isolation was completed by enrichment in broth containing mannitol and sodium chloride, followed by culturing to chromogenic agar. Isolates were tested for coagulase production, mannitol fermentation and resistance to oxacillin and cefoxitin. Detection of penicillin-binding protein 2a (PBP2') by latex agglutination was used to confirm methicillin-resistance. A multiplex PCR assay was used to differentiate between coagulase-positive staphylococci of veterinary significance: S. aureus, the S. intermedius group, and S. hyicus. Strain types were determined by sequence analysis of the X region of protein A (spa typing). Culture results from 195 samples (56 of each chicken, beef and pork, and 27 turkey) have been completed to date. The overall prevalence of MRSA in raw retail meat was 2.1% (4/195), while the prevalence in chicken was 5.4% (3/56), in beef 1.8% (1/56) and in pork and turkey 0%. All isolates displayed typical phenotypic characteristics of MRSA, and were PBP2' positive. Molecular typing is being completed and will be presented. These results are in contrast with a Canadian study where MRSA was primarily isolated from pork, with samples of chicken being the least likely to be MRSA positive. Determination of the strain type will provide insight to whether the MRSA isolated is human or livestock associated, and will lend evidence to the source of the contamination. Factors associated with MRSA contamination of retail meat will be explored.

7A

SLAUGHTERHOUSE PERSISTENCE OF LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA CC398)

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During the last decade livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) clonal complex (CC) 398 has become pandemic. Pigs are usually asymptomatic carriers, but MRSA CC398 can spread from animals to humans and therefore has a zoonotic potential. Three studies on MRSA in swine in 2008, 2008 and 2012, documented a very low prevalence of MRSA CC398 in Norwegian swine holdings with 0, 0 and 0.6% positive herds, respectively. However, in 2011, 1033 pigs from 207 different farms were sampled at ten different slaughterhouses. From one slaughterhouse, MRSA CC398 was

detected in six pooled samples (3%). An attempt to identify positive swine holdings was performed unsuccessfully. Follow-up sampling of the environment in the slaughterhouse barn showed that MRSA CC398 was present in the environment, and thereby may have contaminated the pigs during housing at the slaughterhouse giving an overestimation of herd prevalence. The objective of the present study was to perform a follow-up sampling at the slaughterhouse now two years after to investigate whether the slaughterhouse environment still is contaminated with MRSA CC398. In addition, the other ten largest slaughterhouses in Norway (handling more than 95% of all pigs slaughtered in Norway) were to be screened for MRSA the same way. MRSA was detected in one slaughterhouse, the same that was positive in 2011. Samples from the remaining ten slaughterhouses were negative. The positive slaughterhouse yielded nine positive samples out of a total of ten samples. Genotyping identified MRSA CC398 *spa* type t034. Norway probably still has a low MRSA prevalence in pigs as ten of the 11 slaughterhouses were negative for MRSA, and also based on the previous performed surveys. Slaughterhouse sampling could be a convenient and less expensive way for early detection of MRSA CC398 status in a country or new area, but the results cannot be used to give estimations on herd prevalence.

8A

INTRODUCTION, CIRCULATION AND MAINTENANCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN CONTACT SURFACES AT A LARGE EQUINE HOSPITAL: YEARLONG MOLECULAR EPIDEMIOLOGY OF ENVIRONMENTAL CONTAMINATION

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The role that environmental contamination might play as a reservoir and a possible source of Methicillin-resistant *Staphylococcus aureus* (MRSA) for patients and personnel at equine veterinary hospitals remains undefined, as the environment is only monitored during outbreak situations or for short periods of time. Therefore, the objectives of this study were to determine the monthly presence and distribution of MRSA in the environment of an equine hospital during one year, to characterize MRSA strains circulating in this setting, and to establish patterns of contamination over time using molecular epidemiological analyses. We hypothesized that if MRSA is present in both humans working at an equine hospital, as well as in horses admitted to such practice, then this bacterium will be found frequently contaminating different contact surfaces across the hospital throughout the year. For this purpose, a yearlong active MRSA surveillance was performed, including a molecular epidemiological analysis of environmental and equine-origin MRSA isolates. Antimicrobial susceptibility testing, SCC*mec* typing, PFGE typing, and dendrographic analysis were used to characterize and analyze these isolates. Overall, 8.6% of the surfaces were contaminated, and 5.8% of the horses sampled were positive for MRSA. The most common contaminated surfaces were: computer (16.7%), feed and water buckets (16.7%), and surgery tables and mats (15.6%). Characterization of all MRSA isolates showed that 90.1% of the isolates were carrying SCC*mec* type IV, and 47.9% were classified as USA500, reflecting a low diversity among the strains circulating the hospital. Molecular analysis showed that new pulsotypes were constantly introduced into the hospital throughout the year. However, maintenance of MRSA in the environment was also observed when unique clones were detected for 2 consecutive months in the same surfaces. It was also detected that MRSA pulsotypes were circulating throughout several areas and different contact surfaces of the hospital. Based on these results, it is evident that MRSA is constantly introduced and frequently found in the equine hospital environment. In

addition, there were contact surfaces that could act as "hot spots", where either MRSA was capable of surviving over time or continuously reintroduced throughout the year. These contaminated surfaces should be actively targeted for strict cleaning and disinfection as well as regular monitoring. **Key Words:** MRSA, Surveillance, Environment, Equine, Molecular Epidemiology, Veterinary Hospital

10A

PRESENCE OF LIVESTOCK-ASSOCIATED MRSA ON BELGIAN PORK

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Since the first description of livestock-associated MRSA (LA-MRSA), high prevalence rates were observed in pigs. However, the importance of this reservoir for public health remains obscure. To investigate one possible route of transmission, the prevalence of LA-MRSA on Belgian pork was determined. Meat samples (chops, bacon, minced pork, ribs, forelimbs and ears; n=137) originating from four butcheries (A to D) were collected weekly for six weeks. Twenty-five grams of chops, bacon and minced meat were 10 times diluted with salt-enriched broth. From the ribs, forelimbs and ears the cm^2/sample was determined and a 1/1 dilution was performed. After homogenization, the dilutions were spread plated on ChromID™ MRSA plates both before and after overnight enrichment. Suspect colonies were confirmed using a MRSA-specific triplex PCR and a CC398-specific PCR. The isolates (n=147) were further characterized using SCC*mec* typing, multiple-locus variable-number tandem-repeat analysis (MLVA) and antimicrobial susceptibility testing. On a selection of isolates, Pulsed Field Gel Electrophoresis (PFGE) and *spa* typing was performed. After direct plating of the dilution series, a general MRSA prevalence of 8% on pork was observed. The cfu/g or cfu/cm^2 ranged from 6.10^0 to 8.10^4. After enrichment, MRSA was isolated from 98 out of 137 samples (72%), mainly originating from rib, ear and forelimb. There was a large genetic diversity amongst the isolates within and between butcheries, which indicates that the obtained isolates were of various origins. Twenty CC398 isolates did not show acquired antimicrobial resistance, except to β-lactam antibiotics, which is unusual for LA-MRSA isolates. In conclusion, MRSA was found in a lower percentage of the pork samples after direct plating in comparison to the high percentage of positive samples after enrichment, which indicates that enrichment is recommended for examination of MRSA on pork. The genetic diversity of the isolates indicated that a butchery can be considered as a reservoir that acts as a potential contamination source for the general human population.

11A

EMERGENCE AND EPIDEMIOLOGY OF LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CC398 IN HUMANS, DENMARK, 2004-2011

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Over the past decade, a livestock-associated methicillin-resistant *Staphylococcus aureus* strain belonging to clonal complex 398 (LA-MRSA CC398) has been increasingly

identified in food-producing animals, retail meat, and humans. In particular, LA-MRSA CC398 has been identified as the predominant strain in pigs and also as an important human pathogen in Europe. The main objective of this study was to investigate the epidemiology of LA-MRSA CC398 in humans, Denmark. During 2004-2011, we noted a significant increase in the incidence of LA-MRSA CC398 carriers, including patients with LA-MRSA CC398 infections, as well as in the percentage of LA-MRSA CC398 carriers/patients among all MRSA carriers/patients. In addition, our findings strongly suggested that spread of LA-MRSA CC398 into the community is increasing due to repeated spillover from an expanding livestock reservoir, rather than to emergence and geographic expansion of distinct community-adapted LA-MRSA CC398 isolates. Consistent with this hypothesis, geographic information system-based analysis showed that LA-MRSA CC398 carriers/patients with no livestock exposure predominantly lived in close proximity to LA-MRSA CC398 carriers/patients with livestock exposure in rural areas where pig densities were high. These findings underscore the need for new strategies to control the spread of LA-MRSA CC398 in the livestock setting and from there to the community.

12A

DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN LIVE CHICKEN AND POULTRY PRODUCTS IN THE BUEA DISTRICT, CAMEROON.

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen in the Public Health sector as diseases caused by this agent are more difficult to treat. There is increasing evidence on the occurrence of MRSA in several livestock animals and the possible transmission of MRSA strains to humans. Although MRSA has been detected in poultry, there is paucity of information on the occurrence of MRSA in both live poultry and poultry products especially in sub-Saharan Africa. The aim of this study was to determine the prevalence of MRSA in both live chicken and poultry products from five poultry farms in the Buea District in Cameroon. **Materials and methods:** A total of 100 live chickens were sampled; including 50 broilers, 25 layers and 25 free-ranged birds. Samples included cloacal and nasal swabs from live chicken, meat from slaughtered chicken and swabs from eggs. Samples were inoculated unto mannitol salt agar (MSA) and isolates were identified using biochemical tests. Antimicrobial susceptibility was tested by disk diffusion. **Results:** MRSA was detected in chicken from all farms including the free-ranged birds; however, the prevalence was higher in broilers (24/50, 48%) than in free-ranged birds (5/25, 20%). MRSA was not detected in any of the layers' samples. Out of the 100 cloacal and nasal samples, MRSA was detected in 51 nasal swabs and 47 cloacal samples. The isolation rates of MRSA from chicken meat and egg swabs were 12/25, 48% and 8/25, 32%; respectively. Results of susceptibility test revealed that all isolates showed multiple resistance against antibiotics such as penicillin, erythromycin, oxacillin, amoxicillin, trimethoprim-sulfamethoxazole, clindamycin and tetracycline. On the contrary, most isolates were susceptible to chloramphenicol, ciprofloxacin and rifampin. **Discussion:** Results from this study confirms the occurrence of MRSA in live chicken and other poultry products as reported by previous studies. Thus, live chicken, eggs, poultry meat as well as other poultry products might be a potential source of infection in humans. The highest isolation rate of MRSA was observed in broilers while MRSA was not found in layers. In the present study, free-ranged birds had lower prevalence of MRSA than battery-caged birds. This difference might be due low exposure of free-ranged birds to antibiotic-containing feed. Further research is required on a larger flock to investigate the colonization of free-ranged and battery-caged poultry and their role in the

transmission of multi-resistant pathogens to humans.

13A

PERSISTENCE OF LIVESTOCK-ASSOCIATED METHICILLIN AND MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG INDUSTRIAL HOG OPERATION WORKERS IN NORTH CAROLINA

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Background. Whether livestock-associated (LA) *Staphylococcus aureus* is a persistent nasal colonizer of individuals frequently exposed to livestock remains unclear. Persistence studies have focused mainly on clonal complex (CC) 398 and methicillin-resistant *S. aureus* (MRSA) - not methicillin-susceptible or multidrug-resistant (MDRSA) *S. aureus* - and limited LA *S. aureus* persistence information is available from the United States. We conducted a 14-day study of nasal carriage of LA *S. aureus*, LA MRSA, and LA MDRSA among industrial hog operation (IHO) workers in North Carolina. **Methods**. Healthy volunteer IHO workers anticipating ≥24 hours away from work self-collected nasal swabs and completed an exposure and work activity journal in the evening on day 1, and in the morning and evening on days 2-7 and 14. Nasal swabs were incubated overnight in enrichment broth at 37°C, then plated on CHROMagar™ Staph aureus and incubated for 24 hours at 37°C. Resulting *S. aureus* isolates were confirmed by biochemical testing and assessed for presence of the 16S, *nuc*, *mecA*, and *scn* genes, susceptibility to 12 antibiotic classes, and spa-type. LA *S. aureus* were defined as any isolate demonstrating ≥1 of the following: absence of the *scn* gene, tetracycline resistance, or CC398. **Results**. 327 nasal swabs from 22 workers were analyzed. During the 14-day period, 10/22 participants were persistently carrying LA *S. aureus*, among which one persistently carried LA MRSA and six LA MDRSA. An additional six workers carried LA *S. aureus* intermittently, 5/6 of whom carried LA MDRSA. No worker carried LA MRSA intermittently. CC398 was detected among eight workers, of whom seven were persistently carrying. LA CC9 was detected among nine workers, of whom two were persistent carriers. Non-LA CC30, CC8, CC15, CC20, and CC779 were also observed among participants. Despite all but two workers experiencing ≥24 hours away from work, we observed null associations between work-related and non-work-related exposures and carriage of LA *S. aureus* and LA MDRSA. No symptoms indicative of infection were reported during the study period. **Conclusion**. Although limited by a small sample size, we observed evidence of persistent nasal carriage of LA *S. aureus*, LA MRSA, and LA MDRSA among workers at IHOs in the United States even after ≥24 hours away from work. Future studies should examine dynamics of LA *S. aureus* persistence in IHO workers' noses over longer periods of time with greater time away from work.

14A

THE HUMAN AND COMPANION ANIMAL MICROBIOME OF HOUSEHOLDS WITH METHICILLIN-RESISTANT STAPHYLOCOCCAL INFECTIONS

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Methicillin-resistant Staphylococcus aureus (MRSA), a skin pathogen in pets and people, produces high rates of re-infection even after

antibiotic treatment. In companion animals, species such as S. aureus, S. pseudintermedius, and S. schleferi, are major pathogens that produce a wide array virulence factors and result in many disease syndromes. Humans and pets live in the same environments, share skin-to-fur contact, and share microbiota; both pathogens and commensals. We hypothesize that companion animals share microbiota with humans, and that the microbiome has a role in modulating MRSA colonization and infection in households with and without pets. Methods: Under approved IRB and IACUC protocols, we enrolled 25 households where there was a primary human MRSA infection for a total of 63 pets (cats, dogs, etc) and 30 humans. We collected animal swab samples (mouth, nose, and lesion if applicable) for microbiome analysis while the humans self-sampled (a combined inguinal crease/axilla swab, a nasal swab, and a lesion swab for the index patient). We obtained samples at two time-points: enrollment and 3 months later, for a total of 329 samples. To investigate the microbiota present, we used next-generation sequencing technologies (Illumina MiSeq) to amplify the V4 region of the 16S rRNA gene from the extracted genomic DNA. The resulting reads were quality filtered, binned, assigned taxonomy, and analyzed with bioinformatics software (QIIME and mothur). We identified bacterial taxa present and analyzed them with alpha and beta-diversity metrics with respect to time, species, sample location, household, carriage of coagulase-positive Staphylococcus (CPS), and the presence of the mecA gene as determined by PCR. Results: The six most abundant bacterial taxa of the total oral and nasal companion animal microbiomes are: Streptococcus, Pasteurellaceae, Moraxellaceae, Clostridiales, Fusobacterium, and Staphylococcus. The greatest determinant of microbial composition is the species and location of sampling (Weighted Unifrac metric, Anosim test, R=0.58, p=0.001). Intriguingly, the human MRSA lesion samples had a high level of diversity and staphylococcal species were present at a low level (an average composition of 20% in the human lesion samples), indicating that the total microbial community may play a key role in Staphylococcal disease modulation. Conclusion: The results of this study describe the human and animal microbiomes longitudinally and indicate that there is transmission of microbes and methicillin-resistance genes between family members and their pets.

15A

THE EVALUATION OF MOLECULAR EPIDEMIOLOGICAL RELATEDNESS WITHIN ST5 *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SWINE VETERINARIANS IN THE USA

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The livestock associated *Staphylococcus aureus* (LA-MRSA) ST 398 has been isolated frequently from livestock, especially pigs, worldwide since first detected from pigs and pig farmers in the Netherland in 2005[1]. Subsequent studies are revealed greater genetic diversity in LA-MRSA using molecular epidemiologic characteristics. Several studies reported that ST9 isolates are predominate among MRSA isolates from pigs in Asian countries, while both ST398 and ST5 lineages have been found in pigs and livestock workers in North America[3,4]. Unlike ST398, which is rarely involved in significant human infections, the occurrence of ST5 sequence type in pig industry is of some public health concern as this ST5 lineage has long been associated with human MRSA infections related the USA100 group (defined by sma1 PFGE)[2,5]. In a pilot study of pig farms in Minnesota, the predominant spa types found were t034 *(ST398*), and t002 (ST5), comprising 37% and 29% of isolates respectively (all MSSA). The aim of this study was to investigate the prevalence of ST5 *S. aureus* nasal swabs in US swine veterinarians, and to evaluate the diversity of spa type t002 isolates using PFGE with *Sma*I. Similar to our findings in pigs, the most prevalent spa types found in US swine veterinarians were t034-ST398 and t002-ST5. ST9 spa types

(t337, t3446, t2498) were also common. Sixty (19%) of 308 MSSA isolates and 6 (19%) of 32 MRSA isolates detected were *spa* type t002 (ST5). Other ST5 MSSA spa types found included t045 (14), t062 (6), t570 (3) and t2049 (1). Sma1 PFGE analysis of 23 isolates indicated that t002 isolates from the swine veterinarians were not clonal but heterogeneous between isolates. Fifteen distinct pulsotypes were seen, and eight isolates were classified as USA100. Given previous reports of t002-ST5 MRSA in North American pigs, our findings of diverse ST5 spa types as well as genetic diversity within spa type t002, provide improved understanding of ST5 lineage, which may have long association with pigs overtime after introduction from human. Further investigation of ST5 lineage is warranted to better understand their potential implications for human health.

16A

MOLECULAR CHARACTERIZATION OF *S. AUREUS* ASSOCIATED WITH PIG ENVIRONMENT IN POLAND, 2010-2013

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Objectives: The objective of the study was to investigate the nasal carriage of S. aureus among Polish pig farmers, veterinarians working on selected farms and at the same time/place among pigs, and to compare on molecular level recovered S. aureus. **Material and methods:** The study was performed on 339 and 2070 nasal swabs from humans and pigs, respectively, taken in Polish 125 farms, between 2010 and 2013. For all isolates re-identification by standard microbiological methods, the detection of mecA and lukS/lukF-PV genes was performed. They were characterized by spa-typing, RM test typing, and SCCmec typing. MLST and dru-typing were performed for selected isolates. **Results:** In total, 192 isolates were identified as S. aureus, including 128 from pigs (58 MRSA and 70 MSSA) and 64 from humans (total carriage 19%; 14 MRSA and 50 MSSA). All characterized isolates belonged to 10 genetic lineages: CC398 (n=140), CC9 (n=26); CC30 (n=14, CC22 (n=3), and CC1, CC8, CC15 (n=2 each), and CC12, CC5, ST182 (n=1 each). Among identified genetic lineages, five (CC398, CC9, CC30, CC22 and CC1) were represented by isolates originated both from pigs and humans, four (CC5, CC8, CC15, and ST182) by isolates from humans, while CC12 by one isolate from pig. lukS/lukF-PV genes were not detected. MRSA isolates belonged to two clonal complexes only (CC398 and CC30), with CC30-MRSA isolates observed exclusively in pigs. CC398, the most represented CC comprised 94 isolates from pigs (50 MRSA and 44 MSSA) and 46 from humans (14 MRSA and 32 MSSA). Among CC398-MRSA, six spa types were assigned: t011 (n=31), t034 (n=25), t108 (n=4), t4389 (n=2), and t2582, t5452 (n=1 each); and three SCCmec types were determined: Vc[zinc] (n=55), V (n=8) and IVa (n=1). The most prevalent dru type was dt11a (n=26 out of 30), followed by dt11ap, dt9s, dt6j and dt5e represented by single isolates. Among CC398-MSSA, 13 spa types were assigned, with the most prevalent t034 (n=36), followed by t4389 (n=13) and t1928 (n=8). All CC398 isolates represented sequence type ST398. The second numerous CC was CC9, gathering only MSSA isolates, both of pig (n=21) and human (n=5) origin. Among this CC 8 spa types were assigned, with the most common t1430 (n=9), t337 (n=7) and t1670 (n=4). The third CC, in terms of the number of isolates, was CC30, that encompassed 10 isolates from pigs (8 MRSA and 2 MSSA) and 4 MSSA from humans. MRSA isolates were characterized by spa type t318, SCCmec IVa and dru type dt10a. CC30-MSSA were assigned to five spa types: t318 (n=2) and t018, t021, t1333, t9034 (n=1 each). **Conclusion:** It seems that isolates of CC398 lineage are most prevalent and well settled in pig farm environ-

ment in Poland. Polish S. aureus belonging to CC398 are genotypically similar to the strains observed worldwide.

17A

GENOTYPE AND PHENOTYPE ANALYSIS OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ST 398 FROM HUMANS, ANIMALS AND RETAIL MEAT.

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Background: Methicillin resistant *Staphylococcus aureus* is a significant pathogen of healthcare, community and livestock associated infections. Among strains of interest are those identified as multi-locus sequence type 398 because of their emergence in swine and human disease. MRSA ST 398 is unique in the inability to sub-type these strains by pulse field gel electrophoresis (PFGE) using SmaI, due to a methylase which modifies the clevage site of the enzyme rendering the strain indigestible. ***Methods:*** A collection of MRSA ST398 strains (n = 68) from humans, swine, sheep, and retail meats were assessed using molecular tools to subtype them. All strains examined were identified as MLST ST398, and failed to restrict when SmaI was used for PFGE Analysis. Molecular profiling was carried out using *spa* typing, SCC*mec* typing, multi locus variance analysis (MLVA), virulence genotyping, biofilm assay and PFGE using EagI, Cfr9I and BstZI. ***Results:*** Variable results were observed but a combination of tools appeared to allow better differentiation of the strains examined. *spa* types identified included types t011, t034, t693, t1430 and t4389, some of which were found in both human and animal host strains, and raw meat. SCC*mec* typing for strains identified as possessing *mecA*, were found to be positive for types I, II and IV. MLVA analysis produced limited results and most isolates gave patterns with 5 bands but could not differentiate the strains as most appeared to have identical patterns. Best results were observed using novel restriction enzymes and PFGE. Restriction patterns for isolates examined using EagI had typically 12 bands ranging in size from 30 kb to < 300 kb; Cfr9I resulted in patterns with 16-20 fragments ranging in size from 20 kb to 1100 kb, and BstZ1 analysis yielded approximately 20 fragments of sizes from 20 kb to 700 kb. Virulence gene analysis produced limited patterns but the presence of adhesion genes appeared to correlate well with biofilm assays. ***Conclusion:*** Multiple molecular tools provide value in assessing host source of MRSA and offer alternative strategies for strain differentiation. Ongoing work is validating the use of these tools for blinded isolates to determine traits for rapidly distinguishing sources of MRSA.

18A

RELATIONSHIP OF MRSA IN VARIOUS SAMPLES FROM INSIDE AND OUTSIDE OF DIFFERENT POULTRY FARMS

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Objective: The occurrence of methicillin resistant Staphylococcus aureus in different animal species has been investigated during the last years. MRSA has a mobile genetic element called staphylococcal cassette chromosome mec which carries the mecA gen being responsible for the resistance against ß-lactam antibiotics. In this study different samples from animals, the barns´ interior and from the vicinity outside were taken. The aim of the presented study is the detection of epide-

miological relations between the strains of different origin. **Material and Methods:** Five turkey and two broiler farms were analyzed three and four times, respectively, within one fattening period. Samples were collected from individual animals (swabs of choana and skin), their direct surroundings (boot swabs, feces, dust, feed, air) as well as from the farms´ environment by taking air samples and samples of the ground surfaces around the barns up to distances of 500m. Selected isolates from in- and outside were spa typed and grouped as Livestock associated (LA)-MRSA according to their association to the clonal complex (CC) 398. Additionally, the isolates were compared by using pulsed field gel electrophoresis (PFGE). **Results:** The investigated isolates predominantly belonged to the clonal complex 398 and are therefore assigned to livestock associated (LA) MRSA. We detected up to five different spa types occurring in and around one investigated barn, however, the spa types of isolates from inside were the same as these from outside. This is underlined by the preliminary results of the PFGE where we could find a close relationship between samples taken from animals, the barn interior and the vicinity of one barn. However, until now this relationship was not found when comparing the isolates from different barns with each other although they belonged to the same company. **Conclusion:** Since we found the same PFGE patterns in isolates from in- and outside of one farm the results show that an emission of MRSA from broiler farms definitively occurs. A preliminary comparison of the results between spa typing and PFGE show a high relationship. However, the PFGE method is necessary for deeper epidemiological analysis. Until now it seems that the relationship of the isolates originating from different sample matrices occurs within one barn and is different between different farms even when they were located in the same region and belonged to the same company as it was the case in both broiler fattening farms. Maybe one MRSA clone is selected and then distributed within the barn. More results will be presented on the conference.

19A

LONGITUDINAL QUANTITATIVE STUDY OF NASAL MRSA CARRIAGE IN PIGS

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Introduction: The current knowledge of nasal methicillin-resistant *S. aureus* (MRSA) carriage in pigs is based on the use of enrichment methods that do not provide any quantitative information about MRSA distribution within individual pigs and herds. **Objective:** To determine in-herd prevalence and nasal load of and MRSA in adult pigs. **Methods:** A longitudinal quantitative study was performed in 16 randomly selected Danish production farms. Within each farm, 24 individual pigs in the finishing sections were ear tagged and sampled three times at weekly frequency using cotton swabs. Swabs were suspended in 1ml saline and serial dilutions (10^{-1} to 10^{-4}) were enriched in Mueller-Hinton broth 6.5% NaCl. Following incubation, aliquots of the enrichment were plated onto MRSA2 Brilliance agar (Oxoid, UK) and MRSA load (CFU) in swabs was estimated by the most probable number (MPN) method. Data were analysed statistically using a logistic regression model for either multinomial or binary data (proc glimmix, SAS Inst.), with sampling round, swab cleanliness and MRSA-positivity for herd as fixed effects when appropriate, and farm, room and pig as random effects, when appropriate. **Results:** Eleven of the 16 farms were MRSA-positive. Among positive farms, 58 pigs (22%) were permanent MRSA carriers, 139 (54%) were transient carriers and 62 (24%) were non-carriers. MRSA load in positive nasal swabs was on average 2.53 $\log_{10}$ cfu per swab (s.d. 0.45) in permanent carriers which was significantly higher than 2.36 $\log_{10}$ cfu per swab (s.d. 0.52) observed in intermit-

tent carriers (OR 1.5, p=0.005). **Conclusions:** This study provides the first data on MRSA load in the nose of pigs, which are comparable to previously observed MRSA load in humans. Prevalence of positive farms was markedly higher compared to previous data based on screening of slaughter pigs in Denmark. Our data showed a significant association between MRSA load and stability of carriage where permanent carriers harbour significant higher amounts of MRSA.

20A

PERSISTENCE AND DIFFUSION OF *MECC*-POSITIVE CC130 MRSA ISOLATES IN CATTLES IN MEURTHE-ET-MOSELLE DEPARTMENT (FRANCE)

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Background: Recently, mecC gene has been described in methicillin-resistant Staphylococcus aureus (MRSA) isolated from cattle. This mecA variant has been mainly found in Northern Europe. In France, animal cases were detected only in two cows in 2008 and 2011 in two farms distant by less than 15 kilometers in the Meurthe-et-Moselle department, France. In 2013, the two same dairy farms were screened again for mecC-positive MRSA as well as farms in the surrounding area to study epidemiological dynamics of these strains. **Methods:** Seventeen dairy farms were tested from January to March 2013. We collected nasal swabs (NS), rectal swabs (RS) and milk (M) from ten randomly-choosen cows in each farm. Farmers (n=21) and domestic animals (n=7) were also sampled, respectively in 12 and 7 farms. Swabs and milk were inoculated into a pre-enrichment medium containing Brain-Heart broth with 5% NaCl broth. After overnight incubation at 37°C, hundred microliters were streaked on SAID agar (bioMérieux) and BrillianceTM MRSA agar (Oxoid). Suspected colonies were subcultured on blood agar and identification was confirmed by MALDI-TOF (Vitek MS, bioMérieux). All S. aureus isolates were tested by PCR for the presence of nuc (a species-specific marker), mecA and mecC genes. MecC positive strains were further characterized using DNA microarray StaphyType Kit (Alere Technologies). **Results:** Whereas S. aureus were isolated in 10 out of 17 farms, including 39 isolates (NS (n=9), RS (n=4), M (n=18), co-colonisation (NS + M; n=4)) from 35 cows, only seven isolates were MRSA. All of them harboured mecC gene. These mecC-positive MRSA isolates were detected only in two specific farms and included respectively two out of the 10 tested cows (NS (n= 2), M (n=1)) and four out of the 10 tested cows (M (n=3), RS (n=1)). The two farms corresponded to those in which mecC-positive isolates had been detected in 2008 and 2011. All MRSA strains belonged to the clonal complex CC130. None of the farmers and domestic animals (including those from farms with mecC-positive isolates in animals) were positive for MRSA. **Conclusion:** Our data suggest that mecC-positive strains are able to persist in farms for a long period (> 5 years), but also to colonize noses, digestive tract and/or to be involved in sub-clinical mastitis in cows as well as to disseminate and to induce outbreak within cattles. Diffusion from cattle to neighbouring cattle and from animals to farmers appears to be limited and to be restricted to specific dairy farms. A larger study in this geographical area with inclusion of a higher number of farms, sampling of all cows in mecC-positive screened farms and clinical human MRSA isolates are underway to further explore the epidemiology and dynamic of mecC-positive clones.

21A

SUSCEPTIBILITY OF METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS*, ON VOLCANOES NATIONAL PARK PERSONAL PROTECTING ENDANGERED MOUNTAIN GORILLA IN RWANDA - 2012

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Humans are natural reservoir of Staphylococcus aureus germs 30 to 40% of healthy adults are carriers. Staphylococcus aureus can be found temporarily or permanently in the skin and mucous membranes (nasal, throat, perineum, groin, navel, and armpit). Following the excessive use of antibiotics and hygienic measures insufficient, some percentage of Staphylococcus aureus become resistant to antibiotics, the objective of this study is to evaluate the susceptibility and prevalence of antibiotics on Staphylococcus aureus isolated from Volcanoes National Park, personal protecting endangered mountain gorilla. In mountain gorilla population, Staphylococcus aureus, can be identified as a secondary infection during respiratory virus infection. 173 anterior nares samples were collected in microbiology laboratory with a sterile swab (BBL Culture swab, Becton Dickinson and Company, Sparks, Maryland, USA) was inserted approximately 1 cm inside the nostrils of each employee before the process bacteriological culture, the sample was enriched in 2 ml of sodium chloride (NaCl) for 24 to 48 hours, then inoculated on fresh blood agar medium at 37 ^{0} C for 24 hours in an aerobic environment. The susceptibility of pathogenic bacteria was performed by the method of diffusion on the solid medium Muller Hilton, as proposed in the recommendations of the committee susceptibility of the French Society of Microbiology (CASFM) and interpreted by comparing the diameters obtained critical diameters proposed by CASFM.les antibiotics (code, load the disc). For the susceptibility test the antibiotics discs use are: cefotaxime, Oxacillin, imipenem, ampicillin, gentamicin, penicillin, Erytromicine, and Methicillin. The anterior nares swabs were taken from 173 Volcanoes National Park personal protecting endangered mountain gorilla 4/173 (2.3%) had Staphylococcus aureus, $_{\beta}$ lactamase positive, antibiotic sensitivity reveals: Cefotaxime was 75% sensitive, Oxacillin was 25% sensitive, Imipenem: was 75% intermediate and 25% , Ampicillin: was 25% sensitive, Gentamicin: was 100% sensitive, Penicillin: was 75% sensitive , Erytromicine: was 75% sensitive and Methicillin was 100% resistant. The strain of Staphylococcus aureus was isolated from (2.3%) Volcanoes National Park, personal protecting endangered mountain gorillas, has active resistance with $_{\beta}$ lactamase production, Methicillin resistant at 100%, this aspect calls us to deepen research in molecular biology, polymerase chain reaction (PCR) for specific identification and epidemiological surveillance of this organism present in the mountain gorillas and humans.

22A

RELIABILITY OF METHODS FOR DETECTING METHICILLIN-RESISTANT *STAPHYLOCOCCI* (MRS) IN ANIMALS DURING ROUTINE MICROBIOLOGICAL DIAGNOSTICS

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Objectives: Routine microbiological diagnostic service assumes that results are available within 48 to 72 h, significantly interfering with detection of MRS strains, especially when laboratory processes large number of samples. Broth microdilution recommended by EUCAST and CLSI, as well as PCR, are time consuming, expensive and technically demanding procedures to be applied in routine diagnostic, especially when most laboratories do not have access to PCR. In Serbia MRS strains are usually reported only upon resistance of staphylococci to cefoxitin detected with disc

diffusion. The subject of this research was to test if animal MRS strains can be detected with certainty during routine diagnostic work by using disc diffusion cefoxitin susceptibility, E test and Slidex MRSA Detection kit. **Materials and methods:** MRS strains were isolated from ear, eyes, skin, and nose swabs originated from different animal species and identified with ID32 STAPH (bioMerieux). Although amoxicillin with clavulanic acid (A/CL) is not recommended due to unreliability of results, since 2012 our routine laboratory work does use A/CL discs besides cefoxitin (Becton Dickinson) for the purpose of better interpretation of staphylococcal susceptibility to β-lactams. MIC values were determined with E test (bioMerieux). Detection of PBP2a was done with Slidex MRSA agglutination kit (bioMerieux). Confirmation of presence of a mecA gene was done by PCR using MRSA ATCC 43300 as control. **Results:** From 2007-2013, 35 MRS strains were detected in 21 dogs, 8 cats, 5 pigs and one donkey sample submitted to routine diagnostics. Until 2012, disc diffusion revealed 31 strains with cefoxitin resistance, E test showed MIC cefoxitin values >4 µg/mL, 22 strains were positive for PBP2a in Slidex MRSA test, and 9 CoNS were false negative. Presence of mecA gene in all strains was detected with PCR. However, in 2012, the finding of 4 CoPS that were sensitive to cefoxitin (>22 mm zones, EUCAST 2103) and resistant to A/CL (zones <20 mm, CLSI 2011), was unclear since this is impossible resistance phenotype. E test of these strains showed sensitivity to cefoxitin with MIC values of <1.5 µg/mL, except for 1 strain of MIC = 3µg/mL, and resistance to A/CL with MIC values of >8/4 µg/mL. These strains were clearly positive on Slidex MRSA detection test and had mecA gene confirmed by PCR. **Conclusions:** Use of cefoxitin alone in disc diffusion can fail to detect MRS. Based on results presented above, it is suggested that A/CL is used alongside cefoxitin, because strains that show cefoxitin sensitivity, but also display A/CL resistance may be MRS. Further studies using larger number of samples are warranted to confirm the results of this study. In addition, E test was deemed applicable to routine diagnostics, since there were no differences in susceptibility results from E test and disc diffusion. Slidex MRSA detection kit is proper for PBP2a detection in CoPS, not for CoNS.

23A

TRENDS IN ANTIMICROBIAL RESISTANCE IN CLINICAL *STAPHYLOCOCCI* ISOLATED FROM COMPANION ANIMALS OVER A 8-YEAR PERIOD

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Staphylococci are a group of bacteria with clinical, agricultural, and economic importance because of their ability to become resistant to antimicrobials. This alarming feature should be considered and antimicrobial susceptibility testing (AST) is important to monitor the spread of resistant organisms/genes and the rise of multidrug-resistant staphylococci. The aim of this study was to investigate the evolution of Staphylococcus spp. resistance to antibiotics between 1999-2006. The 384 clinical samples were received by the Laboratory of Clinical Analysis of the FMV-UL and were obtained from various animal species admitted to several hospitals in the Lisbon's district. AST was performed by disk diffusion using 26 antibiotics (AB). Staphylococcal species were identified by PCR amplification of the respective nuc gene and by 16S rRNA sequencing. The mecA and mecC genes were screened by PCR. Statistical analyses were performed using SAS 9.3 and differences were considered relevant if $P \leq 0.05$. In total, 251 isolates were resistant to at least 1 AB (65.36%), with higher frequencies of resistance to penicillin and ampicillin (53.13%) followed by tetracycline (31.77%). Overall, 75 isolates (19.53%) were multidrug resistant and only 34.64 % of the isolates were susceptible to all the AB tested. Resistance increased over time, with the

highest level observed in 2006 (79.55%). The highest resistance to 1 AB was found in S. aureus and S. pseudintermedius (nearly 73% of the isolates) followed by coagulase-negative staphylococci (CNS) and S. schleiferi (about 49%). Eleven CNS were resistant to oxacilin, but only 8 were mecA-positive. One S. aureus had the mecA gene and was cefoxitin-resistant. When compared to dogs, cats had lower odds to have a resistant isolate (P=0.001, OR=0.307, [0.152-0.619]) and a CNS resistant to at least 1 AB (P=0.004, OR=0.170, [0.051-0.560]). S. pseudintermedius originating from otitis had higher odds of being resistant to at least 1 AB than those from pyoderma (P=0.013), OR=0.452, [0.241-0.845]). Our results confirmed that antimicrobial resistance is very frequent in staphylococci, and the presence of methicillin-resistant staphylococci in sick animals highlights the importance of horizontal transfer of different SCCmec elements and might favor the transmission of resistance genes from staphylococci present in animals to those from humans. Changes in antibiotic resistance require continuous monitoring for adjustment of antimicrobial strategy.

24A

EPIDEMIOLOGY OF METHICILLIN RESISTANT COAGULASE POSITIVE *STAPHYLOCOCCUS* IN AUSTRALIA

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Coagulase-positive staphylococci are recognised as major zoonotic pathogens causing serious infection in both humans and animals. Recently, there has been an increase in the prevalence of methicillin-resistant Staphylococcus spp. in both humans and animals around the globe. Australia currently has no national network of surveillance for monitoring antimicrobial resistance in animals. We recently commenced a study sponsored by Zoetis to determine the prevalence of antimicrobial resistance among an Australia-wide collection of coagulase positive staphylococci causing clinical infections in animals. The survey will focus on coagulase-positive staphylococci (n=1500) identified as causes of infection by all veterinary diagnostic laboratories (n=22) across Australia from January-September 2013. All isolates will be subjected to CLSI disc diffusion susceptibility testing for 16-18 antimicrobials of importance to veterinary and public health. Here we report the antimicrobial resistance profiles on the first 193 isolates (S. pseudintermedius n=120 and S. aureus n=73). S. pseudintermedius isolates were all obtained from dogs and cats whereas S. aureus isolates were from dogs (n=16) and cats (n=7), horses (n=9) and bovine mastitis (n=41). The antimicrobial resistance profiling of S. pseudintermedius revealed that 10% of the isolates were resistant to oxacillin. In addition, resistance to penicillin (80%), tetracycline (20.8%), azithromycin (12.5%), gentamycin (11.6%), clindamycin (11.6%) trimethoprim-sulfamethoxazole (9.2%) and ciprofloxacin (7.5%) was also observed. The majority of the oxacillin-resistant isolates exhibited a multidrug-resistant phenotype (resistance to clindamycin, trimethoprim-sulfamethoxazole, azithromycin, gentamicin and ciprofloxacin). A slightly higher proportion of S. aureus isolates from dogs, cats and horses were resistant to oxacillin (12.5%). The S. aureus isolates were resistant to penicillin (78.1%), tetracycline (28.1%), gentamicin (9.4%), azithromycin (3.1%) and ciprofloxacin (3.1%). The S. aureus from cases of bovine mastitis were sensitive to all antimicrobials tested with the exception of penicillin (19.5%). This study demonstrates that methicillin-resistant S. pseudintermedius isolates with a multidrug resistant phenotype have emerged in Australia but currently represent a small proportion of total isolates. Whilst a higher proportion of S. aureus isolates showed methicillin resistance these isolates were sensitive to a greater number of antimicrobial classes. Whilst the study is only in its preliminary stages, it does provide the first

national data on antimicrobial resistance in coagulase positive staphylococcus for evaluating the public health impact of veterinary use of antimicrobials in both livestock and companion animals in Australia.

25A

STAPHYLOCOCCUS SPP. ANTIBIOTIC RESISTANCE ON THE RISE; A SOUTH EAST USA PERSPECTIVE

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The global rise of Staphylococcal infection (Staph) poses a veterinary challenge as increases in Meticillin resistant staphylococcus (MRS), indicating general beta-lactam resistance, often corresponds with resistance from other drug classes making treatment prolonged and/or difficult. We characterized the Staph infections submitted to the University of Georgia's (UGA) Athens Diagnostic Laboratory (ADL) from clinically ill patients over the years 02, 05, 08 and 11 from UGA's small and large animal hospitals and those submitted from private clinics. We hypothesized that the prevalence of MRS increased annually as has the prevalence of multi-drug resistance (MDR) within them. We aimed to identify differences in drug resistance between primary and tertiary care centers and hypothesized a higher prevalence of MRS in tertiary care settings who often perform surgical interventions and take referrals from failed primary care treatment posing higher infection risks. Over the study, 3406 of the phenotypically identified Staph infections had antimicrobial sensitivity testing. Oxacillin was used as a proxy for Meticillin resistance and Tetracycline, Trimethoprin-sulfa and Fluoroquinolones as indicators for MDR. S.psuedintermedius comprised 66% (2235/3406) of the total infections & 50% (293/580) of MRS infections. Undifferentiated coagulase (-) infections comprised 21% (727/3406) of all infections & 33% (191/580) of MRS. S.aureus made up 9% (305/3406) of total infections and 11% of MRS (64/580). Undifferentiated coagulase (+) infections comprised the rest; the relative proportion of staph subtypes remained consistent over the years. Year tested was associated with MRS ($p<.0001$); the prevalence of MRS increased from 6% (45/722) in 2002, 11% (98/857) in 2005, 19% (181/977) in 2008 and 32% (256/794) in 2011. The prevalence of MDR Staph infections also increased linearly and was associated with year ($p<.0001$). In 2002, 4% (2/45) of all MRS were resistant to all three drug classes used to indicate MDR compared to 36% (91/256) in 2011. Conversely, in 2002, 36% (16/45) of all MRS cases were sensitive to the three drug classes; this dropped to 19% (48/256) by 2011. Care center was associated with MRS ($p<.0001$). Primary care contributed 79% (2091/2639) of the cases and had an overall MRS prevalence of 18% (367/2091) while tertiary care centers contributed 21% (548/2639) of cases but had a MRS prevalence of 31% (168/548); 767 cases did not specify location and were removed from this analysis. Companion animals (dog, cat, horse, ferret, guinea pig, rabbit) comprised 97% (3301/3406) of all cases which is concerning for practitioners in this population. Treatment and prevention options must be carefully evaluated as resistance spreads. This is paramount within tertiary care settings which hosts a larger prevalence of MRS and often contain patients in close proximity to one another for longer time periods.

26A

DETECTION OF MRSA DURING THE BACTERIOLOGICAL ROUTINE EXAMINATION

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Because of their antimicrobial resistance situation and the risk of transmission to other species and contamination of the environment, infections with Methicillin resistant

Staphylococcus aureus-species constitute a big challenge for human and veterinary medicine. Therefore, the early detection of MRSA infections is important for the early initiation of the optimal therapy and for interrupting transmission pathways. Thereby, the quick and certain diagnosis in the laboratory is of special significance. This study shows retrospectively, how many MRSA could be identified during the microbiological routine examination of veterinary sample materials in 2012. S. aureus are identified in clinical samples by means of their culture morphology, hyaluronidase activity and, if applicable, by using MALDI-ToF mass spectrometry or latex agglutination (best bion dx GmbH, Cologne, Germany). S. aureus-isolates that show complete resistance against beta-lactam-antibiotics and cephalosporins in the antibiogram are selected and analyzed with an MRSA-detection test (Biomerieux, Marcy L'Etoile, France). If this test shows a positive result, all isolates are examined via PCR (McDonald, Antonishyn et al., 2005) for the presence of the mecA gene. In 2012, S. aureus-species were diagnosed in a total of 1.333 samples of companion animals, horses and farm animals. 5,93 % of these isolates showed resistances to beta-lactam-antibiotics and cephalosporins and a positive agglutination result. 84,81 % of these resistant S. aureus-isolates had the mecA gene. 53,16 % of the MRSA-suspicious S. aureus-isolates came from dogs, 27,85 % from cats, 15,19 % from horses and 3,80 % from birds/rabbits. The highest percentage of mecA positive S. aureus-species among all suspicious isolates was found in horses. Wounds were the main localization in all animal species. This study reveals that a multi-resistant antibiogram can give certain indication of the presence of the mecA gene. Even during the bacteriological routine examination, such S. aureus-species are positively detected and therefore give the practitioner an early possibility of initiating an optimal therapy and adequate hygiene management.

27A

GENETIC RELATEDNESS, ANTIMICROBIAL AND BIOCIDE SUSCEPTIBILITY PATTERNS AMONG HUMAN, ANIMAL AND ENVIRONMENTAL METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN PORTUGAL

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The extension of the host range of methicillin-resistant Staphylococcus aureus (MRSA) to different animal species has raised the concern for the role of animals in the epidemiology of MRSA infection and colonization in humans. The aim of this study was to characterize the genetic relatedness, antimicrobial and biocide susceptibility patterns of human, animal and environmental MRSA isolates (n=60). Infection (i) and colonization (c) isolates were obtained from pigs (n=17 i+c), environmental dust samples from breeding pig sheds (n=14), humans in contact with animals (n=11 c), calves (n=6 c), dogs (n=5 i+c), cats (n=5 i+c) and horses (n=2 c). MICs towards 26 antimicrobial agents, 3 biocides and 1 dye were determined by broth microdilution according to CLSI recommendations. PCR amplification was used to detect antimicrobial resistance and efflux-pump genes. The promoter region of the norA gene was sequenced. All strains were subjected to PFGE and spa typing. Overall, eleven spa types were identified. Four of these spa types were associated with CC398 (n=40), while five were related to CC22 (n=17) and only three associated with CC5 (n=3). Three different clusters, corresponding to CC5, CC22 and CC398, were identified by PFGE (> 80% similarity). All CC398 strains were resistant to tetracycline due to the presence of tet(M),

tet(K), tet(L) or a combination of these genes. All CC22 and bovine CC398 strains were fluoroquinolone-resistant. One porcine and the six bovine CC398 MRSA, were resistant to chloramphenicol and florfenicol due to the fexA gene. Genes dfrK and vga(A) were present in almost all porcine and environmental MRSA CC398 strains. Twelve strains had high MICs to ethidium bromide (32 mg/L in three strains and 16 mg/L in nine strains) and benzalkonium chloride (1 mg/L in four strains, 2 mg/L in seven strains and 4 mg/L in one strain) when compared to S. aureus ATCC 29213 and all carried the qacG gene. Two of these strains had an insertion of sequence CAAT in the -10 motif of the norA promoter gene. All strains had a low MIC to triclosan (≤ 0.125 mg/L). This study showed that MRSA are important reservoirs of antimicrobial and biocide resistance genes. Animals and humans that are in close contact with them share the same MRSA clone. Moreover these strains can stay in the environment, which can perpetuate the colonization/infection cycle. This raises the concern for the successful treatment of human infections and the effective control of MRSA dissemination.

28A

ANTIMICROBIAL RESISTANCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM PIGS IN BELGIUM

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During the last decade, an increasing number of studies reported the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in animals. Most studies have focused on the asymptomatic carriage of MRSA among pigs, in which clonal complex (CC) 398 is the dominant lineage. During this year, we are performing a survey in different pig farms randomly selected over Belgium, with the aim of monitoring the current prevalence and antimicrobial susceptibility of MRSA among asymptomatic pigs. Detection and identification of MRSA was confirmed by selective chromogenic agar (ChromID MRSA) and 16S rRNA-*mecA-nuc* triplex PCR. MRSA isolates were characterized by susceptibility testing by a microbroth-dilution method using epidemiological cut-off values (Eucast), *spa*-typing and *sau1-hsdS1* CC398 PCR. Currently, a total of 111 farms, out of the 141 tested, were positive (78.7%) for MRSA. One MRSA isolate was recovered from each farm. At present, a total of 87 isolates have been characterized by susceptibility testing. They showed a high prevalence of resistance to tetracycline (98.9%) trimethoprim (97.7%), clindamycin (75.9%), ciprofloxacin (71.3%), erythromycin (64.4%), and gentamicin (50.6%). Lower resistance levels were detected against to kanamycin (46.0%), tiamulin (40.2%), quinupristin/dalfopristin (32.2%), streptomycin (34.5%), sulfamethoxazole (28.7%), rifampicin, fusidic acid (both 20.7%), chloramphenicol (18.4%), mupirocin (10.3%), and linezolid (1.2%). Most isolates (98.9%) were multi-resistant (resistance to 3 or more antibiotics), but susceptible to vancomycin. A total of 67 MRSA isolates were typed by the CC398 PCR, and all them belonged to this lineage. Moreover, 43 CC398 isolates have been already analysed by *spa* typing. Six different *spa* types were identified, being t011 the most common (86%) in the series. Few isolates have other *spa* types: t034 (2 isolates), t1451, t1456, t1985 and t4872 (one isolate each). These preliminary results suggest that MRSA prevalence remains high among Belgian pigs, and worrisomely most isolates are multi-resistant. This survey is on-going and more isolates are been analysed. **Acks:** M.A. Argudín is supported by Fundación Alfonso Martín Escudero.

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IN VITRO ANTIMICROBIAL SUSCEPTIBILITY OF *S. AUREUS* STRAINS FROM DAIRY HERDS IN KWAZULU-NATAL

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Staphylococcus aureus is one of the most important causes of bovine mastitis and is responsible for significant economic losses to the dairy industry worldwide. One of the principal approaches used in treating intramammary infections is the administration of antimicrobials. Due to the propensity of *S. aureus* to develop resistance, antimicrobial susceptibility monitoring is necessary to ensure that treatment regimens are effective. As part of this investigation, a collection of 90 *S. aureus* strains, isolated from mastitis cases submitted to Allerton Provincial Veterinary Laboratory during 2008 and 2009, was evaluated for their susceptibility to a panel of 10 antimicrobials. Only 8 of the 90 *S. aureus* isolates tested (8,9 %) were found to be susceptible to all of the antimicrobials evaluated. A very high level of resistance to the beta-lactam antibiotics was noted: 47,8 % resistance to penicillin and 65,6 % resistance to ampicillin. Minimal resistance to oxacillin, cephalothin and trimethoprim-sulfamethoxazole (1,1 %) was found. Seventeen (18,9 %) of the isolates tested were found to resistant to 3 or more antimicrobials. The need for vigilant monitoring of bacterial resistance trends in the dairy industry is warranted as the potential public health implications hereof are significant.

30A

CHARACTERIZATION AND EPIDEMIOLOGY OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN BOVINES

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as a growing problem in human and veterinary medicine. The aim of this study was to determine the molecular epidemiology of MRSA in healthy bovines and to compare different rearing practices. Therefore, in 2012, nasal swabs were collected on 432 randomly chosen Belgian farms and tested for the presence of MRSA. Among these farms, 141 were dairy farms, 187 reared beef cattle and 104 veal calves. MRSA was isolated from a pool of 20 nasal samples using an enrichment method. Identification of *S. aureus* and methicillin resistance was confirmed by a triplex PCR. MICs were determined using microbroth dilution test and MRSA isolates were characterized by means of SCC*mec* typing, *spa*-type, CC398 PCR and MLST. Eighty-two farms (19.0%, 95% CI [15.3% -22.7%]) were positive for MRSA. Among these, 14 (9.9%, 95% CI [5.0% -14.9%]) were dairy farms, 19 (10.2%, 95% CI [5.8% -14.5%]) were farms holding meat cows and 49 were (47.1%, 95% CI [37.5% -56.7%]) farms rearing veal calves. One isolate did not regrow after conservation and was lost. More than 90% of the isolates were resistant to tetracycline and trimethoprim. A high prevalence of resistance was also observed to clindamycin, erythromycin, kanamycin and gentamicin. More than half of the isolates were also resistant to streptomycin. Lower resistance levels were detected to fusidic acid, sulfamethoxazole, quinupristin/dalfopristin, tiamulin, ciprofloxacin, rifampicin, chloramphenicol and mupirocin. No resistance was observed to linezolid and vancomycin. Among the 81 MRSA recovered, 78 were

ST398. One isolate was ST8 and two ST239. Ten different *spa*-types were identified. Sixty-four were *spa*-type t011, and others were t037, t121, t388, t1451, t1456, t1985, t3423, t6228 or non-typable (NT). MRSA *spa*-type t121 was associated to MLST type ST8, t388 and t037 to ST239. Forty-six isolates carried SCC*mec* type IV (2B) and eleven SCC*mec* type IV (2B&5). Sixteen isolates carried SCCmec type V (5C2) and two SCC*mec* type III (3A). Six isolates were non typable (NT) using Kondo's protocol. Both ST239 isolates carried the NT SCC*mec* cassette while the ST8 isolate harboured SCC*mec* type IV (2B). In summary, MRSA has been found at low prevalence in the nares of dairy and meat cows, but at elevated levels in veal calves. Most isolates were resistant to tetracycline and trimethoprim. The prevalence of acquired resistance to erythromycin, clindamycin, kanamycin and gentamicin is extremely high compared to what has been found in MRSA from other origins in Belgium. Although SCC*mec* type III was previously described in CC398 using Zhang's protocol, these isolates were eventually found to carry a novel type of SCC*mec* type V. Therefore, further analyses have to be performed in order to confirm the SCC*mec* type for these isolates.

Ackowledgment: Dr. M. A. Argudín has received a research grant from the Fundación Alfonso Martín Escudero.

31A

MULTIPLE DRUG RESISTANT *STAPHYLOCOCCUS AUREUS* (MDR) DETECTION IN METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AND *STAPHYLOCOCCUS AUREUS* DERIVED FROM BOVINE MASTITIS CASES IN TURKEY

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The aim of this cross-sectional study was to find out MRSA presence in milk samples derived from cows with clinical mastitis and reveal their potential Multiple Drug Resistance patterns. For that purpose, 480 milk samples with clinical mastitis out of 1600 cattle were examined in the province of Northwestern Turkey. API-Staphy® (bioMèrieux) detection kit was used for identification and the results were evaluated by API-web® system. The isolates were also confirmed by Staphaureux Plus® (remel) Latex Agglutination Test. Phenotypic methods i.e. disk diffusion test with cephoxitin® (OXOID), PBP2 ' Latex Agglutination Test® (OXOID), Nitrocephin Disk® (remel) and reproductive characteristics in ORSAB medium (Oxacillin Resistant Screening Agar Base-OXOID ®) were carried out to determine the methicillin resistance properties of the isolates. Kirby-Bauer disk diffusion test was performed to the isolates using 20 antimicrobials belonging to 7 different groups. A PCR method was applied to the isolates in terms of carriage of the mecA gene. Additionally, MIC Evaluators (Oxoid-Remel®) Penicillin G, Vancomycine, Tetracycline, Oxacillin, Ciprofloxacin and Gentamycin were used for the accurate detection of minimal inhibitory concentration (MIC) of 10 selected isolates which were carrying mecA. The zone diameters and MIC breakpoints were both evaluated by the directives according to EUCAST Clinical Breakpoint Table Version 3,0, 2013. In this study, S. aureus ATCC 25923 and the methicillin-resistant strains (MRSA) ATCC 33 591, ATCC 43 300 and ATCC 700 699 were used as reference strains. As a result, 151 (31.45 %) S. aureus were isolated from 480 milk samples. Several numbers of isolates (10 (6.62%) by PBP2 '; 40 (26.49%) by ORSAB and 36 (23.84%) by Nitrocephin Disc) showed positivity at ß-lactamase activity. Twenty four of 151 (15.89%) S. aureus isolates were detected as mecA gene carrier. Sixty two (41.05%) isolates were resistant to cefoxitin by disc diffusion testing. Broad range of antibiogram and their resistance patterns of the isolates are; Penicillin group of antibiotics, cephalosporins, fluoro-

quinolones, glycopeptides, macrolide group of antibiotics, tetracyclines and the other groups at the rates of 32.4, 38,5, 18.6, 2, 34, 44, and 10.6 %, respectively. Seventy six of isolates (50.33%) showed resistance more than one and 23 (15.23%) were more than three groups of antibiotics. MIC results were ranged between 0.12-256 and five isolates showed MDR patterns as follows; one was 6, two were 5 and two were 4 different groups of antimicrobials. Additionally, four isolates were accepted as borderline Glycopeptide Intermediate Staphylococcus aureus (GISA) and two were GISA. As a conclusion, a high rate of antibacterial resistance was observed in S. aureus isolates from bovine milk samples under large-scale investigation. This is remarkable both in terms of animal and public health.

32A

USING ORAL FLUIDS TO DETECT METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN PIGS AND PORK PRODUCTION FARMS

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global health concern and reservoirs of specific types have been found in swine and pork production facilities. This has led to concern about possible transmission of livestock-associated MRSA (LA-MRSA) to swine workers and their contacts. MRSA can be detected from a variety of sample matrices including nasal swabs and environmental sponges. Oral fluids are an efficient, cost-effective method of collecting diagnostic samples for several swine diseases. Use of oral fluids to detect MRSA would allow an effective means of determining the status of specific swine facilities as well as aiding the investigations into the prevalence of MRSA in swine. **Objectives:** 1) evaluate level of detection of MRSA from oral fluids with and without enrichment broth, 2) compare detection of MRSA from pork production facilities using oral fluids, environmental sponges and nasal swabs, and 3) assess prevalence of MRSA from oral fluid samples submitted for routine diagnostic testing. **Methods:** MRSA negative swine oral fluids were spiked with three strains of MRSA (ATCC 43300, spa t034, spa t002) at various levels. Colony counts from oral fluids and phosphate buffered solution (PBS) spiked with same levels were compared after streaking onto chromogenic media (BioRad MRSASelect). Additionally aliquots (50 µl) from spiked oral fluids were added to 5ml of enrichment broth (10g tryptone/L, 75g NaCl/L, 10g mannitol/L and 2.5g yeast extract/L), incubated, streaked onto chromogenic media and presence of MRSA determined. Samples (nasal swabs, environmental sponges, and oral fluids) were collected from pork production facilities with known and unknown MRSA status. These samples were tested for MRSA as described using enrichment broth. Aliquots of oral fluids were taken from diagnostic samples submitted for routine testing and tested for MRSA as described using enrichment broth. In all samples *S. aureus* isolates were confirmed as MRSA with *mecA* PCR and selected isolates forwarded for spa typing. **Results:** There was no difference in level of recovery of MRSA from PBS versus oral fluids. After enrichment MRSA could be detected in spiked oral fluids at <1 CFU/ml. MRSA could be detected from all samples of oral fluids, nasal swabs, and environmental swabs collected at a known MRSA positive pork production farm. Paired oral fluids and environmental sponges were collected from 15 pork production farms of unknown MRSA status. Four farms were positive for MRSA (3 from oral fluids, one from environmental sponge). Thirty one (31) of 513 diagnostic oral fluids (6.0%)were positive for MRSA. Spa types found in this study included: t002, t034, t548, and t111.**Conclusion:** The use of oral fluids appears to be a sensitive, efficient and cost-effective method for detecting MRSA in swine.

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SCC*MEC* METAL RESISTANCE GENES AMONG ANIMAL *STAPHYLOCOCCUS AUREUS* ISOLATES FROM GERMANY

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Methicillin resistant *Staphylococcus (S.) aureus* (MRSA) has emerged as a zoonotic pathogen in the animal production worldwide. Most of the livestock associated (LA) MRSA belong to the clonal complex (CC) 398, although other CCs (non-CC398) have also been described. The reason for the LA-MRSA emergence is unknown, but it has been suggested that tetracycline resistance (R) could have driven selection of MRSA CC398. Besides antimicrobial agents used for therapy, other substances with antimicrobial activity are used in animal feed, including metal containing compounds used for prevention of gastrointestinal diseases. Recently, metal R-genes have been found in various novel SSC*mec* cassettes. The aim of this study was to assess the occurrence of metal R-genes among LA-*S. aureus*. A total of 478 isolates from animals (pigs, bovine, poultry and companion animals), dust from farms, milk and meat products and humans between 2004 and 2012, were selected from the collection of the National Reference Laboratory for coagulase positive staphylococci including *S. aureus*. They were assigned to CCs using *spa* or MLST typing, and analysed for their SCC*mec* type and the presence of R-determinants via PCR, including: *tetK* (tetracycline-R, SCC*mec* III, V (5C2&5) c and plasmid associated), *arsA* (arsenate-R, SCC*mec* IX), *cadD* (cadmium-R, SCC*mec* IX and X), *copB* (copper-R, SCC*mec* IX and X), and *czrC* (cadmium and zinc-R, SCC*mec* V (5C2&5)c). 75% of the isolates carried at least one metal-R gene. Among the CC398 isolates (n=406), 6%, 25% and 73% were positive for *arsA*, *copB* and *czrC* genes, respectively. In contrast, only 5.6% and 4.3% of non-CC398 isolates (n=72) were positive for *copB* and *czrC* genes, respectively. The *czrC* gene was associated with the presence of SCC*mec* V in both CC398 and non-CC398 MRSA isolates. 95% of SCC*mec* V and *czrC* positive isolates carried *tetK*, indicating that they may have SCC*mec* V (5C2&5)c. Interestingly, one CC398 isolate with a possible new SCC*mec* V variant carried also *czrC*. *czrC* was found in CC398 isolates from all origins, whereas among non-CC398, all *czrC* positive isolates were from pig origin, only. The gene *copB* was present in isolates with different SCC*mec* types and was observed in pigs, cattle, and poultry. The *arsA* gene was found in one MSSA and in isolates with SCC*mec* V, mainly from poultry. This study shows that genes conferring metal-R are frequently present in LA-CC398, suggesting that the use of metal containing compounds in animal feed may contribute to the selection of MRSA. **Acks:** M. A. Argudín is supported by Fundación Alfonso Martín Escudero. Part of the work was carried out in the EMIDA-ERA NET Project "LA-MRSA" (Grant-No. 0315868A).

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PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN ITALIAN DAIRY HERDS.

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Methicillin-resistant Staphylococcus aureus (MRSA) has been reported in human medicine as a cause of nosocomial and community-associated infections. In veterinary medicine,

the microorganism has been identified in a wide range of animals and diseases, including dairy cows mastitis. Therefore, MRSA is considered an emerging threat with a high zoonotic potential. **AIM:** In the present study, we investigated the diffusion of MRSA in Italian dairy herds and the prevalence of infected animals at herd level. **MATERIALS AND METHODS:** Quarter milk samples were aseptically taken from all lactating cows of 83 S. aureus infected herds and bacteriological analysis was performed by culture on blood-agar plates. The hemolytic, Gram-positiv and coagulase-positive colonies of growth were identified as S. aureus. At least four isolates morphologically representative for each herd were tested by oxacillin disk-diffusion method to detect methicillin-resistance. DNA of oxacillin resistant isolates was extracted and the presence of mecA gene was investigated by PCR. **RESULTS:** All oxacillin-resistant isolates were confirmed as MRSA by mecA positivity. Prevalence of S. aureus infected cows and frequency of MRSA isolation were registered at herd level. Overall prevalence of mammary infections by S. aureus ranged 0,7 - 62%: in 28 herds (33.7%) the prevalence was higher than 25%, while in 27 herds (32.5%) it was lower than 4.7%. Methicillin-resistant strains were detected in 11 farms (13,25%), all belonging to the low-prevalence group. In 5 out of such 11 herds, all S. aureus isolates were MRSA, while in the remaining 6 herds both MRSA and methicillin-susceptible S. aureus (MSSA) were present. **CONCLUSIONS:** Our data confirmed the potential risk of subclinical mastitis caused by MRSA and suggest a low diffusiveness of these strains in infected herds. This characteristic could reduce the zoonotic potential of bovine MRSA, but the real prevalence in dairy farms could be underestimated, since bacteriological analysis of milk is usually performed only when a clear problem of mammary infections affects the herd.

35A

HIGH PREVALENCE OF TETRACYCLINE RESISTANCE IN *S. EPIDERMIDIS* FROM OVINE AND BOVINE SUB-CLINICAL MASTITIS: EVIDENCE FOR ADAPTATION TO ANIMALS?

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S. epidermidis is a human commensal bacterium that can cause opportunistic infections mainly associated to medical devices in hospitals and community. In humans, the rates of resistance to antimicrobials are lower in the community setting when compared to hospitals. In animals, colonization with S. epidermidis appears to be sporadic and dependent on the contact with humans; however S. epidermidis is frequently found as a cause of mastitis in bovine and ovine, suggesting that they are adapted to these hosts. In the veterinary setting huge doses of antibiotics are used as growth promoters and in the treatment of infections, but the frequency of antimicrobial resistance as well as the epidemiology of S. epidermidis from animals is not well understood. To evaluate the role of S. epidermidis as a reservoir of antibiotic resistance genes in the veterinary setting and study its epidemiology, we analyzed a collection of 87 S. epidermidis isolates collected from the milk of sheep and cows with sub-clinical mastitis located in two different farms in Portugal, over a period of eight years (1996-2003). Antimicrobial susceptibility testing to a panel of six antibiotics (oxacillin, vancomycin, ciprofloxacin, tetracycline, erythromycin and chloramphenicol) and molecular characterization by pulsed-field gel electrophoresis (PFGE) were performed for representative isolates. Moreover, isolates were screened for methicillin-resistant determinant (mecA/SCCmec) and biofilm-associated genes (atlE and aap) by PCR. The great majority of the isolates (70%) belonged to only 5 out of 11

clonal types identified by PFGE. Almost half of the isolates analyzed (48%) were resistant to tetracycline and 18% were resistant to oxacillin. However, the mecA was present in only 11 out of 87 isolates (12%). SCCmec type IV was frequent (n=6), but SCCmec III (n=1) and non-typable cassettes (n=4) were also found. The atlE and aap were found in a high proportion of isolates (76% and 44%, respectively), many of which were also resistant to methicillin and tetracycline. S. epidermidis from bovine sub-clinical mastitis appear to have increased resistance to tetracycline (48%) when compared to human isolates (<10%) from the same geographic region, suggesting long-term colonization and adaptation of S. epidermidis to animal host.

36A

PREVALENCE OF *STAPHYLOCOCCUS AUREUS* AND MRSA IN BULK TANK MILK OF BOVINE AND GOAT HERDS OF NORTHERN ITALY

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AIMS: *S.aureus* is the most important causative agent of subclinical mastitis in cattle and goats. Methicillin-resistant strains (MRSA) may have zoonotic importance, especially in case of occupational exposure and were repeatedly isolated from bovine mastitis in Italy and other countries, but very rarely in goat herds. The aim of our study was to evaluate the prevalence of MRSA in bulk milk of bovine and goat dairy herds from Lombardy region (Northern Italy) whose production of cow's milk represents 38% of national production and to identify the main circulating genotypes. **METHODS:** 673 and 197 bovine and goat bulk tank milk samples from different herds were examined respectively. Samples were plated directly on Blood agar and the *S.aureus* count was determined on Baird Parker Agar + RPF. For each sample, at least 5 colonies suspected to be *S. aureus* were tested for susceptibility to oxacillin by disk diffusion test. The same milk samples were examined by enrichment in MH broth + 7.5% NaCl and TSB + 5mg/l of oxacillin and plating on Brilliance MRSA agar. The presence of the *mecA* gene was confirmed by PCR. Genotyping was performed by Multilocus Sequence Typing (MLST). **RESULTS:** A total of 291 bovine (43.2%) and 85 caprine (43.1%) samples tested positive for *S.aureus*. Among these, MRSA were demonstrated in 29 bovine and 4 goat samples (10.0 and 4.7% of the isolates respectively). Five out of 33 MRSA were isolated by both by direct plating and after enrichment, 12 only by enrichment and 16 only by direct plating,. MLST analysis of 20 bovine MRSA isolates found twelve belonged to Sequence Type ST398, four to ST1, three to ST97 and one to ST5. MLST of the 4 caprine MRSA isolates identified three ST398 and one ST1. **DISCUSSION:** The high prevalence of positive *S. aureus* bulk tank milk samples underline the importance of the infection in both bovine and caprine farms in the investigated area. MRSA were detected with significant prevalence in bovine samples and were also found in caprine bulk milk. The lower MRSA positivity rates observed when testing isolates by selective enrichment may be due to higher susceptibility of clones to the salt concentration employed. The genotyping of bovine and goat MRSA strains showed that the more frequent lineage was ST398, while ST97 and ST1, both associated with cattle and pigs in Italy, were also confirmed to be common among herds from Northern Italy. The application of more stringent control measures against MRSA mastitis in cattle and goat herds, seems appropriate in order to minimize the risk of transmission of MRSA to humans by occupational exposure or through the consumption of raw milk or products made of un-pasteurized milk.

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Proposal for quality control ranges of *Staphylococcus aureus* ATCC® 25923 for agar disk diffusion using 30 µg tylosin disks

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Objective: Tylosin is a 16-membered macrolide that is licenced for the treatment of bovine mastitis, pneumonia and arthritis in calves, bronchitis in dogs, pneumonia, ileitis and erysipelas in pigs, and various diseases in poultry. When testing bacteria for their susceptibility/resistance, approved quality control (QC) ranges are important parameters for the internal validation of antimicrobial susceptibility test systems. For agar disk diffusion, there have been available only QC ranges for the 60 µg tylosin disks. These disks, however, are rarely if at all commercially available while the 30 µg tylosin disks are commonly used in routine diagnostics. The aim of the present study was to determine QC ranges for 30 µg tylosin disks for the reference strain *Staphylococcus aureus* ATCC® 25923. **Materials and methods:** An interlaboratory trial including eight laboratories was performed according to the recommendations given in the CLSI document M37-A3. Each laboratory tested the *S. aureus* reference strain ten times by agar disk diffusion using two different lots of tylosin 30 µg disks (Biolab, Mast) and three different Mueller-Hinton (MH) agar lots (Oxoid, Roth, Mast). A 15 µg erythromycin disk (Oxoid) was tested on the MH agar from Roth as quality control. The tests of all eight laboratories resulted in 480 data points for the tylosin 30 µg disks. The analysis of the results was performed by using the RangeFinder software based on the method described by Turnidge and Bordash. **Results:** All results of the testing of *S. aureus* ATCC® 25923 with the erythromycin 15 µg disk were within the CLSI-approved QC range of 22-30 mm. For the tylosin 30 µg disks, zone diameters of 18 to 26 mm, 18 to 27 mm, and 19 to 26 mm were observed for the MH agar lots from Oxoid, Roth and Mast, respectively. The data revealed a mean of 22.16 mm and a median of 22 mm (20-23 mm) for all participating laboratories. Based on the results obtained with the RangeFinder software, we recommend to use 18 to 26 mm for the 30 µg tylosin disk as QC range for *S. aureus* ATCC® 25923. This proposed QC range includes 475/480 (99.0%) of the observed zone diameter values for the 30 µg tylosin disk. **Conclusions:** The proposed QC range has been recently approved by the CLSI. This QC range will help the routine diagnostic laboratories to validate their results when using 30 µg tylosin disks. Moreover, this QC ranges can contribute to a harmonization and standardization of tylosin susceptibility testing.

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Proposal for cefoperazone quality control ranges for *Staphylococcus aureus* ATCC® 25923 for agar disk diffusion using 30 µg disks

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Objectives: The third generation cephalosporin cefoperazone is commonly used for the therapy of bovine mastitis. Quality control (QC) ranges are important parameters for the internal validation of antimicrobial susceptibility testing in routine diagnostics. So far, there are only QC ranges for the 75 µg cefoperazone disks available. The aim of the present study was to determine QC ranges for 30 µg cefoperazone disks for the QC reference strain *Staphylococcus aureus* ATCC® 25923

based on the recommendations given in the document M37-A3 of the Clinical and Laboratory Standards Institute (CLSI). **Material and Methods:** According to the recommendations of CLSI document M37-A3 an interlaboratory trial was performed. Each of the eight participating laboratories tested the *S. aureus* ATCC® 25923 reference strain ten times using two different cefoperazone 30 µg disk lots (Oxoid, Mast) and three different Mueller-Hinton (MH) agar lots (Oxoid, Roth, Mast). A 75 µg cefoperazone disk (Oxoid) was tested on one MH agar lot (Oxoid) as quality control. The test results obtained from all eight laboratories resulted in 480 data points. The analysis of the results was performed by using the RangeFinder software based on the method described by Turnidge and Bordash. **Results:** The results of the testing of *S. aureus* ATCC® 25923 for the cefoperazone 75 µg disk were within the CLSI-approved QC-range of 24-33 mm. The testing of *S. aureus* ATCC® 25923 with the cefoperazone 30 µg disk revealed zone diameters of 23 to 38 mm, 25 to 34 mm and 25 to 34 mm for the MH agar lots from Oxoid, Roth and Mast. The zone diameters for the disk lots from Oxoid and Mast ranged from 23 to 38 mm and from 23 to 35 mm, respectively. The 30 µg cefoperazone results revealed a mean value of 28.71 mm for all laboratories. The median value for all laboratories was 28 mm (26 to 31 mm) and the overall mode value was 28 mm. Six of the laboratories had mode values of 27 to 30 mm, whereas the remaining two laboratories had 31 and 32 mm, respectively. **Conclusions:** Based on the data, we recommend the use of 23 to 34 mm as QC range for *S. aureus* ATCC® 25923 and the 30 µg cefoperazone disk. This new QC range has recently been approved by the CLSI and will help routine diagnostic laboratories to validate their cefoperazone susceptibility testing results when using 30 µg disks. Moreover, they will contribute to a harmonization of the cefoperazone susceptibility testing.

39A

CHLORAMPHENICOL AND FLORFENICOL MIC DISTRIBUTIONS, GENETIC RESISTANCE MARKERS AND TIME-KILL KINETICS IN CANINE CLINICAL ISOLATES OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

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Introduction: The emergence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has led to an urgent need for alternatives to conventional antibiotic agents in small animal practice. Chloramphenicol and florfenicol may be alternative drugs for treatment of MRSP infections. Although florfenicol is currently not specifically approved for dogs and cats, this veterinary drug may be used under Animal Medicinal Drug Use Clarification Act (AMDUCA) or similar regulations. **Objective:** The aim of the study was to elucidate the antibacterial activity of chloramphenicol and florfenicol on *S. pseudintermedius* and to evaluate the potential use of these phenicols in small animal medicine. **Methods:** MICs were determined by broth microdilution according to CLSI guidelines in 170 canine clinical isolates of *S. pseudintermedius* from Denmark (n=93), US (n=30), Italy (n=28) and Germany (n=19), including 30 MRSP. Isolates with MIC-values >8 µg/ml were screened by PCR for the presence of the chloramphenicol resistance genes cat_{pC194}, cat_{pC221} and cat_{pC223}. Time-kill kinetics at concentrations corresponding to 0.5-16 x MIC was determined for one isolate with MICs of 8 µg/ml (chloramphenicol) and 4 µg/ml (florfenicol). **Results:** The florfenicol MIC-distribution was unimodal with MICs in the range 1-8 µg/ml. The chloramphenicol MIC distribution was bimodal with MICs of 4-16 µg/ml for susceptible isolates (n=141), and 32-64 µg/ml for resistant isolates (n=29).

Five MRSP isolates were resistant to chloramphenicol. All resistant isolates harbored the gene cat_{pC221}. At concentrations corresponding to 1-2 x MIC, a 0-1 log unit reduction in cfu/ml was seen after 24 hours for both drugs. At 4-16 x MIC concentrations, a 2-3 log unit reduction in cfu/ml was observed. **Discussion/conclusion:** The antibacterial action of both drugs on *S. pseudintermedius* was bacteriostatic and time-dependent, although moderately enhanced killing was observed at increasing drug concentrations. The absence of florfenicol resistance and the rare occurrence of chloramphenicol resistance indicate that these antibiotics could potentially be useful against MRSP. Further studies, including *in vivo* PK-PD target determination and Monte Carlo simulation applying MIC values and target animal pharmacokinetics, are needed to optimize the use of these drugs in small animal practice.

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MUPIROCIN RESISTANCE IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATED FROM DOGS.

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In the United States, veterinary use of mupirocin is primarily limited to the treatment of canine pyoderma caused by methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). In this study, only 1 of 580 (0.002%, 1/580) *S. pseudintermedius* isolates tested was resistant to mupirocin. Further evaluation of this isolate indicated that it carried the high-level mupirocin resistance gene, *ileS2* on a plasmid. The isolate was collected from a patient without clinical staphylococcal infection that was presented for orthopedic evaluation. The isolate was not methicillin-resistant. All isolates were collected prior to February 15, 2012 in a large referral practice in Texas. A total of 155 isolates (27%, 155/580) were methicillin-resistant. These results indicate that mupirocin resistance in the patient population of the referral practice is low.

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TWO CASES OF CO-INFECTION WITH METHICILLIN RESISTANT AND METHICILLIN SUSCEPTIBLE STAPHYLOCOCCAL ISOLATES IN ANIMALS.

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Co-infection with different isolates of the same species, including *Staphylococcus aureus* with or without the *mec*A gene, has been described from infections in humans. Current knowledge about such infections in veterinary medicine is limited. In this study we have characterized isolates involved in co-infection with two different staphylococcal isolates; methicillin resistant and methicillin susceptible, in a pig (*Staphylococcus aureus*) and a dog (*Staphylococcus pseudintermdius*). From a pig admitted to autopsy two different *S. aureus* were isolated after culturing from the spleen. The autopsy findings and histological examination showed that the pig suffered from meningitis, probably as consequence of tale biting. The *S. aureus* isolates were investigated for their antimicrobial resistance profiles, *mec*A status (PCR), *spa*type and multi locus sequence type (MLST). One of the *S. aureus* was an MRSA, with *spa*type t034, belonging to ST398. The other was *mec*A negative (MSSA), belonged to *spa*type t1430 and ST9. The two *S. aureus* isolates displayed different resistance profiles, with the MSSA being fully susceptible and the MRSA resistant to a broad range of antimicrobial agents. From a dog with otitis externa two different *S. pseudintermedius* were isolated. The staphylococcal isolates occurred in a mixed flora also containing *Pseudomonas aeruginosa* and *Malassezia pachydermatis*. The staphylococcal isolates were subjected to species determination and were further tested for antimicrobial resistance, *mec*A content

and subjected to MLST. These investigations confirmed presence of two *S. pseudintermedius*, one with *mec*A (MRSP) and the other without (MSSP). The strains had different resistance profiles and belonged to different STs; The MRSP to ST129 and the MSSP to ST120. In both cases there was no clear difference in colony morphology on blood agar. The presence of different isolates was discovered when performing confirmation tests (*S. aureus*/MRSA) and susceptibility testing by the use of disc diffusion (*S. pseudintermedius*). Our findings show that co-infection with MSSA/MRSA and MSSP/MRSP can occur in animals. It is important that the diagnostic laboratories are aware of this as methicillin resistant and susceptible isolates may mask each other and challenge correct diagnostic. A co-infection may also enable exchange of genetic elements like resistance and virulence genes. This is particularly relevant for the *S. aureus* strains from swine as MRSA ST9/t1430 is considered a possible new emerging livestock associated MRSA.

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DIRECT REPEAT UNIT (DRU) TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* FROM DOGS AT A DIAGNOSTIC LABORATORY IN ATLANTIC CANADA.

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Methicillin-resistant Staphylococcus pseudintermedius (MRSP) has emerged as major pathogen in dogs, being primarily isolated from skin, ears, surgical site infections, and wounds. The Atlantic Veterinary College (AVC) Diagnostic Bacteriology Laboratory is a principle reference laboratory for Atlantic Canada, and has seen a significant increase in the recovery of MRSP from samples submitted for routine culture and sensitivity. Currently, there are no reports investigating MRSP in dogs in this region. Sequence analysis of the mec-associated direct repeat units (dru typing) is a preferred method for strain typing MRSP. A significant difference in the distribution of predominant dru clusters has been reported between Europe, 5 U.S. states, and Ontario, Canada, with suggestion that dru types may be unevenly distributed in a single country. The objective of this study was to strain type the MRSP cultured at the AVC Diagnostic Bacteriology Laboratory using dru typing. Staphylococcal isolates recovered from dogs between January 2010 and December 2012 were tested. Staphylococci were identified using biochemical testing and methicillin-resistance was confirmed by the presence of penicillin-binding protein 2a (PBP2'). A multiplex PCR assay was used to identify coagulase-positive staphylococci to the species-level. Isolates were typed by analyzing sequence data from the direct repeat units. To date, 115 MRSP isolates have been typed. 17 different dru types have been identified with the majority belonging to type dt11a (n=33, 28.7%), dt10h (n=27, 23.5%), dt9a (n=27, 23.5%), and dt11af (n=12,10.4%). The remaining 16 (13.9%) isolates were distributed between 13 different dru types, nine of which have not been previously identified: dt5k, dt6t, dt8ag, dt9ba, dt9bd, dt10bz, dt10cc, dt10cj, and dt11ca. Each of the previously unidentified dru types was represented by one isolate. The predominant dru types identified in this study are similar to those found in Ontario, Canada; however, a cluster analysis is needed to make further conclusions. Further work will include epidemiologic analysis of dru types and patient information. Results from this study will provide information on the MRSP situation in Atlantic Canada, furthering the understanding of the dissemination of this pathogen.

43A

Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Animals in Finland

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Background: In Finland, there is no systematic surveillance for MRSA in animals, but it is recommended to send suspected isolates for verification. Intermittent surveys in pigs have revealed MRSA positive farms and slaughterhouses. MRSA have also been found from clinical and screening specimens in hospitalized horses. Among cattle, MRSA have been detected from two dairy farms, both originating from a mastitis case. Among small companion animals, MRSA findings have been sporadic including only a few isolations from cats and dogs. The aim of the present study was to characterize the Finnish veterinary MRSA isolates and to compare the *spa* types with those of human isolates. **Materials and methods:** Animal MRSA strains isolated between 2005 and 2013 (N=44) were *spa* typed. The SCC*mec* type and the presence of *czrC* gene, conferring resistance to cadmium and zinc, were determined. MLST analysis was performed to selected strains. **Results:** Sixteen different *spa* types were found, twelve of which were common with human isolates. In Finnish pig farms, livestock-associated (LA) MRSA CC398 (*spa* types t034, t108, t3933, t5103) is the most common type but MRSA t127 (CC1) is also present. The porcine MRSA isolates were mainly of SCC*mec* type V. Five different *spa* types were found among equine MRSA: t026, t064, t1399 (ST125), t011 (ST398), t6867 (ST398). MRSA isolates from horses were of SCC*mec* type IV or V. The two cattle isolates were of *spa* type t172 (ST375, SCC*mec* IV) and t3256 (ST130), the latter being the only *mecC* positive MRSA finding in animals in Finland so far. In addition, three MRSA isolates have been isolated from dogs (t008, t067 and t073, SCC*mec* IV and nontypeable) and two from cats (t324, ST72, SCC*mec* nontypeable). All the porcine and one equine MRSA had the *czrC* gene. *Spa* types t3256, t3933, t5103 and t6867 were unique for animal isolates. **Conclusions:** Several different *spa* types were present among animal MRSA isolates and most of them were also found in human isolates. SCC*mec* types V and IV were most commonly encountered. The *czrC* gene was mainly associated with porcine MRSA.

44A

Characterization of a Catalase-Negative Methicillin-Resistant *Staphylococcus aureus* Isolate from a Dog

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Introduction: Catalase-negative staphylococci are characterised by mutation and/or deletions of the katA gene that alter the functionality of the enzyme, and are thought to be less virulent. However, catalase-negative S. aureus strains, either resistant or susceptible to methicillin, have been associated with systemic infections in human patients. A dog tested positive for a catalase-negative MRSA strain and a methicillin resistant S. pseudintermedius strain (MRSP). To our knowledge, this is the first report in animals on a MRSA strain with a catalase-negative phenotype. **Methods and results:** Samples from a dog with a severe form of pododermatitis were subjected to bacteriological analysis. Pure cultures of staphylococci were isolated, with two distinct populations on Mannitol Salt Agar. The mannitol-positive isolate (164/13a) was shown to be catalase

negative, and identified as S. aureus by species-specific PCR. The mannitol-negative isolate (164/13b) was catalase positive and it was identified as S. pseudintermedius with a species-specific PCR. Both strains were shown to be methicillin resistant by PCR detection of mecA. Strain 164/13a was subsequently typed as t002 by spa-typing and as ST5 by Multilocus Sequence Typing. This isolate harboured Staphylococcal cassette chromosome (SCCmec) type I, and tested negative using a PCR targeting Panton Valentin Leukocidin (PVL) genes. The full-length katA gene of strain 164/13a was amplified as described previously and the sequence was determined, revealing a 99.6% nt identity in the catalase gene to the reference strain S. aureus ATCC 12600 (accession AJ000472). A deletion at position 487 caused a frame shift and introduced a premature termination codon (TAA) downstream at position 536-538, which altered the structural and functional integrity of the enzyme. **Discussion:** In strain 164/13a the altered functionality of the catalase was related to a deletion but it is not clear whether and to which extent that strain played a primary role as pathogen. In fact a MRSP was also isolated from the cutaneous lesions of the dog. The clonal type ST5 t002 has been reported in dogs in USA and in France, and also in human patients from the same regions. SCCmecI and PVL negative pattern are characteristic of Hospital-acquired MRSA in man. Thus, a human source of that strain may be hypothesized. **Conclusion:** The catalase test is a rapid assay routinely employed for the identification of staphylococci in clinical microbiology laboratories. While providing firm evidence, for the first time, that catalase-negative MRSA can be harboured in animals, the findings of this study may also suggest that the presence of staphylococci with this uncommon phenotype may be underestimated. Moreover, further studies are needed to assess the virulence of catalase-negative phenotypes.

45A

ISOLATION A NOVEL METHICLLIN *STAPHYLOCOCCUS AUREUS* T11469 STRAIN BELONGING TO MULTILOCUS SEQUENCE TYPE STS IN CHICKEN

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Background: In the last decade, a distinctive type of methicillin resistant *Staphylococcus aureus* (MRSA) emerged in livestock and companion animals. Its isolation in chicken has been reported in some countriesand its propensity for zoonotic transmission represents potential risk for poultry farm workers and the general population. **Methodology**:Nasal and cloacae swabs of 1800 birds selected at random but equally from 9 poultry farms in Ebonyi State, Nigeria were screened and analyzed using standard microbiological techniques to determine the prevalence of MRSA amongst chicken population in the State. Antibiotic resistance profiles of the isolates were determined using the Kirby-Bauer disc diffusion (DAD) method. Molecular epidemiology of the isolates were determined using Polymerase Chain Reaction (PCR) based techniques. All the MRSA were screened for methicillin resistance gene (*mecA*), Panton Valentin leukocidin determinants (*lukF-lukS*), a tetracycline resistance gene (*tetM*) and *spa*-sequence typing of the polymorphic region of proteinA. Staphylococcal Cassette Chromosome mec (SCC*mec*) and Multilocus sequence typing (MLST) of the novel isolates were also carried out. **Result**: Out of 247 (13.7%) *Staphylococcus aureus*from 1800 chicken screened, 15 wereresistant to cefoxitin, representing an MRSA prevalence of 0.83%. All the MRSA isolates were susceptible to vancomycin and carried the *mecA* gene by multiplex PCR. Spa typing analysis show that 3 spa types; t002, t2304 and t11469 were circulating amongst poultry birds in Ebonyi State and all belonged to SCC*mec* type V. A new strain of MRSA t11469 that has not been described anywhere, to the best of our

knowledge, was isolated from poultry birds in Ebonyi State. **Conclusion:** The presence of MRSA in this study represents a potential health risks to the poultry farm workers, their families and the general public. The isolation of a new strain of MRSA t11469 shows the versatility and the changing epidemiology of this agent.

46A

EPIDEMIOLOGY OF *MECC* MRSA IN GREAT BRITAIN

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Our group recently described a novel *mecA* homologue in MRSA from humans and dairy livestock in the United Kingdom and Denmark. This homologue, originally named $mecA_{LGA251}$ and since designated *mecC*, has 69% identity to *mecA* at the DNA level. This divergence results in *mecC* MRSA producing negative results in molecular tests to identify or confirm MRSA despite being resistant to methicillin (oxacillin/cefoxitin). This has important implications for the correct diagnosis of individual patients and for the surveillance of MRSA.To better understand the epidemiology of *mecC* MRSA in Great Britain we conducted prevalence studies among human MRSA isolates and bovine bulk tank milk samples. In the case of human prevalence, six hospital laboratories supplied three hundred and fifty sequential MRSA isolates in 2011-12 which were tested by PCR to determine their *mec* gene status. From a total of two-thousand one hundred isolates, nine were positive for *mecC* (0.43%) with the remainder being *mecA* positive. Eight of these *mecC* MRSA isolates belonged to clonal complex 130 with the ninth belonging to ST425. In the bovine prevalence survey, four hundred and sixty-five bulk tank samples in England and Wales and six hundred in Scotland were tested for *mecC* MRSA. Ten farms (2.15%) in England/Wales were positive for *mecC* MRSA but none in Scotland were positive. Seven of these isolates belonged to ST425 with the other three being CC130. *mecC* MRSA therefore appears rare among human MRSA isolates in GB at present with CC130 the predominant lineage. In bovine milk ST425 appears the predominate lineage. In the UK we have also identified veterinary isolates of *Staphylococcus xylosus* and *Staphylococcus sciuri* which are *mecC* positive and have found *mecC* MRSA from domestic dog, chaffinch and common seal. The presence of *mecC* MRSA in other host species and the presence of *mecC* in staphylococcal species other than *S. aureus* needs to be considered in our understanding of *mecC* MRSA epidemiology.

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MSSA SPA TYPES IN US PIGS AND SWINE VETERINARIANS CORRESPOND WITH MRSA SPA TYPES REPORTED GLOBALLY IN SWINE

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Since the detection of ST398 MRSA in pigs in Europe, research worldwide has identified diverse MRSA types in swine, both within the ST398 lineage and across MLST types. Studies in Asian pigs generally report a predominance of ST9 MRSA, and ST398 and ST5 variants appear most common in pigs in North America, where ST9 MRSA have also been found. Other MLST types detected in pigs in various countries include ST49, ST97, ST39, and ST1. Despite active research on MRSA in livestock, there are few systematic studies of generic S. aureus ecology in swine herds. We are conducting 3 studies of S. aureus (MSSA and MRSA) in pigs and swine veterinarians in the USA: Study A - an intensive longitudinal

study of 2 multiple site production systems in Minnesota; Study B - a cross-sectional study of 36 herds in 15 states; Study C - an 18 month longitudinal study of 67 swine veterinarians in the same states. We report the distribution of spa types and MLST sequence types among 513 isolates from Study A (completed); 135 isolates from 8 farms in study B, and 254 isolates from 6 months of sampling in Study C (both ongoing). In study A, no MRSA were detected in 384 samples from pigs (192) or the environment (192) of the two systems. Among 513 S. aureus isolates, 15 spa types were detected and the most common were ST398/t034 (36%); ST9/t337 (28%); ST9/t7331 (13%);ST9/t3446 (7%); ST9/t2462 (6%) and ST5/t002 (5%). Only 1 isolate (ST72) did not belong to ST398 (39% of isolates), ST9 (55%), or ST5 (6%). S. aureus was isolated from 65% of 395 veterinary samples (57% MSSA; 8% MRSA) over 6 months, with 12 veterinarians (18%) consistently positive for a specific spa type. Predominant spa types were ST398/t034 (38%); ST5/t002 (18%); ST9/t337 (13%); other ST398 (13%); other ST5 (7%); other ST9 (4%). Overall, 93% of spa types from the veterinary samples belonged to ST398, ST9, or ST5. All MRSA isolates belonged to ST398 (t034, t2330, t011) or ST5 (t002, t2049). In 8 farms sampled in study C, ST398, ST9 and ST5 were the predominant lineages on all farms and collectively accounted for more than 85% of isolates (all MSSA). The absence of MRSA in all 10 swine herds tested, together with the low prevalence (8%) among swine veterinarians supports previous data suggesting a relatively low prevalence of MRSA in the US swine industry. The consistent predominance of the ST398, ST5, and ST9 lineages across all 3 studies is striking, and diversity of spa types was evident within all 3 major MLST lineages. We infer that the normal S. aureus flora of US commercial swine, and occupationally exposed veterinarians, is dominated by diverse swine adapted MSSA largely clustered within the ST398, ST9 and ST5 lineages. The same 3 lineages predominate in MRSA in pigs globally, implying they may be widespread in pigs in many countries. The apparently recent emergence of MRSA in swine likely reflects sporadic acquisition of the mecA gene by preexisting flora of pigs.

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THE ASSOCIATION BETWEEN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) CARRIAGE AND THE GENE EXPRESSION OF GROWTH FACTORS AND IMMUNOMODULATORY AGENTS IN PIGS

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Although very prevalent among swine herds, rarely caused disease in pigs. However, it is unclear whether MRSA carriage elicits any immune response. The objective of this study was to determine whether MRSA colonization in pigs is associated with differential gene expression of C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp), interferon- α (IFN-α), interferon- γ (IFN-γ), tumor necrosis factor- α (TNF-α), interleukin (IL)-1β, IL-6, IL-10, and IL-18 in addition to several growth factors, including insulin-like growth factor-1 (IGF-1), IGF binding protein-3 (IGFBP-3), and growth hormone receptor (GHR). Liver tissue, nasal swabs, and serum samples were collected from healthy 9-week-old pigs (n=168) from 8 commercial farrow-to-finish operations in southern Ontario, Canada. Data on growth performance and herd-level management was also collected. Nasal swabs were cultured for MRSA and serum samples were tested for porcine reproductive and respiratory syndrome virus (PRRSV) using real time PCR. Reverse transcription quantitative PCR (RT-qPCR) was used to quantify the gene expression in liver of 74 samples from 4 farms. The pig-level and herd-level prevalence of MRSA was 41.5% (68/164) and 75% (6/8), respectively. For PRRSV, the pig-level and herd-level prevalence was 28.0% (46/164) and 50% (4/8), respectively. Univariable analysis revealed a significant association between the

herd-level prevalence of PRRSV and MRSA (R2=0.635; P=0.018). MRSA colonization did not affect expression of the studied cytokines, acute-phase proteins, and growth factors (all P>0.05). In contrast, PRRSV was found to be inversely associated with IGF-1 expression in liver tissue (OR=0.034, P<0.001). There was no association between MRSA carriage and farm antibiotic use (P=0.98), with MRSA found on 3/4 antibiotic-free farms, with prevalences ranging from 33-71%. The association between PRRSV and MRSA has not been previously reported. This may be the result of poor farm biosecurity or an indicator of movement of pigs from other herds; however, factors such as a reduction in bactericidal activity of porcine macrophages from PRRSV exposure cannot be excluded. The lack of association between MRSA and immunomodulatory gene expression was expected since MRSA is thought to be a relatively host-adapted commensal in pigs. However, further research could whether there are differences with different MRSA strains as well as the relationship between MRSA carriage and local immunity. These data provide more indication that MRSA carriage is not associated with a demonstrable systemic immune response in pigs, as opposed to subclinical carriage of PRRSV. The association of MRSA and PRRSV requires further study because of the commonness of both pathogens, the potential influence of PRRSV on farm MRSA status and the potential impact of PRRSV control on MRSA.

49A

STAPHYLOCOCCUS PSEUDINTERMEDIUS INFECTIONS AFTER DOG BITES IN HUMANS MISDIAGNOSED AS *STAPHYLOCOCCUS AUREUS*

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After the first isolates of methicillin resistant *Staphylococcus (S.) pseudintermedius* (MRSP) were confirmed in Sweden in 2006 and the number of cases started to increase during the upcoming years, worried dog owners contacted the National Veterinary Institute (SVA), Uppsala, Sweden, about MRSP. A frequently asked question was how dangerous MRSP were for themselves and their family members. *S. aureus* and *S. pseudintermedius* are common coagulase positive staphylococci in human and veterinary medicine. Both are pathogens and can cause similar types of infections. *S. pseudintermedius* belong to the normal bacterial skin flora of dogs and is common cause of wound and skin infections.. However, infections in humans are rare but with the uncertainty that these two species can be mixed up in routine laboratory diagnostics. There is also a probability for MRSP to be mixed up with in methicillin resistant *S. aureus* (MRSA) since the *mec*A gene mediating the methicillin resistance is the identical. In this study, we wanted to investigate if *S. pseudintermedius* could cause wound infection after dog bite and how commonly *S. pseudintermedius* were misdiagnosed as *S. aureus* in these cases We choose dog bites because of the higher likelihood of having an *S. pseudintermedius* involvement. The hypothesis was that *S. pseudintermedius* can be misdiagnosed as *S. aureus* by routine laboratory diagnostics in human medicine. *S. aureus* isolated from dogs bite wound infections in human medicine laboratories were sent to SVA for species confirmation with PCR. *S. aureus* and *S. pseudintermedius* were also analyzed with *spa*-typing. In total, 102 suspected *S. aureus* were sent to SVA for further analyses from 22 different laboratories in seven different counties. Of these, 13 (12.7%) were misdiagnosed as *S. aureus* and were instead *S. pseudintermedius.* The *S. aureus*- isolate belonged to 58 different *spa*-types where one isolate represented one *spa*-type besides nine isolates that all belonged to t015. They were isolated in different counties. *Spa*-typning was not an useful tool for analyzing relationships between the *S. pseudintermedius* isolates. This study showed that *S. pseudintermedius* can cause wound infections after dog bites and that

they can be misdiagnosed as *S. aureus*. There are therefore needs for better species characterization and methods for bacterial relationship to improve epidemiological tracing and treatment together with communication between human and veterinary medicine.

50A

MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CHICKENS AND CATTLE IN MALAYSIA

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Methicillin resistant Staphylococcus aureus (MRSA) was recently reported from a diverse group of food producing animals. The aim of this study was to investigate the genetic background of thirty one MRSA isolates using different types of typing methods pulse field gel electrophoresis (PFGE), for multilocus sequence typing (MLST) and spa typing were carried out on twelve isolates. Pyrogenic toxin genes screening was carried out on 27 MRSA and three methicillin resistant staphylococci. MLST characterized 12 MRSA isolates into 11 sequence types, namely ST9, ST15, ST14, ST537, ST190, ST194, ST795, and ST1279 from chickens while ST59, ST35 and ST573 from cattle. These 12 isolates were grouped into five spa types' t437, t442, t360, t189 and t5696. The analysis of PFGE macrorestriction patterns percentage of similarity identified from the dendogram at 80% similarity coefficient was used to define pulsotypes. The PFGE analysis identified 22 pulsotypes with nine sub types and the most common cluster is C which appeared to be present in four farms. Cluster B was similar albeit having different spa types. Diversity ensued among the isolates from chickens due to occurrence of more than two pulsotypes,' no genetic diversity was observed among the cattle isolates. Thirty staphylococcal isolates (including 27 MRSA and 3 MRS) were screened for the presence of 10 pyrogenic toxin genes. Nine of the 27 (90%) (27/30) MRSA harbored 1 to 5 toxin genes. One organism (ST537, t437) possessed five genes sed + seg + sei + sea + sej; the most predominant toxin genes are seg + sei (20%) (egc cluster). Toxic shock syndrome toxin genes (tsst-1) were found in two (2/30) (6.67%) MRSA and one MRS isolate (1/30) (3.33%). No toxin genes found in three cattle isolates. This study highlighted that food animals could serve as a vehicle for the transfer and disseminations of antibiotic resistant bacteria with enterotoxigenic potential to the public thereby making clinical treatment difficult and expensive.

51A

COPS OTHER THAN *S. AUREUS* IN CLINICAL MICROBIOLOGY LAB: COULD NEW TECHNOLOGIES PROVIDE USEFUL INFORMATIONS?

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Background: Coagulase positive staphylococci (CoPS) different from Staphylococcus aureus (SA) have gained importance not only in veterinary medicine, but also in medical microbiology. Among the microorganisms belonging to the Staphylococcus intermedius group (SIG), S. intermedius (SI) and S. pseudintermedius (SP) are sometimes described as cause of diseases in humans. With standard clinical microbiology techniques, it is very difficult to correctly identify these isolates at species level; only molecular techniques allow to do that. Moreover, different reports emphasized that methicillin-resistant (MR) SP isolates, if evaluated according to the CLSI/EUCAST criteria for SA, are susceptible to cefoxitin but resistant to oxacillin (cefoxitin is widely used in clinical microbiology as a marker of methicillin-resistance). Aim of

this study was to evaluate if two molecular techniques that are used directly on positive blood cultures, the Staphylococcus QuickFISH test (QF - AdvanDx, USA - an in situ fluorescence), and Gene Xpert MRSA/SA Blood Culture (Cepheid, USA), a real time PCR that is able to identify SA and to detect MR strains, can provide information useful to discriminate among CoPS. **Study Design:** The ATCC strains of S. delphini and of SI and 8 strains of SP (7 MRSP, one methicillin susceptible), previously identified through molecular methods, were analyzed using QF and Xpert. **Results:** All the strains analyzed through QF gave a red fluorescence that is characteristic, for this test, for coagulase negative staphylococci (CoNS). Xpert technology identified the isolates as different from SA. It was able to detect the mecA gene for all the 7 MRSP but their SCCmec regions were not amplified. **Discussion:** When automated instruments identify CoPS as SI, it is very difficult for the clinical microbiologists to confirm this classification. Our experience, even if based on a limited number of strains, demonstrates that QF and Xpert technologies could provide ambiguous results if used directly on clinical samples (it is possible to suppose the presence of CoNS in the specimens). However, if they are used on cultured strains that have been known to be coagulase positive, they are both able to exclude SA. Xpert seems also to be able to detect the mecA gene in MRSP, solving the problem of the cefoxitin susceptibility if SA breakpoints are used. Both techniques, if available in clinical labs, could provide useful information for a reliable SIG identification.

52A

EPIDEMIOLOGY OF *STAPHYLOCOCCUS SCHLEIFERI* ISOLATES FROM DOGS IN THE UNITED STATES (2003-2013)

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Background: Staphylococcus schleiferi is a Gram positive, coagulase variable bacterium that has been isolated from the skin, ears, and nasal mucosa of healthy dogs and from the pre-axillary skin of healthy humans. In humans it has been shown to be the causative agent in a variety of infections, including prosthetic infections, wound infections, bacteremia, and endocarditis. In dogs it has been isolated from lesions of pyoderma, otitis externa and media. Reports of infection caused by S. schleiferi are increasing in both human and veterinary medicine, but the true incidence may still be unknown due to the potential for misidentification of S. schleiferi as S. aureus due to their very similar biochemical profiles. Multiple studies have determined the prevalence of methicillin resistance amongst S. schleiferi isolates to be between 40-60%. While many of these isolates are susceptible to other classes of drugs multi-drug resistance strains have begun to emerge. **Aims:** To determine the relatedness of S. schleiferi isolates obtained from dogs from across the United States. **Methods:** Four hundred canine isolates that were determined to be S. schleiferi on the basis of biochemical testing were used in this study. Isolates were obtained from clinical samples that were collected by Dermatologists in the United States and submitted for routine diagnostic purposes. Isolates were frozen in Microbank Tubes prior to analysis. All isolates were tested with the multiplex PCR assay described by Sasaki et al (2010) to confirm the phenotypic identification. Phenotypic methicillin resistance was confirmed using the Oxoid PBP2a latex agglutination test. Pulsed-field gel electrophoresis was performed with SmaI according to the CDC Pulse Net protocol for molecular typing of S. aureus. BioNumerics software was used to determine pulsed-field profiles based on Dice coefficients of similarity. A similarity coefficient of 80% was selected to define pulsed-field profile clonal clusters. **Results:** Three clonal clusters were identified that comprised the majority of isolates. Isolates in clonal clusters 1 and 2 were predominantly methicillin resistant but clonal cluster

3 comprised isolates that were predominantly methicillin sensitive. The rate of methicillin resistance was 64% overall. **Conclusions:** As the incidence of infections caused by S. schleiferi increases in the veterinary and medical fields an understanding of the population structure is important. The data presented here is the most expansive performed on canine isolates of S. schleiferi to date. Methicillin resistant isolates were recovered from dogs across the United States and these data suggest the emergence and spread of at least two successful multidrug resistant S. schleiferi clones.

53B

METHICILLIN RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* FROM HEALTHY DOGS ATTENDING SMALL ANIMAL CLINICS IN NORWAY

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Methicillin resistant *Staphylcoccus pseudintermdius* (MRSP) is of growing concern in small animal veterinary medicine. In Norway the first MRSP was detected in 2008 and since that time the number of MRSP infections in dogs has increased steadily. The current knowledge about MRSP carrier prevalence among healthy dogs is limited. In this study we wanted to investigate the occurrence of MRSP among healthy dogs without infections and with no recent history of antimicrobial therapy. Detected MRSP isolates were genotyped and compared with clinical MRSP isolates. A total of 189 dogs, attending ten small animal clinics in Oslo and the surrounding counties during the period February to April 2013, were included in the study. The clinics were invited to participate in advance and instructions on how to sample and include dogs for the study was given. The dogs were sampled at two body sites by swabbing: the mouth and perineal region. Detection of MRSP in the laboratory included one enrichment step in Mueller Hinton broth with 6.5% NaCl overnight at 37°C, followed by plating out on MRSA Brilliance agar and incubation at 37°C. Presumptive MRSP isolates were subjected to PCR for species identification and PCR for *mec*A detection. All MRSP isolated from healthy dogs were subjected to multilocus-sequence typing (MLST) based on a seven-locus technique. MRSP was recovered from five dogs (2.6 %), sampled at three different small animal clinics located in geographically distinct areas. MLST showed that the isolates belonged to three different STs: ST89 (one isolate), ST71 (two isolates) and the novel ST252 (two isolates). MLST of clinical MRSP isolates (n=35) isolated in our diagnostic laboratory has shown that MRSP ST89 is most frequent followed by ST71; the same STs as detected among three of five MRSP carrier isolates. Our findings showed that MRSP could be isolated from healthy dogs, but the carrier rate was low. The MRSP population, including carrier and clinical isolates, contained MRSP belonging to several different STs. The most prevalent STs of clinical MRSP were also represented among the MRSP isolates recovered from healthy carriers. Risk factors for MRSP colonization and possible ways of eradication of MRSP in carriage in dogs should be further investigated.

54B

A CASE REPORT OF A CANINE LONG-TERM PATIENT SUFFERING FROM RECURRENT INFECTIONS DUE TO INDISTINGUISHABLE METHICILLIN RESISTANT AND -SUSCEPTIBLE *S. PSEUDINTERMEDIUS* GENOTYPES

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Introduction: The skin and mucosa of dogs is the natural habitat of *S. pseudintermedius* and individuals may carry one or more different strains over a long time. As an opportunistic

pathogen, *S. pseudintermedius* is an important cause of purulent infections in dogs, with skin, ear and soft tissue as main infection sites. Infections with methicillin resistant *S. pseudintermedius* (MRSP), which have been increasingly reported during the last years, are of particular concern. This case report describes recurrent infections of a canine patient with two distinct *S. pseudintermedius*-lineages over a five year period. **Material and Methods:** In total, 21 *S. pseudintermedius*-isolates from wound infection swabs of the canine patient were sampled between August 2008 and April 2012. Species identity was confirmed by polymerase chain reaction-restriction length polymorphism (PCR-RFLP) and methicillin resistance was confirmed by PCR-detection of the *mec*A-gene. The clonal relationship of all strains was investigated by pulsed field gel electrophoresis (PFGE) using BioNumerics 6.5 (Applied Maths, Sint-Martens-Latem, Belgium). **Results:** Investigation of wound swabs from different time points during five years revealed twelve MRSP- and six MSSP-positive cultures. Both, MRSP and MSSP were detected simultaneously twice. PFGE-analysis confirmed the occurrence of two distinct patterns. While all MRSP showed identical profiles (pattern A), MSSP clustered in pattern B, including five undistinguishable strains (subtype B1) and a single isolate belonging to subtype B2. MSSP-infections occurred initially and recurring infections with this strain were detected at least once a year. Between each time period of MSSP-detection, several infections accounted for MRSP. **Discussion:** This case report describes a canine patient with recurring *S. pseudintermedius*-infections during a five year period, mediated by strains belonging to two distinct PFGE-types. The alternation between MRSP and MSSP infections associated with two distinct genetic lineages (PFGE-A and -B) over a long period points towards several independent re-infection processes with either MRSP or MSSP in this patient. Both lineages seem to be potent pathogens for wound infections in dogs. Recurrent auto-infection or/and a re-infection source in the proximity of this patient seems to be a reasonable explanation. Further investigation including whole genome sequencing is needed to reveal critical genomic alterations among the strains reported on here.

55B

METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATION FROM COLOSTRUM AND MILK OF BITCHES IN A BREEDING KENNEL

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Dogs are natural hosts of *Staphylococcus pseudintermedius* and methicillin-resistant strains (MRSP) have been isolated with increasing frequency; antimicrobials use is a known predisposing factor for the selection of resistant strains. This study is part of a larger investigation on subclinical mastitis in the bitch and is focused on the pattern of isolation of MRS strains from colostrum and milk of postpartum bitches in a breeding kennel, also in relation to antimicrobials use. The study included 12 Staffordshire bull terrier/American Staffordshire terrier bitches, aged 2 to 5 years, belonging to a breeding kennel in Northern Italy. The incidence of Cesarean deliveries is rather high in the kennel, especially on Staffordshire terrier bitches, and antimicrobials (either amoxicillin/clavulanic acid or cephazolin) are administered in the following 5-7 days. In our study, six bitches underwent Cesarean section and were treated with antimicrobials; six bitches had a natural delivery, but the same agents were administered to two of them, because of endometritis risk and of generalized dermatitis, respectively. On day 1, 7 and 15 postpartum, drops of secretion were collected from the last abdominal mammary glands. Coagulase-positive staphylococci were identified using conventional biochemical analysis and MALDI-TOF MS; antimicrobials susceptibility and presence of mecA gene were

also tested. Methicillin-resistant staphylococci were isolated from 6 out of 12 bitches, and all the strains resulted *mec*A positive. Two MRS species were identified: *Staphylococcus pseudintermedius* and *Staphylococcus sciuri*. Methicillin resistant *S.sciuri* was isolated from a single bitch, in the second and third samples, together with MRSP. MRSP colonization was highest at D7 postpartum and involved 5/6 bitches under antibiotic treatment, while MRSP was isolated from a single bitch at D1. At D15 only 1 bitch out of 4 still hosted MRSP. Among non-antibiotic-treated bitches, MRSP strains were isolated at every sampling time from a single animal, that had been brought in the kennel only for parturition. Notwithstanding the limited number of animals, some interesting observations can be made. The presence of MRSP strains appears associated with antimicrobials use and it looks sporadic, not persisting overtime in the majority of cases. The rather high frequency of Cesarean deliveries in this kennel, and the following antimicrobials administration, induced the selection of methicillin-resistant strains and then the diffusion of MRSP appears rather high. The presence of MRSP did not affect neonatal health and survival, and neonatal mortality rate was low, especially in the naturally delivered puppies (2.2% versus 11.1%). Genetical characterization of the MRSP strains is under way, in order to assess whether they belong to the major clone lineage dominating in Europe, the ST71-J-t02-II-III.

56B

METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN A VETERINARY TEACHING HOSPITAL IN ATLANTIC CANADA.

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Methicillin-resistant (MR) staphylococci have been implicated as an important cause of nosocomial infections in human and veterinary hospitals. Since 2010 an infection control program at the Atlantic Veterinary College Veterinary Teaching Hospital (AVC-VTH) has focused its efforts on reducing transmission and persistence of MR Staphylococcus pseudintermedius (MRSP) in patients and the hospital environment. The objectives of this study were (1) to determine if MRSP isolates recovered from hospital environments are clonal to isolates recovered from patients associated with that area, and (2) if epidemiologically related patients with MRSP have clonal strains. It was hypothesized that hospital and patient strains and isolates from epidemiologically associated patients would be clonal. Environmental specimens were collected using an electrostatic cloth. Culture of cloths was accomplished by using an enrichment broth, followed by routine plating methodology. Patient and environmental isolates were identified by colony morphology, coagulase production, and resistance to oxacillin. Methicillin-resistance was confirmed by penicillin-binding protein 2a (PBP2') latex agglutination. Species-level identification was achieved by using a multiplex PCR assay for coagulase-positive staphylococci. MR isolates were typed by sequence analysis of the mec-associated direct repeat units (dru typing.) Epidemiologically related isolates of the same dru type were compared using pulsed-field gel electrophoresis (PFGE). Preliminary analysis of isolates from 2010 to date indicated that 35 patients were positive for MRSP at the AVC-VTH and MRSP was isolated from 9 environmental samples. Molecular work has been completed for all isolates from 2010 to 2012. All isolates were confirmed to be S. pseudintermedius by PCR. Typing results have shown that strains isolated from VTH areas were the same dru type as temporally associated patient strains. PFGE provided further evidence to suggest that patients were contaminating the hospital environment. There were two instances where hospital-acquired infections were suspected. In one instance, the dru types of the isolates were the same. PFGE analysis of these isolates revealed clonal strains, further

suggesting hospital-acquired transmission. Results from this study will provide support for the development and application of policies in veterinary infection control programs.

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THE SUITABILITY OF A COMMERCIAL CONTRAST BROTH MEDIA FOR DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN DOGS

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Background: A number of different enrichment broths exist to increase the sensitivity of screening of methicillin resistant Staphylococcus aureus (MRSA) in humans, but very little data is available about their usefulness for screening of methicillin resistant Staphylococcus pseudintermedius (MRSP) in dogs. Some broths, as the one studied here, contain selective agents that suppress growth of other bacteria, and an indicator that changes the color of the media when the target bacterium ferments sugars. This allows for a more rapid discrimination between negative and suspected positive samples. **Aims and objectives:** The purpose of this study was to investigate the suitability of a commercial contrast enrichment broth (CB) (Contrast MRSA Broth, Oxoid, UK) to detect MRSP in screening specimens from dogs by using a trypticase soy broth (TSB) with 6.5% NaCl (MRSA broth, Tammer-Tutkan Maljat Oy, Finland) as the reference. If the CB proved efficient it would reduce the reply time of negative samples, and decrease the amount of samples requiring further testing. **Methods:** The functionality of the CB was tested by a set of bacterial isolates including S. aureus (MRSA and MSSA), S. pseudintermedius (MRSP and MSSP), methicillin resistant coagulase negative or other staphylococci (MRS), Escherichia coli and Enterococcus spp. MRSP screening specimens were taken from the perianal, nasal and oral mucosa of 106 dogs. The CB and TSB were inoculated in parallel. After incubation all TSB and positive CB tubes (=yellow/orange color) were plated onto selective media (MRSA Select, Bio Rad, USA). Identification of suspected MRSP-isolates was done by phenotyping, oxacillin resistance and mecA detection. **Results:** All tested MRSP (n=36) and MRSA-strains (n=6) produced a positive color reaction in CB. E. coli (n=1), MRS (n=5) and MSSP (n=9) isolates were negative (=red color). However, all tested enterococci (n=6) and MSSA-isolates (n=6) caused a false positive color reaction. 22 of the 106 (21%) patient specimens were found to be MRSP-positive by the TSB-method, while 16 (15%) were positive with the CB-method. In 3 cases the CB-method revealed MRSP when the TSB-method did not. The sensitivity and specificity of the CB was 59% and 96%, respectively. The positive predictive value was 81%, while the negative predictive value was 90%. **Conclusions:** We cannot recommend the studied CB media for MRSP screening in dogs due to its low sensitivity.

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COMPARISON OF OXACILLIN, CEFOXITIN AND CEFPODOXIME DISK DIFFUSION SCREENING TESTS FOR DETECTION OF *MEC A* GENE-MEDIATED METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATES FROM DOGS

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The question posed in this study was: How do oxacillin, cefoxitin and cefpodoxime disk diffusion susceptibility test results compare

when used singly or in combination to detect methicillin resistance in *Staphylococcus pseudintermedius*? A retrospective analysis was performed on cumulative data from 1841 isolates of *Staphylococcus pseudintermedius* from dogs. Each isolate was from specimens submitted to the Clinical Bacteriology and Mycology Laboratory at the University of Tennessee College of Veterinary Medicine (Tennessee, USA) from 2006 to 2012. Multiple isolates from a single patient were possible; however, each isolate was unique with respect to year isolated, body site from which it was isolated and antimicrobial susceptibility profile. Species identification was made by signature biochemical profile and/or MboI restriction endonuclease fragment profile of a polymerase chain reaction (PCR)-amplified locus of the phosphate-acetyltransferase (*pta*) gene. Disk diffusion susceptibility tests were performed with oxacillin (1 µg disk), cefoxitin (30 µg disk) and cefpodoxime (10 µg disk) according to Clinical and Laboratory Standards Institute (CLSI) standard reference methods. Diameters of the zones of growth inhibition were recorded. PCR assays for detection of the *mec A* gene, performed by either conventional or real time methods, were recorded as positive or negative. Receiver-operator- characteristic analysis, was performed using commercial software. Epidemiological cutoff values (ECVs), that optimized both sensitivity and specificity, were 16, 29 and 21 for oxacillin, cefoxitin and cefpodoxime, respectively. Error rates obtained when using these ECVs were unacceptable. When adjusted to ensure very major error rates of < 2%, ECVs of 19 for oxacillin, 33 for cefoxitin and 26 for cefpodoxime were recommended. However, total error rates exceeded 10% and will require further attention in the future. Senstitvities and specificities of multiple disk diffusion tests analyzed in series or in parallel were not significantly greater than those observed for the single disk assays. The results of this study, in general, support previously recommended ECVs for oxacillin and cefoxitin and provide new information regarding the correlation of cefpodoxime disk diffusion test results with *mec A* gene detection.

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MIC VALUES OF SELECTED ANTIBIOTICS FOR MRSP STRAINS ISOLATED FROM DOGS IN POLAND

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Introduction: Infections caused by methicillin resistant *Staphylococcus pseudintermedius* (MRSP) in small animal practice has been observed with increasing frequency. Most MRSP strins are multidrug resistant, including resistance to fluoroquinolones, macrolides, tetracyclines, which are commonly used to treat *S. pseudintermedius* infections. Veterinarians are limited to a few effective antibiotics that require careful selection based on the results of antimicrobial susceptibility testing making prudent use of "last resort" antibiotics for human treatment. **Objective:** The objective of this study was to determine the MIC values for randomly selected 16 MRSP isolated from clinical infections in Poland against 12 antimicrobials that are widely used in small animals. **Methods:** Sixteen clinical MRSP of canine origin isolated at the Diagnostic Laboratory of the Division of Microbiology, Faculty of Veterinary Medicine at the Warsaw University of Life Sciences were examined. Methicillin resistance was confirmed by the detection of *mec*A. Additionally, all strains were SCC*mec* typed. E-test® strips (bioMerieux) were used for determining MIC values of enrofloxacin, ciprofloxacin, chloramphenicol, mupirocin linezolid, clindamycin, kanamycin, amikacin, doxycycline, vancomycin, rifampicin and gentamicin according to the procedures defined by the CLSI and Eucast. **Results:** The predominant SCC*mec* type was II-III (n=15).

One strain carried a novel SCC*mec*; *mec* A, *ccr* C. This *mec*A-positive isolate was susceptible to all tested antibiotics with the exception of kanamycin. All strains were susceptible to mupirocin, linezolid, amikacin and vancomycin. Resistance to rifampicin and chloramphenicol was observed in one (MIC = >32 µg/ml) and two (MICs = 32 µg/ml and 64 µg/ml) strains, respectively. Most of the MRSP were resistant to 6 out of 12 tested antibiotics: kanamycin MIC_{90} >256 µg/ml (n=16, 100%); enrofloxacin MIC_{90} >32 µg/ml (n=15, 93%); ciprofloxacin MIC_{90} >32 µg/ml (n=15, 93%); clindamycin MIC_{90} >256 µg/ml (n=15, 93%); gentamicin MIC_{90} >192 µg/ml (n=15, 93%), and doxycycline MIC_{90} 12 µg/ml (n=13, 81%). **Discussion and conclusion:** Our results demonstrate the high-level of resistance to commonly used antibiotics among MRSP and complicating current empiric treatment strategies of *S. pseudintermedius* infections. Treatment should be based on antibiotic resistance profiles. In our study, susceptibility to: mupirocin, linezolid, vancomycin and rifampicin was observed but these antibiotics are not licensed for use in veterinary medicine. Chloramphenicol and amikacin look promising but they are not widely available in veterinary hospitals in Poland, and they have serious side effects.

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HIGH GENETIC DIVERSITY AMONG METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATED FROM CANINE INFECTIONS IN DENMARK

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Background: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) have emerged globally in companion animals in recent years. In Denmark, MRSP constitute approximately 2% of *S. pseudintermedius* obtained from clinical specimens of dogs. The objective of this study was to study diversity of clinical MRSP isolates obtained from dogs, including five dogs sampled on multiple occasions, in Denmark over a five-year period. **Methods**: A total of 38 MRSP isolates obtained from clinical specimens of 29 dogs between 2008 and 2013 were tested for resistance to 22 antibiotics using broth microdilution, and were subjected to multilocus-sequence typing (MLST) and SCC*mec* typing. **Results:** Sixteen sequence types (STs) were identified. The most common STs were ST71 (n=11 in 11 dogs), ST258 (n=7 in 2 dogs), ST45 (n=2 in 2 dogs), ST269 (n=2 in 2 dogs), and ST273 (n=2 in 1 dog). ST118, ST265, ST267, ST268, ST270, ST271, ST272, and five of six new STs were all detected once. The isolates harbored SCC*mec* types IV (n=17), II-III (n=11), V (n=3) or non-typeable (n=7). Isolates belonging to ST71 and the related ST270 and ST272 displayed the most resistant phenotypes (resistant to 20 of 22 antibiotics). Four out of the five dogs, from which MRSP were repeatedly isolated, harbored two distinct strains. **Conclusions:** The European MRSP clone ST71-SCC*mec* II-III is the most common lineage in Denmark. However, the overall genotypic MRSP diversity is much higher than reported in other countries. Our results indicate multiple introductions of distinct MRSP strains into Denmark. SCC*mec* type IV was associated with 9 STs, suggesting enhanced mobility of this genetic element across distinct *S. pseudintermedius* lineages. The isolation from four dogs of distinct STs on different sampling events suggests repeated exposure and that some patients may be predisposed to MRSP acquisition. Alternatively, since the isolates obtained from these dogs were single locus variants of the same ST, this finding may indicate short-term genetic evolution of MRSP strains within individual patients.

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CHARACTERIZATION OF CLINICAL CANINE METHICILLIN-RESISTANT AND METHICILLIN-SUSCEPTIBLE *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN FRANCE

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Introduction: *S. pseudintermedius* is an opportunistic pathogen in dogs, and was originally susceptible to most antibiotics available for treatment. Since 2006, methicillin-resistant *S. pseudintermedius* (MRSP) has emerged as an important pathogen resistant to nearly all classes of antibiotics. Here, we conducted a one-year survey in order to gain insight into the antimicrobial susceptibility patterns and genetic diversity of MRSP and methicillin-susceptible *S. pseudintermedius* (MSSP) clinical isolates in France. **Materials and Methods**: A total of 268 non-replicate coagulase-positive staphylococci were collected from diseased dogs at a veterinary referee clinic from January to December 2010. Species were identified by *kat* PCR-RFLP. Antimicrobial susceptibility testing was performed by disc diffusion and methicillin resistance was confirmed by a *mecA*-specific PCR. Genetic diversity was assessed by *spa* typing on all isolates, plus SCC*mec* typing, PFGE and MLST on MRSP isolates. **Results**: *S. pseudintermedius* was identified in 91% of isolates, and 41 *S. pseudintermedius* were further confirmed to be MRSP. MSSP mainly presented resistances to penicillin (71%), tetracycline (52%), kanamycin (44%) and macrolides (38%), whereas MRSP presented numerous co-resistances to macrolides, lincomsamides, aminoglycosides, fluoroquinolones and tetracyclines. No resistance was observed to pristinamycin, glycopeptides and florfenicol. Only a few *spa*-types were identified amongst the MRSP, with 27/41 isolates having *spa* type t02 associated with SCC*mec* II-III. Conversely, MSSP were diverse with 19 different *spa* types. PFGE on MRSP isolates showed the presence of two main clusters: cluster A (n=34) associated with ST71, and cluster B (n=7) associated with non-ST71. **Conclusion**: This study confirms the presence of the common multi-resistant European MRSP ST71 clone causing infections in French dogs. Also of interest is the multi-resistant phenotypes observed amongst genetically diverse MSSP clinical isolates. Together, these data support the continuous surveillance of both MSSP and MRSP, as well as the development of new therapeutic alternatives.

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VIRULENCE FACTORS, AGR GROUPS (ALLELES), BIOFILM-FORMING ABILITY IN METHICILLIN-RESISTANCE AND METHICILLIN-SUSCEPTIBLE *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

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Staphylococcus pseudintermedius is the most common opportunistic pathogen in dogs. Several virulence factors have been described in S. pseudintermedius, however little is known about its epidemiology. The aim of this study was to investigate the diversity of virulence factors, agr groups and the biofilm-forming ability among 20 methicillin-resistant and 20 methicillin-susceptible Staphylococcus pseudintermedius (MRSP and MSSP, respectively). Isolates were typed by MLST. The agr group and the presence of 18 virulence genes were analysed by PCR, and the biofilm-forming ability was evaluated by Congo red agar (CRA) plates and by the capacity to adhere to polystyrene microtitre plates using brain heart infusion broth (BHIB), BHIB+1% glucose and BHIB+4% NaCl as the growth mediums. The isolates were relatively unevenly distributed

among the four agr groups, but agr group III was significantly associated with MRSP strains (p=0.014). All MRSP clonal complex (CC) 71 (n=17) isolates belonged to agr group III. All the MSSP strains belonged to different sequence types. Five virulence genes were detected among all strains, but two genes only appeared in MSSP isolates. spsO gene was found significantly more associated with MSSP than with MRSP (p=0.04). Using CRA 14 strains (35%) were identified as biofilm-producers. All 40 strains produced biofilm in BHIB+4% NaCl. Two and nine strains did not produce biofilm on BHIB and BHIB+1% glucose, respectively. Biofilm production in the BHIB and BHIB+1% glucose media was significantly higher in MSSP than in MRSP isolates (p=0.03 and p=0.02, respectively). There was a high association between results of the biofilm-producing ability on polystyrene using BHIB+4% NaCl and the presence of ica operon. The high frequency of virulence genes among the S. pseudintermedius strains explains the high adaptation of these bacteria to its host. Furthermore the association between CC71, agr allele III and certain virulence factors found in this study may explain how this clone expanded so rapidly over the past few years. The dissemination of multidrug resistant clones and highly virulent strains may be a serious problem in public health. Understanding the biological basis of S. pseudintermedius virulence might allow veterinarians to more successfully treat and prevent MRSP infections.

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CARRIAGE OF CELL WALL-ASSOCIATED PROTEINS IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATES FROM DOGS.

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Recent work demonstrated that 4 cell wall-associated (CWA) proteins were variable in *Staphylococcus pseudintermedius* (Bannoehr et al. 2011). We hypothesized that carriage of the genes that encode these four proteins would vary between isolates from patients with clinical staphylococcal infections and those without clinical infections. Between September 22, 2010 and February 15, 2012 we conducted a study to determine prevalence of methicillin-resistant *Staphylococcus* spp. in dogs that presented for orthopedic evaluation to our hospital. A total of 188 isolates of *S. pseudintermedius* were recovered from the nares (n = 82) and perineum (n = 106) of 133 dogs. Of these isolates, 14 (7%; 14/188) were methicillin-resistant of which 8 were recovered from the perineum and 6 from the nares from 10 dogs. Three of these 10 dogs carried methicillin-resistant *S. pseudintermedius* (MRSP) at both the nares and perineum. Of the 188 isolates, 62% (117/188) did not carry *spsF*, *spsO*, *spsP*, or *spsQ*. Carriage of these genes was 20% (37/188) for *spsF*; 12% (22/188) for *spsO*; 10% (19/188) for *spsP*, and 14% (26/188) for *spsQ*. At the same time as this prevalence study, we collected a convenience sample of each isolate of *Staphylococcus* spp. from the infection site in canine patients with clinical infections. A total of 193 isolates of *S. pseudintermedius* were collected from 164 dogs. Of the isolates from clinical infections 55 (28%; 55/193) were MRSP. Of the 193 isolates, 65% (125/193) did not carry *spsF*, *spsO*, *spsP*, or *spsQ*. Carriage of these genes was 18% (34/193) for *spsF*; 7% (14/193) for *spsO*; 24% (46/193) for *spsP*, and 24% (47/188) for *spsQ*. There were only 2 (4%; 2/55) MRSP isolates that carried *spsO* as compared to 12 (9%; 12/55) methicillin-susceptible isolates. There were 24% (46/193) carrying *spsP* and 24% (47/193) carrying *spsQ*. These data suggest that there were differences in carriage of *spsO*, *spsP*, and *spsQ* between *S. pseudintermedius* isolates from patients with clinical infections and those without. The primary limitations of this study were that the isolates from patients with clinical infections were a convenience sample and the demographics of these animals may differ from those of patients presented for

orthopedic evaluation. Further study is needed to resolve these differences. Following these studies, we retrospectively analyzed a historical collection of 199 *S. pseudintermedius* isolates collected between 2007 and September 22, 2010 and found that 37% (74/199) did not carry *spsF*, *spsO*, *spsP*, or *spsQ* while 44% (88/199) carried *spsF*, 11% (22/199) carried *spsO*, 27% (53/199) carried *spsP*, and 24% carried (48/199) *spsQ*. This suggests that there have been temporal changes in carriage of *spsF* in *S. pseudintermedius* isolates from our patient population.

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COMPARATIVE ADHERENCE TO CORNEOCYTES AND HOST SPECIFICITY BETWEEN METHICILLIN-SUSCEPTIBLE AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATED FROM DOGS AND HUMANS

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Introduction: *Staphylococcus pseudintermedius* is the most common opportunistic bacterial pathogen associated with otitis and pyoderma in dogs. Multidrug methicillin-resistant *S. pseudintermedius* (MRSP) are increasingly reported with two dominant clonal lineages recognized worldwide: ST71 and ST68. Bacterial adherence to the host epithelium is an essential step to colonization and subsequent infection. Corneocytes constitute the outermost layer of skin epithelium and represent a passive barrier between external environment and internal organs. **Objective:** To investigate the epidemiological success of specific MRSP lineages, in vitro adherence properties of methicillin-resistant and methicillin-susceptible *S. pseudintermedius* (MSSP) strains were compared with special focus on the epidemic lineage ST71. **Methods:** Corneocytes were collected from five dogs and six humans by applying 25-mm diameter adhesive discs on the donor skin. The adherence of two MSSP isolated from dogs and one from human, two MRSP non-ST71 from dogs, one dog MRSP ST71, and one human MRSP ST71 were evaluated by an *in vitro* assay. Experiments were carried out at two different growth phases; mid-exponential and late stationary. Adherent bacteria were counted manually at × 1000 magnification. Mean bacterial counts for all combinations were compared using a negative binomial regression model. **Results:** Mean bacterial counts were affected by corneocyte donor type and strain genotype whereas strain host origin was not statistically significant, in both growth phases. The adherence of *S. pseudintermedius* was significantly greater to canine corneocytes than to human corneocytes ($p<0.0001$). MRSP ST71 showed greater adherence than MSSP and MRSP non-ST71 ($p<0.0001$). Overall MRSP ST71 of human origin was the best strain adhering to both canine and human corneocytes. **Discussion/Conclusion:** Our study supports the general notion that *S. pseudintermedius* is a bacterial species adapted to dogs and indicates that under *in vitro* conditions MRSP ST71 adheres better compared to MSSP and MRSP non-ST71. The enhanced adherence properties of ST71 may facilitate colonization and transmission between dogs, thereby contributing to the epidemiological success of this MRSP lineage. The unexpected ability of MRSP ST71 to adhere to human corneocytes suggests possible adaptation to the human host as exemplified by the greater adherence of the human ST71 isolate to human corneocytes.

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METHICILLIN AND ZINC RESISTANCE IN *STAPHYLOCOCCUS HYICUS* FROM PIGS WITH EXUDATIVE EPIDERMITIS

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Staphylococcus hyicus is the leading cause of exudative epidermitis (EE), an imporatnt disease in young pigs. Anecdotally decreased response of EE to beta-lactams has raised concern about the emergence of methicillin-resistance (MR) in S. hyicus (MRSH). Additionally, there has been recent concern about zinc and cadmium resistance, conferred by czrC, in MR staphylococci. The objective of this study was to evaluate the prevalence of methicillin-resistant S. hyicus (MRSH) in pigs with EE and the presence of czrC. Skin swabs were collected from 186 pigs with EE from 30 conventional and antibiotic-free farms. Staphylococcus hyicus was isolated using enrichment culture and characterized by PBP2a LAT, mecA PCR, SCCmec typing and PCR detection of czrC. S. hyicus was isolated from 124 (68%) pigs. 28 (23%) were MRSH, for an overall MRSH pig prevalence of 15%. MRSH was found on 15 (50%) farms. 18 MRSH contained SCCmecV. The rest were untypable as they only contained recognized ccr or mec complex, not both. 4 possessed classA mec, 2 had classC mec, and 3 had ccr5. SCC 14 (50%) MRSH from 8 farms carried czrC. czrC was found in 14 isolates. All 14 czrC positive isolates possessed SCCmecV versus only 4/14 (29%) of czrC negative isolates ($P<0.0001$). There was an inverse association between antimicrobial exposure and czrC ($P<0.027$), with 64% of MRSH-positive pigs being raised without exposure to antimicrobials. MRSA possessing SCCmecV were isolated from 5/13 (38%) farms that harboured SCCmecV MRSH. Both MRSA (t571) and MRSH containing SCCmecV were isolated from one pig. MRSH was common, which is consistent with anecdotal evidence of poor response of EE to beta-lactams. The high prevalence of zinc resistance in MRSH is similar to reports involving MRSA, as is the association with SCCmecV, since crzC is known to reside within SCCmecV. The finding that czrC is common amongst MRSH, inversely associated with antimicrobial exposure and distributed across multiple Ontario farms raises concern because of the commonness of supplementation of swine starter ration with high levels of zinc oxide (≥2500 ppm) to prevent post-weaning diarrhea, particularly on antibiotic-free farms. Exposure to zinc oxide in the starter ration may be selecting for MRSH in newly weaned pigs and the unintended consequences of zinc supplementation on MR staphylococci deserves further study. Whether MRSH could act as a potential source of SCCmec for other staphylococci is unknown.

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VALIDATION OF APPROPRIATE REFERENCE GENES FOR QUANTITATIVE POLYMERASE CHAIN REACTION (QPCR) STUDIES IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* AND PRELIMINARY ASSESSMENT OF GENE EXPRESSION IN BIOFILM-EMBEDDED BACTERIA.

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Quantitative PCR (qPCR) is rapidly becoming the standard method for analyzing gene expression in a wide variety of biological samples. It is extremely sensitive and specific, but can suffer from significant bias and error if stably expressed reference genes are not identified. Here, three reference genes in Staphylococcus pseudintermedius were identified and validated from a set of 8 potential genes (proC, gyrB, rplD, rho, rpoA, ftsZ, recA, sodA). Two strains of Staphylococcus pseudintermedius were used, and primer specificity and efficiency were confirmed and measured. Ranking of the genes with respect to expression stability revealed a combination of gyrB, rho and recA as suitable reference genes for qPCR. This combination was used to quantify expression of a single biofilm associated target,

icaA, in logarithmic, stationary and biofilm growth phases; there was significant upregulation of expression of this gene in the biofilm growth phase in both strains. We also report a modified nucleic acid harvesting technique affording significantly increased yields, particularly from biofilms.

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ASSESSMENT OF EXPRESSION OF SEVERAL POTENTIAL VIRULENCE GENES IN THE CANINE PATHOGEN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN PLANKTONIC AND BIOFILM PHASES ON A VARIETY OF SURFACES USING QPCR.

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Reasons for the rapid dissemination of methicillin-resistant Staphylococcus pseudintermedius (MRSP), an important canine pathogen, and the leading cause of surgical site infections, are unknown. Biofilm formation may be an important factor, but evaluation of regulation of virulence factors and biofilm-associated genes in MRSP is lacking. The objective of this study is to evaluate expression of a series of biofilm-associated and antimicrobial resistance genes during the planktonic and biofilm growth phases using qualitative polymerase chain reaction (qPCR). qPCR is emerging as the standard for examining gene expression, but requires the identification of constitutively expressed reference genes to obtain valid results. Previously, reference genes have been identified for MRSP, providing the basis for qPCR expression studies in this species. Following development and validation of suitable primers for qPCR, expression of a number of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) including elastin, fibrinogen/fibronectin and fibronectin binding proteins (ebpS, spsE, fnbB), a bifunctional autolysin (atl), penicillin binding protein 2a (mecA), biofilm associated genes in the intracellular adhesion operon (icaA, icaC, icaD), and the accessory gene regulator quorum sensing system (agrA, agrB) were assessed in logarithmic, stationary and biofilm phases for two strains of MRSP (ST68, ST71). Testing was performed on a broad spectrum of clinically relevant surfaces; stainless steel, titanium, polystyrene, polymethylmethacrylate, latex, silicone, polydioxanone and glass. One-way ANOVA followed by Tukey's pairwise comparison (where appropriate) was used to identify any differences due to surface for each gene / strain combination. GyrB, rho and recA were used a reference genes. Genes in the ica family tended to be upregulated in biofilm phases, especially on glass. MSCRAMMs also tended generally to be upregulated in biofilm phases, however there were notable strain variations such as where atl was significantly upregulated in the planktonic phase in one sequence type. MecA expression varied both with strain and surface. There was some variation identified, but no trend in expression of quorum sensing genes. Upregulation of biofilm and MCRAMMs in biofilms is not unexpected, as these genes are necessary for the initiation and proliferation of biofilms. The other genes studied may have more complex determinants of expression.

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COMPLETE SEQUENCE OF A STAPHYLOCOCCAL RESISTANCE PLASMID OBTAINED FROM A SMALL ANIMAL CLINIC

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Objective: During analysis of methicillin-resistant staphylococci from samples taken in a small animal clinic, nine *S. epidermidis* (MRSE) isolates showed the same molecular characteristics and resistance to antimicrobial agents belonging to ten different classes. The aim of this study was to investigate these MRSE isolates for the presence and organization of plasmid-borne resistance genes.
Materials and Methods: The nine MRSE

isolates had SCC*mec* type IV, *dru* type dt10a, belonged to multi locus sequence type ST5 and were resistant to aminoglycosides/aminocyclitols, β-lactams, fluoroquinolones, lincosamides, macrolides, phenicols, pleuromutilins, sulfonamides, tetracyclines and trimethoprim. Plasmids were extracted by alkaline lysis and transferred by protoplast transformation into *Staphylococcus aureus* RN4220. Transformants were subjected to suceptibility testing using broth micro- or macrodilution according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI). Resistance genes were detected by PCR and plasmids were subjected to restriction analysis. One representative plasmid was cloned, completely sequenced and analyzed. **Results:** Transformants carrying the large plasmid showed increased MICs to clindamycin, tiamulin, trimethoprim, tobramycin, tetracycline and minocycline and harboured the corresponding resistance genes *vga*(A), *dfrK*, *aadD*, *tet*(L) and *tet*(M). They showed indistinguishable restriction patterns with BglII, EcoRI and HindIII. The sequence of the 28,743 bp plasmid pSWS47 confirmed the presence of the five antimicrobial resistance genes. Sequence analysis identified *tet*(M) as part of a ΔTn*916*-like element. Furthermore, a functionally inactive Δ*blaZ* gene as part of a ΔTn*552* was detected. Moreover, several complete or incomplete plasmid maintenance genes (*rep*, *pre*, *mob*, *rlx*), as well as six complete or incomplete insertion sequences (IS*257*, IS*431*, IS*1310*) were identified. Sequence analysis showed in part high identity (≥98%) to other described resistance plasmids, which suggests recombination processes. **Conclusions:** The presence of several resistance genes on the multi-resistance plasmid pSWS47 from a feline MRSE isolate ensures the persistence of the plasmid under the selective pressure of different antimicrobial agents used. Moreover, to the best of our knowledge, this is the first report of a staphylococcal plasmid carrying the tetracycline/minocycline resistance gene *tet*(M).

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DISTRIBUTION OF BIOFILM FORMATION GENES IN LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM SWINE AND SWINE SLAUGHTERHOUSE WASTEWATER

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Background: Livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) are found in different animals and meat products. LA-MRSA ST9 has emerged as a potential zoonotic pathogen for humans and animals. Biofilms mediate MRSA colonization and are associated with host infection. The occurrence of biofilm formation genes and the biofilm formation ability in LA-MRSA ST9 from different ecological niches have not been reported. The aims of the study were to investigate the dynamics of biofilm formation genes (intercellular adhesion [ica], and staphylococcal accessory gene regulator [SarA]) and biofilm expression in LA-MRSA ST9 from asymptomatic nasal swine and swine slaughterhouse wastewater. Methods: A total of 259 MRSA isolates were recovered between March 2010 and August 2011, including 147 isolates from asymptomatic LA-MRSA-carrying swine (the colonization group) and 112 isolates from swine slaughterhouse wastewater (the environmental group). The distributions of ica (icaR and the icaADBC operon) and SarA loci for the colonization and environmental group were investigated independently by PCR. Biofilm formation was quantified by microtiter plate assay. Data were analyzed by chi-square test for homogeneity of two-group proportions. Comparisons of biofilm formation between groups were performed using the Mann-Whitney U test. Results: The ica and sarA locus were detected in 64.9% (168/259) and 82.6% (214/259) of the MRSA isolates, respectively. The detection rate of the ica and sarA loci in the environmental group (48.2% and 65.2%)

were significantly lower than in the colonization group (77.6% and 95.9%) ($P < 0.05$, $\chi 2$). Of the 259 MRSA isolates, 61.8% (n=160) MRSA isolates exhibited the biofilm formation ability. Biofilm production in the colonization group was higher than in the environmental group ($P < 0.05$, MWU test). Conclusions: This study characterized the distribution of ica, and sarA in LA-MRSA isolates from different ecological niches and in biofilm formation. The high prevalence of biofilm formation gens and biofilm production in LA-MRSA ST9 from the colonization group may improve the persistent MRSA in the swine population and present a threat to the health of livestock animals as well as farm workers.

70B

HOST RANGE AND DISTRIBUTION OF *MECC* POSITIVE CC130 MRSA

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Recently, a novel methicillin resistance gene was discovered in Staphylococcus aureus and designated mecC. There is growing evidence for a zoonotic background of mecC-positive MRSA although it also has been found in humans. In order to obtain an insight into the prevalence and distribution of mecC, clinical isolates from a university hospital in Dresden, Saxony, veterinary screening isolates from Thuringia and Bavaria and wildlife isolates from Germany and Sweden were screened for the presence of mecC /SCCmec XI elements using DNA microarrays (Alere Technologies). 1000 human MRSA isolates, collected in Dresden between 2000 and 2013, were tested and their strain/clonal complex affiliations were determined, but no mecC-isolates were identified. However, three human isolates of CC130-MRSA-XI were referred by external units for characterisation, one originating from a county hospital nearby and two from North-Eastern Germany (Frankfurt/Oder). Screening of approximately 600 veterinary isolates of S. aureus from Thuringia, mostly from cattle and a few from goats and sheep, did not reveal any mecC-positive isolates. In Bavaria, milk samples from approximately 2000 cows were screened for the presence of S. aureus and MRSA. This resulted in the detection of CC130-MRSA-XI in milk samples from 16 cows and an udder swab of one additional cow in one herd of 56. Regarding wildlife, mecC was identified in two ill hedgehogs (Erinaceus europaeus) from Sweden as well as in one fox (Vulpes vulpes) and one fallow deer (Dama dama) from Germany. S. aureus isolates from other wildlife and belonging to other CCs, namely CC1 from fallow deer and mouflon sheep (Ovis aries), CC133 from a mute swan (Cygnus olor), ST425 from roe deer (Capreolus capreolus), red deer (Cervus elaphus), wild boar (Sus scrofa) and badger (Meles meles), CC692 from a white-tailed eagle (Haliaeetus albicilla), ST2279 from lynx (Lynx lynx) and reindeer (Rangifer tarandus), ST2425 from hare (Lepus europaeus), ST2691 from moose (Alces alces) as well as coagulase-negative staphylococci from roe deer, wild boar, least weasel (Mustela nivalis), fox and racoon (Procyon lotor) all were found to be mecC-negative. All detected mecC-positive isolates, both from humans and animals, were virtually identical to the initially described human CC130-MRSA-XI strain from Ireland lacking enterotoxin genes, PVL genes and lukM/lukF-P83, but harbouring edinB and an exfoliative toxin gene homolog etD2. CC130-MRSA-XI is rare, but it can be found sporadically in humans, livestock and wildlife, and it has the potential to cross host species barriers and

to cause outbreaks. Thus, MRSA screening procedures and diagnostic tests need to be evaluated and redesigned to ensure detection of isolates harbouring mecC.

71B

OPTIMIZING PROTEIN FRACTIONATION AND CHARACTERIZING THE WHOLE PROTEOME OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

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Pyoderma caused by Staphylococcus pseudintermedius is one of the most common infections seen in small-animals veterinary practice worldwide. The recent emergence of methicillin-resistant S. pseudintermedius (MRSP) strains has renewed the interest in the pathogenesis and virulence of this species. The proteomic approaches can be useful to identify new potential antigens, in order to develop new therapeutic strategies, namely vaccines. The aim of this study was to optimize the protein fractionation and characterize the proteome of S. pseudintermedius through 1-DE-LC-MALDI-TOF/TOF and 2-DE MALDI-TOF/TOF approaches. The studied strain of S. pseudintermedius (5819/10) was isolated from a dog with pyoderma. Bacterial cells were treated with lysostaphin, from which the supernatant, enriched in cell wall proteins (fraction I), was obtained. After an osmotic shock, proteins were ultracentrifuged and two fractions were attained: the pellet was enriched in membrane proteins (fraction II) and the supernatant was enriched in cytoplasmatic proteins (fraction III). The proteins were precipitated either with trichloroacetic acid (fraction I) or trifluoroethanol/chloroform (fractions II and III). Total protein in each fraction was quantified and separated by 1-DE and 2-DE gels. All fractions differed in their protein content as visualized by both electrophoretic separations. The most abundant five to eight proteins from each 1-DE gels were identified by MALDI-TOF/TOF mass spectrometry. Those identifications revealed that enrichment on the proteins of each cellular fraction was achieved. Nevertheless, some proteins were identified in all fractions, which could indicate that they had multiple subcellular locations or that they can transit between the cytosol and the surface compartments, depending on the physiological and/or environmental conditions. The established fractionation protocol was shown to be efficient and adequate for the characterization of S. pseudintermedius proteome. The protein identification by mass spectrometry is being performed and will help us understand the cell physiology and, consequently, the pathogenesis of these bacteria.

72B

GENETIC CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCI* IN WALLABIES

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Methicillin-resistant staphylococci (MRS), defined as staphylococci harboring the *mecA* gene, have been isolated from a number of farm and companion animals in addition to free-living birds, rodent species, deer, boars and ibexes. Few studies have examined the prevalence of MRS in native endangered fauna. The objective of this study was to determine and characterize the prevalence of beta-lactam resistance in commensal staphylococcal species isolated from healthy captive and wild wallabies from two distinct environments in South Australia, namely indigenous land and Monarto Zoo. Here, 89 staphylococ-

cal isolates recovered from 98 wallabies were studied. These isolates were characterized by 16S rRNA sequencing, Kirby-Bauer disc diffusion and growth on oxacillin-selective media. Minimum inhibitory concentrations for oxacillin were determined by broth microdilution for MRS. These 89 isolates from 15 staphylococcal species were screened for the presence of *blaZ* and *mecA*. Sequencing of the 23 *blaZ* genes revealed three signature types dominated by signature type 3 and, according to Bush classification, the amino acid sequence has inferred plasmid origins for these elements. The sequencing of *mecA* from 11 isolates show a 97-99% identity to that of the prototype N315 strain. Multiplex PCR was undertaken to classify the *mecA* determinants into various SCC*mec* types, revealing one SCC*mec* type III and two SCC*mec* type V isolates. However, eight strains of *Staphylococcus sciuri* harboring a class A *mec* region were unable to be typed by conventional methods. These eight isolates contained 19 single-nucleotide polymorphisms and were subjected to *ccrB* typing which found a potential novel allele of *ccrAB* allotype 1 in a single isolate. Interestingly, *ccrB* typing also revealed a novel combination of a *ccrAB* allotype 6 with the single SCC*mec* type III element. Studies into determining the presence of *mecC* in staphylococci which exhibited a resistance phenotype for oxacillin and cefoxitin, yet were negative for *mecA, blaZ* and *ccrB*, are currently being undertaken. Thus, genetic characterization of the *mec* elements revealed two SCC*mec* types being identified in three MRS. Additionally, a combination of the novel *ccrB* allele with a class A *mec* region could constitute a novel variant of the SCC*mec* type I element being isolated from healthy wallabies in South Australia. Importantly, irrespective of captivity status, a similar carriage rate of MRS was found in these populations.

73B

ANTIMICROBIAL RESISTANCE AND ARRAY ANALYSIS OF *MECA* POSITIVE COAGULASE NEGATIVE STAPHYLOCOCCI FROM VEAL CALVES IN BELGIUM

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Non *S. aureus* staphylococci are usually more resistant to antibiotics than *S. aureus*, and are considered a reservoir of resistance genes. The aim of this work was to study the prevalence of typical *S. aureus* resistance genes among 58 *mecA* positive coagulase negative staphylococci-CNS recovered from nasal swabs of veal calves in Belgium. tRNA intergenic spacer PCR was used for species identification. Isolates were characterized by susceptibility testing by a microbroth-dilution method using clinical breakpoints (Clinical Laboratory Standards Institute and Eucast) and SCC*mec* PCR typing. Presence of *S. aureus* antimicrobial resistance genes was analysed via microarray (Alere). CNS were identified as: *S. lentus*-22, *S. sciuri*-20, *S. epidermidis*-11, *S. haemolyticus*-4, and *S. equorum*-1. All isolates were multi-resistant, and they showed a high prevalence of resistance to erythromycin, clindamycin-both 86%, tetracycline-84%, tiamulin-76%, fusidic acid-69%, and streptomycin-67%.
A lower prevalence of resistances was seen against trimethoprim-59%, kanamycin-52%, ciprofloxacin-48%, sulfamethoxazole-47%, synercid-45%, chloramphenicol-38%, gentamicin-24%, and linezolid-5%. Phenotypic resistance to penicillin-93% and cefoxitin-76% was lower than expected based on the positive results for *mecA*. All CNS were susceptible to rifampicin, mupirocin and vancomycin. Most isolates-69% carried the SCC*mec* III and few carried other *mec* cassettes (7%-SCC*mec* IV, 9%-SCC*mec* V, 15%-non-typeable) as shown by the combined SCC*mec* PCR and array as-

say. Regarding to the antimicrobial resistance genes selection detected by the array, in most isolates one or more genes conferring resistance to macrolides, lincosamides and streptogramins B-83%, aminoglycosides-78% and tetracyclines-76% were detected, with the individual percentages of resistance genes being: *ermA*-19%, *ermB*-22%, *ermC*-55%, *mpbBM*-22%, *mphC*-19%, *msrA*-7%, *vgaA*-5%, *lnuA*-10%, *aacA-aphD*-45%, *aphA3*-40%, *aadD*-40%, *sat*-40%, *tetK*-64% and *tetM*-43%. 40% of the isolates carried chloramphenicol resistance genes (*cat*-17%, *fexA*-24%). Less isolates carried the *bla* operon-28%, trimethoprim *dfrS1*-19%, fusidic acid *far1*-5%, fosfomycin *fosB*-3%, mercury *merA-merB*-5%, quaternary ammonium compound *qacA*-14% and *qacC*-5% resistance genes. Three isolates carried the multi-resistance *cfr* gene. In conclusion, although the CNS carried mainly different SSC*mec* types than those found in the typical livestock associated CC398 MRSA, they share a large number of resistance genes with *S. aureus.* Hence, they might be an important reservoir of antimicrobial resistance genes. There is a large discrepancy between the presence of *mecA* and the expression of β-lactam resistance indicating that phenotypic data are an underestimation of the presence of *mecA* in CNS from veal calves. **Acks:** M.A. Argudín is supported by Fundación Alfonso Martín Escudero.

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MICROARRAY-BASED GENOTYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM PRIMARY PRODUCTION IN GERMANY

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Methicillin-resistant Staphylococcus aureus (MRSA) especially of clonal complex 398 (CC398), are considered an occupational health problem for farmers, veterinarians and other livestock professionals.
The aim of this study was to characterise MRSA strains isolated from farm animals or their environs in Germany with respect to their virulence and resistance genes. In total, 125 German MRSA strains isolated between 2008 and 2012 at primary production from pig (n = 56), cattle (n = 36) and turkey (n = 34) were analysed using a commercially available microarray (StaphyType, Alere Technologies, Jena, Germany). The array covers about 300 target sequences corresponding to approximately 185 distinct genes and their allelic variants including, among others, virulence and resistance genes, SCCmec-, agr-, and capsule typing markers. Further characterisation of SCCmec-, spa- and in some cases MLST-type was done by means of PCR and sequencing, respectively. Most of the isolates tested were associated to CC398 (90%), while the remaining belonged to CC9, CC5, CC30, CC97, CC8 and CC12. Isolates mainly harboured SCCmec type V (80%) and exhibited spa type t011 (39%) or t034 (31%). Altogether 54 different resistance gene patterns were detected. The most frequent resistance genes beside mecA and blaZ (100% each) were tet(M) (94%), tet(K) (73%), ermC (30%) ermA (28%) and aadD (28%). In contrast, the virulence gene content was low. For 87% of the strains the virulence pattern lukF-lukS-hlgA was found. All of the strains were negative for PVL, exfoliative toxins and the ACME locus. Few strains carried the gene tst1 (2 strains), genes for the phage associated immune modulators sak (5 strains), scn (3 strains), chp (1 strain) or for enterotoxin genes like sea and seb (1 strain each), sec (3 strains), sek, sel and seq (3 strains each) or the egc-cluster (9 strains). As expected, almost all of the MRSA tested harboured a high prevalence of resistance encoding genes. In contrast, virulence genes were predominantly found in MRSA not associated with CC398. Whether these Non-CC398 strains were introduced by humans into livestock or represent particular adapted animal strains is not known yet. The results of the study underline, that

molecular typing of MRSA is essential to closely characterize the risk associated with MRSA in livestock and that a focus should be on those strains that are not associated with the CC 398. **Acknowledgement:** This work was carried out in the EMIDA-ERA NET Project "LA-MRSA" (Grant-No. 0315868A)

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GENETIC ENVIRONMENT OF THE MULTI-RESISTANCE GENE *CFR* IN METHICILLIN-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI FROM CHICKENS, DUCKS, AND PIGS IN CHINA

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Objective: During a previous study, the multidrug-resistance gene *cfr* was detected in coagulase-negative staphylococci (CoNS) from 3/401 pigs, 15/305 chickens, and 3/78 ducks obtained from 31 different farms and one slaughterhouse in 2 provinces of China. Twenty of the 21 *cfr*-positive isolates were methicillin-resistant. The gene *cfr* was detected on plasmids in the majority of the isolates. The aim of this study was to investigate the genetic environment of the *cfr* gene in the 20 methicillin-resistant CoNS (MRCoNS) and the single methicillin-susceptible CoNS from chickens, ducks and pigs in China. **Materials and methods:** The *cfr*-carrying plasmids in 13 CoNS were transferred into *Staphylococcus aureus* RN4220 by electrotransformation. The transformants were screened for their plasmid content and resistance phenotypes. The sizes of the *cfr*-carrying plasmids were determined by BglII restriction analysis. The partial nucleotide sequences of the *cfr*-carrying plasmids or chromosomal regions were determined by a modified random primer sequencing walking strategy. **Results:** Plasmid-borne *cfr* genes were found on either previously described plasmid types pSS-02 and pSS-02-like (n= 7), pSS-03-like (n=1), pJP1-like (n=3) or novel plasmid types pSS-04 (n=1) and pJP2 (n=1). These plasmids ranged in size between 35 and 50 kb. Analysis of the *cfr*-flanking regions on plasmids revealed that IS sequences (IS*21-558*, IS*256*, IS*257*, IS*1216E*) and other resistance genes (*aacA-aphD*, *aadD*, *ble*, *fosD*, *erm*(B), *erm*(C) and/or *fexA*) coexisted on the respective plasmids. Chromosomal copies of *cfr* were identified in eight *S. lentus* isolates. The *cfr* gene was also bracketed either by IS elements (IS*256-cfr*-IS*256* or IS*Enfa5-cfr*-IS*Enfa5*) or by a combination of a composite transposon and an IS element (Tn*4001-cfr*-IS*256*) in the chromosome of the eight *S. lentus* isolates. The chromosomal *cfr*-containing region, which may have a plasmid origin, can be looped out via IS element-mediated recombination. **Conclusions:** This is the first time that the genetic environment of *cfr* has been identified on plasmids or in chromosome of CoNS isolates from chickens and ducks. IS*256* and IS*Enfa5* elements may play an important role in the dissemination of *cfr*, not only on plasmids, but also in the chromosome. The co-location of *cfr* with several other antimicrobial resistance genes bears the risk of co-selection and persistence of the *cfr* gene under the selective pressure imposed by these other antimicrobial agents.

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COMPLETE GENOME SEQUENCE OF THE METHICILLIN-RESISTANT PATHOGEN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* NA45

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Introduction: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is an important zoonotic pathogen commonly found

in canines, but has been shown to infect a variety of species including humans. Similar to methicillin-resistant *Staphylococcus aureus* (MRSA), multiple drug resistance phenotypes exist within the species and cause significant clinical infection. MRSP isolate NA45 is a clinical canine isolate with high drug resistance and virulence characteristics. By sequencing the genome of this isolate and comparing it to other *S. pseudintermedius* genomes, we hope to elucidate the genetic components responsible for these pathogenic qualities. Here we present the complete, circular genome, in comparison with methicillin-susceptible (MSSP) isolates ED99 and HKU10-03, and methicillin-resistant (MRSP) isolate E140. **Methods:** *S. pseudintermedius* NA45 was isolated in the United States from canine urine captured by cystocentesis and sub-cultured into pure stock cultures. Whole Genome Sequencing (WGS) was performed using Illumina©, Ion Torrent™ and 454© paired-end sequencing, and *de novo* assembly of contigs was performed using Geneious©. De novo contigs were aligned against a whole genome map acquired on the Argus® system and a combination of BLAST alignments and extension read mapping resulted in a complete, circular genome without the use of a reference genome sequence. Annotation was performed using the RAST annotation server, and comparative genomic analysis was performed using progressiveMauve and GCview. **Results:** *S. pseudintermedius* NA45 has a genome content of 2,841,212 base pairs encoding 2812 CDS, 76 RNAs and an average GC content of 37.3%. It contains a novel SCC*mec* cassette type and has 4 prophage insertions, 3 of them unique sites incorporating ~200Kb. Phage and other mobile elements are elevated in NA45 and other MRSPs relative to MSSPs, and a ~120Kb prophage has inverted GC content relative to lagging strand DNA, indicating recent incorporation. Unique genomic regions contain genes for sporulation, erythrocyte binding, heavy metal resistance, aminoglycoside resistance and a superantigen pathogenicity island, among others. Many of these are not taxonomically conserved and have been found only in other genera such as *Streptococcus* and *Bacillus*. **Conclusion:** Whole genome sequencing has revealed that mobile genetic elements are responsible for most of the genomic differences between NA45 and the other genomes, and contain genes involved in drug resistance and pathogenicity. As several of them are not conserved within the *Staphylococcus* lineage, movement of these elements appears to cross species and genus level boundaries. As more genomes are sequenced, these elements can be better tracked and used for functional and epidemiological studies to determine their contribution to the evolution of drug resistance and virulence in this important pathogen.

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EFFECT OF OPSONIZATION ON BACTERICIDAL ACTIVITY OF CANINE AND HUMAN POLYMORPHONUCLEAR LEUKOCYTES AGAINST *S. PSEUDINTERMEDIUS*

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Introduction: *S. pseudintermedius* is a common pathogen in dogs. Human infections are rare but have recently gained attention due to several reports of methicillin-resistant *S. pseudintermedius* (MRSP) in human patients. Very little is known about the ability of this staphylococcal species to evade killing by

polymorphonuclear leukocytes (PMNs) in diverse hosts. PMNs are part of the innate immune system and phagocytosis by PMNs is facilitated by opsonization that allows leukocytes to readily recognize bacteria. **Objective:** To study the effects of opsonization on bactericidal activity of canine and human PMNs against *S. pseudintermedius*. **Methods:** PMNs were isolated from heparinized venous blood from two healthy dogs and ten healthy humans using Hystopaque-1077 (canine blood) or Hystopaque-Ficoll (human blood) gradient centrifugation. Purity and cell viability were determined by flow cytometry. Three *S. pseudintermedius* (two MRSP, one methicillin-susceptible *S. pseudintermedius*) were tested by an *in vitro* phagocytosis assay using a ratio of 10 bacteria per PMN. For each strain, both unopsonized and opsonized cultures were used. Opsonization was obtained by incubating the strains in 50% autologous serum at 37°C for 30 min prior to addition of PMNs. PMNs were allowed to phagocytise bacteria for 1 or 3 hours. Survival of bacteria was evaluated by comparing mean colony forming units (CFUs) of bacteria exposed to PMNs and bacteria alone using paired *t* test. **Results:** *S. pseudintermedius* (serum opsonized and unopsonized) had significantly greater capacity to survive following phagocytic interaction (1h) with canine PMNs compare to that with human PMNs ($p<0.05$). However, following extended time points (3h) after phagocytosis with either canine and human PMNs, survival was significantly greater for opsonized *S. pseudintermedius* compared to unopsonized bacteria ($p<0.05$). No significant differences were found between bacterial strains. **Discussion/Conclusion:** This is the first study evaluating the interaction between human and canine PMNs and *S. pseudintermedius* and the effects of serum opsonization. Although our results are based on limited number of test subjects, they reveal an unexpected ability of opsonized *S. pseudintermedius* to evade phagocytic killing by human or canine PMNs. Future research should investigate whether this property is associated with specific bacterial surface-associated proteins or whether survival at 3 h is related to lysis of PMNs, thereby reducing their phagocytic effect. Regardless of opsonization, *S. pseudintermedius* had reduced ability to survive in the presence of human PMNs, confirming that this staphylococcal species is not adapted to the human host.

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FIRST DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ST398 STRAIN WITH ARGININE CATABOLIC MOBILE ELEMENT (ACME)

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Recently, a specific clone of methicillin resistant *Staphylococcus aureus* (MRSA) has been identified in farmers and food producing animals. This so called livestock associated MRSA (LA-MRSA) belongs to multilocus sequence type 398 (ST398) or closely related STs associated with clonal complex 398 (CC398). Acquisitions of mobile genetic elements which harbor genes that can enhance the transmission of LA-MRSA CC398 in humans have the potential to threaten public health. As part of the CAM study aiming to identify determinants of community-associated MRSA (CA-MRSA) in the Netherlands, we identified an MRSA isolate of ST398 that was positive for the arginine catabolic mobile element (ACME). The presence of ACME has been reported to enhance colonization of the skin and the mucosal surfaces by neutralizing the acidic pH of human sweat. The objective of this study was to determine the genetic organization of ACME- staphylococcal cassette chromosome *mec* composite island (SCC*mec*-CI) in

the ACME-positive isolate by whole-genome sequencing (WGS). A total of 125 MRSA isolates recovered from patients between 2009 and 2011 in The Netherlands were screened in the current study. The isolates were analyzed by DNA microarrays and the LA-MRSA ACME-positive isolate of ST398 was investigated by WGS. Sequence analysis revealed a new organization of the ACME-SCC*mec*-CI in the ST398 isolate. ACME-SCCmec-CI was composed of the J1 region of SCC*mec* I adjacent to *orfX*, followed by a truncated form of ACME type II and SCC*mec* type IVa. Moreover, in the J1 region of SCC*mec* I the gene encoding plasmin sensitive protein (Pls) was found, which has been implicated in adherence to desquamated nasal epithelial cells and as a virulence factor in murine septic arthritis. This is the first report of the ACME element in MRSA with the genotype ST398. This finding may have implications for the transmissibility of ST398 isolates, and thus public health.

79B

NOVEL SCC*MEC* CASSETTE IN A *MECB*-CARRYING *MACROCOCCUS CASEOLYTICUS* ISOLATE FROM A SINUSITIS INFECTION OF A DOG.

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Background: The methicillin-resistant *mecB* gene has been only detected in *M. caseolyticus* in three occasions, always located within a *mec* transposon, which contains the *mec* complex (*mecRm-mecIm-mecB-blaZm*), with either a plasmid or chromosomal location. We characterised the SCC*mec* cassette of a *mecB*-positive *M. caseolyticus* strain isolated from a clinical sample of a dog with sinusitis. **Material and Methods:** An alpha-hemolytic methicillin-resistant *mecB*-positive *M. caseolyticus* strain, named KM450, was isolated from the nares of a dog with sinusitis in May 2013 in Bern (Switzerland). Minimum Inhibitory Concentration (MIC) was performed to 22 different antimicrobials. Southern-Blot hybridization was conducted to determine the chromosomal or plasmid location of the *mecB* gene. High-throughput sequencing of KM450 was performed with Roche 454 GS Titanium chemistry and protocols (GS Junior Szytem, Roche Diagnostics) and sequence reads were assembled *de novo* using newbler 2.6. **Results:** Whole Genome Sequencing (WGS) of *M. caseolyticus* strain KM450 as well as Southern-Blot hybridization experiments permitted the identification of a novel chromosomally located SCC*mec* cassette. The novel SCC*mec* element was 39 kb in size and only presented two discontinuous regions of homology (SCC*mec* coverage of 46%) to the single chromosomal SCC*mec* element described to date in the genus *Macrococcus* (*M. caseolyticus* strain JCSC7096): (i) the *mec* complex (98.8% identity) and (ii) the *ccr* region (91.8%), which also covered several *orfs* and a *radC* gene. The novel SCC*mec* element, named SCCmec_{KM450}, was integrated at the 3' end of *orfX* and was delimited at both ends by imperfect direct repeats (GA[AG] TCGTATCATAAGTGA). SCCmec_{KM450} contained a *mecRm-mecIm-mecB-blaZm* complex in the 3' end of the cassette Neither transposons nor transposase genes were detected in the whole cassette. SCCmec_{KM450} contained the recombinase genes *ccrA* and *ccrB* 7.5-kb distant from the *orfX*. **Conclusions:** A novel *mecB*-carrying SCC*mec* cassette is detected in a clinical alpha-hemolytic *M. caseolyticus* strain. This observation underlines both the role of commensal bacteria as opportunistic animal pathogens and as reservoirs for novel SCC*mec* elements.

80B

NEW SEQUENCE TYPE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM LIVER INFECTION IN AN ALPINE CHAMOIS

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Methicillin resistant *Staphylococcus aureus* (MRSA) is a well-known cause of skin and soft tissue infections in humans, both in hospital and community settings. Companion animals (dogs, cats, horses) can experience such infections as the consequence of penetrating wound and surgery. Nasal and skin carriage are proved predisposing factors. Production animals are frequently healthy carriers of the Livestock-associated sequence type 398 (ST398) at nasal and other body sites level. Cattle mastitis can be caused by MRSA. Wild animals are thought to be less exposed to MRSA due to presumed uncontaminated environment and lack of antibiotics selective pressure. Yet, MRSA was recently detected in free-ranging species in aquatic and terrestrial environment in North America. In wild ruminant, a low prevalence of ST398 MRSA carriage was recently reported in healthy Iberian Ibex and red deer. *S. aureus* isolates included were obtained from a kid chamois (*Rupicapra r. rupicapra*), that was euthanized by gamekeepers, due to walking impairment and painful status, in autumn 2011 in north-western Italian Alps. A *post-mortem* examination was performed. Samples for bacteriological analysis were swabs collected from nasal cavities and organs (brain, lung, liver, spleen and kidney). After an overnight incubation in Brain Hearth Infusion at 37°C, 100 µl of the pre-enrichment were plated onto 5% sheep blood agar. Bacteria were identified according to colony morphology, hemolysis, Gram-stain and reaction to catalase and coagulase tests. They underwent confirmation by PCR, antimicrobial susceptibility test and typing. *S. aureus* was isolated from nasal mucosa and liver. No other relevant bacterial growth was detected. At the *post-mortem* examination, animal showed a good kidney fat deposit and regular contents of stomach compartments. No macroscopic lesions were observed. PCR revealed *mecA* gene in the liver isolate. Antimicrobial susceptibility test confirmed resistance to all beta-lactams tested (amoxicillin-clavulanic acid, penicillin, ampicillin, ceftiofur, cefoxitin) besides resistance to fluoroquinolones (ciprofloxacin, enrofloxacin, marbofloxacin) and susceptibility to tetracycline. This resistance profile is unusual for MRSA commonly isolated from livestock and poultry, due to coexistence of sensibility to tetracycline and resistance to fluoroquinolones. No antimicrobial resistance was detected in the nasal isolate. Multi Locus Sequence Typing revealed two different new ST, namely ST 2716 for MRSA from the liver and ST2715 for nasal mucosa isolate. This accidental isolation of MRSA in a free-living wild animal opens new perspectives in MRSA spread. Surveillance on lineages and their prevalence should be implemented among wild animal population to clarify host specificity and to assess zoonotic potential of *S. aureus*.

81B

NASAL CARRIAGE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN CAPTIVE CHIMPANZEES (PAN TROGLODYTES)

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Methicillin resistant *Staphylococcus aureus* (MRSA) in non-human primates within research facilities is reportedly widespread; however, this is not reflected in the literature. At the Keeling Center for Comparative Medicine, we identified a trend of culturing MRSA

from clinical cases within our chimpanzee colony. Over a period of two years, sixty percent of our *S. aureus* cultures were methicillin resistant. Based on this information, we investigated the overall prevalence of MRSA in chimpanzees, hypothesizing that the rate for captive chimpanzees may be higher due to lack of chimpanzee personal hygiene, close contact while in captivity and antibiotic practices. Chimpanzees were trained to present their nostrils for sample collection via nasal swabs that were subsequently tested for the presence of MRSA. Isolates were characterized by spa typing and PCR detection of Panton Valentine leukocidin genes. Of the 158 chimpanzees housed at our facility, we were able to sample 125 chimpanzees within a one-month period. MRSA was isolated from 86 (69%) chimpanzees. Three chimpanzees were sampled twice for a total of 89 positive samples. Fifty-seven (66%) isolates were PVL positive t008, consistent with the sequence type (ST) 8 USA300 clone. All but two of the remaining isolates corresponded to six spa types that were related to t008 (t818 (19, 22%) t024 (4, 4.7%), t197 (2, 2.3%), t2030 (2, 2.3%) and 1 (1.2)% each t9141, t682 and t6172. Single isolates of t116 and t1754, which are related to each other but distinct from ST8, were also found. In the three chimpanzees that were sampled twice, two of the chimpanzees had the same strain in both cultures (t008, t818) and one chimpanzee had two different but related strains on each culture(t24, t818). MRSA positivity did not correlate with age or sex. The housing location of chimpanzees within our facility may have had an effect. Testing identified groups that were completely positive, other groups that were completely negative and some groups that showed a mixed pattern. Further evaluation is being completed to determine which groups are more volatile or affiliative and if this factor correlates with the number of positive MRSA cultures. Overall, this project represents the first prevalence study for MRSA carriage within captive chimpanzees, indicating both a striking prevalence of MRSA and the predominance of an important human epidemic clone. Recent literature noted similar strains of *S. aureus* between human sanctuary workers and chimpanzees in Africa; however, none of the strains were methicillin resistant. Further research is ongoing to determine the prevalence of chimpanzee caretakers that carry MRSA and if the strains are similar to the chimpanzees.

82B

FIRST IDENTIFICATION OF CC398 METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN IRISH LIVESTOCK: VALUABLE LESSONS

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Methicillin-resistant Staphylococcus aureus (MRSA) belonging to multilocus sequence type (MLST) clonal complex (CC) 398 have not been reported previously in Ireland although methicillin-susceptible S. aureus CC398 have been identified in Irish pigs. The aim of the study was to characterize the first CC398 MRSA isolates recovered from horses and pigs in Ireland and to identify their probable source of introduction. Isolates were investigated using antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE) using the restriction enzyme ApaI, spa typing and DNA microarray profiling using the StaphType kit (Alere, Germany) to assign isolates to MLST CCs/sequence types, SCCmec types and to detect a range of virulence and resistance genes. Selected isolates were also analysed using whole genome mapping. In June 2012 MRSA was isolated from an umbilical abscess in a foal admitted to the UCD veterinary hospital. A second isolate obtained from a nasal swab of one of the attending clini-

cians was indistinguishable based on PFGE and microarray analysis and differed only by 1.1% on whole genome mapping, the difference being the acquisition of a novel genomic island by the human isolate. Both isolates were assigned to CC398-MRSA-IV, agr type I, spa type t011, and harboured the immune evasion cluster (IEC) genes sak, chp and scn, and a range of resistance genes. CC398-MRSA-IV are common in Belgium, the country of origin of the attending clinician. CC398 MRSA was not isolated from any other horses in the hospital or from nasal swabs collected from horses in the stable of origin. No evidence of onward spread of this strain was found. Coincidentally, in September 2012 CC398-MRSA-V was isolated from a joint abscess in a pig submitted for post mortem examination to the UCD veterinary hospital. The farm had recently depopulated and restocked with Irish pigs and a small number of gilts imported from Germany. Additional CC398-MRSA-V isolates were subsequently obtained from nasal swabs of pigs from the farm and spread to pigs on a second farm was also documented; this farm had bought pigs from the original farm. The MRSA isolates were typical of livestock strains and displayed characteristics consistent with a German origin including SCCmec V, agr type I, spa types t011 and t034, carriage of a variety of resistance genes and lack of IEC and enterotoxin genes. Whole genome mapping showed a very close relationship between these isolates and other published CC398 strains. These findings clearly illustrate two distinct routes of introduction of LA MRSA into a country previously free of infection: human and animal carriers. Animal infections caused by CC398 MRSA are not notifiable in Ireland and animals being imported into this country are not required to be tested for this organism. Inadequate biosecurity has resulted in the introduction of CC398 MRSA to Ireland. Countries still free of CC398 MRSA should take note.

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PERSISTENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ST 398 IN AN INDUSTRIAL RABBIT HOLDING AND FARM RELATED PEOPLE

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Livestock associated MRSA belonging to the clonal complex CC398 was described in animals and farm or slaughterhouse workers of swine, bovine and poultry industrial primary production. So far, despite *S. aureus* being commonly found in rabbits and importantly affecting production, the clone was not reported in rabbits raised for meat. In 2012-2013 forty Italian industrial rabbit farms were selected by convenience according to staphylococcosis severity to investigate on the genetic markers of *S.aureus* virulence. Within farm 60 female breeders were chosen by systematic sampling and clinically examined. Between 20 and 35 rabbits were also sampled by skin swabbing and tested for *S. aureus*, ten isolates were selected to be phenotypically and genetically characterized within the primary research scope, this included *mecA*, *mecC*, *tetM* and *tetK* genes detection. After detecting MRSA all *S. aureus* isolates of one holding were genetically characterized and nasal swabs of farmworkers and relatives were tested for MRSA. The sampling of rabbits was repeated after five months and extended to farm environment by surface swabs and air samples. MRSA isolation, detection and confirmation was carried out using standard cultural, phenotypic and molecular methods. PCR was used to detect *mecA* and *mecC*. Although it was not described in rabbits so far, the latter was added to avoid MRSA misclassification. MRSA were typed with MLST and *spa* typing. With the exception of air samples, where *S. aureus* and MRSA were counted, only one *S. aureus* isolate per rabbit/person/environment sample was characterized. Among 40 industrial holdings

tested only the rabbits of one holding were found MRSA colonized/infected. MRSA was detected in 11 (48%) of 23 *S. aureus* isolates detected in 25 rabbit skin swabs during the first sampling and in 17 (28%) of 59 *S. aureus* isolates detected in 60 rabbits sampled after five months in same holding. Five persons (four farm workers and one farmer's relative) were carrying MRSA in their nose. MRSA collected during the first rabbit sampling were ST398, *spa*-type t034 and t5210 and the MRSA isolates from the humans belonged to same *spa*-types and were ST398 as well. Two out of ten surface samples, and two out of three air samples were contaminated with MRSA. Air samples were carrying 5 and 15 cfu/m^3 MRSA counts. All MRSA isolated had *tetM* and *tetK* genes and none *S. aureus* from animal or environment carried the *mecC* gene. This paper reports MRSA ST398 t034 and t5210 circulating in rabbits raised for meat production and involving also people in contact with rabbits. After five months from first sampling MRSA was still detected in animals and farm environment. The case described was the only one out of a considerable farm sample and may represent the initial spreading of the clone among the rabbit meat primary production sector. Clonal spreading of CC398 from breeders to slaughter rabbits should be prevented.

84B

IDENTIFICATION OF THE NOVEL SPECTINOMYCIN RESISTANCE GENE *SPW* IN MRSA AND MSSA OF HUMAN AND ANIMAL ORIGIN

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Background: Spectinomycin resistance in staphylococci is mostly mediated by a spectinomycin 9-O-adenyltransferase encoded by the gene *spc*. Analysis of MRSA ST398 and MSSA ST9 isolates of human origin from Spain, but also MRSA ST9 isolates from swine in China identified multi-resistance gene clusters, which were most likely of enterococcal origin. A closer look at these clusters revealed the presence of a reading frame for a putative spectinomycin resistance gene. Database searches showed identical or closely related proteins in *E. faecium*, *E. faecalis* and *L. johnsonii*. The corresponding proteins were referred to as either streptomycin 3"-adenyltransferase, spectinomycin 9-O-adenyltransferase, a putative toxin-antitoxin system, or as hypothetical proteins. **Material and Methods:** A first PCR assay was developed which amplified the entire gene. This amplicon was cloned into the *E. coli-S. aureus* shuttle vector pLI50 and transferred into the recipient strain *S. aureus* RN4220. Susceptibility testing was conducted by broth macrodilution for spectinomycin and streptomycin. To see whether *spw* is present in other staphylococci, a second PCR assay was developed that amplified an internal *spw* segment. This PCR assay was applied to four spectinomycin-resistant, but *spc*-negative MRSA CC398 isolates from fresh turkey meat or turkey meat products. **Results:** In comparison with *S. aureus* RN4220 and *S. aureus* RN4220 carrying the shuttle vector pLI50, *S. aureus* RN4220 transformants carrying the putative spectinomycin resistance gene exhibited an at least 16-fold increase in the MIC of spectinomycin. In contrast, the MIC values for streptomycin were 4 mg/L for all tested isolates. Of the four isolates from fresh turkey meat or turkey meat products, three were also negative for *spw* while the remaining isolate from a seasoned turkey breast schnitzel was positive in the *spw*-specific PCR. **Conclusions:** The gene in the multiresistance gene clusters of MRSA CC398 as well as MSSA/MRSA ST9 of human and porcine origin, respectively, represents a novel spectinomycin resistance gene, designated *spw*. Moreover, the

spw gene is likely of enterococcal origin and thus represents another example of the gene flux between enterococci and staphylococci. More expanded analyses of spectinomycin-resistant staphylococci are needed to determine how widespread is the *spw* gene in staphylococci of different clonal lineages and different geographical and host origins.

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STAPHYLOCOCCUS AUREUS CARRIAGE IN CAPTIVE NON-HUMAN PRIMATES AND IN CAREGIVERS AT THE "LE PARC DE LA TÊTE D'OR" ZOO IN LYON, FRANCE.

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During the last decade, the emergence of livestock-associated methicillin-resistant staphylococcus aureus (LA-MRSA) in human population has raised questions about their host specificity and animal/human transmission. If Staphylococcus aureus has been reported in horses, pigs, cattle, poultry and companion animals, only few data are available about carriage in captive wildlife at zoos. The goal of our study was i) to determine staphylococcus aureus carriage in a non-human primate population of the "Le parc de la Tête d'Or" zoo, Lyon, France; ii) to compare Staphylococcus aureus carriage isolates from wild animals and from their caregivers and veterinarians; iii) to investigate potential transfer between both groups. Nasal (N), throat (T) and rectal (R) swabs were collected on 22 lemurs belonging to different species (Lemur catta, n=6; Varecia variegata variegata, n=6; Varecia variegata subcincta, n=6; Varecia rubra n=2, Eulemur rubriventer, n=2). Nasal samples were collected from all caregivers and veterinarians (n=22). All swabs were inoculated into a pre-enrichment medium containing Brain-Heart broth with 5% NaCl broth. After overnight incubation at 37°C, hundred microliters were streaked on SAID agar (bioMérieux). Suspected S. aureus colonies were subcultured on blood agar and identification was confirmed by MALDI-TOF (Vitek MS, bioMérieux). All S. aureus isolates were tested by PCR for the presence of nuc gene (a species-specific marker), mecA and mecC genes. Genetic characterization was performed using DNA microarray (StaphyType, Alere). Fourteen out of the 22 lemurs were positive for S. aureus (N, n=8; T, n=8; R ,n=6). All were methicillin-susceptible (MSSA), belonged to the clonal complex CC49 and harboured the same microarray profile. Fourteen out of the 22 caregivers and veterinarians were detected as S. aureus carriers. Surprisingly, 12 were methicillin-resistant S. aureus (MRSA), among which 11 belonged to the CC5 pediatric clone, and one belonged to CC398. The two remaining human MSSA were identified as CC398 MSSA and CC30 MSSA, two clones highly prevalent in MSSA French carriers in the community. Our study showed that S. aureus is able to colonize captive lemurs and is associated to nasal, oral and/or rectal carriage. A single CC49 clone, classically considered as well adapted to animal, has disseminated in lemurs, but no evidence of transmission to caregivers was observed. Conversely, the worrisome diffusion of a CC5 MRSA clone within the zoo worker population is surprising and could be potentially due to another animal reservoirs, even if this genetic background is classically rather a human-related clone. Further epidemiological investigations are underway to determine the sources and the reasons for this high prevalence of MRSA in caregivers and veterinarians at the "Le parc de la Tête d'Or" zoo.

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COMPLETE SEQUENCE OF THE MULTI-RESISTANCE PLASMID PV7037 FROM A PORCINE MRSA INCLUDING TWO NOVEL RESISTANCE GENES

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Background: The two novel resistance genes *lsa*(E) and *spw* have been identified as parts of a multi-resistance gene cluster in MRSA/MSSA. In the porcine MRSA ST9 isolate SA7037, this multi-resistance gene cluster was identified on the ca. 41-kb plasmid pV7037 of which only 17.5 kb have been sequenced previously. The aim of this study was determine the complete sequence of plasmid pV7037 to gain insight into its structure and organization. **Material and Methods:** In addition to the previously sequenced 17.5-kb XbaI fragment, the three remaining XbaI fragments of ca. 4.5, 6.5 and 12.5 kb cloned in the shuttle vector pLI50 were sequenced by primer walking. The entire plasmid sequence was assembled and investigated for reading frames. In addition, two reading frames, one coding for an ABC transporter and the other for a rRNA methylase, were also cloned into pLI50 and expressed in *S. aureus* RN4220 to investigate their potential role in antimicrobial resistance. Antimicrobial susceptibility testing of *S. aureus* RN4220 carrying each of the two recombinant shuttle vectors was performed by broth microdilution. **Results:** Plasmid pV7037 proved to be 40,971 bp in size. Besides the previously determined resistance gene cluster, it carried a functionally active tet(L) gene, a complete *cadDX* operon and also a variant of the β-lactamase transposon Tn*552*. Two single bp deletions, which resulted in frame shifts, functionally deleted the genes for the BlaZ β-lactamase and the signal transducer protein BlaR1 in this Tn*552* variant of pV7037. Comparative susceptibility testing of *S. aureus* RN4220 and *S. aureus* RN4220 carrying the recombinant shuttle vectors did not show differences in the MIC values suggesting that the ABC transporter and rRNA methylase genes have no function in antimicrobial resistance. **Conclusions:** The co-location of seven functionally active antimicrobial resistance genes together with a cadmium resistance operon bears the risk of co-transfer and co-selection of resistance genes, but also persistence of resistance genes even if no direct selective pressure by the use of the respective antimicrobial agents is applied. The sequence of plasmid pV7037 confirms the ability of *S. aureus* to acquire genetic material from other bacteria and to generate novel mosaic plasmids with numerous resistance genes from different sources.

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NOVEL PSEUDO SCC*MEC* ELEMENT (ΨSCCmec_{57395}) IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* CC45

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Genetic characterization of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from Thailand and Israel revealed the presence of a predominant atypical clonal lineage which was not typeable by SmaI-PFGE and SCC*mec* typing. A novel pseudo staphylococcal cassette chromosome (ΨSCCmec_{57395}) element was identified in MRSP strain 57395 (ST45) by whole genome sequencing. The 12,282-bp ΨSCCmec_{57395} element contained a class C1-like *mec* gene complex but no *ccr*

genes. In addition to the methicillin resistance gene *mecA*, ΨSCC*mec*$_{57395}$ also carried determinants of resistance to heavy metals, such as arsenic, cadmium, and copper. Bsu36I restriction analysis of the ΨSCC*mec*$_{57395}$ element amplified by long range PCR revealed the presence of a of genetic elements similar to ΨSCC*mec*$_{57395}$ in 29 additional isolates of MRSP ST45, and in 2 MRSP ST179, 1 MRSP ST57, and 1 MRSP ST85 isolate(s), which all belonged to clonal complex CC45. The strains originated from healthy and diseased dogs and cats, as well as from the environment of one clinic. Cfr9I-PFGE and *dru* typing allowed to further distinguish between CC45 isolates from the two different countries. Microarray analysis also identified genes that confer resistance to β-lactams (*mecA*; *blaZ*), aminoglycosides [*aac(6')-Ie–aph(2')-Ia*; *aph(3')-III*; *ant(6)-Ia*], macrolides and lincosamides [*erm*(B)], tetracyclines [*tet*(M)], trimethoprim [*dfr*(G)], streptothricin [*sat4*], and chloramphenicol [*cat*$_{pC221}$]. Fluoroquinolone resistance was attributed to specific amino acid substitutions, namely Ser-84Leu in GyrA and Ser80Ile and Asp84Asn in GrlA. The ΨSCC*mec*$_{57395}$ element represents a new class of SCC*mec* and has been identified in MRSP of CC45, which is a predominant clonal lineage in Israel and Thailand.

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METICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CARRYING *MECC* IN CAPTIVE MARA

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Introduction: *Staphylococcus aureus* sequence type ST130 was isolated from seven captive mara (*Dolichotis patagonum*) at a Danish Zoo in 2010. After ST130 was associated to *mecC* carriage in 2011, we investigated the presence of this novel methicillin resistance determinant in the isolates from mara, a large rodent species native to South America. **Objective:** To identify *mecC* in *S. aureus* ST130 isolates from mara and characterize their antimicrobial susceptibility patterns, virulence and resistance gene content. **Material and methods:** All seven isolates were screened by *mecC* PCR. Whole genome sequencing was performed on two randomly selected *mecC*-positive isolates to analyze SCC*mec* sequence and presence of virulence and resistance genes. Antimicrobial susceptibility was tested by broth microdilution using commercial MIC plates (Sensititre, TREK diagnostics). **Results:** All isolates harboured *mecC*. The *mecC* sequences in two sequenced strains were 100% identical to that described in *S. aureus* LGA251. Both strains carried SCC*mec* XI with 23 SNPs compared to the prototype of SCC*mec* XI in LGA251. The only putative resistance gene detected in addition to *mecC* and *blaZ* (as part of the *mec* complex class E) was *norA*. The following virulence factors were detected: *hla*, *hlb*, *hlgACB*, *lukED*, *eta*, *etb*, *edin-B*, *set2, set3, set4, set5, set7, set10* and a previously described variant of *etd*. A 3.3 kb deletion was observed in the collagen-adhesin gene (*cna*) as previously described in ST130. The two strains were resistant to cefoxitin but susceptible to oxacillin according to current breakpoints for MRSA detection, and displayed susceptibility to all non-beta-lactam agents tested. **Discussion and conclusions:** This study provides further evidence of the broad host range of MRSA ST130. The strains from mara had a set of virulence factors and genomic features previously observed in ST130 originating from other host species, suggesting that the genome of this lineage is conserved. Methicillin resistance was not accompanied by resistance to antimicrobial classes other than beta-lactams and susceptibility data matched with the resistance genotype since *norA* is a housekeeping gene that confers fluoroquinolone resistance only when overexpressed.

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) SCREENING AT A TERTIARY VETERINARY HOSPITAL: IS TESTING COST-BENEFICIAL FROM AN INTEGRATED HUMAN-ANIMAL PERSPECTIVE?

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Methicillin-resistant Staphylococcus aureus (MRSA) is an antimicrobial resistant organism of international significance to human and veterinary medicine. In human medicine, policies proposed and tested for MRSA control include variations of universal patient screening and surveillance programs. In veterinary medicine information on MRSA control is scarce. Objective: To develop a cost-benefit (CB) analysis from an integrated human-animal that could be used for policy development and including all animals admitted for hospitalization to NCSU CVM Veterinary Teaching Hospital. Materials and methods: A model was developed to estimate the costs of MRSA of all animals admitted to the hospital taking into consideration the projected economic benefits of the prevention of human infections. Results: The baseline model used the most plausible inputs and different scenarios were considered in the sensitivity analysis. The cost of the screening policy was estimated at $320,104 and exceeded the savings of about $183,409. Variations in the input assumptions, most notably the additional cost for treating a human case, rendered a variety of possible outcomes. Conclusions: Our study suggests that it is not beneficial to implement MRSA screening programs in veterinary hospitals from an integrated economic human-animal perspective.

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A NOVEL PEPTIDE-PEPTOID HYBRID ACTIVE AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

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Introduction: Limited treatment options are available against methicillin-resistant Staphylococcus pseudintermedius (MRSP) infections in small animals. In recent years, much research has been directed towards antimicrobial peptides as potential antimicrobial agents. More recently, the focus of this research has been extended to peptoids, synthetic analogs of peptides whose structure improves pharmacological properties of natural peptides. Objective: To identify novel antimicrobial peptide-peptoid hybrids for topical use against pyoderma and otitis in dogs. **Methods:** Ten peptoids and peptide-peptoid hybrids were obtained by solid phase synthesis. Minimum inhibitory concentrations (MICs) of the purified products were determined for 50 clinical S. pseudintermedius isolates by broth microdilution. The most potent candidate was tested against 10 methicillin resistant strains, and its hemolytic activity on human erythrocytes was measured by determining the half maximal effective concentration (EC50) after 1 hour of exposure. Time kill kinetics of the lead compound was studied at 1x, 2x and 4x MIC. **Results:** The MICs of the ten compounds ranged from 2 to 32 µg/ml. Compound B1, a peptide-peptoid hybrid, showed the highest antimicrobial activity (MIC=2 µg/ml). The hemolytic activity of B1 was low (EC50>128 µg/ml). The compound showed ability to kill MRSP within 2 hours at 2x MIC and 1 hour at 4x MIC. No difference in MIC values was observed between MRSP and MSSP. **Discussion/Conclusions:** We identified a peptide-peptoid hybrid which is active against MRSP and potentially may be used in the treatment of canine pyoderma and otitis. This molecule was

selected as the lead candidate for further development. Optimization of drug formulation and in vivo toxicity and efficacy studies using animal models will be the next steps.

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MECC AND CEFTAROLINE SUSCEPTIBILITY IN CLINICAL *S. AUREUS* ISOLATES

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Objectives: Ceftaroline fosamil (ceftaroline) is a novel cephalosporin with broad-spectrum activity against Gram-positive pathogens, including methicillin-resistant Staphylococcus aureus (MRSA). It was approved in the EU in August 2012 for the treatment of complicated skin and soft tissue infections and community acquired pneumonia. The activity of ceftaroline against MRSA is attributed to its ability to inhibit the biochemical activity of PBP2a more efficiently than other presently available ß-lactams. Previous studies suggested that the relatively low minimal inhibitory concentration (MIC) towards oxacillin found in mecC positive S. aureus is most likely a result of a higher affinity of the mecC encoded PBP2a for oxacillin compared to the cephalosporin cefoxitin. Thus, susceptibility of these isolates towards ceftaroline appeared as an interesting question. So far, there is only limited data on susceptibility of mecA and mecC positive MRSA towards ceftaroline, especially with respect to the German S. aureus population. **Material and methods:** We investigated the susceptibility of mecC positive S. aureus towards ceftaroline using a collection of 76 human isolates originating from all over Germany. Based on spa-typing, the majority of isolates was affiliated to clonal lineage ST130. Initially, all isolates were tested for their susceptibility towards cefoxitin and ceftaroline by disk diffusion methodology followed by broth microdilution according to EUCAST guidelines. Selected isolates were further tested for their ceftaroline susceptibility using Etest. **Results:** Our collection included 23 (30%) mecC positive, phenotypically oxacillin susceptible isolates (MIC range, 0.5-2 mg/L) and all of them were resistant towards cefoxitin using disk diffusion test. With regard to ceftaroline susceptibility testing by disk diffusion, only four isolates revealed "borderline" zone diameters of 21 mm and subsequent broth microdilution revealed a susceptible phenotype for all of them (MIC range, 0.5-1 mg/L). Among the 72 isolates isolates with zone diameters above 22 mm, we detected three non-susceptible isolates (zone diameters: 22-23 mm; MICs: 2 mg/L). MICs for the ceftaroline susceptible isolates were 0.25 mg/L (n=3), 0.5 mg/L (n=32) and 1 mg/L (n=38), respectively. Subsequent Etest for selected isolates revealed MICs which were generally 2- to 3-fold lower than those generated by broth microdilution. **Conclusion:** Preliminary results demonstrate a low prevalence of ceftaroline non-susceptibility among mecC positive S. aureus (4% of all isolates investigated).

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ANALYSIS OF SOCIETAL BENEFITS AND COSTS OF PREVENTING INTRODUCTION OF MRSA CC398 AMONG PIGS IN SWEDEN

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Background: A reservoir of MRSA CC398 in pigs implies a risk for transmission to humans in contact with live pigs. The Swedish nucleus- and multiplying herds are considered free from MRSA and monitoring in fattening

pigs at slaughter has only revealed one positive finding. If MRSA CC398 is introduced and spread among pigs in Sweden and persons in contact with live pigs would be considered a risk group for carriage, costs would arise when people in the risk group visit health care institutions. Trade of live animals is the most important risk factor for MRSA CC398 in pigs and the favourable situation in Sweden is probably mainly due to limited import of live pigs. Since imports of live animals are expected to increase due to structural changes in breeding practices in Sweden, the Swedish Farmers' Disease Control Programme (SDS) has issued recommendations to prevent MRSA from being introduced through import of live animals or semen. The breeding companies operating in Sweden abide to the recommendations. The present study ventures to investigate if the societal benefits of abiding to the recommendations are large enough to outweigh their societal costs. **Methods:** The risk group, i. e. persons in contact with live pigs, was established as pig farmers, slaughterhouse workers, pig transporters, pig veterinarians and household members to these groups. It was assumed that following the import recommendations would preserve Sweden free of MRSA CC398 in pigs. The societal benefits were considered as the value of health care expenditures and production losses caused by MRSA CC398 in the risk group that would be avoided if MRSA was not introduced among Swedish pigs. These were estimated using Swedish data on the incidence of skin infections in primary- and hospital care for the period 2001 - 2011, Swedish data on treatment costs and wage costs, and assuming that human prevalence of MRSA CC398 in Sweden, without the recommendations, would have been the same as in the Netherlands or, alternatively, in Denmark. The societal costs were considered as costs of abiding to the recommendations including quarantine procedure, sampling of animals, laboratory analyses, culling of colonized animals, destroying of contaminated semen and adverse effects on profits due to the delay of genetic progress caused by the recommendations. These were estimated using Swedish data on the expected number of live boars (batches of semen) imported annually, duration and costs of the quarantine period, costs of collecting and analyzing bacteriological samples and prices of boars and semen. **Results:** A model for estimation of the annual societal benefits and costs has been created and values are under calculation.

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THE EFFECT OF ROUTINE ANTIMICROBIAL THERAPY ON CANINE COMMENSAL STAPHYLOCOCCI

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Meticillin resistance is emerging at an alarming rate among staphylococci. Antimicrobial therapy, particularly with either ß-lactams or fluoroquinoles, has been associated with carriage of and/or infection with meticillin resistant staphylococci in man and other animals. This study was undertaken to examine the canine mucosal staphylococcal populations before and following therapy with systemic antimicrobials. Dogs (n=126) requiring routine systemic antimicrobial therapy either with cephalexin (CFX; n=31), amoxicillin-clavulanate (AC; n=29), cefovecin (CVN; n=25), clindamycin (CD; n=28) or a fluoroquinolone (FL; n=13) without prior antimicrobial treatment for 3 months were enrolled. Mucosal swabs (nasal and perineal) were collected from each dog before therapy (D0), end of therapy (End) and one and three months after therapy (M1 and M3 respectively). Staphylococcus spp. were isolated and identified phenotypically and tested for antimicrobial susceptibility by disc diffusion. Isolates with an oxacillin resistant phenotype were further investigated for the carriage of the mecA gene by PCR assay. Outcome measures were oxacillin resistance and carriage of the mecA gene (mecA), multidrug resistance (MDR; ≥3 antimicrobial

classes), and carriage of coagulase positive (CoPS) and negative staphylococci (CoNS). There was an overall trend for increased carriage of mecA gene positive staphylococci at End following general antimicrobial therapy and in the CFX, AC, CVN and FL groups followed by a decline towards pre-treatment levels at M3, particularly for CoNS. However these findings were only statistically significant for general antimicrobial therapy. MDR staphylococci also tended to increase at End following general antimicrobial therapy and in the CFX, CVN and FL groups and decline by M3 but these findings were not statistically significant. Carriage of CoPS decreased at End in the majority of antimicrobial groups but recovered by M3, particularly in the CFX and CD groups. These changes were significant in the CD group. On the other hand, CoNS carriage increased at End in all groups other than CFX and CD and decreased again by M3 in the majority of the groups but did not reach significance (Cochran and McNemar; $P \leq 0.05$). This study demonstrated an association between antimicrobial therapy and increased carriage of MDR and oxacillin resistant and mecA positive canine commensal staphylococci and their subsequent recovery to near pre-treatment levels three months after the end therapy. Interestingly, whilst there was an increase in carriage of CoNS during therapy, CoPS carriage decreased. Increased carriage of the mecA gene and MDR was more common among CoNS than CoPS. It is therefore likely that the increase in CoNS populations during therapy is due to the fact that CoNS are generally more antimicrobial resistant than CoPS populations.

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ISOLATION AND CHARACTERIZATION OF PHAGES WITH LYTIC ACTIVITY AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS BELONGING TO THE CLONAL COMPLEX 398

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Some years ago, Methicillin-resistant Staphylococcus (S.) aureus (MRSA) emerged in livestock worlwide. In Germany, 90% of these livestock-associated MRSA (LA-MRSA) isolates can be attributed to the clonal complex 398 (CC398). People with occupational livestock contact are at high risk of colonization hence, invasive infections with CC398 MRSA have already been reported in several countries. This calls for intervention strategies focusing on a prevalence reduction among livestock. A reduction of MRSA CC398 in livestock might be achieved by application of virulent phages. However, there have yet no reports been published on phages lysing MRSA CC398 strains. In this study three virulent phages (PSa1, PSa2 and PSa3) with lytic activity against MRSA CC398 strains were isolated from German pig husbandries. Morphologically the phages are members of the family Podoviridae and exhibited an identical host range. They lysed 52 (60%) out of 86 tested MRSA CC398 strains representing 18 different but most common spa types. While the PSa1 and PSa3 genomes have a similar size of approximately 17.5 kb, the PSa2 genome is somewhat larger (ca. 18.5 kb). Southern hybridization revealed strong DNA homologies between the phages confirmed by sequence analysis of cloned restriction fragments and PCR products. Moreover, the whole PSa3 genomic sequence (17,602 bp) showed close relationship to 44AHJD-like phages, which are not known to contain virulence-associated genes. To assess whether these phages

might be suitable candidates for applications in vivo, in vitro experiments were carried out in which the number of MRSA CC398 could be reduced by up to four log10 units. The phages were stable at a wide range of temperatures and pH values. Further experiments will be conducted to assess the potential of these lytic phages to reduce the total LA-MRSA burden for public health. This work was accepted for publication: Kraushaar, B. et al. (2013). Isolation and characterization of phages with lytic activity against methicillin-resistant Staphylococcus aureus strains belonging to clonal complex 398. Arch Virol, in press: DOI: 10.1007/s00705-013-1707-6 **Acknowledgement:** This study has been partially supported by a grant from the Bundesministerium fuer Bildung und Forschung, "MedVet-Staph" (01KI1014C).

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MODELING THE TRANSMISSION OF LIVESTOCK ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ALONG THE PIG SLAUGHTER LINE

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The study introduces a new approach for a qualitative transmission assessment of MRSA throughout the pig slaughter process. Based on prevalence data found in literature the MRSA contamination and elimination rates of each individual slaughter step were estimated. The rates were used to set up a Monte Carlo simulation for modeling the propagation of MRSA along the process chain and to quantify the impact of a variable initial prevalence on the outcome prevalence on carcasses. Sensitivity analyses for the model as well as three different scenarios were performed to estimate the impact of cross contamination during slaughter and to determine the process stages where hygiene interventions are most effective. Regardless of the initial extent of MRSA contamination low outcome prevalences ranging between 0.15 and 1.15 % were achieved among pig carcasses indicating that the pig slaughter chain generally includes process steps with the capacity of limit carcass contamination. Especially scalding and singeing can lead to a significant reduction of superficial MRSA contamination during the first half of the slaughter process. Nevertheless, scenario analyses showed that the low MRSA outcome prevalence can only be guaranteed if recontamination during the ongoing slaughter process is obviated. In order to ensure a low MRSA load on pig carcasses at the end of slaughter the abattoir should primarily concentrate on controlling the process parameters of scalding and singeing and avoiding recontamination on subsequent process steps. **Acknowledgements:** This work was carried out within the Project MedVet-Staph funded by the German Bundesministerium für Bildung und Forschung, Grant Nr. 01KI1014C.

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THE EFFECTIVENESS OF BACTERIOPHAGES AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL COLONIZATION IN PIGS IN VITRO, EX VIVO AND IN VIVO

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Background Methicillin-resistant *Staphylococcus aureus* (MRSA) is widely spread among animals. Humans in contact with animals are at increased risk of becoming colonised with this opportunistic pathogen. Bacteriophages are generally specific for subsets of strains within a species and would be a promising candidate therapeutic agent for eliminating colonization by MRSA. The aim of this study was to compare the efficacy of bacteriophage treatment on porcine nasal colonization with

MRSA *in vitro*, *ex vivo*, and *in vivo*. **Methods** Phages Fred*710 and Felix (developed for use against human MRSA strains) were provided in a proprietary gel formulation by Novolytics Ltd. (Warrington, UK). The *in vitro* effectiveness of these phages was assessed by incubating phages with MRSA strain V0608892/1 (ST398) at 10^6 CFU/mL, measuring the OD_{600} hourly. The strain was grown on a porcine mucosa explant and phages were applied to investigate the *ex vivo* efficacy of treatment. To study the *in vivo* effect, phages were administered for 5 days to caesarean-derived piglets (N=8), which were experimentally colonized with the aforementioned MRSA strain to assess *in vivo* effectiveness. Eight piglets received a placebo. MRSA was enumerated from explants and nasal swabs by bacterial culture. Six days after the last phage administration 8 piglets received a nasal ointment with mupirocin for 5 days, 2 doses per day. **Results** MRSA did not grow after incubation with phages *in vitro*. On the explants colonization with MRSA was established with approx. 10^8 CFU/explant. However, after application of phages no reduction of colonization was observed. In 16 piglets, which were colonized with MRSA with approx. 10^5 CFU/nasal swab, the numbers of MRSA recovered from the nose were not reduced after application of the phages or the placebo. Phages that were re-isolated from pig's noses were still effective against the original and the re-isolated MRSA strain. Mupirocin ointment eradicated MRSA from the nose and the mucosal explants. **Conclusions** i) The MRSA strain was not able to grow in the presence of the Fred*710 and Felix phages *in vitro*. ii) Phages did not reduce porcine nasal colonization *ex vivo* and *in vivo*, in contrast to mupirocin. This may be due to physiological properties of the pig mucosa rather than lack of efficacy of the phages. iii) The correlation between the results from the *ex vivo* and *in vivo* experiments suggests the potential of the explant model for pre-animal screening.

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IMPACT OF GENTAMICIN AND SILVER ON METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* BIOFILM FORMATION ON POLYMETHYMETHACRYLATE

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Methicillin-resistant Staphylococcus pseudintermedius (MRSP) has emerged as the leading cause of surgical site infections (SSIs) in dogs. Biofilm formation has been suspected as a possible virulence factor and reason for its rapid emergence. This is of particular concern for orthopedic procedures involving implants, since biofilm formation on these foreign materials can complicate infection. Polymethylmethacrylate (PMMA) is commonly used in total joint arthroplasty and a variety of antimicrobial additives have been used in an attempt to prevent bacterial adhesion and SSI. The objective of this study was to evaluate the impact of addition of gentamicin or silver to PMMA on MRSP biofilm formation. Six MRSP isolates from dogs were evaluated. PMMA beads were prepared with 1.25% gentamicin, 1% microsilver, their combination or no additive, then inoculated into MRSP suspension. Beads were rinsed to remove non-adhered (planktonic) bacteria and then sonicated for 5 minutes to remove biofilm-embedded bacteria. Serial dilutions of the sonicate were plated on Columbia blood agar and colony forming units (CFU) counted. Analysis of variance (ANOVA) was used to compare biofilm growth (log10 CFUs) between the four PMMA groups. A p value of <0.05 was considered significant. None of the PMMA additives completely inhibited MRSP biofilm formation. Gentamicin-loaded PMMA significantly reduced log10 CFUs compared with no additive ($p = 0.04$). Silver-loaded PMMA did not significantly reduce log10 CFUs compared with PMMA alone ($p =$

0.97). The combination of gentamicin + silver loaded PMMA did not significantly reduce log10 CFUs compared with gentamicin alone ($p = 0.85$). The results of this study indicate that gentamicin-loaded PMMA was effective in reducing in vitro MRSP biofilm formation. Silver-loaded PMMA had no effect on reducing MRSP biofilm formation in vitro. This may be a result of the concentration of microsilver tested in this study. Further in vitro and in vivo study into PMMA additives that prevent MRSP biofilm formation is warranted.

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ANTIBIOTIC EFFECT OF YERBA MATE TEA EXTRACTS ON METHICILLIN-RESISTANT *STAPHYLOCOCCUS* ISOLATES FROM DOGS

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The emergence of multidrug resistance in staphylococci and other bacterial pathogens has led many investigators to seek alternative antimicrobial compounds that might circumvent resistance through potentially unique mechanisms of action. In this study extracts of yerba mate tea were tested with modified agar disk diffusion and microbroth dilution methods for their antimicrobial activity on recent clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), *S. pseudintermedius* (MRSP) and *S. schleiferi* subsp. *coagulans* (MRSS) from dogs. Preliminary testing with the quality control strain, *S. aureus* ATCC 29523, gave reproducible growth inhibition over a linear dynamic range of disk contents from 40 mg to 160 mg. Disk content of greater than 20 mg of yerba mate tea extract was required to produce measurable zones of growth inhibition. Each of the canine isolates was also inhibited by these concentrations of extract. Zone of growth inhibition diameters were greatest among *S. aureus* isolates and smallest for *S. schleiferi* subsp. *coagulans* isolates. The minimum inhibitory concentrations of each *Staphylococcus* species was less than 20 mg/ml and it appeared that after the 24 hr contact period at which the microbroth dilution test was read, this concentration resulted in killing of all isolates. Yerba mate extracts or compounds purified from such extracts may be of potential use for topical treatment of canine infections caused by multiresistant *Staphylococcus* species.

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IN VITRO ANTIBACTERIAL AND ANTIBIOTIC-POTENTIATION ACTIVITIES OF PIPER NIGRUM AND TELFAIRIA OCCIDENTALIS AGAINST MULTIDRUG-RESISTANT BACTERIA

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Background and Aim: Methicillin-resistant Staphylococcus aureus (MRSA) is Multi-drug resistant pathogen (MDR), over-expressing, different efflux pumps which causes many health problems in human, animals and even in agriculture. Because of this problem of resistance to conventional antibiotics, attention is now being shifted towards biologically active components from plant species used as herbal medicine, as these plants may offer a new source of antibacterial. The present study was designed at evaluating the antibacterial activities of the methanol extracts of black Piper nigrum and Telfairia ociidentalis, two plants locally used in Cameroon to treat microbial infections, and their synergistic effects with antibiotics against a panel of twenty nine Gram negative bacteria including some phenotypes expressing active efflux pumps. **Methods:** The broth microdilution method was used to determine the minimum inhibitory concentrations (MIC) of the extracts alone and in the presence of Phenylalanine-Arginine β-Naphtylamide (PAβN), an efflux pumps

inhibitor (EPI), as well as the MIC of antibiotics in association with two of the most active extracts, Piper nigrum and Telfairia occidentalis. The preliminary screening of the extract composition was conducted according to the standard phytochemical methods. **Results:** Phytochemical analysis showed the presence of alkaloids, phenols, flavonoids and tannins in both plant extracts. Other chemical classes of secondary metabolites were selectively present. The results of the MIC determination indicated that the extract from P. nigrum were able to inhibit the growth of all the twenty nine studied bacteria within a concentration range of 32 to 1024 µg/ml meanwhile T. occidentalis prevented the growth of 93.1% of such microorganisms. At MIC/2 and MIC/5, synergistic effects were noted with both extracts on more than 70 % of the tested bacteria for seven antibiotics, namely tetracycline (TET), doxycyclin (DOX), ciprofloxacin (CIP), norfloxacin (NOR), kanamycin (KAN), chloramphenicol (CHL) and erythromycin (ERY). **Conlusions:** The overall results of the present study provide information for the possible use of the studied edible plants extracts in the control of bacterial infections involving MDR phenotypes. They also provide a good basis for further investigations with the plant extracts for the control of MRSA.

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GROWTH DYNAMICS OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* USA300 AND ST398 EXPOSED TO THERAPEUTIC CONCENTRATIONS OF CEFUROXIME AND CEFOTAXIME

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) are resistant to traditional penicillins and cephalosporins. As an alternative to new antimicrobial drugs, resistance could be overcome by development of "helper drugs" that reverse methicillin resistance or have a synergistic effect with β-lactams. In order to explore the latter option, it is important to understand MRSA response to therapeutic concentrations of β-lactams. **Objective:** To investigate the growth response of MRSA to therapeutic and sub-therapeutic concentrations of cephalosporins widely used in clinical practice, cefuroxime and cefotaxime. **Methods:** Two clinical strains representing epidemic MRSA lineages, ST8 (USA300) and ST398, were selected. MICs of cefuroxime and cefotaxime were determined by Etest® (bioMérieux). Two *in vitro* growth experiments were performed using the Bioscreen® in microtitre plates for 48 hr and in traditional flasks for seven hr. In both experiments, cultures in early exponential phase (Bioscreen: 10^6 CFU/mL, flask: 10^7 CFU/mL), were exposed to either cefuroxime, cefotaxime or no antibiotic as a control. A therapeutic concentration of 30 µg/mL was selected for both drugs based on PK calculations estimating the serum concentrations achieved 1 hr after standard dosage (1.5-3 g q8 hr). The sub-therapeutic concentration (0.3 µg/mL) was arbitrarily defined as 1/100 of the therapeutic concentration. Optical density (OD_{600}) was measured every 20 min (Bioscreen) or 30 min (flask). In addition, bacterial counts were determined for each OD_{600} reading in the flask experiment. All experiments were performed in triplicate. **Results:** The USA300 strain displayed cefuroxime and cefotaxime MICs of 16 and 24 µg/mL, respectively. Slightly lower MICs were observed for the ST398 strain (6 and 8 µg/mL, respectively). Growth was not inhibited and resembled that of the control following exposure to the sub-therapeutic concentration of both cephalosporins. Both strains exhibited a marked reduction in growth 3.5 hr after exposure to the therapeutic concentration. However based on bacterial counts, such reduction was 2.5-fold for ST398 and 0.8-fold for USA300 regardless of the cephalosporin used. **Conclusions:** Under *in vitro* conditions, both MRSA strains were inhibited by cefuroxime and cefotaxime concentrations achieved

during therapy, even though their MICs were above the clinical breakpoint. This observation suggests that MRSA, even if regarded as clinically resistant to cephalosporins, are stressed in the presence of therapeutic concentrations of these drugs. These data support our hypothesis that β-lactam "helper drugs" may be developed to target MRSA under such stressful conditions. Gene expression profiling studies are underway to identify potential target genes that are up-regulated in MRSA during therapy with cephalosporins.

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VIDEO OBSERVATION OF HAND HYGIENE PRACTICES DURING ROUTINE COMPANION ANIMAL APPOINTMENTS AND THE EFFECT OF A POSTER INTERVENTION ON HAND HYGIENE COMPLIANCE

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Background: Hand hygiene is considered the most important infection control measure for preventing transmission of hospital-associated pathogens, including methicillin-resistant staphylococci, but there is little information available regarding hand hygiene frequency and technique used in veterinary clinics. The objectives of this study were to describe hand hygiene practices associated with routine appointments in companion animal clinics in Ontario, and the effectiveness of a poster campaign to improve hand hygiene compliance. **Results:** Observation of hand hygiene practices was performed in 51 clinics for approximately 3 weeks each using 2 small wireless surveillance cameras: one in an exam room, and one in the most likely location for hand hygiene to be performed outside the exam room following an appointment. Data from 38 clinics were included in the final analysis, including 449 individuals, 1139 appointments before and after the poster intervention, and 10894 hand hygiene opportunities. Overall hand hygiene compliance was 14% (1473/10894), while before and after patient contact compliance was 3% (123/4377) and 26% (1145/4377), respectively. Soap and water was used for 87% (1182/1353) of observed hand hygiene attempts with a mean contact time of 4 s (median 2 s, range 1-49 s), while alcohol-based hand sanitizer (AHS) was used for 7% (98/1353) of attempts with a mean contact time of 8 s (median 7 s, range 1-30 s). The presence of the posters had no significant effect on compliance, although some staff reported that they felt the posters did increase their personal awareness of the need to perform hand hygiene, and the posters had some effect on product contact times. **Conclusions:** Overall hand hygiene compliance in veterinary clinics in this study was low, and contact time with hand hygiene products was frequently below the recommended 15 s. Use of AHS was low despite its advantages over hand washing and availability in the majority of clinics. The poster campaign had a limited effect on its own, but could still be used as a component of a multimodal hand hygiene campaign. Improving the infection control culture in veterinary medicine would facilitate future campaigns and studies in this area.

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PENICILLIN, THE FIRST CHOICE FOR INFECTIONS IN HORSES IN SWEDEN

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Introduction: The presence of antimicrobial-resistant bacteria (ABR) in veterinary medicine might bring unpremeditated use of broad spectrum antimicrobials in infected animals. Current data of causative pathogens and their antibiogram then becomes important. Therefore equine surgical site infections (SSI) and traumatic wounds with symptoms of infection on admission to hospital (TW) were sampled for culture and antibiogram testing. **Material and methods:** During approximately one year each, between May 2009 and October 2011, three Swedish equine hospitals collected

samples of SSI and TW. Culture, conventional typing of the bacterial species, antibiogram testing (VetMIC) and when relevant, extended genotyping i.e. for MRSA were performed according to standard routine methods (National Veterinary Institute, Uppsala, Sweden). SSI were defined according to Centers for Disease Control and Prevention, USA (CDC) criteria, except for allowance to exceed the deadline of 30 days if there was a clear link to the surgery, i.e. funikulitis after castration. TW was defined as lesions caused by trauma having clinical symptoms of infection (CDC) on admission to hospital. **Results and discussion:** If antimicrobial treatment is considered necessary to clear an infection a narrow spectrum antimicrobial is most ideal. To guide veterinarians of first choice antimicrobial pending the culture report, current local data of causative pathogens and their antibiogram should be continuously collected. In this study 63 of 83 isolates (76%) from sampled wounds were susceptible to penicillin (Figure 1). Another common disease in horses in Sweden is respiratory infections, predominantly caused by virus and/or beta-haemolytic streptococci, the latter constantly susceptible to penicillin. **Conclusion:** Penicillin is still an adequate first choice in many infected horses in Sweden if antimicrobials are considered necessary. Figure 1: Bacterial species cultured from SSI (n=45) and IW (n=38) in three Swedish equine hospitals. * Penicillin susceptible except for one penicillinas producing *Actinobacillus*; NG/USP = no growth or unspecific growth; A = *Actinobacillus* spp; BHS = beta-haemolytic streptococci; CPS = coagulase positive staphylococci; EC = *E. coli*; O = other species

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