

# Is There a LA-MRSA Transmission from Hospital Rmplyees to Hospital Environment

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ASM CONFERENCES

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

# 4th ASM-ESCMID Conference on

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

November 2 – 5, 2015
Chicago, Illinois

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ASM CONFERENCES

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# Acknowledgments

The American Society for Microbiology and the European Society of Clinical Microbiology and Infectious Diseases gratefully acknowledge the following sponsor of the 4th 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 Atlantic Ballroom Foyer at the Radisson Blu Aqua Hotel during posted registration hours. Participants may collect name badges and program materials at the registration desk. A name badge is required for entry into all sessions and meals.

## GUEST REGISTRATION

Registered participants may also register an accompanying guest (age 16 and older) to attend the Welcome Reception for an additional fee of $40. 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.

## NETWORKING MEALS AND SOCIAL EVENTS

Registration includes attendance at the Welcome Reception on Monday evening, Networking Lunches on Tuesday and Wednesday. Ample time has also been scheduled for participants to network during coffee breaks.

## GENERAL SESSIONS

All general sessions will be held in the Atlantic Ballroom in the Radisson Blu Aqua Hotel.

## POSTER SESSIONS

Poster boards are located in the Atlantic Ballroom at the Radisson Blu Aqua Hotel.

Posters will be available for viewing informally throughout the conference, with official poster sessions scheduled on Tuesday and Wednesday.

All posters should be mounted on Tuesday morning, November 3, between 7:30 and 9:00 am, and should be removed by 12:30 pm, Thursday, November 5. Posters which are not removed in time will be discarded.

Odd-numbered posters (1,3,5…) will be officially presented in Session A on Tuesday, November 3, and even-numbered posters (2,4,6…) will be officially presented in Session B on Wednesday, November 4.

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

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## STUDENT TRAVEL GRANTS

ASM encourages the participation of graduate students and new postdocs at ASM Conferences. To support the cost of attending the conference, ASM has awarded travel grants of $500 to each of the following individuals:

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Min Tao Wan

# Scientific Program

## Monday, November 2, 2015

| | |
|---|---|
| 7:00 pm – 8:00 pm | **Opening Keynote Session** |
| 7:00 – 7:15 pm | Welcome Remarks |
| 7:15 – 8:00 pm | Keynote Lecture<br>***Maryn McKenna**, Independent Journalist and Author; Senior Fellow of the Schuster Institute for Investigative Journalism, Brandeis University* |
| 8:00 pm – 9:00 pm | **Welcome Reception** |

## Tuesday, November 3, 2015

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|---|---|
| 9:00 am – 12:15 pm | **Session 1: Epidemiology of LA–MRSA** |
| 9:00 – 9:45 am | Comparison of LA–MRSA in Europe and North America<br>***Michael David**, University of Chicago, Chicago, IL* |
| 9:45 – 10:00 am | *Staphylococcus aureus* Nasal Carriage is Associated with Symptoms of Skin and Soft Tissue Infection Among Industrial Hog Operation Workers in North Carolina<br>***Christopher Heaney**, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD* |
| 10:00 – 10:15 am | *Staphylococcus aureus* and MRSA Colonization and Infection in US Swine Veterinarians: An 18 Month Longitudinal Study<br>***Jisun Sun**, Univ. of Minnesota, Saint Paul, MN* |
| 10:15 – 10:30 am | Species and Staphylococcal Cassette Chromosome Mec (SCCmec) Diversity Among Methicillin–resistant Non–*Staphylococcus aureus* Staphylococci Isolated from Broilers Chickens<br>***Patrick Butaye**, Ross Univ. School of Veterinary Medicine and Ghent Univ., Basseterre, SAINT KITTS and NEVIS* |
| 10:30 am – 11:00 am | **Break** |

| | |
|---|---|
| 11:00 – 11:15 am | Risk Factors for Methicillin–resistant *Staphylococcus aureus* in Pig Herds in Ontario, Canada<br>***Mackenzie Slifierz**, Ontario Veterinary College, Univ. of Guelph, Guelph, ON, CANADA* |
| 11:15 – 11:30 am | MRSA Spread in the Intensive Farming Animals (i.e. Veal Calves, Swine and Dairy Cow) in North–Eastern Italy<br>***Michela Corro**, Istituto Zooprofilattico Sperimentale delle Venezie, Padova, ITALY* |
| 11:30 – 11:45 am | High Prevalence of *mecA*–positive Methicillin–resistant *Staphylococcus aureus* (MRSA) in European Hares (*Lepus europaeus*) Population on the German Island Pellworm<br>***Igor Loncaric**, Institute of Microbiology, Univ. of Veterinary Medicine, Vienna, AUSTRIA* |
| 11:45 – 12:00 pm | Recurrent *Staphylococcus aureus* t571/ST398 Infection in an Iowa Farmer<br>***Tara Smith**, Kent State Univ., Kent, OH* |
| 12:00 – 12:15 pm | Prevalence of Antibiotic–resistant *Staphylococcus aureus* Nasal Carriage Among Children Living with Industrial Hog Operation Workers<br>***Sarah Hatcher**, Univ. of North Carolina Gillings School of Global Public Health, Chapel Hill, NC* |
| 12:15 pm – 1:15 pm | **Lunch** |
| 1:15 pm – 2:15 pm | **Poster Session A** |
| 2:15 – 4:00 pm | **Session 2: Epidemiology of MRSP and MRSA in Companion Animals** |
| 2:15 – 3:00 pm | MRSA and MRSP in Companion Animal Practice: Where are the problems?<br>***Annette Loeffler**, Royal Veterinary College, Hatfield, UNITED KINGDOM* |
| 3:00 – 3:30 pm | Lessons from Pets and Environmental Transmission of Staphylococci (PETS): A Longitudinal Household Study Nested in a Randomized Clinical Trial of People with Methicillin–resistant *Staphylococcus aureus*<br>***Meghan Davis**, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD* |

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|---|---|
| 3:30 – 3:45 pm | Skin or Soft Tissue Infections in Companion Animals Residing with a Person Diagnosed with Community Methicillin–resistant *Staphylococcus aureus* Infection<br>***Dan Laucks**, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD* |
| 3:45 – 4:00 pm | Methicillin–resistant *Staphylococcus aureus* Isolated from Equine Patients at a Veterinary Teaching Hospital in Atlantic Canada<br>***Matthew Saab**, Atlantic Veterinary College – Univ. of Prince Edward Island, Charlottetown, PE, CANADA* |
| 4:00 – 4:30 pm | **Break** |
| 4:30 – 6:00 pm | **Round Table Discussion/Debate I: Do Antimicrobial Restriction Regulations Help Reduce MRSA on Farms?** |

## Wednesday, November 4, 2015

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|---|---|
| 9:00 am – 12:15 pm | **Session 3: Genomics and Molecular Epidemiology** |
| 9:00 – 9:30 am | Typing of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP)<br>***Kristina Kadlec**, Inst. of Farm Genetics, Friedrich–Loeffler–Institute, Neustadt–Mariensee, GERMANY* |
| 9:30 – 9:45 am | Changing Characteristics of LA–MRSA Isolated from Humans in the Netherlands. Emergence of a Subclade Transmitted without Livestock Exposure<br>***Thijs Bosch**, National Inst. For Public Health and the Environment (RIVM), Bilthoven, NETHERLANDS* |
| 9:45 – 10:00 am | Plasmid Location of the Multiresistance Gene *cfr* and its Integration into a SCC*mec* Cassette in MRSA from Pigs<br>***Stefan Schwarz**, Inst. of Farm Genetics, Friedrich–Loeffler–Institute, Neustadt–Mariensee, GERMANY* |
| 10:00 – 10:15 | Methicillin–resistant *Staphylococcus aureus* in the Same Household in the Context of Clinical Disease in a Person and a Dog<br>***Meghan Davis**, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD* |

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|---|---|
| 10:15 – 10:30 am | Microevolution of *S. pseudintermedius* Isolated from Infections of One Canine Patient at 25 Different Time Points Between 2008 and 2014<br>***Szilvia Vincze**, Inst. of Microbiology and Epizootics, Centre for Infection Medicine FU–Berlin, Berlin, GERMANY* |
| 10:30 – 11:00 am | **Break** |
| 11:00 – 11:45 am | Evolution of ST398 in Humans and Animals<br>***Anne-Catrin Uhlemann**, Columbia Univ., New York, NY* |
| 11:45 – 12:00 pm | Population Structure of MRSP and MSSP in Germany – A Genomic Approach<br>***Torsten Semmler**, Robert Koch Inst., Berlin, GERMANY* |
| 12:00 – 12:15 pm | Whole Genome Sequencing a Promising Additional Tool in Epidemiologic Investigations of Transmission of Methicillin–resistant *Staphylococcus pseudintermedius* (MRSP)<br>***Ulrika Windahl**, National Veterinary Inst., Uppsala, SWEDEN* |
| 12:15 – 1:15 pm | **Lunch** |
| 1:15 – 2:15 pm | **Poster Session B** |
| 2:15 – 4:45 pm | **Session 4: Treatment, Prevention and Control** |
| 2:15 – 2:45 pm | Search and destroy…does it still work for LA–MRSA?<br>***Robert Skov**, Statens Serum Inst., Copenhagen, DENMARK* |
| 2:45 – 3:15 pm | Control of LA–MRSA in Swine – is it Possible? Lessons Learned from Outbreaks and Eradication in Norway<br>***Carl Andreas Grontvedt**, Norwegian Veterinary Inst., Oslo, NORWAY* |
| 3:15 – 3:30 pm | Assessment of Methods to Quantify Livestock Associated MRSA in Pig Herds<br>***Julie Elvekjaer Hansen**, Technical Univ. of Denmark, Frederiksberg, DENMARK* |

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|---|---|
| 3:30 – 3:45 pm | Antibacterial Actions of Antimicrobial Peptides Derived from Different Animal Species Against Methicillin–resistant *Staphylococcus aureus* and the Effect of Bacterial Efflux Pumps<br>***Stefanie Blodkamp**, Univ. for Veterinary Medicine, Hannover, GERMANY* |
| 3:45 – 4:15 pm | **Break** |
| 4:15 – 4:30 pm | The Antibacterial Dressing, Acticoat, Becomes Bactericidal Against MRSA When Wetted with Surfactants and Antimicrobial Peptides<br>***Josh Ravensdale**, Curtin Univ., Perth, AUSTRALIA* |
| 4:30 – 4:45 pm | Mupirocin Susceptibility in Environmental Methicillin–resistant *Staphylococcus aureus* from Homes with and without Pets in the Context of a Mupirocin–based Randomized Clinical Trial<br>***Phillip Hahn**, Univ. of Florida, Gainesville, FL* |
| 4:45 – 6:00 pm | **Round Table Discussion/Debate II: Are MRSA and MRSP in Companion Animals Relevant Zoonotic Disease Threats?** |

## Thursday, November 5, 2015

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| 9:00 am – 12:00 pm | **Session 5: Diagnostic Testing and Epidemiology** |
| 9:00 – 9:45 am | MRSA Diagnostics: Advances, Controversies, and Conundrums<br>***Fred Tenover**, Cepheid, Sunnyvale, CA* |
| 9:45 – 10:00 am | Pig–2–Bac as a Biomarker of Occupational Exposure to Swine and to *Staphylococcus aureus* and Methicillin Resistance<br>***Nora Pisanic**, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD* |
| 10:00 – 10:15 am | Co–colonization Dynamics and Clonal Diversity of Methicillin–sensitive and Methicillin–resistant *Staphylococcus aureus* in Sows<br>***Alexandra Fetsch**, Federal Inst. for Risk Assessment, Berlin, GERMANY* |

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|---|---|
| 10:15 – 10:30 am | Characterisation of Methicillin Resistant and Susceptible *Staphylococcus* Infections in Australian Animals<br>***Kate Worthing**, Univ. of Sydney, Sydney, AUSTRALIA* |
| 10:30 – 11:00 am | **Break** |
| 11:00 – 11:15 am | Characterization of Methicillin–resistant *Staphylococcus aureus* (MRSA) from Primates, Personnel and Environment at a US Primate Center<br>***Marilyn Roberts**, Univ. of Washington, Seattle, WA* |
| 11:15 – 11:30 am | Temporal and Spatial Trends in the Distribution of Methicillin–resistant *Staphylococcus schleiferi* from Companion Animals in the Mid–Atlantic United States<br>***Shelley Rankin**, Univ. of Pennsylvania School of Veterinary Medicine, Philadelphia, PA* |
| 11:30 – 11:45 am | Novel Variants of Staphylococcus Intermedius–like Organisms Isolated from Black Bears in the Great Smoky Mountains National Park, Tennessee, USA<br>***David Bemis**, Univ. of Tennessee, Knoxville, TN* |
| 11:45 am – 12:00 pm | **Conference Wrap Up** |

# Speaker Abstracts

## S1:1

### COMPARISON OF LA-MRSA IN EUROPE AND NORTH AMERICA

***M. Z. David;***
*The University of Chicago, Chicago, IL.*

## S1:2

### STAPHYLOCOCCUS AUREUS NASAL CARRIAGE IS ASSOCIATED WITH SYMPTOMS OF SKIN AND SOFT TISSUE INFECTION AMONG INDUSTRIAL HOG OPERATION WORKERS IN NORTH CAROLINA

*M. Nadimpalli[1], J. R. Stewart[1], N. Pisanic[2], D. C. Love[3], D. Hall[4], J. Larsen[5], A. Krosche[6], T. Tekle[7], T. Ross[7], K. Carroll[7], T. M. Perl[7],* ***C. D. Heaney[2];***
*[1]Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, [3]Center for a Livable Future, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, [4]Rural Empowerment Association for Community Help, Warsaw, NC, [5]Statens Serum Institut, Copenhagen, DENMARK, [6]Johns Hopkins University, Baltimore, MD, [7]Johns Hopkins Hospital, Baltimore, MD.*

Since the early 2000s, antibiotic-resistant strains of Staphylococcus aureus, including methicillin- (MRSA) and multidrug- (MDRSA) resistant S. aureus, have been detected in the noses of individuals exposed to livestock. It remains unclear whether carriage of these livestock-associated strains is associated with disease. Here we examine whether S. aureus nasal carriage is associated with symptoms of skin and soft tissue infection (SSTI) at the baseline visit of a cohort of industrial hog operation (IHO) workers and household members in North Carolina. Baseline data were collected from IHO workers and their household contacts via questionnaire to assess risk factors for S. aureus nasal carriage and symptoms of SSTI in the prior three months. All participants also provided a nasal swab that was analyzed for S. aureus, including presence of mecA (indicating MRSA), resistance to ≥3 classes of antibiotics (indicating MDRSA), and absence of scn (indicating livestock association). Six of 103 (6%) IHO workers, 6 of 54 (11%) minor household members and none of 26 (0%) adult household members reported recent symptoms of SSTI. Overall, participants who carried S. aureus (PR: 4.5, 95% CI: 1.4, 14.9) or MDRSA (PR: 3.1, 95% CI: 1.2, 7.8) at baseline were more likely to report SSTI compared to those who did not have these bacteria in their noses. Among workers, MDRSA (PR: 6.3, 95% CI: 1.5, 38.4) and scn-negative S. aureus nasal carriers (PR: 5.1, 95% CI: 1.2, 22.2) were more likely to report SSTI. Prevalence of MDRSA (PR: 3.9, 95% CI: 1.0, 15.2) and scn-negative S. aureus (PR: 5.0, 95% CI: 1.2, 21.4) was elevated among those who reported never vs ever wearing a face mask at work. Among the 37 of 100 IHO workers who always wore a face mask during work, none reported SSTI. This study suggests that S. aureus nasal carriage may be associated with increased prevalence of symptoms of S. aureus infection and that infrequent mask usage may increase IHO workers' exposure to antibiotic-resistant S. aureus.

## S1:3

### STAPHYLOCOCCUS AUREUS AND MRSA COLONIZATION AND INFECTION IN US SWINE VETERINARIANS: AN 18 MONTH LONGITUDINAL STUDY

***J. Sun****, M. Yang, P. Davies;*
*University of Minnesota, Saint Paul, MN.*

Although high risk of exposure to MRSA is known for people working with pigs, the nature of nasal culture positivity for MRSA and

its health implications are poorly understood. A cohort of swine veterinarians was recruited for an 18 month study to describe patterns of S.aureus (SA) colonization and infection. The veterinarians, resident in 15 US states, were given instructions for self-collection of nasal swabs by video. From July 2012, they were contacted monthly to collect a nasal swab and complete an on-line survey to record recent pig exposure, events of physical injury focusing on skin wounds, and selected health events (occurrence of skin or soft tissue infections, or confirmed staphylococcal infections). A compliance rate of >98% was achieved for swabs and surveys from 66 participants. The prevalence of SA (64% overall) and MRSA (9%) varied monthly from 58 to 82%, and from 6 to15%, respectively. The frequency of culture positivity for individual veterinarians varied from 0% (1 vet) to 100% of sampling events. The likelihood of positivity did not vary with the interval from sample collection to processing, but decreased significantly with the interval between sampling and last pig contact. There was no suggestion of seasonality or an increase in prevalence over time. Predominant spa types were t034 (ST398, 50%), t002 (ST5, 25%) and t337 (ST9, 18%), which were also the most common spa types found in a concurrent study in pigs. Cocolonization with two spa types was observed in 71 positive samples. Based on detection patterns, veterinarians were classified into three groups: Persistent carriers (PC, 24), Intermittent carriers (IC, 41) and Non-carriers (NC, 1). One-time quantitative testing of nasal swabs without enrichment indicated that PC veterinarians carried significantly higher numbers of SA than IC. The same spa type was detected at all sampling events in 9 (14%) veterinarians. There was some evidence suggesting that ST9 variants were less likely to be persistently carried than ST398 and ST5 variants. No confirmed cases of S. aureus infection were reported. The elevated prevalence of SA and MRSA in US veterinarians relative to the general population appears to be a consequence of acquisition of SA variants from pigs during occupational exposure, however MRSA prevalence (9%) was much lower than a similar study in Holland (44%). Repeated occupational exposure did not lead to prolonged colonization in most subjects, and the higher numbers of SA in PC subjects suggests that host factors strongly influence the likelihood of prolonged colonization by SA of livestock origin. Although the period of follow up was limited, the absence of clinical infections, consistent with the sparsity of published reports of serious clinical SA infections in swine workers, suggests that major health concerns for healthy workers due to livestock associated SA are uncommon.

## S1:4

### SPECIES AND STAPHYLOCOCCAL CASSETTE CHROMOSOME MEC (SCCMEC) DIVERSITY AMONG METHICILLIN-RESISTANT NON-STAPHYLOCOCCUS AUREUS STAPHYLOCOCCI ISOLATED FROM BROILERS CHICKENS

*S. Bousiaali[1], W. Vanderhaeghen[2], M. Argudín[3],* ***P. R. Butaye[4];***
*[1]Veterinary and Agrochemical Research centre, Brussels, BELGIUM, [2]Ghent University, Merelbeke, BELGIUM, [3]Université Libre de Bruxelles, Brussels, BELGIUM, [4]Ross University School of Veterinary Medicine and Ghent University, Basseterre, SAINT KITTS AND NEVIS.*

In Belgium poultry, methicillin resistant Staphylococcus aureus (MRSA) and methicillin-resistant S. sciuri (MRSS) prevalence (5% and 13%, respectively) is low compared to pigs and veal calves1, 2. Information on other methicillin-resistant non-S. aureus staphylococci (MRNAS) is scarce. Therefor we assessed the carriage and species distribution of MRNAS on healthy broilers farms in Belgium. A total of 200 nasal swabs (10 broiler farms, 20 animals per farm) were collected as previously described2. 16SrRNA-mecA-nuc triplex PCR identified MRNAS (mecA and

16SrRNA positive). Identification at species level was done by the tRNA intergenic spacer PCR. SCCmec type was determined and susceptibility was tested by a microbroth-dilution method using epidemiological cut-off values (Eucast). In total 62 MRNAS were recovered from 51 animals (26%) belonged to 9 farms. They were identified as S. carnosus (2), S. cohnii (6), S. epidermidis (9), S. haemolyticus (9), S. lentus (11) and S. sciuri (25). Most MRNAS isolates were non-wild type (NWT) for clindamycin (76%), synercid, trimethoprim (both 68%), erythromycin, tiamulin (both 60%), fusidic acid (58%) and tetracycline (48%). Few MRNAS isolates were NWT for sulfamethoxazole (19%), ciprofloxacin, mupirocin (both 18%), linezolid (5%), and rifampicin (2%). All MRNAS isolates were wild type (WT) for kanamycin, gentamicin, streptomycin, chloramphenicol and vancomycin. Most isolates (2 S. cohnii, 6 S. lentus, 23 S. sciuri and 2 S. carnosus) carried SCCmec III(3A). The remaining S. sciuri isolates (2) carried the SCCmec III variant•3A&5 (SCCmec III with SCCHg). Most S. epidermidis isolates carried SCCmec IV variants: IV(2B&5) (4), IVa(2B) (2) and IVc(2B) (1). The remaining isolates carried non-typeable (NT) cassettes. Most of these NT variants contained known ccr and mec complex types in new arrangements: NT(2B&4) (1 S. lentus, 1 S. epidermidis), NT(5C&2) (1 S. lentus), NT(5A) (8 S. haemolyticus, 1 S. cohnii). The remaining isolates (3 S. cohnii, 3 S. lentus, 1 S. epidermidis, 1 S. haemolyticus) carried a NT variant with the mec complex A but negative for all the ccr complex types investigated. A total of 16 MRNAS isolates were WT for cefoxitin, but they carried the mecA gene associated to different cassettes [SCCmec III (9), NT(5C&2) (1), NT (-A) (5), NT(5A) (1)]. MRNAS were detected on most broilers farms and almost one quarter of the sampled broilers was positive. Among six different MRNAS species, MRSS predominated. Few isolates carried SCCmec variants associated with livestock associated MRSA. Most MRNAS carried possible new variants that should be further studied to elucidate the mechanisms explaining the presence and spread of SCCmec cassettes in animal staphylococci. 1Nemeghaire et al. Avian Pathol. 2013, 42:342-6. 2Nemeghaire et al. J Antimicrob Chemother 2014, 69:2928-34. Acks: MAA was supported by Fundación Alfonso Martín Escudero.

## S1:5

### RISK FACTORS FOR METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PIG HERDS IN ONTARIO, CANADA

***M. J. Slifierz**, R. Friendship, S. Weese; University of Guelph, Guelph, ON, CANADA.*

Methicillin-resistant Staphylococcus aureus (MRSA) carriage by swine has emerged as a public health concern but our understanding of risk factors for MRSA carriage among nursery herds is lacking. The purpose of this study was to identify risk factors associated with MRSA carriage in commercial nursery herds. A prospective cohort study of 390 pigs from 26 farms was conducted in southern Ontario. Pigs were screened for MRSA by enrichment culturing of nasal swabs and farm-level parameters surveyed. Resistance genes were identified by PCR and susceptibility determined by microbroth and agar dilutions. Six (27.3%) cohorts carried MRSA just prior to weaning and 8 (36.4%) were positive 3-weeks later. The MRSA-positive nursery cohorts were more likely to receive zinc therapy (P=0.022), routinely disinfect the nursery pens for every batch (P=0.022), and have a higher stocking density (P=0.048). Multivariate analysis revealed that pigs were more likely to carry MRSA when consuming a high zinc diet (OR=12.4; 95% CI: 3.04-50.25; P<0.001) and when raised in a system that routinely disinfects the nursery for each incoming batch (OR=14.12; 95% CI, 4.36-45.77; P<0.001). The use of antibiotics was not associated with MRSA carriage. Molecular analysis of MRSA isolates revealed that 90% (36/40) of isolates were phenotypically resistant to zinc

and 62.5% (25/40) carried the zinc resistance gene czrC. All isolates carried at least 1 gene associated with resistance to quaternary ammonium compounds (QACs) and 75% of isolates carried ≥2 QAC-resistance genes. The most common genotype was gacG-qacH-smr. The isolates in this study belonged to spa types t034 (57.5%), t571 (37.5%), t3075 (2.5%), and t002 (2.5%). All isolates were negative for Panton-Valentine leukocidin and scn. The results of this study indicate that resistance determinants for zinc and QACs are widespread among MRSA of porcine origin and many of these resistance determinants are co-located with other antibiotic-resistance genes. Coupled with the finding that MRSA is associated with a high zinc diet and disinfection practices, it is possible that zinc and QAC-based disinfectants may be co-selecting for antibiotic resistance genes. In conclusion, these findings raise the concern that zinc and QAC-based disinfectant usage on farms may be contributing to the emergence and persistence of MRSA within swine herds.

## S1:6

### MRSA SPREAD IN THE INTENSIVE FARMING ANIMALS (I.E. VEAL CALVES, SWINE AND DAIRY COW) IN NORTH-EASTERN ITALY

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The presence of methicillin resistant *Staphylococcus aureus* (MRSA) in farm animals has been reported since 1970's. During the last years in some countries of northern Europe the reports have described a concerning situation with high MRSA prevalence especially in swine and veal calves farming. The aim of this study was to evaluate the prevalence of MRSA in farm animals (dairy cows, veal calves and swine) of the North-Eastern Italy with intensive farming situations, and to investigate their genetic characteristics. In this study we sampled 48 veal calves farms, 58 swine farms and 219 dairy cows farms. In all the farms a nasal swab was collected from approximately 30 animals; in dairy cows composite milk samples and bulk tank milk sample were also collected. Questionnaires on farm management and use of antibiotics were administered to livestock farmers. The samples were pre-enriched in NaCl 6.5% Muller-Hinton broth for 18-24 hour and then plated in a MRSA selective plate. Suspected colonies were identified with a multiplex PCR targeting *nuc* gene (for *S. aureus* species identification) and *mec*A gene (to detect methicillin resistance). Genetic typing (*spa*-typing, and multilocus sequence type), detection of some antimicrobial resistance genes and Panton-Valentine Leucocidin gene and slime-forming attitude were performed. The MRSA prevalence varied greatly among species, 66.7% in veal calves farms vs 62.0% in swine and 3.2% in dairy cows. The most frequent strain was CC398 in all the three animal categories, less represented were CC97 and CC1. A new codified ST 3071, closely related to ST 398 was discovered in a veal calves farm suggesting continuous modifications in the predominant ST. 15 different *spa*-types were present, with t899, t034 and t011 as the most frequent ones. High prevalence of genes encoding resistance to Macrolides and Tetracycline were found in MRSA isolates, but no strain was positive for Vancomycin resistance genes nor PVL gene. The isolates slime-forming attitude varied between the species: low prevalence in swine and veal calves (respectively 4.7% and 1.6%) and higher prevalence in dairy cows (29%). The results obtained in this study may reflect differences in the antibiotic usage in intensive farming. Veal calves and swine are routinely treated at the introduction in the farm or when regrouping occur as a preventive measure with oral administration of antibiotics to the whole group, while this doesn't happen in dairy cows. Questionnaires reported use of

multiple antimicrobial classes per productive cycle in veal calves and swine (respectively 3.56 e 2.69) while in dairy cows the number was lower (1.2). These results confirm the high prevalence of MRSA in intensive farming animals (i.e. swine and veal calves) in the North-Eastern Italy and the need of a continuous monitoring and new policies on antimicrobial use in order to prevent a wider spread of this emerging pathogen.

## S1:7

### HIGH PREVALENCE OF MECC - POSITIVE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN EUROPEAN HARES (*LEPUS EUROPAEUS*) POPULATION ON THE GERMAN ISLAND PELLWORM

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**Background:** In 2011/2012, *mecC*- positive methicillin-resistant (MR) *Staphylococcus* (*S.*) *aureus* (MRSA) were isolated for the first time from abscesses and lungs of European hares (*Lepus europaeus*) on the German island Pellworm. In 2013, *mecA* and *mecC*- positive MRSA were isolated from livestock sharing their habitat with hares. The objectives of the present study were: i) to assess the prevalence of MR staphylococci (MRS) in the hare population and livestock, and ii) to understand/track transmission of MRS between hares and livestock on Pellworm. **Materials and Methods:** During the hunting seasons in 2013 (n=39) and 2014 (n=41), 80 hares were sampled. Small intestine and nasal swabs from all hares and in some cases feces were examined. In spring 2015, 30 nasal swabs from livestock and one hare found dead were included in the study. MRS from animals were characterized by *MecA/mecA1/mecC* PCRs, SCCmec typing and DNA microarray analysis. MRSA isolates were genotyped by spa typing, MLST and MLVA, and were further phenotypically biotyped and investigated for capsular polysaccharide (CP) expression using FTIR spectroscopy. Species assignment of methicillin-resistant coagulase-negative staphylococci (MRCoNS) was conducted by *rpoB* sequencing. Susceptibility testing was performed by agar disk diffusion and broth microdilution. **Results:** In 2013, 3/39 hares were MRSA-positive. Two *mecC*-positive isolates were tNT (non-typeable)-ST2944-XI-CP8 while the single *mecA*-positive isolate was t011-ST398-V-CP5. MR *S. haemolyticus* was found in one hare. MRSA isolates were present in 20/41 hares examined in 2014. Solely nasal colonization with MRSA was observed in seven, a combination of nasal/intestinal colonization in six, intestinal/fecal colonization in two and solely intestinal colonization in five hares, respectively. Among these MRSA isolates, only one MRSA was *mecA*-positive and belonged to t011-ST398-V-CP5. The remaining *mecC*-positive MRSA isolates displayed two MLST types (ST130, ST2944), two spa types (t843, t4335), four MLVA types, and five FTIR biotypes (CP5, CP8 and NT). In 2015, MRSA was detected in eight livestock examined, belonging to t011-ST398-V-CP5 (n=3) or t4335-ST2944-XI-CP8 (n=5). Two *mecC*- positive t4335-ST2944-XI-NT MRSA isolates from abscesses of a hare found dead in spring 2015 belonged to

two different FTIR biotypes. **Conclusion:** We observed a strong increase in the prevalence of *mecC*-positive MRSA in the hare population. Multi-drug MRCoNS remains a sporadic observation. Our typing data strongly suggest transmission of different MRSA types between wildlife and livestock animals. More systematic studies on hares as potential reservoirs for MRSA and the role of MRSA as a pathogen for hares are required.

## S1:8

### RECURRENT STAPHYLOCOCCUS AUREUS T571/ST398 INFECTION IN AN IOWA FARMER

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*Staphylococcus aureus* strain ST398 has emerged in the past decade, largely in individuals with contact to swine or other livestock. While colonization with ST398 seems to be common in livestock workers, infections are less frequently documented. We report a repeated infection in an Iowa farmer in contact with swine and cattle. Molecular analyses of isolates collected from this individual over a period of 13 months demonstrated matching *spa* types and antibiotic susceptibility profiles of the infection isolates with samples taken from the individual's nose and throat, as well as a colonization isolate from his spouse, at enrollment in July 2011. An incident infection isolate provided in November 2011 showed identical characteristics. A third infection swab from August 2012 showed isolates with two different susceptibility patterns: one identical to previous isolates, while another demonstrated borderline oxacillin resistance. Whole-genome sequence analyses confirmed that the isolates from this case were from the livestock-associated ST398 lineage (CC398-IIa) and that the same strain was present over the course of the study. It is unclear whether the farmer's recurrences arose from repeated acquisitions from exposure to livestock or the result of long-term colonization. This case demonstrates the ability of livestock-associated *S. aureus* ST398 in the US to cause repeated skin and soft tissue infections in a manner similar to community-associated strains, and the necessity to screen for methicillin-sensitive as well as resistant strains when studying the spread of these organisms.

## S1:9

### PREVALENCE OF ANTIBIOTIC-RESISTANT STAPHYLOCOCCUS AUREUS NASAL CARRIAGE AMONG CHILDREN LIVING WITH INDUSTRIAL HOG OPERATION WORKERS

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Antibiotic use in industrial hog operations (IHOs) supports the emergence of antibiotic-resistant (ABR) *Staphylococcus aureus* and may lead to ABR *S. aureus* exposure among IHO workers. It remains unclear whether adult household member occupation at an IHO is associated with ABR *S. aureus* carriage in children. We investigated the association between adult occupation in an IHO and carriage of ABR *S. aureus* in their household members under seven years of age. One hundred and ninety-eight IHO worker-child household pairs and 202 non-occupationally exposed community referent (CR) adult-child household pairs were recruited from the top ten hog-producing counties in North Carolina. Adults and children provided nasal swabs and completed a questionnaire. Nasal swabs were assessed for the presence of *S. aureus* and methicillin-re-

sistant *S. aureus* (MRSA) using culture-based and molecular methods. Multidrug-resistant *S. aureus* (MDRSA; non-susceptibility to ≥3 antibiotic classes) was confirmed by Kirby-Bauer disk diffusion. Confirmed *S. aureus* isolates were genetically typed using Staphylococcal protein A (*spa*)-typing and assigned to clonal complexes (CCs) 398, 9, or neither based on the existing scientific literature. Lack of *scn*, a putative indicator of livestock adaptation in *S. aureus*, was measured using a multiplex polymerase chain reaction (PCR) assay. We then examined the association between occupational exposure to IHOs and *S. aureus* nasal carriage prevalence in adults and children using log binomial regression. The crude prevalence of *S. aureus*, MRSA, and MDRSA among children living in IHO households was 1.6 (95% Confidence Interval [CI]: 1.2, 2.1), 2.4 (95% CI: 1.3, 4.6), and 2.7 (95% CI: 1.6, 4.6) times that of children living in CR households, respectively. In addition, the crude prevalence of *scn*-negative *S. aureus*, and CC9 *S. aureus* was greater among IHO workers compared to CR adults (*scn*-negative *S. aureus* PR: 5.1, 95% CI: 2.0,13.1; CC9 *S. aureus* PR: 6.1, 95% CI: 1.4, 27.0). These results suggest that children living in IHO worker households may have a greater risk of exposure to *S. aureus*, including MRSA and MDRSA, compared to children living in households with no occupational exposure to IHOs. Additional molecular characterization may better elucidate the source of *S. aureus* carriage in this population and additional research is necessary to evaluate infection and potential health risks.

## S2:1

### MRSA AND MRSP IN COMPANION ANIMAL PRACTICE: WHERE ARE THE PROBLEMS?

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The clinical presentation
MRSA, MRSP and other MRS infections continue to present clinical challenges to small animal veterinarians worldwide due to their reduced antimicrobial susceptibility. However, as for other staphylococcal infections, most MRS infections involve the skin, ears and wounds and the prognosis for resolution of infection is typically good provided underlying causes can be addressed successfully at the same time. Topical antibacterial therapy can be highly effective for superficial infection and overall mortality remains low.
Prevalence and geographical distribution
MRSA infection and carriage in pets has remained infrequent over the past 15 years, generally at less than 10% of studied populations, with the highest occurrence reported from countries with high levels of human hospital-associated MRSA. In contrast, the first reports on MRSP already indicated a prevalence of 15-30% amongst S. pseudintermedius clinical submissions found in North America and Europe. Since then, reports have continued to emerge from different countries including Asian, Australian, African and South American areas. Figures for MRSP rates amongst infection isolates have reached more than 50% in some countries, carriage rates amongst healthy dogs remain low.
The zoonotic implications
Many studies over the past 20 years have presented good, although indirect, evidence supporting that methicillin-susceptible staphylococci and MRS can be transmitted between humans and pets in both directions. According to the WHO website on zoonoses (http://www.who.int/zoonoses/en/, October 2015), "any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa is classified as a zoonosis". Both MRSA and MRSP can therefore be considered zoonotic but main direction and emphasis vary with the primary host. It is widely accepted that for MRSA, human-to-animal is likely to be the most frequent direction of transmission based on the predominance of human-healthcare associated lineages found in canine infection and carriage. This is compatible with the low prevalence of MRSA infections amongst pets and the lack of epidemic spread amongst

pets over the past 15 years. For MRSP, a mirror image epidemiology is likely with human infection cases still remaining rare despite its successful spread amongst animals. While risk factors for zoonotic transmission have not been reported yet, MRSP exposure and immune status of the human host will play a role.

The future

MRS are no longer the only multidrug-resistant pathogens of concern in small animal practice emphasizing that control strategies need to be improved urgently, including hygiene and antimicrobial stewardship. For MRS in particular though their zoonotic potential combined with the ease of transmission between hosts and their recently demonstrated ability to evolve on and to hosts remain of great concern.

## S2:2

### LESSONS FROM PETS AND ENVIRONMENTAL TRANSMISSION OF STAPHYLOCOCCI (PETS): A LONGITUDINAL HOUSEHOLD STUDY NESTED IN A RANDOMIZED CLINICAL TRIAL OF PEOPLE WITH METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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**Background:** Households are increasingly recognized as drivers of community-onset methicillin-resistant Staphylococcus aureus (MRSA) infections; however, less is understood about the role that pets or contaminated homes play in the context of intervention strategies. **Methods:** Index patients recently treated for MRSA skin or soft-tissue infection (SSTI) and their household members (HHM) were enrolled in a randomized clinical trial to test household mupirocin/chlorhexidine decolonization. These homes were eligible for the PETS study to evaluate home surfaces and pets over time; home visits were made at enrollment (before randomization) and three months later (after decolonization). Humans provided samples for MRSA culture every two weeks. Samples from eight surface sites and all pets collected at each home visit were evaluated for MRSA, S. pseudintermedius, and other staphylococci using broth-enrichment culture with PCR confirmation. Logistic regression modeling was used to evaluate household risk factors for human MRSA colonization (index & HHM) and pet health outcomes cross-sectionally and longitudinally. In a subset of homes, microbiome analysis of pets (nares, mouth) and people (nares, skin) was conducted. **Results:** MRSA prevalence rates at enrollment were: 34% of 88 index patients and 26% of 301 household members; 68% of homes were MRSA contaminated and 10% of homes had at least one MRSA-positive pet. MRSA prevalence rates at three months were: 31% of 55 index patients and 18% of 238 household members; 49% of homes were MRSA contaminated and 9% of homes had at least one MRSA-positive pet. MRSA-positive homes and MRSA-positive pets were associated with increased odds of MRSA colonization in people at enrollment and longitudinally. Pets carrying S. pseudintermedius were present in 19% of homes; this was associated with protection against human MRSA colonization at enrollment and longitudinally. S. pseudintermedius was the most common pathogen associated with observed SSTIs in pets. Having two or more pets at the three-month visit, controlling for Staphylococcus carriage status of the pet and other risk factors, conferred a protective effect against human MRSA colonization. Pets were found to carry diverse microbial communities and to enhance microbial sharing among human HHM. **Conclusions:** MRSA-contaminated homes and MRSA-positive pets contribute to human

MRSA colonization. MRSA-negative pets and S. pseudintermedius positive pets may offer protection against human MRSA colonization, perhaps via microbial competition or contributions to microbial diversity.

## S2:3

### SKIN OR SOFT TISSUE INFECTIONS IN COMPANION ANIMALS RESIDING WITH A PERSON DIAGNOSED WITH COMMUNITY METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS INFECTION

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**Background:** Human infections caused by community-onset methicillin-resistant Staphylococcus aureus (MRSA) have been on the rise. Numerous studies have described household pets as potential MRSA reservoirs; fewer studies have evaluated the animal health impacts from pet exposure to MRSA infected people in the home. **Goals:** 1) To examine risk factors for prevalent and incident dermatologic disease in pets residing in households with a person diagnosed with MRSA infection; 2) To compare the rates, anatomic site carriage and Staphylococcus species between pets with and without evidence of skin or soft tissue infection (SSTI). **Population:** One hundred eighty two (182) companion animals, predominantly dogs and cats, residing in households with a person recently diagnosed with MRSA infection. **Methods:** Owners were queried regarding individual pet and household factors at enrollment and at a second home visit three months later. All pets except fish were examined for SSTI's, and the nares, mouth, inguinum, perineum and lesional sites were cultured to isolate S. aureus (MRSA/MSSA), S. pseudintermedius (MRSP/MSSP), and S. schleiferi. Risk factors were evaluated using logistic regression modeling. Results: At the enrollment visit, 11/168 (6.5%) pets exhibited SSTIs, of which six were dogs with MSSP-positive lesions. At the three-month visit, 7/112 (6.3%) exhibited SSTIs, of which six were dogs with MSSP-positive lesions. The incidence rate was 1.4 lesions per 100 pets per month over the three-month period. Potential allergens in the household, specifically mold (adjusted OR 8.95, p=0.01) and fleas (aOR 7.36, p=0.02), were significantly associated with the presence of SSTIs. An association with feeding a raw meat diet was also observed (aOR 49.6, p=0.002); however the exposed population was small. Dogs were more likely than other pets to carry MSSP (aOR 62, p<0.001) with carriage typically at the mouth or perineum; no differences were noted between pets with and without SSTIs, controlling for pet species. **Conclusions:** Dogs were most likely to develop SSTIs. Despite exposure to a MRSA-infected person, many SSTIs were associated with the veterinary pathogen MSSP. Risk factors for SSTIs among pets recently exposed to a MRSA-infected person were similar to those reported for a veterinary care-seeking population. A potential association with feeding a raw meat diet needs to be further evaluated in future studies.

## S2:4

### METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM EQUINE PATIENTS AT A VETERINARY TEACHING HOSPITAL IN ATLANTIC CANADA

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Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as an important pathogen in Canadian horses over the last 10 years. In an Ontario study, 2.7% of horses at admis-

sion had MRSA detected in a nasal swab. It has been reported that CMRSA-5 (USA500) is the predominate clone in North American horses. Prior to the current study at this tertiary hospital in Atlantic Canada, MRSA was isolated from a hospitalized equine patient with catheter-related thrombophlebitis. This isolate was spa type t011 associated with ST398, a livestock-associated MRSA strain common in European horses. Previous to this case, studies from this region and this hospital have not detected MRSA in healthy or hospitalized horses. It was hypothesized that MRSA would be detected in equine nasal swabs at a veterinary teaching hospital (VTH) in Atlantic Canada. The objective of this study was to estimate the prevalence of MRSA in equine patients at a VTH in Atlantic Canada upon admission and at discharge. Nasal swabs from hospitalized horses were collected at admission and discharge. Swabs were placed in enrichment broth and incubated for 24 h at 35°C. Ten microliters of broth were plated on mannitol salt agar and incubated for 48 h at 35ºC. Suspect colonies were cultured to Columbia agar with 5% sheep blood for further identification. Methicillin-resistance was detected using the disk diffusion test for cefoxitin or oxacillin as appropriate, and confirmed using a latex agglutination to detect penicillin-binding protein 2a. Isolates were frozen in a broth with 15% glycerol for future molecular work. To date, 371 samples were collected from 319 patients between July 1, 2014 and July 31, 2015. Admission swabs were collected from 299 patients, and discharge samples were collected from 72 patients. Methicillin-susceptible S. aureus was detected in 36 (12.0%) samples at admission. MRSA was detected in 6 (1.6%) samples and 1.9% patients tested. Admission and discharge samples were collected for three MRSA positive patients, and MRSA was only detected in the discharge sample. Two of the remaining three MRSA patients had one sample collected at least two days into hospitalization, while the remaining patient's sample was taken at an unknown point during a nine day hospitalization. This is the first report of MRSA in horses in Atlantic Canada. The prevalence (1.9% of horses) indicates that MRSA is uncommon in hospitalized horses in this region. For three patients with MRSA, it can be suggested that the horses likely became exposed in the hospital environment. It is difficult to infer whether the remaining three MRSA positive patients were colonized upon admission or if they acquired MRSA during hospitalization. Molecular analysis of all isolates will be completed and will provide insight to the distribution of clones in Atlantic Canadian horses. Future work will include analysis of epidemiologic factors associated with MRSA in these horses.

## S3:1

### TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* (MRSP)

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## S3:2

### CHANGING CHARACTERISTICS OF LA-MRSA ISOLATED FROM HUMANS IN THE NETHERLANDS. EMERGENCE OF A SUBCLADE TRANSMITTED WITHOUT LIVESTOCK EXPOSURE

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**Introduction:** Since its first detection in 2003, livestock-associated MRSA (LA-MRSA) has become the predominant MRSA clade isolated from humans in the Netherlands since 2007. We characterized all submitted human LA-MRSA isolates to assess possible temporal changes. **Methods:** Over 9,000 LA-MRSA isolates submitted from 2003 to 2014 for the

Dutch MRSA surveillance were characterized by multiple-locus variable number of tandem repeat analysis (MLVA) and *spa*-typing. The MLVA also includes the detection of the *mecA* and *mecC* genes and of the *lukF* gene, indicative of Panton Valentine leucocidin (PVL) production. Furthermore, next generation sequencing (NGS) was performed on 118 isolates and the presence of bacteriophage φ3 was determined for 1,538 isolates. Nearly 6,000 questionnaires provided epidemiological data. **Results:** Over 80% of the Dutch LA-MRSA isolates belonged to one of three predominant MLVA/*spa*-types; MT398/t011, MT572/t108 and MT569/t034. After an initial rapid increase with a peak in 2009 ($n$ = 1,368), the total number of submitted LA-MRSA isolates has been slowly decreasing to 968 in 2014. However, the number of MT569/t034 isolates increased since 2008, surpassing MT572/t108 as the second most frequently found LA-MRSA in 2014. NGS showed that MT569/t034 isolates were genetically more diverse than MT398/t011 and MT572/t108. PVL positive LA-MRSA were only found in 23 isolates (0.3%) with 14 different MLVA/*spa*-types. The overall prevalence of φ3 among LA-MRSA was 2.2%, with the highest prevalence among MT569/t034 isolates (6.6%). Concurrent with the decrease in LA-MRSA in the Netherlands, fewer people reported having contact with livestock and this was most prominent for people from whom MT569/t034 LA-MRSA was isolated. The proportion of LA-MRSA isolated from infection-related materials increased from 6.3% in 2009, to 13% in 2014 and most of these isolates originated from patients older than 50 years of age. Remarkably, 83% of these patients reported not having contact with livestock. **Conclusion:** There is an ongoing change in the genotypic and epidemiological characteristics of Dutch LA-MRSA isolated from humans with the emergence of a LA-MRSA subclade independent of livestock exposure. This suggests LA-MRSA starts to resemble non-LA-MRSA in terms of transmissibility and pathogenicity.

## S3:3

### PLASMID LOCATION OF THE MULTIRESISTANCE GENE *CFR* AND ITS INTEGRATION INTO A SCC*MEC* CASSETTE IN MRSA FROM PIGS

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**Objectives:** The gene *cfr* confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A and reduced susceptibility to certain macrolides, such as josamycin and spiramycin. Although *cfr*-carrying methicillin-resistant *Staphylococcus aureus* (MRSA) have been reported in human medicine, *cfr*-carrying livestock-associated (LA-) MRSA of animal origin have been detected rarely. The aim of this study was to investigate MRSA isolates from pigs for the presence and the location of the *cfr* gene. **Material and Methods:** A total of 231 porcine MRSA were obtained from pigs at farms and slaughterhouses or from an animal hospital in four different regions in China. The *cfr*-carrying isolates were subjected to SCC*mec* typing, MLST, *spa* and *dru* typing, and SmaI PFGE. Moreover, all *cfr*-positive isolates were tested for their susceptibility to antimicrobial agents and the presence of resistance genes. The *cfr* genes and their flanking regions were sequenced. **Results:** The multiresistance gene *cfr* was found in 8/231 porcine MRSA isolates. They were characterized by MLST, *spa*, *dru* and SCC*mec* typing as ST627-t002-dt12w-IVb, ST6-t304-dt12w-IVb, ST9-t899-dt12w-IVb, ST9-t899-dt12ae-IVb, or ST63-t899-dt12v-IVb. All eight MRSA isolates were multiresistant and carried the resistance genes *fexA* and *mecA* while the genes *tet*(L), *vga*(A)v, *aadE*, *spw*, *lsa*(E), *lnu*(B), *erm*(33), and *spc* were seen in 1-6 isolates. S1-nuclease PFGE and

Southern blot analysis showed that the gene *cfr* was located on ~35-kb plasmids in 7 isolates. Plasmid pSS-02, carrying the intact insertion sequence IS*21-558* immediately upstream of *cfr*, was detected in 6 isolates whereas a pSCFS7-like plasmid, in which the *cfr* gene was preceded by a largely truncated IS*21-558* element, was found in a single isolate. The remaining isolate carried the *cfr* gene in its chromosomal DNA. Analysis of a 26,737-bp contig identified the *cfr* gene flanked by a copy of IS*Enfa4* and a copy of IS*256*, both located in the same orientation. This IS*Enfa4-cfr*-IS*256* segment was inserted into the *hp1* gene located in the joining (J) region 1 of a type IVb SCC*mec* element. **Conclusions:** This is to the best of our knowledge the first time that *cfr* was detected in a SCC*mec* element. Bearing in mind that oxazolidinones are last resort antimicrobial agents for the control of serious infections caused by MRSA in humans, the location of a *cfr* gene together with the *mecA* gene in the same SCC*mec* cassette is an alarming observation.

## S3:4

### METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN THE SAME HOUSEHOLD IN THE CONTEXT OF CLINICAL DISEASE IN A PERSON AND A DOG

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**Background:** Home surfaces and pets have been implicated as potential reservoirs of community MRSA. Previous case reports have strongly implicated pets in maintenance or recurrence of human MRSA colonization or infection. **Methods:** We conducted a randomized clinical trial testing household-wide mupirocin/chlorhexidine decolonization of people in the homes of an index patient recently treated for MRSA skin or soft-tissue infection (SSTI). People were tested for MRSA every two weeks; home visits were made at enrollment (prior to randomization) and three months later (following decolonization). Home surfaces and pets were sampled and samples cultured for MRSA at each home visit. One dog in the study developed a MRSA surgical site infection; multiple genetic methods were used to describe isolates found in the dog, the environment, and in colonizing isolates identified subsequently in both people in the household. Briefly, 454 whole genome sequencing (WGS) was performed on isolates following inconclusive typing according to pulsed-field gel electrophoresis and spa-typing. WGS data were compared using single nucleotide polymorphism (SNP) techniques. **Results:** At enrollment, the people, dog, and eight surfaces in the home were negative for MRSA; the pet's bed was positive. The dog underwent cruciate ligament repair two days later and presented with a MRSA surgical site infection (SSI) four weeks later. Both dog bed and dog SSI MRSA isolates were USA300/ST8/t121. The dog was prescribed clindamycin; concurrently, both household members were randomized to decolonization treatment. Subsequent human samples were negative until the three-month visit, when the index patient and household member were MRSA positive; the dog and environment were negative. Of the four MRSA isolates identified from people at the three-month visit, one was USA100/t002 and three were USA300/ST8/t008 (t121 is a single-deletion variant of t008). The dog bed and dog SSI were closely related by WGS SNP analysis; the subsequent USA300 isolates in people differed by more than 100 SNPs. **Conclusions:** This report describes laboratory-confirmed MRSA infections in a person and a dog in spatial and temporal proximity, but fails to implicate the pet in subsequent human MRSA colonization.

Whole genome sequencing was necessary to confirm that dog isolates were not related to subsequent colonizing human isolates. The apparent introduction of diverse MRSA strains into the household within six weeks of cessation of decolonization treatment of people and harmonized treatment for infection in the dog suggests that community sources outside the home may be important for recurrent MRSA colonization or infection.

## S3:5

### MICROEVOLUTION OF *S. PSEUDINTERMEDIUS* ISOLATED FROM INFECTIONS OF ONE CANINE PATIENT AT 25 DIFFERENT TIME POINTS BETWEEN 2008 AND 2014

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**Introduction:** The opportunistic pathogen *S. pseudintermedius* mainly causes purulent infections in dogs. Recurrent infections have been described in the past. In order to unravel the microevolution as well as the phenotypic diversity of this pathogen within one patient phenotypic and genotypic characterization was performed for methicillin-susceptible (MSSP) and methicillin-resistant *S. pseudintermedius* (MRSP) isolated from multiple wound infections of a dog during a seven-year time period. **Material and Methods:** Between 2008 and 2014, S. *pseudintermedius* was isolated from 25 wound swabs from one patient. In total, 38 isolates (up to eight colonies / swab) were sequenced. Clonal relationship was determined based on the allele diversity of 1845 target genes (Ridom SeqSphere + 2.3.1). Variability within isolates of distinct genotypes (gene content and SNPs [single nucleotide polymorphisms]) was analyzed (geneious 6.1.5). To display the phylogenetic relationship for isolates of each lineage neighbor-joining trees were built. The binding capacity to fibrinogen and fibronectin as well as biofilm formation was determined for eleven isolates. **Results:** MLST+ typing revealed three distinct genotypes (I, II, III). All MRSP (n=21) belonged to genotype I. MSSP clustered into two genotypes II (n=13) and III (n=2). Lineages I and II contained the majority of isolates. Within each of these two predominant lineages only minor variations were detected regarding the gene content and SNPs. Despite the low number of identified SNPs (MRSP-I n=27; MSSP-II n=17), an accumulation was observed over the time for both lineages. While MRSP-I showed only weak adherence to fibrinogen, MSSP-III had a moderate and MSSP-II a strong binding capacity. Strong biofilm formation was observed for all MRSP. Isolates sharing the same genetic background displayed a comparable phenotypic profile. **Discussion:** Sampling of 25 wound infections from one patient revealed two different successful genetic lineages. Interestingly, isolates sharing the same genetic background showed only minor genetic variation even though the strains were isolated over seven years. While mixed infections with MRSP-I and MSSP-II were determined twice, exchange of mobile genetic elements was not detected. Phenotyping revealed opposing abilities for MRSP-I and MSSP-II regarding adherence to fibrinogen and biofilm formation, indicating that none of these tested mechanisms is essential for *S. pseudintermedius* to successfully infect dogs. The lack of phenotypic variability of isolates sharing the same genetic background is in accordance with the stable genome of these strains. A reasonable explanation for the lack of variability within the identified lineages might be recurrent autoinfections or a persistent infection rather than re-infections due to an external source.

## S3:6

### EVOLUTION OF ST398 IN HUMANS AND ANIMALS

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S. aureus sequence type (ST)398 has emerged as a pandemic clone affecting both humans and livestock. Since the original description MRSA ST398 quickly spread amongst pigs and other types of livestock and has been associated with MRSA infections in farmers. In parallel, animal-independent human colonization and infections with ST398 MSSA have emerged and are now been encountered worldwide. The recent advent of high-throughput whole genome sequencing has allowed us to gain a deeper understanding of how acquisition and loss of mobile genetic elements, the acquisition of antibiotic resistance traits as well as core genomic adaptations have shaped the evolution of this highly successful lineage. Phylogenetic analyses have predicted that human-associated ST398 MSSA diverged from a most common recent ancestor about 40 years ago. We will compare and contrast recent studies on how MRSA ST398 has evolved in animals, adapts in settings of close human and animal contact and lastly discuss the short-term evolution and transmission patterns of MSSA ST398 in community households.

## S3:7

### POPULATION STRUCTURE OF MRSP AND MSSP IN GERMANY - A GENOMIC APPROACH

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**Introduction:** In veterinary medicine, infections with methicillin resistant Staphylococcus pseudintermedius (MRSP) are a global therapeutic challenge. MRSP are often associated with a multidrug resistant phenotype and therefore difficult to treat successfully. In addition, MRSP are typical nosocomial pathogens tending to spread within veterinary clinical settings. During a risk assessment study in Germany, 100 MRSP and 100 methicillin susceptible S. pseudintermedius isolates have been collected from various geographic origins, clinical settings and animal patient histories. Based on PFGE pattern and epidemiological data we chose 108 representative strains from both groups for whole genome sequencing (WGS) to get an insight into the composition and phylogenetic relationship of clinical relevant S. pseudintermedius strains in Germany. **Methods:** Species identity was confirmed by NCBI-blast for housekeeping genes pta and cpn60. Based on the Maximum Common Genome (MCG, von Mentzer et al., Nat. Gen. 2014) a maximum likelihood (ML)-phylogeny was calculated with the maximum resolution. In addition, the amount and distribution of recombination events was analyzed with Bayesian Recombination Tracker (BRAT) and the distribution of the accessory genes clustered for similarity within the selected genomes. Further zoom into the MRSP-ST71 group was performed to gain insight into the microevolution of this successful lineage. **Results:** The MCG determined for 108 S. pseudintermedius (55 MRSP, 53 MSSP) isolates comprised 1.909 orthologous genes. Bootstrap analysis indicated a diverse phylogenetic background: Beside genomes of isolates sharing the same ST, no significant clustering could be detected. All genomes showed a more or less comparable distance to a common ancestor. This observation was also confirmed by distribution analysis of the accessory gene content. Overall, the genomes showed rather limited recombination together with an evenly distribution of these events over the genome, lacking a certain cluster-specific variation.

Among MRSP reported on here, those belonging to sequence type (ST)71 predominated (> 80%). These MRSP-ST71 genomes share a MCG of 2.308 orthologous genes and were not as similar as one would expect. Although numerous genetic variations were detected, they did not unravel any significant host or geographic association. **Discussion:** The investigated S. pseudintermedius population is remarkably diverse. Within this diversity the genomic ST71 background seems to have an evolutionary advantage as these strain are the most frequent detected population subgroup. As all ST71 strains contain the mec complex, but are clonally divers, their spread is not due to clonal expansion. Whether the content of mobile genetic elements or other factors are responsible for their successful spread requires further investigation.

## S3:8

### WHOLE GENOME SEQUENCING A PROMISING ADDITIONAL TOOL IN EPIDEMIOLOGIC INVESTIGATIONS OF TRANSMISSION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS (MRSP)

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Longitudinal studies of MRSP carriage and transmission between dogs are scarce. Furthermore, development of rapid, discriminatory and standardized methods for genetic characterization, as well as of online databases for use in epidemiological investigations would be highly useful in furthering understanding of the epidemiology of this bacterial species. In the present study, carriage of MRSP was investigated over several months in 11 dogs living in four separate multiple dog households (household A, B, C & D), each with a clinically infected dog. In total 25 MRSP- isolates were detected. Whole-genome sequencing (WGS), a methodology not previously used in published longitudinal MRSP studies was applied for genetic characterisations of these MRSP- isolates, and thereby evaluated for future use in epidemiologic investigations of MRSP transmission. Whole-genome sequences were compared with MLST+, a core genome MLST approach. The genome-comparison software Gegenees was used to create a phylogenomic overview of the isolates. Whole-genome sequence reads for each isolate are deposited at the European Nucleotide Archive. Study accession number PRJEB8361. The results of the MLST+ analyses supported transmission of MRSP occurring within each family group, as the isolates from the respective groups were shown to be genetically separated. Pairwise comparisons of isolates within each household showed ≤ 6 allele differences, with the exception of household B where ≤18 allele differences were shown. The number of allele differences between the four family groups was 18-30; 18 differences between isolates from family B and C, and 30 differences between isolates from family B and D. In conclusion, the WGS methodology appeared when applied to isolates of known origin to be a promising additional tool in epidemiologic investigations of MRSP transmission. Follow-up studies are warranted. Notably, breakpoints for allele differences for what is to be considered the same strain are difficult to set without knowledge of the mutation rate of MRSP over time. Furthermore the relatively large divergence of allelic profiles between the isolates from family B indicates a potential risk of overlapping results between individual isolates originating from separate family groups in future, larger data sets.

## S4:1

### SEARCH AND DESTROY…DOES IT STILL WORK FOR LA-MRSA?

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## S4:2

### CONTROL OF LA-MRSA IN SWINE - IS IT POSSIBLE? LESSONS LEARNED FROM OUTBREAKS AND ERADICATION IN NORWAY

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The prevalence of LA-MRSA in the Norwegian pig population is low. This is well documented in the monitoring program for antimicrobial resistance in the veterinary sector; NORM-VET, and in pig population screening studies. The first traceable findings of CC398 occurred in 2013 detecting *spa*-type t034 in a pig submitted for post-mortem examination and a separate detection of a *spa*-type closely related to t034 in a hospitalized farm worker. These findings initiated epidemiological investigations and subsequent population screenings. Measures were imposed by the Norwegian Food Safety Authority (NFSA) to eradicate MRSA from positive herds to avoid reservoirs for transmission between farms and from pigs to humans. Pooled cloth samples from pig herds (animals and environment) were collected by the NFSA and analyzed at the Norwegian Veterinary Institute using the protocol described by EFSA. Selected isolates from each herd were analyzed by *spa*-typing and the presence of *mec*A at the Norwegian Reference Laboratory for MRSA and by whole genome sequencing (WGS) at Statens Serum Institut in Denmark. A strategy to eradicate MRSA CC398 from positive herds was attempted. This included restrictions on live animal trade, depopulation and thorough washing and disinfection of buildings and equipment. Following the eradication protocol and collection of MRSA negative environmental samples, herds were repopulated with pigs from herds presumed free from MRSA. Follow up testing of animals and environment was performed several times to assess the effect of eradication. The goal of the strategy was to eradicate MRSA from positive herds. All samples and data were collected to control MRSA in the pig population, and not as part of planned scientific studies. From 2013 to 2015, 4 epidemiologically and genetically separate clusters with MRSA CC398 positive herds were identified through contact tracing from initial findings. In total, these clusters included 41 herds positive for MRSA CC398. Epidemiological data strongly indicate humans as the source of MRSA CC398 introductions in all primary case herds (4 herds), and that the predominant route of further transmission was by trade of live pigs. Preliminary results from follow up sampling indicate that MRSA eradication was successful in the first attempt in 22 of 26 herds. Two herds likely reintroduced MRSA with pigs at restocking, whereas no explanation for why the eradication failed was identified for one herd. One herd was closed after depopulation and has not been restocked. The remaining 15 herds have not yet completed the eradication and/or follow up sampling due to short time since detection. The results indicate that eradication of MRSA CC398 from positive herds is possible in Norwegian pig production.

## S4:3

### ASSESSMENT OF METHODS TO QUANTIFY LIVESTOCK ASSOCIATED MRSA IN PIG HERDS

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A high prevalence of LA-MRSA, especially in the pig production, has been reported from many countries. However, there is a lack of knowledge about the actual MRSA loads on infected farms. It is highly relevant to assess the LA-MRSA level on animals and in the environment, to be able to study a potential connection between the level of LA-MRSA and the risk for human exposure, and to estimate the effect of intervention strategies. The present study was undertaken to assess methods to quantify LA-MRSA on animals and in the environment in pig herds. In addition, the role of flies as potential environmental carriers of MRSA was investigated. Sampling was conducted on two different occasions at a confirmed MRSA infected farm. Samples were collected in the farrowing section and the weaner section. Nasal samples from sows (n=5), piglets (n=5) and weaning pigs (n=18) were collected with dry flocked swabs. Additionally, piglets and weaning pigs were sampled behind one ear with a moistened flocked swab. The swab material was extracted in 1 mL liquid Amies medium. Samples were analyzed individually and quantified by direct plating from dilution $10^0$ and $10^{-1}$ onto a MRSA selective agar. Following incubation, colonies were counted, and counts expressed as cfu/swab for each sample. Airborne MRSA in the weaning section was quantified by calculation of cfu/$m^3$ of various air volumes, collected with two different sampling devices for comparison. One sampling directly onto a selective MRSA agar plate (Sampl'air Air sampler) and the other sampling onto a membrane filter (Sartorius, MD8 air sampler). Flies were sampled both individually and in pools of 5 and enriched prior to MRSA determination on MRSA selective agar. All tested animals (28/28) were found to be MRSA positive, with a nasal MRSA load ranging from 6.6-$\times 10^1$-$3.9\times 10^4$ cfu/swab and skin load ranging from $1.1\times 10^1$-$2.6\times 10^5$ cfu/swab. Both air sampling devices were able to detect MRSA. A higher cfu/$m^3$ level was found with Sampl'air, range $5.8\times 10^2$-$1.56\times 10^3$, than with MD8, range $4.1\times 10^2$-$6.3\times 10^2$, however MD8 gave a more stable detection level of MRSA from the different volumes. In sections with animal activity, flies treated individually and in pools, were found to carry MRSA, but in sections with no animal activity both individually treated flies and pooled samples were found MRSA negative. In conclusion: This study demonstrated that fast quantification of the animal MRSA load in pig herds is possible by direct plating of either nasal or skin swab samples. The MD8 air sampler provides fast environmental quantification and it was possible to detect MRSA in flies with animal contact demonstrating their potential as environmental MRSA carriers. Intensive sampling from both animals and environment can be used to estimate an overall farm MRSA level. Our results will now be used to study possible transmission routes and investigate the effect of different intervention strategies.

## S4:4

### ANTIBACTERIAL ACTIONS OF ANTIMICROBIAL PEPTIDES DERIVED FROM DIFFERENT ANIMAL SPECIES AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AND THE EFFECT OF BACTERIAL EFFLUX PUMPS

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Antimicrobial peptides (AMPs) e.g. cathelicidins have recently been discussed as potential new strategy against antibiotic-resistant bacterial infection. The aim of this study was to characterize the antimicrobial action of cathelicidins derived from different animal species against livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA) and to analyze whether S. aureus uses endogenous efflux pumps to evade the antimicrobial functions of different cathelicidins. For this purpose the minimal inhibitory concentrations (MICs) of S. aureus field isolates for the cathelicidins LL-37 (human), mCRAMP (mouse), CAP18 (rabbit), BMAP-27 and BMAP-28 (bovine) in the presence and absence of the resistance-nodulation cell division (RND) efflux pump inhibitor 1-(1-Naphthylmethyl)-piperazine (NMP) were determined. Furthermore, the MICs of mutants lacking a specific RND efflux pump (SecDF) were compared with the MICs of their respective wildtype strain and of the complemented mutant. Distinct differences were detected in the Mode MICs when comparing the activity of different cathelicidins against all field isolates. While MIC values were lowest for bovine cathelicidins (BMAP-27 and BMAP-28), MIC values were highest for the human and mouse cathelicidins (LL-37 and mCRAMP). Interestingly, only the MICs for CAP18 significantly decreased when bacteria were treated with NMP. In good correlation to these data, the sec translocase knockout mutants showed significantly decreased MICs for CAP18 and also for BMAP-27 compared to their respective wildtype and complemented strains. No differences were detected for the cathelicidins LL-37, mCRAMP or BMAP-28. These results indicate an involvement of the efflux pump SecDF in the intrinsic resistance of S. aureus to certain animal-specific cathelicidins, which might contribute to the species-specific colonization of several S. aureus strains. Current experiments are performed that will clarify the role of SecDF in the species-specific host-pathogen interaction.

## S4:5

### THE ANTIBACTERIAL DRESSING, ACTICOAT, BECOMES BACTERICIDAL AGAINST MRSA WHEN WETTED WITH SURFACTANTS AND ANTIMICROBIAL PEPTIDES

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Sepsis, resulting from invasive Staphylococcus aureus or Pseudomonas aeruginosa infection, is the leading cause of mortality in burn patients. To prevent systemic infection, the burn eschar is wrapped in antibacterial gauzes such as the silver nanoparticle loaded bandage Acticoat. Multiple studies have shown Acticoat and similar gauzes can inhibit growth of bacteria but are not bactericidal, which could leave patients vulnerable to sepsis. We have evaluated the bactericidal effectiveness of Acticoat against methicillin resistant Staphylococcus aureus on a pig skin model and investigated whether the addition of solvents or antimicrobial peptides mel12-26 and bac8c can improve the bactericidal activity of Acticoat. Agar inoculated with four different S. aureus strains and overlain with Acticoat strips showed growth inhibition beneath and within a 1 mm perimeter of the gauze after 24 h. Acticoat lifted from the inoculated agar and briefly blotted onto fresh agar plates transferred viable bacteria, but roughly 5-fold less than did filter paper controls. This suggests that Acticoat has bacteriostatic activ-

ity but is not effectively bactericidal. When viewed by scanning electron microscopy, the surface of Acticoat overlaying MRSA for 24, 48 and 72 h showed dense clusters of apparently undamaged cells distributed along the mesh. The highest concentration of cells was found on the nodes between the Acticoat mesh, which protrude beyond the surface of the gauze. This structure could limit contact of the mesh with surrounding areas of the wound surface. The number of surviving bacteria growing on inoculated pig skin, underneath and on the surface of Acticoat, was measured after contact for 10 s, 0.5, 4, 8 and 24 h. The number of surviving bacteria was lower than on the controls for the first 8 h, but after 24 h the number of bacteria on the skin was 2.3-fold greater than that of the untreated controls. In contrast, the number of cells surviving on the Acticoat surface after 24 h was 11.9% of controls. The risk of recurring infection in wounds treated with Acticoat may be greatly reduced if dressings are changed at intervals no longer than 8 h. Acticoat wetted with 10% glycerol with 50 µg/ml mel12-26 or bac8c, reduced the number of bacteria on the gauze and on the skin underneath to below 99.99% of the controls respectively. When lysozyme (1 mg/ml) was added to Acticoat wetted with glycerol and bac8c, the gauze was able to prevent the survival of bacteria on densely inoculated pig skin and on the surface of Acticoat for up to 24 h. It appears that after 24 h MRSA can overcome the bacteriostatic activity of Acticoat and even use the polyethylene mesh as a growth surface. In their native form most AMPs are suitable only for topical applications. From our results it appears possible that biocompatible solvents and AMPs might enhance the bactericidal efficacy of Acticoat.

## S4:6

### MUPIROCIN SUSCEPTIBILITY IN ENVIRONMENTAL METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS FROM HOMES WITH AND WITHOUT PETS IN THE CONTEXT OF A MUPIROCIN-BASED RANDOMIZED CLINICAL TRIAL

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**Background:** Methicillin-resistant Staphylococcus aureus (MRSA) contamination of the home environment may contribute to human recolonization and treatment failure. Mupirocin remains an important topical antimicrobial used for MRSA decolonization, although mupirocin resistance can limit decolonization treatment options. **Goal:** To determine whether the home environment can serve as a reservoir for mupirocin-resistant (mupR) MRSA, and if so, whether pet presence or human treatment influence this reservoir. **Methods:** Previously-collected household environmental MRSA isolates (n=101) from 95 homes of patients diagnosed with community MRSA infection were evaluated. MRSA isolates (n=56) were also evaluated from 65 of these homes re-assessed at a three-month home visit, following randomization of patients with recent MRSA infection to a mupirocin-based decolonization therapy or an education-based control group. Mupirocin susceptibility was evaluated using E-test® (Biomerieux, France) and established real-time PCR methods. **Results:** Household-level prevalence rates of phenotypic mupirocin-resistance were 6% at both visits. Presence of pets in the home was associated with 93% decreased odds of environmental mupR MRSA

at baseline (p=0.02). Pets were not treated and no owners reported pet use of mupirocin prior to or during the study. All mupR MRSA at the baseline home visit were high-level mupR mediated through a genetic mechanism (mupA). Households with mupA-positive MRSA in the environment at baseline were significantly more likely to have it present in the environment at the three-month visit (OR 20.00 p=0.05). Both homes (n=2) with persistent mupA-positive MRSA environmental contamination were associated with mupA-positive MRSA colonization in people and were associated with failure of the index patient to successfully clear MRSA colonization during treatment. Among 39 households randomized to mupirocin treatment, two (5%) had incident low-level mupR MRSA in the home environment at the three-month visit; none of the 26 households randomized to the education control group were found to have mupR MRSA at three-months. **Conclusion:** The household environment can act as a reservoir of mupirocin-resistant MRSA, and presence of mupR MRSA in households may be associated with treatment failure. The potential protective role of pets is novel and should be evaluated more fully in future research.

## S5:1

### MRSA DIAGNOSTICS: ADVANCES, CONTROVERSIES AND CONUNDRUMS

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## S5:2

### PIG-2-BAC AS A BIOMARKER OF OCCUPATIONAL EXPOSURE TO SWINE AND TO STAPHYLOCOCCUS AUREUS AND METHICILLIN RESISTANCE

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Over 50 million hogs are raised annually in the United States for consumption, mostly on industrial hog operations (IHOs). IHO workers are exposed to airborne particulates, zoonotic pathogens, and other workplace hazards, but access to IHOs to assess sources of microbial exposure is restricted in the US, mainly due to biosecurity concerns. Here we examined whether the quantity of a microbial source-tracking marker (Pig-2-Bac) in IHO workers' noses is related to time since last IHO work shift and to IHO worker nasal carriage of S. aureus and mecA (methicillin resistance). Between June-August, 2012, 22 IHO workers in North Carolina provided 316 nasal swabs as part of a 14-day repeated-measures study. Swabs were collected each morning and evening on days 1-7 and also on day 14 of the study. Swabs were collected between <1 and up to ≥3 days after an IHO work shift. DNA extracted from nasal swabs was assessed by real-time qPCR for the S. aureus specific gene femA, the methicillin resistance gene mecA, and for Pig-2-Bac. Fixed effect linear regression models were used to estimate beta coefficients and standard errors (SEs) of associations between time since last IHO shift and Pig-2-Bac qPCR estimates and between Pig-2-Bac and femA and mecA qPCR estimates. Pig-2-Bac was detected in 61% of nasal swab samples (mean±SE Pig-2-Bac log10 ΔCT/swab=1.5±0.8). Fixed-effects linear regression modeling showed that log10 Pig-2-Bac decreased -0.35 (SE: 0.07) for each additional day since last IHO work shift ($p<0.0001$). femA was present in 67% of nasal swab samples (mean±SE log10 copy number/swab=4.2±1.3) and mecA was present in 93% of nasal swab samples (mean±SE log10 copy number/swab=4.7±0.9). femA and mecA increased by 0.14 (SE: 0.06; $p<0.03$) and 0.09 (SE: 0.04; $p<0.06$), respectively, for each one-unit increase in Pig-2-Bac. The association between Pig-2-Bac and time since last

work shift suggests that Pig-2-Bac may have utility as an objective biomarker of intensity of occupational exposure to pigs and their fecal waste. Associations between Pig-2-Bac and femA and mecA indicate that the source of exposure to femA and mecA among IHO workers is intensive contact with pigs and their fecal waste. These associations may help with identification of specific occupational exposure risk factors in future epidemiologic studies of IHO workers, particularly when access to the IHOs is restricted.

## S5:3

### CO-COLONIZATION DYNAMICS AND CLONAL DIVERSITY OF METHICILLIN-SENSITIVE AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN SOWS

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Methicillin-susceptible Staphylococcus (S.) aureus (MSSA) and methicillin-resistant S. aureus (MRSA) are colonizers of skin and mucosa and may cause severe infections both, in humans and animals. In humans, some studies have shown that MSSA and MRSA co-colonization of the anterior nares is common and one clone can be found rather than differing types of MSSA and MRSA. In the last decade, MRSA were frequently detected in healthy farm animals and livestock professionals. In this context MSSA carriage in pig farmers was postulated as a possible protective effect against the colonization with MRSA. This might also be interest for possible interventions in pigs. The objective of our study was to investigate the colonization dynamics and clonality of both, MSSA and MRSA in pigs over a longer time period. Eighteen sows were nasally sampled three times every ten weeks. Additionally, 50 environmental samples from walls, toys, brushes, watering places, and troughs were taken, together with boot swab samples and a pooled dust sample. Samples were investigated for MSSA and MRSA, respectively. Spa-typing was done with up to five MRSA and MSSA isolates found per sample and time point (total: 133 MSSA and 120 MRSA isolates, respectively); 59 selected isolates were further investigated by microarray. 16.7 % of sows were MSSA/MRSA non-carriers and permanent carriers of MSSA, respectively at all sampling times; 38.9 % of sows infrequently carried both, MSSA and MRSA; 66.7 % of sows showed a changing carriage status over time. CC398 (t034, t011) and CC9 (t337, t1430, and t13816) associated spa-types were exclusively found among MRSA and MSSA, respectively. In 44.4 % of sows up to two different types of MSSA were present at the same time and sample. Strains of the same clonal lineage showed a high genetic identity despite their origin. Highly identic clones were present in sows and their environment. As conclusion, MSSA/MRSA may not exclude each other in the anterior nares of pigs. Pigs may also carry different clones at the same time. This calls for a critical review of the number of isolates that need to be analyzed, in particular in the course of in-depth epidemiological investigations.

## S5:4

### CHARACTERISATION OF METHICILLIN RESISTANT AND SUSCEPTIBLE STAPHYLOCOCCUS INFECTIONS IN AUSTRALIAN ANIMALS

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**Background:** Methicillin resistant *Staphylococcus* species cause increased morbidity and mortality in humans and animals but their prevalence in the Australian veterinary setting is unknown. **Aim:** To characterise staphylococcal infections in animals in Australia, with a focus on resistant infections. **Methods:** *Staphylococcus* isolates were collected from companion animal submissions to all Australian veterinary diagnostic laboratories. Epidemiological data including animal species, sample site and clinical syndrome were recorded. The antimicrobial resistance profile of each isolate was determined by disc diffusion and minimum inhibitory concentration. Isolates were speciated using phenotypic and molecular tests. Methicillin-resistance was confirmed by identification of the *mecA* gene. Resistant coagulase-positive isolates were further characterised using whole genome sequencing. Data was analysed using multivariable logistic regression. **Results:** A total of 794 clinical *Staphylococcus* isolates were collected from 22 veterinary diagnostic laboratories. *S. pseudintermedius* was the most commonly isolated species in dogs (82%) while *S. felis* (50%) and *S. aureus* (78%) were the most common species in cats and horses respectively. Methicillin resistance was found in 70/723 (10%) canine isolates, 8/71 (11%) feline isolates and 10/67 (15%) equine isolates. There were 14 methicillin-resistant *S. aureus* (MRSA) isolates: six from horses, five from dogs and three from cats. Thirteen MRSA were identified as human-associated MRSA clones including seven isolates as ST22 (EMRSA-15); a common cause of human health care -associated MRSA infections in Australia and Europe. One MRSA isolate from a cat was identified as ST425; a previously reported animal-associated clone. Amongst the 69 methicillin-resistant *S. pseudintermedius* (MRSP) isolates, ST71 (SCCmec II-III) was the most frequently identified clone. ST71 is frequently identified in Europe and Japan. We also identified several new MRSP clones including ST496 (SCCmec V), which was isolated only in dogs on the east coast and was resistant to all tested antimicrobials except rifampicin. Interestingly, there were no isolates in Australia from the ST68 clone, which is common in the USA. **Conclusions:** Methicillin resistance is present in 10% of staphylococcal infections in Australian companion animals. The dominant strains of MRSA and MRSP in Australian animals are similar to those reported overseas, but several novel Australian MRSP strains have also arisen.

## S5:5

### CHARACTERIZATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM PRIMATES, PERSONNEL AND ENVIRONMENT AT A US PRIMATE CENTER

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**Background:** Very little has been published on MRSA colonization or infection in primates. MRSA was first detected at the WANPRC Feb. 2014 in cranial implanted primates. The MRSA+ (positive) animals were originally found on one floor then MRSA+ animals were found in other sections of the center including on-campus and off-campus facilities. The aim

of the study was to characterize selected primate MRSA isolates, determine if the primate center environment was contaminated and if personnel working in the center were also MRSA+. Initial antibiograms of the primate MRSA isolates from in-house primates and animals purchased from a commercial facility had similar highly resistant MICs which varied only in their susceptibility to trimethoprim/sulfamethoxazole suggesting a potential of a single clone at both the commercial and WANPRC sites. **Methods:** Human primate center personnel and primate nasal samples were collected with SANICULT™ and TransPRO™ swabs respectively. Composite environmental samples were collected with SANICULT™ and 3M™ Sponge-Sticks. The isolates were biochemically verified as *S. aureus*, coagulase tested and the *mec*A gene verified using the Oxoid penicillin binding protein latex agglutination test®. Selected isolates were SCC*mec* typed and their MLST type determined using PCR assays. **Results:** A total of 596 primates had nasal cultures done. A total of 105 (17.6%) animals were MRSA+. Two (2.5%) of 79 personnel and 2 (3.6%) of 56 composite environmental sites were MRSA+. Nine MRSA isolates from primates representing each section of the WANPRC and 4 MRSA isolated from purchased commercial animals, 2 human and 2 environmental samples were selected for characterization. The WANPRC primates, personnel and environmental isolates were all SCC*mec* IV. Initial testing indicated that 1 human, both environmental and 2 WANPRC primate MRSA isolates were ST188. The 4 MRSA from the commercial primates were SCC*mec* II with the same novel MLST type. **Conclusion:** The SSC*mec* and MLST data are consistent with a potential clone in the primates, people and environment within WANPRC and a different clone from the commercial primates. This is the first study to type and characterize MRSA isolates taking a One Health approach by screening MRSA from primates, personnel and their environment from a primate center. MRSA ST188 SSC*mec* V human infections have been reported in Asia. It is not clear if the WANPRC strain is related to these isolates.

## S5:6

### TEMPORAL AND SPATIAL TRENDS IN THE DISTRIBUTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS SCHLEIFERI FROM COMPANION ANIMALS IN THE MID-ATLANTIC UNITED STATES

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Staphylococcus schleiferi is an emerging pathogen of people that more typically has been associated with skin disease in dogs (and cats). Little is known about its epidemiology in a veterinary or human health context. In preparation for an investigation of transmission of S. schleiferi between people and pets, we compared the spatial and temporal trends of methicillin-resistant S. schleiferi (MRSS) to methicillin-susceptible S. schleiferi (MSSS) isolates from animals that presented to the Ryan Veterinary Hospital, University of Pennsylvania (VHUP) for treatment of skin or soft-tissue infection. Laboratory-confirmed S. schleiferi infection in pets that presented to VHUP between January 2003 and June 2014 and whose owners lived in the mid-Atlantic U.S. (PA, DE, NJ, or MD) were included. Cases that originated outside this region or lacked street address were excluded. Client address, isolate antibiogram and other data were obtained from the VHUP Hospital Information System and BioNumerics (Applied Maths, Austin TX) software databases. MRSS were defined as isolates resistant to oxacillin. Addresses were geocoded in ArcMap 10.2 (ESRI, Redlands, CA), household locations were mapped, and the distance between each mapped household and VHUP was measured. Odds ratios (ORs) were calculated testing (odds of MRSS) / (odds of MSSS) in Stata 13 (College Station, TX). Household

coordinate data expressed as latitude (x) and longitude (y), a generated xy interaction term, and household distance in kilometers from VHUP were evaluated as spatial predictors of MRSS. 849 cases were identified; 32 were excluded for residence outside the mid-Atlantic, unknown address, or missing antibiogram. Eight additional cases were missing collection date, leading to a total of 817 cases for spatial analysis and 809 cases for temporal analysis. Prevalence of MRSS was 55% (451/817). Odds of MRSS increased 6.7% [95% Confidence Interval: 2.0%, 11.6%] for each year of the study ($p<0.01$); estimates were similar when adjusted for spatial variables. Household coordinate data were not associated with trends in MRSS. Interaction between spatial and temporal variables was not associated with MRSS. Cases one kilometer further from the VHUP were slightly but significantly less likely than cases closer to the VHUP to be MRSS (OR 0.996 [95% CI: 0.993, 0.999], $p=0.05$). This is the first study to evaluate spatiotemporal trends in S. schleiferi. Increasing trends in methicillin resistance among veterinary clinical isolates of S. schleiferi were identified between January 2003 and June 2014. Further analysis is needed to evaluate case proximity to pet-related locations, such as dog parks, to better understand community drivers of methicillin-resistant staphylococci in pets.

## S5:7

### NOVEL VARIANTS OF STAPHYLOCOCCUS INTERMEDIUS-LIKE ORGANISMS ISOLATED FROM BLACK BEARS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK, TENNESSEE, USA

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A survey of Staphylococcus and Staphylococcus-like species was conducted on swap samples taken from nuisance black bears trapped in high public access- and backcountry- areas of the Great Smoky Mountains National Park during the summer of 2010. From over 70 isolates screened by colony morphology on blood agar and partial 16S rDNA sequencing, six isolates, each from a separate individual bear, were identified as probable species belonging to the Staphylococcus intermedius group (SIG). Our hypothesis was that each of these isolates would be identified as one of the three known species within this group. Subsequent testing included analysis of biochemical activities, antimicrobial susceptibilities, MALDI-TOF-MS profiles, cpn gene sequences, and nuc gene PCR profiles in comparison to those of type species of the SIG, namely Staphylococcus intermedius, Staphylococcus delphini, and Staphylococcus pseudintermedius. It was determined repeatedly that the bear isolates were most closely related to members of the Staphylococcus intermedius group but that they were not readily identified to the species level. The isolates also display specific 16S rDNA and cpn sequences, and they did not react in species-specific nuc gene PCR assays. The conclusion of this study was that the six Staphylococcus isolates from bears likely represent a novel species within the SIG group or at least a unique subset of variants within an existing species. It is of clinical, diagnostic and ecological interest to note that each of the bear isolates were tube coagulase-negative, susceptible to multiple antimicrobials, including oxacillin and penicillin, and could not be clearly identified with molecular methods. These bear isolates extend our knowledge of host distribution of S. intermedius-like organisms and with further study may provide insights regarding acquisition of antimicrobial resistance including SCCmec cassettes among naïve populations of organisms within the Staphylococcus intermedius group.

# Poster Abstracts

## 1

### ADHESION AND INVASION OF TAIWAN LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* TO HUMAN LUNG CARCINOMA EPITHELIAL CELL LINE A549

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**Background:** Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been detected in swine, poultry, bovine and other livestock-associated products. The prominent lineage of LA-MRSA in Asia is sequence type (ST) 9 which has emerged as a potential zoonotic pathogen for humans and animals. *Staphylococcus aureus* (S. aureus) has been shown to adhere, invade and induce the death of various host cells. However, only few studies have determined the capacity of adherence and invasiveness in LA-MRSA ST9. The aims of the study were to investigate whether Taiwan LA-MRSA ST9 can adhere and invade to human lung carcinoma epithelial cell line A549. **Methods:** Eight different Taiwan LA-MRSA ST9 clones, collected from swine nostrils, were selected for the study. Human lung carcinoma epithelial cell line A549 (2×105 cells) were exposed to MRSA (8.6×105 cells/mL) for adhesion and invasion assay. The S. aureus ATCC 29213, a known clinical cytotoxic strain, was used for positive control. Each assay was performed in triplicate. The differences of the capability of MRSA adhesion and invasion between positive control and swine origins were compared by using the Student's t-test. **Results:** Of the 8 Taiwan LA-MRSA isolates, all exhibited the ability of adhesion and invasion to A549 epithelial cells. The relative adhesion ability of the Taiwan LA-MRSA (79.8%) was significantly lower than that of the control strain (100%, $P < 0.05$, Student's t-test). But there was no significant difference in the ability of invasion between Taiwan LA-MRSA (97.2%) and the control strain (100%, $P > 0.05$, Student's t-test). **Conclusions:** The study characterizes the capability of adhesion and invasion of Taiwan LA-MRSA ST9 for the first time and the ability of inducing cytotoxicity to A549 epithelial cells by this lineage needs further elucidation.

## 2

### COMPARISON OF TWO CULTURE BASED METHODS FOR DETECTION OF LA-MRSA IN SWINE

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**Introduction:** The prevalence of livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) in the Norwegian pig population is low. Recently, attempts were made to control the occurrence of LA-MRSA and also to eradicate MRSA from positive swine holdings in Norway. An important tool for achieving this goal is to use cost-effective and sensitive screening methods. Currently there is limited information available concerning the sensitivity of the recommended culture based detection method for MRSA in livestock. **Material and Methods:** A total of 921 samples from 120 swine holdings were included in the study. The material consisted of cloth samples from pigs (skin behind ears) and cloth samples from the indoor environment of the pig houses. All samples were analyzed with the protocol described by European Food Safety Authority using two enrichment steps, before plating out on MRSA Brilliace 2 agar plates. The same samples were also analyzed by omitting the second enrichment step (Tryptone Soya

Broth containing 3.5 mg/L cefoxitin/75 mg/L aztreonam) before plating out on the selective agar plates. Presumptive MRSA isolates were confirmed using molecular methods. **Results and Discussion:** In total, 854 of the samples were negative using both methods whereas 47 were positive with both methods. Fifteen of the samples were positive using the single broth enrichment but negative using the double broth enrichment. Five samples were negative using the single broth method, but came out positive with double broth enrichment. These results show that there may be a slight increase in the sensitivity using single broth enrichment, compared to using two enrichment steps. However, a larger number of samples should be analyzed before a firm conclusion can be drawn. There may also be differences between the various MRSA genotypes with regard to detection with different methods. All in all, there was a good agreement between the two methods, but the cost-benefit of including the second enrichment step can be questioned.

## 3

## DETECTION OF PANTON VALENTINE LEUKOCIDIN, ENTEROTOXIN A AND C IN METHICILIN SUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* (MSSA) ISOLATED FROM BOVINE MASTITIS

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The pathogenicity of *Staphylococcus aureus* can increase due to the production of many factors of virulence including a range of extracellular toxin. Panton Valentine is a leukocindin higly citotoxic for polymorphonuclear cells being capable of causing lysis of these cells in bovine organism, with an important role in the induction of clinical and subclinical mastitis. Staphylococcal enterotoxins are recognized as food poisoning agents and may be involved in other types of infections in humans and animals. The present study investigated 119 isolates from cattle with bovine mastitis in São Paulo State, Brazil. The strains were submitted to disk diffusion test for oxacillin and cefoxitin and also for the presence of mecA, sea, sec and Panton Valentine Leukocidin gene, by PCR. The results showed sensibility for the antibiotics tested and the absence of mecA gene, so the strains were classified as methicilin susceptible *Staphylococcus aureus*(MSSA). All the strains were negative to pvl gene whereas 9% (92/119) presented sec and 4.2% (5/119) sea. Both toxins were observed in 3 isolates. The presence of these toxins is an alert to the management measures that are improved, because these are very important for intramammary infections, since they can induce the release of inflammation factors not only affecting the herd as causing disease in humans, in a serious problem for public health.

## 4

## PRESENCE OF MECA GENE IN *S. AUREUS* ISOLATED FROM BOVINE SUBCLINICAL MASTITIS IN A SINGLE HERD IN BRAZIL

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The use of antibiotics for the treatment of mastitis and possible sources of contamination in dairy herds are important controls measures. However, one of the main responsible for bovine mastitis, *Staphylococcus aureus* has shown resistance a lot of antimicrobial. S. aureus methicillin-resistant (MRSA) has been found in cattle, carrying mecA gene, localized in an island genomic called SCCmec which is a mobile region that allows the interchange between different species of Staphylococcus. Due to the great epidemiological importance, the aim this study was to evaluate the antimicrobial susceptibility profile and the presence of mecA gene in S. aureus strains isolated from cows with subclinical mastitis in a Brazilian herd. A total of 65 S. aureus strains was confirmed by the presence of the nuc gene and the

strains were tested using disc-diffusion, according to CLSI (2012). The tested antibiotics were vancomycin (1 ug), streptomycin (10 g), gentamicin (10 g), oxacillin (1 g), cefoxitin (30 µg), tetracycline (30 µg) erithromicina (15 µg), clindamycin (2 µg), tobramycin, trimethoprim-sulfamethoxazole (1.25 / 23.75 µg), tetracycline (30 µg), cephalothin (30 µg). The strains resistant to oxacillin and cefoxitin were subjected to PCR for detection of the mecA gene. We noticed 48 (73.8%) strains sensitive to all antibiotics and 17 (26.2%) presented intermediate susceptibility or resistance to at least one of the antibiotics tested. We found 6 resistant strains to oxacillin, among these, 5 also proved resistence to cefoxitin. The mecA gene was observed in 3 of them. The presence of MRSA strains in dairy herds complicates the control of mastitis and possible contamination of other animals, causing economic losses dairy industries. The resistance in MRSA without the presence of mecA gene can occur due to other genes, like mecC, no tested yet.

## 5

## ANTIMICROBIAL SUSCEPTIBILTIES AND VIRULENCE ASSOCIATED GENES OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PIGS AND IN-CONTACT HUMANS IN JOS, NIGERIA

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*Staphylococcus aureus* is a commensal and pathogenic bacterium that causes a wide variety of diseases in humans and animals with high impact on public health and the livestock industry**.** The aim of this study was to investigate the nasal carriage of *S. aureus* in pigs and in-contact humans and determine the characteristics of the isolates. A total of 300 and 101 nasal swabs were collected from backyard raised pigs and in-contact humans respectively and screened for carriage of *S. aureus*. Ethical approval to carry out the work was obtained from the Ministry of Health, Jos. In total, 7.2% (29/401) samples were found to be positive for *S. aureus* after confirmation with conventional biochemical tests, Microgen (UK) biochemical kit and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) technique. The prevalence in pigs and in-contact humans were 5.6% (17/300) and 11.8% (12/101) respectively. The 29 isolates were subjected to antimicrobial susceptibility testing and 3 different multiplex PCRs to detect enterotoxin genes *sea, seb, sec, sed, see; seg, seh, sei, sej*; and *mec*A, *spa* A, *scn*, and *pvl* genes. By *spa* typing the isolates were assigned to clonal complexes (CC). The isolates were resistant to beta-lactams (97%), tetracycline (62%), sulphonamide (52%), macrolides (24%), aminoglycoside (21%), flouroquinolone (14%), linezolide (7%) and mupirocin (3%). The 29 isolates had multiple antibiotic resistance (MAR) index >0.2, 27 (93%) carried *scn* gene, 6 (21%) the *pvl* gene but none was positive for *mec*A gene. The *spa* types identified included types t084, t355, t2216, t311, t1931, t002, t127, t442 and t304 of which t084, t355 and t311 were found in both in-contact humans and pigs from same farms. The questionnaire survey showed that age, gender, occupation and work experience were not statistically associated ($p>0.05$) with respect to nasal colonization, but medical related occupation of household members was significantly ($p<0.5$) associated with *S. aureus* carriage. This study therefore suggests the presence of multidrug resistant S. *aureus* (MDSR) but absence of methicillin resistant *Staphylococcus aureus* (MRSA) carriage among the study population. A high proportion of *S. aureus* carried *pvl* and enterotoxin genes in both in-contact humans and pigs. Also, the group of isolates from both in-contact humans and pigs shared *sea* and *sei*, and *spa* types that belonged to CC5, CC15 and CC152. This is the first study in Nigeria to report presence of *pvl* and enterotoxin genes and *spa* typing among isolates from pigs and in-contact humans.

## 6

### PRESENCE OF GROUPS ENCODING THE ACCESSORY GENE REGULATOR (AGR) AND TOXIC SHOCK SYNDROME TOXIN GENE IN METHICILLIN SUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* (MSSA) ISOLATED FROM BOVINE MASTITIS IN BRAZIL

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Accessory gene regulator (agr locus) control the expression the most virulence genes in Staphylcoccus aureus and four allelic groups the agr are being associated with bovine mastitis caused for this pathogen, that also produce a Toxic Shock Syndrome toxin (TST), representing a problem for health human. The aim of the study was analyze 283 strains of S. aureus, isolated of subclinic mastitis in different herds in São Paulo State, as the presence of agr groups gene(I, II and III), to TSST-1 gene (toxic shock syndrome toxin-1) and also for the presence of mecA gene by the method of Polymerase Chain Reaction (PCR). The classification for agr groups, showed that 8% (23/283) the isolated were positive to agrI, 48% (136/283) to agrII, 20% (57/283) to agrIII and 24% (67/283) not showed any of them. About of the presence for TSST-1 gene, 105 strains (37%) were positive, with this 19 (18%) belonging to agrI, 52 (49,5%) to agrII and 21(20%) to agrIII and the mecA gene, no strain was positive. This variation observed in a distribuition positive strains for tsst, between different agr groups proves that the virulence factors are not distributed evenly and are not always regulated in the same way showing that S. aureus may have different mechanisms in the establishment of an infection such as bovine mastitis, which is a serious factor in the production chain and also for the health of the population.

## 7

### PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN CATTLE MILK FROM DAIRY HERDS IN OYO STATE, NIGERIA

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Livestock associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is of veterinary and public health (food safety and zoonotic) significance and of global concern. Bulk milk from dairy herds where milk from mastitic cows is included in milking pool could be a major source of LA-MRSA. A survey of bulk raw milk (comprising 165 milk samples) from commercial milk collection centers in Oyo State, Nigeria was carried out to investigate the prevalence and antibiotic susceptibility of MRSA isolates using cultural methods. MRSA was identified using cefoxitin disk diffusion method. Fifty two (31.5%) milk samples yielded *Staphylococcus aureus* while 13 isolates (7.9%) were confirmed to be MRSA. Multiple (48) antibiotics resistance pattern were identified among the *Staphylococcus aureus* isolates with highest resistance (88.5%) to Cloxacillin, follow by Oxacillin and Augmentin (67.3%); Cefuroxine (65.4%); Erythromycin (61.5%); Ceftriazone (51.9%) and Gentamicin (25%) and Ofloxacin with lowest (5.76%) resistance. The results clearly indicate that milk produced for human consumption in Oyo State are contaminated with *S. aureus* that are mostly resistance to β-lactam and other antibiotics that could be a source of food poisoning and zoonotic livestock associated MRSA. Molecular epidemiological tracking of bacterial sources and resistance profile as well as hygienic milking, pasteurization of milk and prudent use of antibiotics in dairy herds are recommended to reduce the incidence of MRSA in milk before human consumption. Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), Bovine milk, Food safety

## 8

### COMPARISON OF PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) CONTAMINATION OF PERSONNEL'S CELLULAR PHONES IN VETERINARY TEACHING HOSPITAL AND MEDICAL CENTRE AND COMPUTER INPUT DEVICES IN THE LIBRARY AND CYBER CAFES AT UNIVERSITY OF NIGERIA, NSUKKA

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a major cause of community-associated S. aureus infections since the turn of the 21st century and strategies to limit the spread of MRSA in communities can only be effective if we understand the common sources of transmission. MRSA have been shown to persist for weeks on many items in the healthcare and non-healthcare settings. This study was intended to compare the prevalence of MRSA on cellular phones of medical and veterinary workers, and on the shared and private computer input devices. Using pre-moistened swabs, we took samples over a two month period (June-July, 2015) from a total of 72 cellular phones of personnel (36 at each of University of Nigeria, Nsukka Medical Centre and the Veterinary Teaching Hospital) and 72 computer input devices (keyboards and mice) at Nnamdi Azikiwe library and private cyber cafes within the campus. Isolates obtained on Manitol salt agar were identified as S. aureus following standard methods. Resistance to methicillin in S. aureus was tested by a Oxacillin disk diffusion method. MRSA was isolated from 33.3% (24/72) of cellular phones and 87.5% (63/72) of computer input devices sampled. Of the total of 24 cell phones contaminated with MRSA, 16 (66.67%) belonged to personnel (nurses and doctors) at the medical centre while 8 (33.33%) were from the veterinary hospital staff. For the computer input devices, a total of 36 samples (100%) collected from the cyber cafes were positive for MRSA while 75% (27/36) of the samples collected from the library were positive. We show that contamination rate of MRSA was significantly higher on phones of medical personnel than for veterinary workers, and conclude that cellular phones and computer input devices represent frequent sites of transmission and are reservoirs of MRSA.

## 9

### CHARACTERISTICS OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM FOOD OF ANIMAL AND NON-ANIMAL ORIGIN IN RETAIL IN THE CZECH REPUBLIC

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**Objectives:** The aim of this study was to examine S. aureus isolated from food of animal and non-animal origin obtained from the retail sale in the Czech Republic for the presence of genes encoding resistance to methicillin and to characterize these strains. **Material and Methods:** 271 of S. aureus strains isolated in the years 2012-2015 from foodstuffs of both the animal (255) and non-animal (16) origin from retail sale were confirmed by PCR for the specific S. aureus fragment SA442 and the presence of mecA and mecC genes encoding resistance to methicillin. Obtained MRSA isolates were characterized using MLST and spa typing methods and further screened for the presence of virulence factors as Panton-Valentine leukocidin, toxic shock syndrome toxin and exfoliative toxins together with the assessment of the resistance to a panel of 11 antimicrobial agents using the disk diffusion method. **Results:** Within this study 13 MRSA strains (5%) were obtained, 11 originated from food of animal (10 raw and 1 processed) and 2 from non-animal origin (1 frozen fruit and 1 raw vegetable). The resistance to methicillin was encoded by mecA gene in all strains with the exception of one isolate from non-pasteurized cow´s milk which harbored the mecC

gene only. MLST method determined that in strains of animal origin 7 MRSA strains (64%) belonged to livestock-associated ST398 (various spa types) and 4 (36%) to other ST types. On the contrary none of the MRSA strains of non-animal origin belonged to ST398. None of the strains carried pvl, tst or exfoliative genes (eta, etb). The strains often showed multiple resistance to antimicrobial agents, mainly to tetracycline (77%), erythromycin (54%), co-trimoxazole (31%) and aminoglycosides (23%). **Conclusion:** Limited data about the occurrence and characteristics of MRSA strains in food of animal and plant origin are available currently in the Czech Republic, EU and USA. This study shows that there is a potential of spread of MRSA strains also via food. The sources of contamination can be either food producing animals or contaminated environment together with the human sources. This study was supported by projects MZCR NAZV KUS QJ1210284 and QJ1510216.

## 10

## PREVALENCE AND CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* FROM LIVESTOCK, LABORATORY ANIMAL AND HUMAN IN BANGLADESH PERSPECTIVE

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Antibiotics are widely used in human and veterinary medicine for the prevention and treatment of infectious diseases. This practice has led to the emergence of antimicrobial-resistant bacteria in both humans and animals. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global human health problem causing infections in both hospitals and the community. The prevalence of infection and antimicrobial susceptibility of *Staphylococcus aureus* in pet dogs, laboratory animals ,frozen chicken ,raw cow milk and hospitalized patients were studied. The PCR was performed to detect these microorganisms and the traditional cultural techniques were performed based on bacteriological analytical manual. Culture sensitivity test and antibiogram study was done to determine the antibiotic sensitivity pattern of Staphylococcus isolates against commercially available antibiotic discs. A total of 58 Staphylococci were isolated from mice, gunia pig ,rabit and livestock among them 33 were coagulase positive *Staphylococcus aureus* and 25 were other Staphylococcus spp. (CNS) Bacteria were recovered from pet dog all nansal swab samples (n=40) which were identified as Staphyococcus spp. All isolates were coagulase negative .Antibiotic sensitivity pattern of Staphytococcat isolates suggested that these were resistant to penicillin. 95.83% of thefrozen chiken ringe samples(n=150) were found to be infected with S. aureus. About 68.53% samples were coagulase positive Staphylococcus and 31.46% were negative. the Antibiotic sensitivity pattern of Staphylococcus isolates against eight commercially available antibiotic discs showed 100% resistant to Ampicillin, more than 80% were resistant to Oxytetracyclin, Doxycycline hydrochloride and Amoxicillin. Ciprofloxacin showed 77.5%, Cephalexin 38.33% and Gentamycin showed the least resistance 13.33%. The isolates were found from raw milk(n=47) as resistant to Penicillin (100%), Erythromycin (75%) and Amoxicillin (100%). On the other hand, the isolates were sensitive to Ciprofloxacin (83.33%), Oxacillin (100%), Cloxacillin(100%) and Neomycin (100%). The isolated S. aureus showed increased resistance to broad spectrum antibiotic (e.g., Ciprofloxacin). As many people have a tendency to drink raw milk and raw milk products,there is high risk of S. aureus infection in human. Out of 160 patient admitted in hospital 131pateint were Staphylococcus positive ,whereas 40 pateient have MRSA. Antibiotic resistant pattern were found 94% to Cotrimoxazole, 84% resistant to Erythromycin, 71% Nalidixicilin acid ,49% Doxyctcyclin, Ciprofloxacin showed 49%, Vancomycin30%, Cephalexin 21%, Lenezolis17%,Oxacilin6%and Penicilin showed the least resistance 1%. But it is clear that MRSA is a potentially important

veterinary and public health concern in Bangladsh that requires a great deal more study to enhance understanding and effective response.

## 11

## WHOLE GENOME SEQUENCING OF MRSA CC398 - INDICATION OF SEVERAL INTRODUCTIONS TO NORWAY

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**Introduction** Until recently, the Norwegian pig population was regarded as nearly free from MRSA. MRSA CC398 was first detected in samples from Norwegian pigs in anonymized surveys conducted in 2011 and 2012, both studies indicating a low prevalence. The first detections of CC398 from an identifiable swine herd occurred in 2013 where MRSA CC398 *spa*-type t034 was isolated. These initiated epidemiological investigations and subsequent population screenings. **Material and methods** From 2013 to 2015, 4 epidemiologically separate clusters with MRSA CC398 positive herds were identified through contact tracing from initial findings. In total, 41 herds were found positive for MRSA CC398. From all positive herds, 2 MRSA isolates were selected for whole genome sequencing and SNP analysis. In addition, CC398 isolates collected from Norwegian slaughterhouses in 2011 and anonymized swine herds in 2012 were included. Finally, 82 CC398 isolates from humans living in Norway were included, among these were isolates from **36** individuals with known contact to the affected swine herds and slaughterhouses. Some of these individuals had earlier been in contact with swine herds outside Norway. The genome sequences were compared to a global reference collection of both MSSA and MRSA CC398 isolates. **Results and Discussion** The SNP analysis showed that the Norwegian CC398 isolates were clearly divided into 4 clusters which completely corresponded to the clusters identified through the epidemiological investigation. Cluster I-II and IV contained isolates of *spa*-type t034 whereas isolates in cluster III belonged to *spa*-type t011. The clusters contained 46, 24, 12, and 19 isolates, respectively, collected from swine herds or slaughterhouses. Clusters I and II contained 26 and 9 isolates from humans, respectively. Cluster III contained one human isolate as well as 20 human isolates more distantly affiliated to this group. Cluster IV did not contain closely affiliated human isolates. Cluster I and II corresponded to outbreaks in Eastern and South-Western Norway, respectively. These clusters were, however, closely related to each other and to a group of isolates collected from swine herds in Denmark. Furthermore, an isolate collected at a slaughterhouse in 2011 was located in cluster I. Cluster III was most closely related to t011 isolates originating from across Central and Southern Europe. Cluster IV contained an isolate collected from an anonymized swine herd in 2012 and was also related to a distinct genotype found in Denmark. The import of live pigs to Norway is negligible. However, there has been a quite extensive use of farm workers from different European countries in addition to Danish farm consultants. The genetic analysis indicate a close relationship between the Norwegian CC398 isolates and isolates from Denmark and also other European countries. Furthermore, the typing of the slaughterhouse isolates indicate that the primary introduction to Norway occurred several years before MRSA-positive swine herds were identified in 2013. These findings indicate four separate introductions of MRSA CC398 to Norway from other European countries. Due to the negligible import of pigs to the country, the bacteria have most probably been by introduced by humans.

## 12

## MRSA CC1 T177, LIVESTOCK ASSOCIATED?

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Since 2013, Norway has practiced contact tracing and extensive sampling when detecting methicillin resistant *Staphylococcus aureus* (MRSA) in samples from pig holdings. From 2014 a national surveillance program for MRSA in pigs was introduced. This presentation describes this first detection of MRSA clonal complex (CC) 1, *spa*-type t177 in Norwegian pig herds and the preliminary results of the following epidemiological and bacteriological investigation. Pooled swab cloths from pigs and the environment of pig holdings were collected by personnel from the Norwegian Food Safety Authority (NFSA) and analyzed at the Norwegian Veterinary Institute using methods previously described by EFSA. Selected MRSA isolates were *spa*-typed and analyzed for *mec*A at the Norwegian Reference Laboratory for MRSA and whole genome sequencing was performed at Statens Serum Institut in Denmark. MRSA CC1 t177 was detected in samples from two finisher pig herds from the southwestern part of Norway. Contact tracing revealed that the two finisher pig herds had received grower pigs from the same sow herd. Samples from the sow herd also demonstrated CC1 t177. The sow herd did not purchase replacement pigs from others, and personnel were considered the most likely source of MRSA introduction to the sow herd. In addition to the two finisher herds described, the sow herd also supplied ten other finisher pig herds. MRSA t177 was detected in samples from six of these ten herds. All the finisher pig farms also kept other livestock and/or companion animals, which were also sampled as a part of the contact tracing. From other animals, MRSA t177 was only detected in samples from sheep housed in the same building as one of the MRSA positive finisher herds. Measures were imposed by the NFSA to eradicate MRSA from all positive pig holdings. Results from WGS will be presented. The preliminary results from this outbreak of MRSA CC1 t177 indicate human introduction of MRSA CC1 t177 to a sow herd and further transmission by the trade of live pigs to eight out of twelve finisher pig herds. These results indicate that it could be relevant to define MRSA CC1 t177 as a livestock associated MRSA.

## 13

## METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AND NON-S. AUREUS PREVALENCE IN BOVINE MILK SAMPLES

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Mastitis infections are frequently caused by Staphyloccocci and S. aureus is a major pathogen. Nevertheless non-S. aureus staphylococci (NAS) are frequently isolated from milk control samples. They may represent a reservoir of antimicrobial resistance genes for S. aureus. Therefore we investigated antimicrobial resistance amongst staphylococcal species isolated from milk samples taken in the control of clinical and subclinical mastitis. We collected 88 staphylococcal isolates recovered in the milk control centre of the Flemish Belgian region. The isolates were classified as methicillin susceptible S. aureus (MSSA), methicillin resistant S. aureus (MRSA), methicillin susceptible NAS (MSNAS) or methicillin resistant NAS (MRNAS) by the 16S rRNA-mecA-nuc

triplex PCR. S. aureus were typed by spa typing, while NAS were identified at species level by the tRNA intergenic spacer PCR. MRSA and MRNAS were also subjected to SCCmec typing and susceptibility was determined by a microbroth-dilution method using epidemiological cut-off values (Eucast). A total of 41 isolates were identified as S. aureus [MSSA (17), MRSA (24)]. MSSA isolates belonged to spa types t008 (1), t011 (2), t224 (1), t337 (4), t524 (1), t529 (5), t1403 (1) or non-typeable (NT) (1). MRSA isolates belonged to spa types t011 (19), t034 (2), t567 (1), t740 (1) or NT (1). All the MRSA carried livestock associated SCCmec types: IV (14) or V (10). Most MRSA isolates were non-wild type (NWT) for tetracycline (23), trimethoprim (22), gentamicin-kanamycin (21), ciprofloxacin (19), streptomycin (17), and erythromycin-clindamycin (14). Few MRSA were NWT for sulfamethoxazole (7), chloramphenicol (4), tiamulin (4), and synercid (2). Of the NAS, MSNAS (41), were identified as S. haemolyticus (12), S. chromogenes/hyicus (10), S. epidermidis (6), S. warneri (2), S. simulans (1), S. arlettae (1), and S. sciuri (1). Eight MSNAS isolates were not successfully typed at species level. MRNAS (6) were identified as S. epidermidis (5) or S. haemolyticus (1). The S. epidermidis carried SCCmec IV, while the S. haemolyticus carried SCCmec V. All S. epidermidis isolates were NWT for, tetracycline, trimethoprim, sulfamethoxazole and streptomycin. Some S. epidermidis isolates were also NWT for ciprofloxacin (4), erythromycin (2), clindamycin (1), chloramphenicol (1), fusidic acid (1), and gentamicin-kanamycin (4). The S. haemolyticus isolate was NWT for ciprofloxacin, erythromycin, clindamycin, synercid, tetracycline, gentamicin, and trimethoprim. Nearly 60% of the S. aureus isolates were MR, which is much higher than the previously found 10% in Belgium. All but a typical hospital clone (t740) and a NT had characteristics of typical LA-MRSA. 13% of the NAS were MR. Seen the high prevalence of MRSA, the NAS do not represent a significant reservoir for methicillin resistance in S. aureus. Acks: MAA was supported by Fundación Alfonso Martín Escudero.

## 14

## STAPHYLOCOCCUS SPP. ISOLATED FROM BOVINE SUBCLINICAL MASTITIS IN DIFFERENT BRAZILIAN STATES: MECA GENE DETECTION AND IDENTIFICATION OF CLONAL PROFILE

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In the treatment of mastitis, methicillin resistance in *Staphylococcus* spp. caused by the *mec*A gene expression is of particular interest because this mechanism imparts resistance to all β-lactam antimicrobial and these resistant strains can be spread in the herd. Thus, the aim of this study was to verify the presence of the *mec*A gene and the characterization of clonal profile of *Staphylococcus* spp. isolated from bovine milk with subclinical mastitis. We studied 181 samples of *Staphylococcus aureus* and *Staphylococcus* negative coagulase isolated from bovine subclinical mastitis in six Brazilian states, including Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais and Pernambuco, representing the South, Southeast and Northeast areas in Brazil, and maintained in the bacteria bank of Embrapa Dairy Cattle. After the DNA extraction of all samples, the detection of *mec*A gene by PCR technique and the clonal relationship of all strains by Pulsed-Field Gel Electrophoresis (PFGE) were made. The results revealed the presence of the *mec*A gene in 8 samples of *S. epidermidis* from different Brazilian states. The PFGE allowed the identification of four major clusters (≥3 samples) *S. epidermidis* with ≥80% similarity. Two isolated from the cluster 1 *S. epidermidis*, from the state of Minas Gerais and São Paulo, showed 88% similarity and both had the *mec*A

gene. The other six strains grouped in clusters 2 and 3, were from Parana, Santa Catarina and Minas Gerais. For the other species studied, there was the formation of six clusters of *S. aureus*, three of *S. chromogenes*, and one of *S. saprophyticus*, revealing a large number of clusters involving isolated samples from different states. The knowledge of clonal profiles and resistance among isolates of *Staphylococcus* spp. is essential, especially for preventing and controlling the spread of antimicrobial-resistant strains. The presence of the *mec*A gene of *S. epidermidis* proves that the bovine can lodge in the milk resistant strains that can be the source of resistance genes for *Staphylococcus aureus*, most virulent species and can be spread to man. The analysis of clonal profile indicated the formation of major clusters, so it is possible to conclude that the low genetic diversity among the isolates, may be related to a possible endemic feature of mammary infections in the herd from different Brazilian states. Financial support: doctoral fellowship - FAPESP n. 2012/24135-0 and FAPESP 2015/01401-4.

## 15

## RESISTANCE TO B-LACTAM MEDIATED BY GENE PRESENCE MECA AND THE B-LACTAMASE HYPER PRODUCTION IN STAPHYLOCOCCUS SPP. ISOLATED FROM BOVINE SUBCLINICAL MASTITIS IN DIFFERENT BRAZILIAN STATES

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*Staphylococcus* spp. present a high level of resistance to antimicrobials, especially to the β-lactams, favoring its persistence in the dairy herd. The detection of the *mec*A gene is considered as a prediction of that resistance. Thus, the aim of this study was to verify the presence of the *mec*A gene and the overproduction of β-lactamase enzyme in *Staphylococcus* spp. isolated from bovine milk with subclinical mastitis. For this purpose, we studied 181 strains of *Staphylococcus aureus* and coagulase negative *Staphylococcus* (CoNS) isolated from bovine subclinical mastitis in six Brazilian states, including Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais and Pernambuco, representing the region South, Southeast and Northeast of Brazil kept in the bank of Embrapa Dairy Cattle, Minas Gerais. The resistance to oxacillin was determined by E-test and detection of *mec*A gene by PCR. The production of β-lactamase was determined by using discs impregnated with nitrocefin (chromogenic cephalosporin Cefinase-BBL). The overproduction of β-lactamase tested with amoxicillin discs (20mcg) and clavulanic acid (10mcg) was performed on all samples that showed resistance to oxacillin through the phenotype method and showed no presence of the *mec*A gene. The determination of Minimum Inhibitory Concentration (MIC) by the E-test revealed 99% of *S. aureus* susceptibility to methicillin, while the CoNS showed 32% resistance to this drug, and of these, 8 (4.4%) contained the *mec*A gene and all belonging to the species *S. epidermidis*. As for the overproduction of β-lactamase of 28 samples tested, 27 (96.4%) showed overproduction of β-lactamase. These results indicate the limitation on the use of beta-lactam antibiotics and attention has to be drawn to the need of using these tests in routine. The production of this enzyme by *Staphylococcus* spp. explains why their survival in an infectious focus, despite the use of β-lactam antibiotics.In addition, other pathogenic micro-organisms, sensitive to β-lactam antibiotics can remain alive in an infectious process in the presence of β-lactam antimicrobial, when concurrently present with strains of *Staphylococcus* spp. β-lactamase producers. Financial support: doctoral fellowship - FAPESP n. 2012/24135-0 and FAPESP 2015/01401-4.

## 16

### CASES OF LIVESTOCK-ASSOCIATED MRSA CC398 INFECTION IN A PERSON WORKING IN A PIGGERY AND MRSA TRANSMISSION TO A HOUSEHOLD MEMBER

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**Introduction:** In the Czech Republic the incidence of LA-MRSA in pig breeding farms is not systematically monitored and reported, actual prevalence is therefore unknown. **Objectives:** The aim of this study was to investigate the incidence of LA-MRSA among staff at the breeding farm and to follow transmission of these strains from animals to personnel with direct and indirect contact with colonized animals. **Material and Methods:** The farm consisted of approx. 100 sows, 2 boars and 3500 piglets per year. On the farm there were two permanent employees with direct contact with animals and five with temporary jobs (one veterinarian and four part timers). Examined samples from animals and humans were manufactured in accordance with the Commission Decision 2008/55/EC using sterile 3MTM Sponge-sticks (3M, USA) or cotton swabs. The sponges and swabs were re-hydrated with Buffered Peptone Water and after transferred in Mueller-Hinton broth with 6.5% NaCl. After incubation at 37 °C overnight the suspension was seeded on BrillianceTM MRSA 2 Agar. Suspect colonies were analyzed by PCR method for the detection of S. aureus specific fragment and screened for the presence of mecA and mecC genes. All MRSA isolates were subjected to the ST398-specific PCR and spa typing. **Results:** Swabs from animals were confirmed to be positive for LA-MRSA CC398. Only the permanent staff constantly coming into direct contact with pigs were positive for MRSA. A man (45 years) working at the farm for 5 years was positive (nose, throat), but without any clinical symptoms. The second personnel a 38 years old female working on a pig breeding farm for more than 5 years, suffering from chronic itching of the face skin, nose and ears. Gradually she has been affected by chronical dermatitis. This woman lives in a household with her husband and two sons. One of the sons is colonized (nose, ears, throat) without any clinical symptoms by the identical strain of MRSA as his mother. **Conclusion:** Mother visited the dermatologist, who without any bacteriological investigation of the etiological agent treated her with penicillin. Infection was suppressed for a short time on the face skin, a positive finding, however, remained in the nose, ears and throat. People who regularly work with pigs are more likely to be exposed to MRSA. They should inform their physician if they suspect an infection or before undergoing a surgery. This study was supported by projects NAZV KUS QJ1210284 and QJ1510216.

## 17

### RECOVERY OF ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM SURFACES IN INDUSTRIAL HOG OPERATION WORKERS' HOUSEHOLDS IN NORTH CAROLINA

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**Background:** Industrial hog operations (IHOs) are a potential source of exposure to antibiotic-resistant (ABR) *Staphylococcus aureus*, including methicillin- (MRSA) and multidrug-resistant (MDRSA) *S. aureus*. We examined whether surfaces in IHO workers' households were contaminated with ABR *S. aureus*, and whether environmental exposure to IHOs might play a role in household contamination. **Hypothesis:** ABR *S. aureus* may be present on household surfaces in IHO workers' homes located in regions of high-density hog production. **Methods:** Electrostatic dust

wipes were collected from eight surfaces per household, including surfaces with high (e.g. pillow) and low (e.g. settled dust on fridge top) human contact. Settled dust samples aimed to differentiate between *S. aureus* present in a household's air column versus *S. aureus* transferred by touching surfaces. *S. aureus* were recovered from samples following enrichment and plating on CHROMagar™ *S. aureus*. PCR was used to assess the presence of *spa* (indicating *S. aureus*), *mec*A (indicating MRSA) and absence of *scn* (indicating livestock association). *S. aureus* were *spa*-typed and assigned clonal complexes (CC). Susceptibility to 12 antibiotic classes was assessed by Kirby-Bauer disc diffusion; resistance to ≥ 3 classes was defined as MDRSA. We examined four indicators of livestock-associated *S. aureus*: lack of *scn*, tetracycline resistance, and strain types CC398 and CC9. For each household, we calculated an "IHO exposure index" that accounted for a) distance to IHOs, and b) the number/type of animals grown on IHOs. We used linear regression to examine associations between household-level risk factors, including IHO exposure index, and the proportion of household surfaces contaminated with *S. aureus*. **Results:** Of 26 households sampled, *S. aureus* was detected in 23/26, MDRSA in 11/26, and MRSA in 3/26. Higher IHO exposure index was associated with a greater proportion of household surfaces positive for *S. aureus* CC398 ($p<0.05$) and MDRSA ($p<0.05$). Living with *S. aureus* nasal carriers, allowing pets on furniture, and living with a worker who reported never wearing a face mask at work were associated with household surface contamination with *S. aureus*-related outcomes. **Conclusion:** This study provides evidence that livestock-associated strains of *S. aureus* can be found in IHO workers' households. Environmental transport and household surface contamination may play a role in *S. aureus* transmission dynamics in this population.

## 18

## PREVALENCE AND DISTRIBUTION OF LIVESTOCK-ASSOCIATED METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ON DIARY FARMS IN SOUTHEASTERN PENNSYLVANIA

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*Staphylococcus aureus* is a pathogenic Gram-positive organism that has emerged as a serious public health concern. Both community-associated and hospital-associated strains of Methicillin Resistant S. aureus (MRSA) have been studied extensively in human populations. In 2005, a new strain emerged in livestock with the first case described on a pig farm in The Netherlands. Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been extensively studied in Europe but little is know about its prevalence in livestock in the USA. LA-MRSA has since been identified in cattle, poultry, and horses. The most common strain is termed Sequence Type 398 (ST398) and has been identified as a belonging to a different clonal lineage than either the community-associated or healthcare-associated strains. No prevalence data currently exist for MRSA on dairy farms in Pennsylvania. Other studies, primarily in Europe, have shown on-farm prevalence rates that range from 4-10%. The aim of this study was to determine the prevalence of LA-MRSA on dairy farms in southeastern Pennsylvania. Bulk tank milk filters were collected from 21 farms, these will be am/pm filters from the same day and an am filter from day two. Milk filters were cultured specifically to enrich for MRSA. A total of 58 milk filters were pre-enriched in Buffered Peptone Water (BPW) and incubated at 37°C for 18-24 hours. A 1 ml aliquot of the BPW was removed, added to Mannitol Salt Broth (MSB) and incubated at 37°C for 18-24

hours. A 10ul aliquot of the MSB was subcultured to Columbia CNA agar with 5% sheep blood, Mannitol Salt Agar with oxacillin and CHROMagar MRSA II plates and incubated at 37°C for 18-24 hours. Presumptive S. aureus colonies on Mannitol Salt Agar with oxacillin, and CHROMagar MRSA II plates were initially tested with for catalase production. Catalase positive organisms were tested for coagulase production and were also tested for the PBP2' protein with a latex agglutination test. A multiplex PCR assay that amplified the mecA and mecC genes was performed directly on all BPW and MSB broth samples using an Applied Biosystems 7500 Fast Real-Time PCR System. A known MRSA isolate and S. aureus LGA251 were used as mecA and mecC positive controls respectively. Of the 58 milk filters from 21 farms tested, none were positive for LA-MRSA. All BPW and MSB samples were negative by PCR and all presumptive colonies were negative. This is the first study to look for LA-MRSA on dairy farms in Pennsylvania. All milk filter samples have been negative, however, more samples will be tested, as the number of farms needed to identify an on-farm prevalence rate between 4 and 10% with 95% confidence is 140 farms. Further analysis is needed in other locations and in other species to better understand the prevalence of LA-MRSA throughout the United States.

## 19

## CHARACTERIZATION OF METHICILLIN RESISTANCE IN S. AUREUS AND OTHER STAPHYLOCOCCI ASSOCIATED WITH PRODUCTION ANIMALS, MEAT AND HUMANS

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**Background:** Antimicrobial resistant bacterial pathogens including *Staphylococcus aureus* is a significant pathogen of healthcare, community and livestock associated infections. Among strains of interest are those identified as methicillin resistant S. aureus (MRSA). **Methods:** A collection of S. aureus strains recovered from meat (n = 75), production animals (n = 717) and humans (n = 150) and non-S. aureus strains (n = 188) were examined for traits associated with methicillin resistance including the mecA gene, and the mecA variant mecALGA251 (mecC). All strains examined were identified as S. aureus, or other Staphylococcus species using Gram positive ID panels (Trek Diagnostics). A selection of strains identified as positive for the mecA gene were subtyped for SCCmec using a multiplex PCR assay. **Results:** The majority of isolates that were methicillin resistant were positive for the mecA gene. Variable results were observed in regards to the SCCmec types with types II, IV, and VI appearing to be the most common types detected. Isolates that were negative for the mecA gene were screened for the mecALGA251 variant using degenerate primers designed to detect the presence of the mecALGA251 but subsequently failed to amplify for the mecC gene. All of the human strains tested positive for the mecA gene except for three strains that failed to amplify for mecA and were also negative for the mecALGA251. **Conclusion:** Methicillin resistance in MRSA and non S. aureus species is linked with the presence of the mecA gene; isolates that were positive for the mecALGA251 variant did not show the presence of mecC suggesting that the use of the degenerate primers may lead to false assumptions. The mecC gene does not appear to be linked with any swine associated strains screened to date, suggesting that methicillin resistance in swine associated staphylococci species is more likely related to the presence of mecA or other similar methicillin resistance mechanism. All three human strains that were mecA and mecC negative were most likely borderline oxacillin resistant S. aureus (BORSA) which is non-mecA mediated. Ongoing work is assessing the nature of methicillin resistance in strains negative for the mecA and mecC genes.

## 20

### EPIDEMIOLOGICAL SCREENING OF LA-MRSA CC398 FOR BACTERIOPHAGE PHI3

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**Introduction:** During the last decade, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) of the clonal complex CC398 emerged in areas with high livestock density and have been introduced in the human healthcare system. Today, little is known about factors enabling the (re-)adaptation of zoonotic MRSA lineages to the human host. Nevertheless, mobile genetic elements such as prophages serve as drivers in bacterial host adaption and pathogenesis. To investigate the role of bacteriophages in the adaptation to the human host, the proportion of LA-MRSA recovered from inpatients at a university hospital in Northwestern Germany and carrying bacteriophage phi3 was systematically assessed for the complete period after the first occurrence of LA-MRSA CC398 isolates among humans in this region. **Materials and Methods:** A PCR-based screening for bacteriophage phi3 associated genes (chp, sak, scn and sea/sep) was developed to test a representative set of LA-MRSA CC398 for the presence of bacteriophage phi3. The LA-MRSA isolates were part of the institutional MRSA collection archiving all MRSA isolates recovered from patients at the University Hospital of Münster. For this study, all MRSA CC398 isolates collected between 2000 and 2006 were included; thenceforth (2007 to 2014), 15 isolates each quarter were tested. Phi3-positive isolates were further screened for the presence of an additional bacteriophage StauST398-5pro. **Results:** Altogether, 572 isolates were screened for a truncated hlb-gene approving for the presence of bacteriophage phi3. Based on repeat pattern (BURP) analysis, 24 different CC398-associated spa-types were identified with t011 (51.2 %), t034 (37.9 %), t108 (3.0 %) and t1451 (1.9 %) detected most frequently. At the beginning of the epidemic (2000 to 2006), 92 isolates were tested. Of these, only one isolate (spa-type t011) carried bacteriophage phi3. Among the 470 LA-MRSA isolates analyzed for the years 2007 to 2014, 17 isolates were found carrying bacteriophage phi3. In phi3-positive isolates, four different immune evasion cluster (IEC) types (IEC B (55.6%); IEC E (22.2%); IEC A (11.1%) and IEC C (5.6%)) and one unidentifiable IEC were identified. Furthermore, five isolates were found to be positive for the bacteriophage StauST398-5pro, which is associated with the human-adapted LA-MRSA subpopulation. **Conclusion:** Within the last 15 years, LA-MRSA clonal lineages have increasingly gained attention as they pose a considerable health risk to humans, especially to persons with direct contact to livestock. The increasing rate of MRSA CC398 isolates from humans carrying an IEC-encoding bacteriophage phi3 could indicate an ongoing re-adaptation process of this zoonotic S. aureus lineage to the human host.

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### LIVESTOCK-ASSOCIATED MRSA IN THE HUMAN GENERAL POPULATION

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**Introduction:** The increasing prevalence of livestock-associated methicillin resistant S. aureus (LA-MRSA) burdens the healthcare system, particularly in those regions with high density of pig farming, such as the North-Western part of Germany. In addition to LA-MRSA transmission by farmers and their families, there is increasing evidence for an introduction of LA-MRSA into healthcare facilities

by patients without known livestock exposure. However, there are only little data available from the human general population regarding colonization with LA-MRSA. Here, data sets were used to comparatively analyze distribution of different spa types and MRSA strains in German individuals with and without regular contact to livestock. **Methods:** In a prospective cohort study, nasal swabs were taken from non-hospitalized persons aged from 7 to 97 years. Up to three consecutive swabs were obtained from the nasal vestibules of each participant (with a time interval of 4-6 months between each swab). S. aureus isolates were subjected to susceptibility testing, spa typing and detection of genes encoding various virulence factors, such as lukS/F-PV, lukE-lukD, lukM, tst, sea-sej, eta-etb, etd, and edinA-edinC. **Results:** The overall prevalence of nasal S. aureus colonization was 41.0% throughout the complete study group of 1,878 individuals. Fifteen participants (0.8%) carried MRSA of which all but two isolates harbored mecA, but no PVL-encoding genes. Within the study group, 61 individuals (3.2%) were employed in jobs with direct contact to livestock (farmers, veterinarians, butchers). While the proportion of persons with nasal S. aureus carriage was similar between individuals with (39.3%; n = 24) and without (40.9%; n = 744) regular livestock contact, the proportion of MRSA on all S. aureus isolates was significantly higher in persons with livestock contact (25%; n = 6 vs. 1.2%; n = 9). In persons with regular livestock contact, MRSA were associated with spa types t011 (n = 4), t127 and t1081 (each n = 1). In the remaining population, spa types t003 (n = 2), t009, t084, t159, t230, t469, t670 and t1017 (each n = 1) were detected. From a total of six LA-MRSA isolates, three were tested positive for at least one of the virulence factors studied (sec, n = 2; seg/sei, n = 2; seh, n = 1; eta, n = 1; edinA, n = 1). **Conclusion:** MRSA carriage was generally rare, but significantly higher in individuals with regular contact to livestock and also mostly associated with spa types indicative for livestock-associated acquisition. In particular, spa type t011, a typical representative within the group of LA-MRSA, accounted for 27% of all MRSA in the general population. The high diversity of MRSA strains regarding genes encoding virulence factors indicates the high capability of S. aureus to quickly adapt to its environment.

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## METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RETAIL MEAT SOLD IN ABAKALIKI, EBONYI STATE, NIGERIA

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Meat and meat products have been an important source of antimicrobial resistant organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA). **Objective:** The aim of this study was to identify and to characterize methicillin-resistant staphylococci (MRS) isolated from retail meat sold in Abakaliki, Ebonyi State. **Materials and Methods:** Three hundred raw meat samples including beef (n=100), chicken(n=100), chevron (n= 100) were collected from Abakaliki abattoir 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. Confirmed *S. aureus* isolates were subject to additional characterization, including antibiotic susceptibility testing using Kirby-Bauer disk diffusion method. **Results:** Out of the 300 samples, 79 representing 29.3% were contaminated with *S. aureus*. The distributions of the bacteria are as follows: beef (n=26), chicken (n=30) and chevron (n=23). Molecular typing is being completed and will be presented. The antibiotic susceptibility

studies showed an alarming level of resistance to all the tested antibiotics reflecting multi-drug resistant strain. **Conclusion:** Our study confirms the circulation of antibiotic resistant pathogens in raw meat sold in Abakaliki abattoir and market, which could possibly play a role in the spread of antimicrobial resistance among food-borne bacteria.

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## CHARACTERIZATION OF METHICILLIN RESISTANT COAGULASE NEGATIVE STAPHYLOCOCCI OF ANIMAL AND HUMAN ORIGIN IN CHENNAI, SOUTH INDIA

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**Introduction:** In recent years, Coagulase negative staphylococci have emerged as important opportunistic pathogens in humans and animals. A great concern related to CoNS is their ability to develop resistance to various antibiotics and serve as a reservoir of antimicrobial resistance. Besides being resistant, CoNS may harbour genes encoding adhesion factors and biofilm formation which promote their colonization, invasion and pathogenesis. Methicillin resistant CoNS from non-hospital related reservoirs, such as animals and animal associated personnel represent a newer challenge in human medicine. Increasing epidemiological and genetic evidence suggest MR-CoNS to be an emerging zoonotic pathogen, with potential for transmission of resistance and virulence genes between animals and humans. Hence, the present study was undertaken to screen for resistance and virulence genes among isolates of MRCoNS from animal and human origin. **Methods:** A total of 47 non duplicate MRCoNS isolates which included clinical isolates of animal origin and carrier isolates of human origin were included in this study. Antibiotic sensitivity testing was carried out for the routinely used antibiotics. Detection of determinants implicating resistance to ß-lactams (blaZ), macrolides (ermA, ermC, msrA), tetracyclines (tetK, tetM) and trimethoprim (dfrA) were amplified using PCR. All isolates were screened for the presence of virulence genes (eno, ebps, bbp, cna, icaAD, aap and atlE) and IS256. Further, the genetic diversity of the study isolates was determined by SCC-mec and ACME typing. **Results:** Amongst the 47 isolates included in the study, highest resistance percentage was observed for tetracycline followed by erythromycin, co-trimoxazole, pristinomycin, ciprofloxacin and clindamycin in both animal and human isolates. All isolates harbored blaZ gene. msrA gene was found in 78% and 30% of human and animal isolates respectively. ermA gene was found in 30% of animal isolates and none carried ermC gene; whereas 11% carried ermC and none harbored ermA gene among human isolates. 67% and 40% of animal and human isolates carried tetK gene; dfrA gene was harbored in 15% and 26% of animal and human isolates respectively. In comparison with animal isolates, human isolates exhibited a higher percentage of virulence determinants viz., eno (97%), aap (56%), atlE (52%) ebp (22%) and icaAD (19%). The prevalence of IS256 in human and animal isolates was 48% and 40% respectively. Among the SCCmec types, type V was predominant in animal isolates, whereas type I was predominant in human isolates. Majority of the study isolates (36%) belonged to ACME type II. **Conclusion:** This is the first Indian study that has demonstrated a wider distribution of resistance and virulence determinants among MRCoNS isolates of both animal and human origin with higher percentage of virulence determinants in human carrier isolates.

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### COMPARATIVE TYPING OF CANINE AND FELINE METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS (MRSP) FROM THAILAND

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**Objectives:** Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) are of major concern in small animal clinics. MRSP are very often multi-resistant and thus, clinical MRSP infections are very difficult to treat. In contrast to other countries, so far little is known about MRSP isolates from Thailand. **Methods:** In total, 39 MRSP isolates from dogs (n=28) and cats (n=11) from Thailand collected from independent clinical cases were included into this study. Species assignment was confirmed by *pta* gene analysis and 16S rDNA sequencing. The isolates were confirmed as MRSP by their oxacillin resistance and detection of the *mecA* gene.The isolates were tested for their susceptibility to additional 29 antimicrobial agents according to CLSI recommendations. In the respective isolates,antimicrobial resistance genes were detected by specific PCR assays. Molecular typing comprised *spa* typing, *dru* typing and macrorestriction analysis with subsequent pulsed-field gel electrophoresis (PFGE). For selected isolates, multi-locus sequence typing (MLST) was performed by sequence analysis of PCR-generated internal parts of seven housekeeping genes. **Results:** All isolates were multi-resistant with resistance to at least six classes of antimicrobial agents. In all cases corresponding resistance genes were detected. In addition to *mecA*, the genes *blaZ*, $cat_{pC221}$, *aacA*/*aphD*, *erm*(B), *dfrG*, *tet*(M) and *tet*(K) were identified. Six *spa* types (t02, t05, t09, t10, t23, t72), eleven *dru* types (dt8ak, dt10ao, dt10cp, dt10cq, dt11a, dt11bo, dt11cb, dt11cj, dt11v, dt11y, dt11z) and 27 PFGE types (designated A-A9, B-B7, C, C1, D-J) were identified. The *dru* types dt11a (n=12) and dt11cj (n=8) were most common. MLST was performed for one isolate of each main PFGE pattern A-J and revealed seven types (ST45 (n=3), ST112, ST155, ST282 as well as the three novel types ST432, ST433 (n=2) and ST434). Merely seven isolates showed the same characteristics: *spa* type t10-*dru* type dt11a-PFGE type B1-resistance to nine classes of agents with six resistance genes (n=2), t02-dt11cj-A-resistance to seven classes with six genes (n=2) and t10-dt11cj-A5-resistance to seven classes with six resistance genes (n=3). **Conclusions:** This study showed that MRSP isolates from clinical cases in individual dogs and cats in Thailand are multi-resistant, harbour the same resistance genes and have similar characteristics as isolates from Europe and from North America.

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### SCCMEC IVG, A COMMON ELEMENT IN MRSA AND MRSP

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Methicillin-resistant *Staphylococcus aureus* (MRSA) and S. pseudintermedius (MRSP) belonging to several different sequence types (ST) have been spreading in many countries around the globe challenging antimicrobial treatment in both human and veterinary medicine. MRSA and MRSP are characterized by the presence of staphylococcal cassette chromosome mec (SCCmec) and are frequently resistant to a large proportion of clinically important antibiotics. Nowadays, several types of staphylococcal cassette chromosome mec (SCCmec) have been completely sequenced. However, apart from SCCmec V which has been found in both MRSA and MRSP, both

species contains unique cassettes. Whole genome sequencing of MRSP strains using Ion Torrent and Illumina technologies revealed for the first time the presence of a 22,737-bp SCCmec IVg element in MRSP ST261 from California and in MRSP ST258 (a single locus variant of ST261) from Norway, both isolated from dogs with otitis. SCCmec IVg from MRSP ST261 and from ST258 were found to be identical and shared 99% identity with SCCmec IVg from MRSA ST5 isolated from bovine mastitis in Korea and from an epidemic community-associated MRSA ST59 from human infection in Taiwan. Only up to 40-bp nucleotide differences distinguished the SCCmec IVg of MRSP from those of MRSA. In both species, SCCmec IVg is integrated at the integration site sequence (ISS) of the species specific orfX. SCCmec IVg is flanked by the two ISS, and belongs to the ccr gene complex 2 (ccrA2B2) and to the class B mec complex (IS431-mecA-ΔmecR1-IS1272). The presence of a common SCCmec element in both MRSA and MRSP indicates that SCCmec IVg has a broad potential for dissemination in different Staphylococcus species from different human and animal sources.

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## THE IMPACT OF METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS CARRIAGE ON SURGICAL SITE INFECTIONS IN DOGS UNDERGOING TIBIAL PLATEAU LEVELING OSTEOTOMY

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**Objective:** To evaluate pre-operative methicillin-resistant Staphylococcus pseudintermedius (MRSP) carriage and its effect on the development of surgical site infections (SSIs) following tibial plateau leveling osteotomy (TPLO) in dogs. **Study Design:** Prospective, multicentre study. Animals: Dogs undergoing TPLO (549 cases). **Procedures:** Dogs admitted for TPLO were swabbed for MRSP in a prospective, multicentre study involving seven hospitals from Canada and the United States. Data collected included pre-operative antimicrobial administration, potential co-morbidities, dog contact and post-operative antimicrobial administration. Univariable analysis was conducted, followed by stepwise backward logistic regression to determine factors associated with pre-operative MRSP carriage, MRSP SSI, overall SSI and post-operative MRSP carriage. **Results:** Of the 549 cases included in the study, 24 (4.4%) were pre-operatively carrying MRSP at one or more body sites. Risk factors associated with MRSP carriage included bulldog breed (OR = 14.06, p = 0.001, 95% CI = 2.974 - 66.426) and increasing weight in kg (OR = 1.094, p = <0.0001, 95% CI = 1.030 - 1.096). Surgical site infection developed in 35 (6.4%) dogs, with MRSP responsible for 11 (29.7%) of SSIs. Pre-operative MRSP carriage was the only identified risk factor associated with increased likelihood of MRSP SSI (OR = 14.8, p = <0.0001, 95% CI = 4.005 - 54.695). A protective effect of post-operative antimicrobials (OR = 0.285, p = 0.007, 95% CI = 0.088 - 0.711) against overall SSI was noted. **Conclusions and Clinical Relevance:** MRSP carriage is a risk factor for MRSP SSI and therefore investigation into measures to rapidly identify MRSP carriers and develop interventions aimed at decreasing the risk of MRSP SSI in carriers are warranted. These data provide further support of the efficacy of post-operative antimicrobials for prevention of TPLO SSI.

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## SURVEY ON MRSCP PREVALENCE IN HEALTHY AND DISEASED DOGS AND CATS

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Methicillin-resistant coagulase-positive *Staphylococci* (MRScp) have been isolated with increasing frequency both from healthy and diseased dogs and may represent a veterinary hazard because of their resistance towards many antimicrobial agents and few available therapeutic options. The objective of this study was to assess the prevalence of MRScp in groups of healthy and diseased dogs and cats in the Triveneto Region (Northern Italy) and to characterize MRScp isolates. Samples examined from July 2012 till July 2014 were classified according to the conditions of the tested animals: a) privately owned dogs and cats with skin and ear infections b) healthy breeding animals housed in kennels or catteries c) healthy stray dogs and healthy cats from feline colonies. Swabs were pre-enriched in NaCl 6,5% Müller-Hinton broth, then plated in a MRSA selective medium (MRSA II ChromAgar, BD). Suspected colonies were identified by Maldi-Tof MS and methicillin resistance was confirmed by a PCR targeting *mec*A gene. Genetic characterization, including *spa*-typing, *SCCmec*-typing, MLST, was performed on 34 dog isolates. A total of 3129 samples was examined: a) 1500 swabs from 1375 dogs and 358 swabs from 249 cats; b) 289 swabs from 97 dogs and 353 swabs from 161 cats; c) 285 swabs from 142 dogs and 344 swabs from 172 cats. The prevalence of coagulase-positive *Staphylococci* and MRScp in the three groups of animals was the following: a) dogs: 361/1375 (26.25%), 19/1375 (1.38%); cats: 33/249 (13.25%), 1/249 (0.40%); b) dogs: 16/97 (16.49%), 11/97 (12.34%); cats: 14/161 (8.69%), 9/161 (5.59%); c) dogs: 41/142 (28.87%), 0/142 (0.00%); cats: 1/172 (0.58%), 1/172 (0.58%). The prevalence of MRScp among the coagulase-positive *Staphylococci* isolates is significantly higher in healthy breeding dogs and cats than in diseased animals ($p<0.05$); healthy stray dogs and cats belonging to colonies showed the lowest prevalence. 48 out of 52 MRScp isolates were identified as MRSP (from 37 dogs and 11 cats), while 4 ones as MRSA (from 2 dogs and 2 cats). Genetic typing of 32 MRSP isolates revealed that most part of the strains belonged to Sequence Type 71, *spa*-type t02 e *SCCmec* type II-III (24 isolates) that represents the main clonal strain in Europe; two isolates belonged to ST71 t06 *SCCmec* type V. Six strains were spa-type non typable (NT): ST258 NT-SCCmec type IV (2 isolates); ST369 NT-SCCmec IV (2 isolates); and ST405 SCCmec type IV and ST301 NT-SCCmec type IV (1 isolate respectively). Two MRSA isolates were *spa*-type t127 SCCmec type IV and t515 SCCmec V. MRSP was the most frequently MRScp isolated, confirming what widely reported in other studies on companion animals. The higher number of MRScp isolated from breeding animals is likely due to the common use of antibiotics in kennels, especially around parturition. Our results confirm that ST71, *spa*-type t02, *SCCmec* type II-III represents the most frequent MRSP lineage identified in dogs in Europe.

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### METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS AMONG DOGS IN SRI LANKA

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Staphylococcus pseudintermedius is a common opportunistic pathogen in dogs and cats, mainly causing otitis media and dermatitis. Its methicillin-resistant variant, MRSP, can pose a therapeutic challenge because it is often resistant against multiple classes of antimicrobials. Much is known about the presence of MRSP in Europe and North America, which have a distinct genotype distribution of MRSP s, but studies outside these continents are sparse. The aim of this study was to investigate the presence and genotypes of MRSP in Sri Lanka. Dogs attending the veterinary hospital in Peradeniya and a few selected private clinics were sampled, with informed consent of the owner. Data about the dogs - including current and recent use of antimicrobials and reason to visit the vet - were collected in a short questionnaire. Samples were cultured by double selective enrichment and both enrichments were plated onto blood agar. Isolates of MRSP were initially identified by Gram stain, coagulase, and mannitol, and confirmed by MALDI-TOF MS. mecA-PCR was performed to test for methicillin-resistance and the isolates were typed by MLST and spa-typing. Furthermore, all isolates will be analysed by whole-genome sequencing to determine SCC-mec types and presence of antimicrobial resistance genes. In total, 199 dogs were sampled in the groin (n=98), ear (n=88), or at the skin when dermatitis was present (n=12). One sample was unidentified. Sampling took place during the course of three weeks. S. pseudintermedius was isolated from 98 samples, 9 of which were MRSP. Isolates of MRSP were of sequence type (ST) 45 (n=3), ST282 (n=3), ST121 (n=1), ST429 (n=1), and one new ST. Spa-types were t09 (n=4), t06 (n=1), t015 (n=1), and several new types (n=3). Whole-genome sequencing is in progress and results will be presented at the meeting. Seventy-four dogs were using one or more types of antimicrobials at the time of sampling. Ceftriaxone, metronidazole, and amoxicillin-clavulanic acid were the most common antimicrobials. In conclusion, MRSP is present among dogs in Sri Lanka. In Europe, the major sequence type is ST71, while in Northern America it is ST68. These genotypes were not found in Sri Lanka. The ratio between MRSP and MSSP is strikingly similar to the ratio in Western countries.

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### ABSENCE OF HUMAN INNATE IMMUNE EVASION COMPLEX IN LA-MRSA ST5 STRAINS ISOLATED FROM PIGS, SWINE FACILITIES, AND HUMANS WITH SWINE CONTACT

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**Background:** Since its first ties to swine, livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has raised public health concerns because livestock maybe the largest reservoir of MRSA outside the hospital setting. In contrast to Europe and Asia, where the primary sequence type (ST) of swine associated LA-MRSA are ST398 and ST9 respectively, US strains are more diverse with ST5, ST398, and ST9 being isolated. The presence of ST5 MRSA in swine increases concern because, unlike ST398 and ST9, ST5 MRSA is among the most prevalent lineages causing

human disease. A primary factor contributing to the clinical impact of ST5 MRSA is the capacity to acquire mobile genetic elements carrying virulence factors and resistance genes. Previous reports establishing the absence of most virulence genes in swine associated ST398 isolates and the lack of disease reports in humans occupationally exposed to swine carrying ST5 MRSA, suggests virulence factors contributing to human disease may also be uncommon in swine associated ST5 strains. **Materials:** ST5 isolates collected from swine, swine facilities, and humans with short- and long-term swine contact were PCR screened for the presence of human-specific virulence factors carried by β-hemolysin converting bacteriophages including: staphylococcal complement inhibitor, chemotaxis inhibitory protein, staphylokinase, enterotoxin A, and enterotoxin P. PCR evaluation of the β-hemolysin gene was used to determine if the gene was intact. **Results:** All LA-MRSA ST5 isolates screened lacked the virulence factors found in β-hemolysin converting bacteriophages. An intact β-hemolysin gene was also found in all isolates indicating the absence of β-hemolysin converting bacteriophages in these isolates. **Conclusion:** The absence of β-hemolysin-converting bacteriophages and their associated human specific virulence factors is consistent with the hypothesis that LA-MRSA strains that are adapted to swine have a reduced capacity to cause significant disease in immunocompetent humans.

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## *STAPHYLOCOCCUS AUREUS* ISOLATED FROM FREE-LIVING RODENTS TRAPPED IN GERMANY: CLONAL BACKGROUND, METHICILLIN RESISTANCE AND SELECTED VIRULENCE FACTOR

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**Introduction:** To date, approximately 3,000 different multi-locus sequence types have been identified for the important opportunistic pathogen *Staphylococcus aureus* (www.mlst.net). However, only a limited number of these sequence types (e.g. ST8) is frequently reported for isolates of different geographic and host origin, including methicillin-resistant and -susceptible variants. Here we report on the prevalence, genetic composition and background of *S. aureus* isolated from the nose of free-living small rodents trapped at different sites in Mecklenburg-Western Pomerania and Thuringia, including one methicillin resistant strain (MRSA) harboring *mec*C. In a pilot study, 100 rodents were trapped by the network "Rodent-Borne Pathogens" in 2011-2013 at different sites in Germany. All animals were frozen at -20 °C after trapping. Nose tissue was homogenized and subsequently cultured in an *S. aureus* enrichment medium for 48 hours and plated on mannitol salt agar dishes in serial dilutions. Then, all morphotypes that could be distinguished visually were subcultured on blood agar plates. *S. aureus* identity was confirmed by *gyrase* gene-specific PCR. All isolates were initially subjected to *spa* typing, and next-generation sequencing (Illumina, MiSeq®) was performed for all 29 *S. aureus* isolates representing diverse origins and trapping sites. Further data analysis was carried out by use of Ridom SeqSphere plus®. **Results:** *S. aureus* was isolated from 29 of 100 rodents belonging to five different species. The majority of the *S. aureus* isolates (24/29) belonged to sequence type ST8-t211. Analysis of altogether 2332 targets based on MLSTplus (n=1811 loci) together with the accessory gene content (n=521 loci) revealed a very close relationship among these isolates from different federal states, trapping sites and host

species. Two isolates belonged to sequence type ST130-t843, including one *mec*C-positive MRSA, harboring a complete *bla*Z-*mec*C-*mec*R1-*mec*I structure (class E *mec* complex) as well as the recombinase genes *crr*A1 and *ccr*B3 and associated loci (e.g. arsenic resistance operon) described for *S. aureus* LGA251. One further *S. aureus* isolate was assigned to ST88-t2311, harboring prophage L54a and genes encoding the LukD/E leukotoxin. Two further isolates belonged to ST890-t1736 and -t1773. **Conclusion:** *S. aureus* ST8 strains, regardless if methicillin resistant or not, seem to be well established in wildlife and companion animals, as well as in hospitals and the human community. The success of certain lineages - denominated as extended host spectrum genotypes (EHSG) - in occupying multiple ecological niches and host species needs to be further evaluated. Furthermore, wild rodents seem to be a potential reservoir for *mec*C-MRSA.

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## PREVALENCE OF EXFOLIATIVE TOXIN GENE CARRIAGE IN *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATES FROM HUMANS AND DOGS

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*Staphylococcus pseudintermedius* is the leading cause of canine pyoderma and is an emerging human pathogen. Methicillin-resistant *S. pseudintermedius* (MRSP) can be harbored by asymptomatic dogs and humans can become colonized with MRSP. Staphylococcal exfoliative toxins (ET) have been implicated in skin disease in humans and dogs by causing degradation and splitting of the epidermis. Four ET genes have been identified in *S. pseudintermedius*: *speta*, *siet*, *exi* and *expB*. Isolates of S. *pseudintermedius* collected from healthy and diseased dogs (n = 515) and diseased humans (n = 45) were analyzed via polymerase chain reaction (PCR) to determine prevalence of ET genes and to determine whether there was an association between ET gene carriage and methicillin resistance. The collection contained 161 and 3 MRSP isolates collected from dogs and humans, respectively. The working hypothesis was that ET gene carriage would be correlated with health status in dogs and carriage of multiple ET genes would be correlated with methicillin resistance in humans and dogs. Five ET genotypes occurred: only *speta*, *speta+siet*, *speta+siet+exi*, *speta+siet+expB* and *speta+siet+exi+expB*. The *exi* and *expB* genes always occurred in conjunction with *speta* and *siet* in isolates from humans and dogs. In humans and dogs, most of the isolates were *speta-siet* (84 and 71%, respectively) with fewer numbers of *speta-siet-expB* (11 and 15%, respectively) and *speta-siet-exi* (4 and 12%, respectively) isolates. The *speta-siet-exi-expB* and only *speta* genotypes were not observed in human-derived isolates. There was no significant difference between ET gene carriage and overall health status in dogs. The proportions of ET genotypes were compared via Pearson's chi-squared and Fisher's exact tests. The *speta+siet* genotype had a higher probability of being methicillin-resistant ($p<0.0001$) in dogs. Contrary to the hypothesis, the *speta+siet+expB* genotype had a higher probability of being methicillin-sensitive ($p<0.003$) than resistant in dogs. There was no significant difference between ET genotype and methicillin resistance in the human isolates, but this is most likely due to low MRSP sample size.

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### THE FINISHED GENOME AND METHYLOME FOR THE VETERINARY PATHOGEN, STAPHYLOCOCCUS SCHLEIFERI

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Third generation DNA sequencing technologies allow the rapid and accurate assembly of whole bacterial genomes. This allows enhanced insight into microbial pathogenesis. Although many *Staphylococcus aureus* strains have been sequenced, there is limited data from species that routinely infect companion animals. Staphylococcus schleiferi is commonly isolated from companion animal infections, particularly pyoderma and otitis externa. Methicillin-resistant strains are common and this can pose a major problem in veterinary clinics and raises concerns about potential zoonotic transmission to humans. Lack of whole genome sequences is a major obstacle in understanding the drug resistance mechanisms, host adaptation, and zoonotic potential of S. schleiferi. Single-molecule, Real-time (SMRT) sequencing technology and de novo genome assembly was performed on four clinical S. schleiferi isolates obtained from canine ear and skin infections. The complete, ungapped genomes were assembled and refined with the SMRT Analysis package, and genome annotation was carried out using the Prokaryotic Genome Annotation Pipeline and Rapid Annotation using Subsystems Technology. The S. schleiferi genome size is approximately 2.5 Mbp, and sequencing resulted in genome coverage between 59X-140X for each isolate. Genome analysis identified adhesins and virulence factors unique to S. schleiferi. Highly strain-specific CRISPR repeat regions and genome-wide methylation patterns were identified and the analysis also identified putative loci associated with multi-drug resistance. The S. schleiferi genome was compared to S. pseudintermedius (the most prevalent staphylococcal species isolated from companion animals) and S. aureus. These whole genome comparisons identified bacterial genes putatively associated with the establishment of S. schleiferi on its canine host.

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### NOVEL PLASMIDS CARRYING A VARIANT OF THE SPECTINOMYCIN RESISTANCE GENE SPD

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**Objectives:** For more than 25 years, the Tn*554*-borne resistance gene *spc* was the only spectinomycin resistance gene known to occur in different *Staphylococcus* (*S.*) species. Since last year, two novel spectinomycin resistance genes, *spw* and *spd*, were identified in *S. aureus*. In the BfT-GermVet study, 179/248 staphylococcal isolates from diseased pigs, dogs and cats belonged to species other than *S. aureus*. Among them, seven staphylococcal isolates showed high MICs (≥512 mg/L) of spectinomycin, including three *S. hyicus* and single *S. chromogenes* and *S. equorum* isolates from pigs as well as two *S. simulans* isolates from cats. The aim of the present study was to identify the genetic basis of spectinomycin resistance among these isolates. **Methods:** Species assignment was confirmed by biochemical profiling using ID32STAPH and 16S rDNA sequencing. The isolates were tested for the presence and the genetic environment of the staphylococcal spectinomycin resistance genes *spc*, *spw* and *spd* by PCR. The plasmid location of spectinomycin resistance genes was tested by transformation into *S. aureus* RN4220 and Southern blotting. Restriction and sequence analysis were conducted to determine the plasmid types of the respective transformants. **Results:** The two feline *S. simulans* isolates, single *S. hyicus* and *S. chromogenes* isolates harboured *spc*. A single *S. hyicus* isolate carried the *spw* gene and another *S. hyicus* isolate and the single *S. equorum* isolate were

only positive for the *spd* gene. The remaining *S. hyicus* and *S. chromogenes* isolates carried the *spc* as well as the *spd* gene. Plasmid profiling followed by Southern blotting and electrotransformation revealed that only the four *spd* genes were located on plasmids with different restriction patterns and sizes of approximately 3.7 - 4.5 kb. Sequence analysis of the *spd* genes showed a 12-bp deletion at the 3'-terminus, which had no impact on the high spectinomycin MIC. **Conclusions:** This study showed that a novel functionally active variant of the spectinomycin resistance gene *spd* is present on a variety of small plasmids in different staphylococcal species. This is the first identification of plasmids carrying a *spd*-like gene in *S. hyicus*, *S. equorum* and *S. chromogenes*. It is also the first identification of the *spw* gene in a *S. hyicus* isolate. Furthermore, this study showed that more than one spectinomycin resistance gene can be present in the same staphylococcal isolate. (2445/2500)

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## DETECTION OF TOXIC SHOCK SYNDROME TOXIN1 AND PANTON VALENTINE GENES IN METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES, RESPONSIBLE FOR BOVINE MASTITIS BOVINE IN BRAZIL

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Mastitis is an inflammation of the mammary gland usually caused by bacterial infection, leading to high economic losses in dairy cattle due to reduced production of milk and the devaluation of the animal. One of the main responsible for bovine mastitis is *Staphylococcus aureus* and its constant presence can select resistant isolates. It is common to find strains of *S. aureus* resistant to antibiotics, which can be explained by the indiscriminate use of these medications. Methicillin-resistant *S. aureus* (MRSA) isolates carry the *mec*A gene located in a mobile genomic island, allowing this gene exchange between different species of *Staphylococcus*. Furthermore, there are several virulence factors involved in pathogenesis of mastitis as Panton Valentine and Toxic Shock Syndrome toxins. Thus the aim of the study was to evaluate the presence of these virulence factors and strains resistant to methicillin in *S. aureus* isolated from milk of cows with mastitis subclinical on a farm in Brazil. A total of 122 strains were collected in twelve months on a farm in Brazil. For MRSA detection, the strains were subjected to disc-diffusion test according to CLSI to antibiotics oxacillin and cefoxitin, when found strains resistance to at least one antibiotic in disc-diffusion test, the strains were subjected to PCR reaction to detect the *mec*A gene. The factors of virulence were evaluated by PCR for detection of panton-valentine and TSST genes. The results showed that 7 strains were resistance at least one antibiotics and 2 showed intermediate resistance. Among these strains, 4 encoded the *mec*A gene. Moreover, the Panton-Valentine gene was observed in 4 strains and the TSST gene was observed in 58 strains. Due to the indiscriminate use of antibiotics, MRSA infections are becoming more common in dairy herds, making it difficult to control this pathogen.

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## DISCOVERING PLASMIDS IN MRSP USING GENOME SEQUENCING

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Staphylococcus pseudintermedius is a pathogen capable of causing infection in a wide variety of animals and is increasingly methicillin- and multidrug-resistant. Often, resistance to antimicrobials and disinfectants are conferred through mobile genetic elements (MGEs) such as plasmids and bacteriophage.

While S. aureus can infect dogs and cats and S. pseudintermedius has been reported to infect humans, these cross-species infections are usually transient. However, co-colonization could provide the opportunity for gene exchange and interspecies transfer of antibiotic resistance and represent an unquantified public health risk. Plasmid carriage rates in Staphylococcus are estimated to be less than 20% and currently there are no complete plasmid sequences for S. pseudintermedius in Genbank. We hypothesized that plasmids conferring resistance should be found frequently as extrachromosomal elements in a majority of antibiotic-resistant S. pseudintermedius isolates. We selected 8 methicillin-resistant Staphylococcus pseudintermedius (MRSP) isolates based on their multi-locus sequence types and antibiotic susceptibility profiles for genome sequencing using a combination of single read, paired end, long read and genome mapping tools. Manual assembly and analysis of high read number contigs was necessary to predict the presence of several low copy number circular plasmids. Surprisingly, 7 of 8 MRSP isolates appeared to harbor MGEs conferring antibiotic or disinfectant resistance, and a total of 12 unique plasmids were assembled. Commercially available plasmid extraction kits were unable to verify the presence of most plasmids per standard protocol. However, predicted plasmid sequences were used to design long-range PCRs that verified the circular and extrachromosomal nature of these elements. A subsequent PCR screen of our MRSP library revealed their presence across multiple Sequence Types. The efficacy of using genomics to identify plasmids involved in antibiotic resistance proved to be higher than common plasmid extraction protocols, potentially due to very low copy number per cell. From genome sequence alone we were able to resolve and confirm the presence of MGEs in 87% of MRSPs tested. Many of these plasmids had high similarity to those found in other Staphylococcus species, suggesting that horizontal transfer of plasmids containing antibiotic resistance is more frequent and potentially more significant than previously realized. Qualifying these findings will be critical to surveillance and efforts to monitor and control the spread of plasmid-mediated antimicrobial resistance.

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## NOVEL BACTERIOCINS: AN EFFICIENT ALTERNATIVE TO COMBAT METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Bacteriocins are ribosomally synthesized antimicrobial peptides (AMPs) and most of them are post-translationally modified. Due to their proteinaceous nature and combined killing mechanisms against target host they become potential alternative to traditional antibiotics. In comparison to enormous microbial diversity, the known numbers of bacteriocins are very less. Therefore, in the current research we made an attempt to isolate, identify and characterize novel bacteriocins from complex soil environments. To accomplish this we have used genomics approach where conserved genes involved in transportation and immunity of the AMPs were tested among different genera by genome mining. We have also used this to compare the genome sequences of our isolates that produced AMPs. Two isolate from each of Brevibacillus and Paenibacillus genera were identified to produce novel AMPs and further characterized in detail. The purified AMPs obtained from these isolates displayed activity against Methicillin resistant *Staphylococcus aureus* and Vancomycin resistant Enterococcus faecium. The gene clusters involved in production of these AMPs were identified from whole genome sequence by using partial N-terminal sequence of purified peptide. While Brevibacillus AMPs characterized as a disulphide containing bacteriocins, the Paenibacillus AMPs found to be type I lantibiotics as the gene clusters involved in

their biosynthesis were found to be present in their genome sequences. Among these, lantibiotics from Paenibacillus strains were found to be more effective against Methicillin resistant *Staphylococcus aureus* as shown by killing kinetics and electron microscopy in a time vs concentration dependent manner. Hemolysis assay (hemoglobin release) revealed these AMPs as non-hemolytic in nature. Thus, they are anticipated to combat drug resistant bacterial infections without exhibiting cytotoxicity. Interestingly, their genetic and structural characterization revealed high similarity with eukaryotic AMPs called as defensins. Therefore, it is worth to investigate on co-evolution of AMPs from prokaryotes and eukaryotes.

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## MULTI-DRUG RESISTANCE OF *STAPHYLOCOCCUS AUREUS* IN PROCESSED CHICKEN AND RESCUING THE EFFECT OF NON-ACTIVE ANTIBIOTICS WITH NATURAL BERRY POMACE EXTRACTS

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While *Staphylococcus aureus* specifically methicillin-resistant S. aureus (MRSA) remains an important public health problem and a most common cause of clinical/nosocomial infections. Multidrug resistant S. aureus has become a common threat to the consumers and the people working in food animal production. Several studies have confirmed higher occurrence of multidrug-resistant S. aureus in poultry meat worldwide, but studies to measure its prevalence in poultry specifically organic/pasture poultry the US are scarce. Moreover, alternative approaches for prognosis and effective treatment against infection are required more than ever. **Purpose:** The purpose of this study was to investigate the prevalence of S. aureus in processed poultry meats collected from farmers markets, organic and conventional retail supermarkets in Maryland and the DC metropolitan area and determine the antibiotic resistance pattern We also evaluate the synergistic effect of bioactive extracts from berry pomaces against S. aureus.isolates including MRSA and rescue the inactive antibiotics supplemented with bioactive berry pomaces extracts. **Methods:** A total of 814 including 405 conventional and 409 organic processed chicken meat samples were collected from farmers markets, organic and conventional retail supermarkets in Maryland and the DC metropolitan area. S. aureus was identified with biochemical tests and confirmed by PCR. Antibiotic resistance of the isolates was determined with agar dilution method as well as evaluated the presence or absence of specific antibiotic resistance genes. Synergistic effect of bioactive extracts of berry pomace and drugs were checked in broth dilution methods. **Results:** A total of 24 S. aureus isolates were identified. S. aureus was detected in 2.93% (12/409) and 2.96% (12/405) of organic and conventional retail chicken meats, respectively, but a higher 3.65% (8/219) from farmers market meats. Resistance to tetracycline, methicillin, vancomycin, ciprofloxacin, erythromycin and gentamycin have been tested. Among the conventional retail meat isolates, 58.33% were resistant to a single or multiple antibiotics tested regardless the presence and absence of antibiotic resistance gene. Only one isolate was resistant to both methicillin and vancomycin. No isolates from the organic retail meats were observed to be resistant against any of the antibiotics tested. We tested the synergistic effect of bioactive extracts from berry pomace on methicillin and erythromycin to inhibit the growth of MRSA isolates and found that bioactive extracts from berry pomaces could reduce the MIC and make sensitive. **Significance:** This study reveals the higher risks of farmers market meat in terms of higher contamination with S. aureus. However, multidrug-resistance and methicillin resistance was only observed in the conventional retail meats isolates.

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### ORANGE OIL TERPENES AS ANTIBIOTIC ALTERNATIVES IN THE TREATMENT OF *STAPHYLOCOCCUS AUREUS* ASSOCIATED MASTITIS

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**Introduction:** Bovine mastitis costs the U.S. dairy industry $2 billion annually, and *Staphylococcus aureus* is one of the major causative agents. Antibiotic therapy has low cure rates and can contribute to antibiotic resistance. Orange oil terpenes have been documented to have antimicrobial effects and should be examined as alternatives to antibiotics. **Hypothesis:** Four orange oil terpenes, citral, decanal, linalool, and valencene, may reduce growth and pre-formed biofilms of *S. aureus* as well as alter interactions between *S. aureus* and bovine mammary cells. **Methods:** Minimum inhibitory concentrations (MIC) were determined for the terpenes against *S. aureus*. The terpenes were then tested in their ability to inhibit *S. aureus* growth over a 72 h period as well as their ability to interfere with biofilm formation. The terpenes were checked for cytotoxicity with an immortalized bovine mammary cell line (MAC-T). The terpenes were used to treat infected MAC-T cells. Associated and invaded *S. aureus* were enumerated and gene expression was measured through qPCR. **Results:** Only citral and linalool were found to have antimicrobial effects on *S. aureus* with an MIC of 0.02% and 0.12% in LB broth, respectively. Concentrations of 0.02% citral and 0.12% linalool reduced growth of *S. aureus* by 3.76 and 2.67 log CFU $mL^{-1}$, respectively, but this difference narrowed by 72 h. After 24 h, *S. aureus* was eliminated when treated with 0.04% citral and 0.24% linalool. All treatments reduced biofilms compared to the control ($A_{540}$ 0.106). The greatest reductions were at 0.08% citral ($A_{540}$ 0.076), 0.24% linalool ($A_{540}$ 0.075), and 0.48% linalool ($A_{540}$ 0.069). Citral and linalool were slightly cytotoxic, but concentrations of 0.02% citral, 0.04% citral, and 0.12% linalool were below CC50 at 29.8%, 47.7%, and 31.8% relative cytotoxicity, respectively. All concentrations used reduced *S. aureus*' association to and invasion of MAC-T cells. For invasion, 0.12% linalool had the greatest impact, a 4.02 log CFU $mL^{-1}$ reduction, while 0.04% citral had the largest decrease, 3.89 log CFU $mL^{-1}$, on association. These concentrations also altered gene expression in response to infection. **Summary:** Citral and linalool inhibit *S. aureus* growth and reduce pre-formed *S. aureus* biofilms. While they are slightly cytotoxic, concentrations below the CC50 may be used. During infection, citral and linalool can reduce pathogenic interactions between *S. aureus* and bovine mammary cells. **Conclusion:** These findings show that citral and linalool are candidates for the treatment of bovine mastitis, warranting further study. As an antibiotic alternative, it would not contribute to resistance.

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### TECHNOLOGY FOR RAPID IDENTIFICATION OF WASTE ANTIBIOTICS IN RAW COW MILK WITH SUBCLINICAL MASTITIS-RIO DE JANEIRO-BRAZIL

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Mastitis is an infection of the mammary gland caused by microorganisms, and is generally classified as clinical or subclinical. The disease affects dairy cattle worldwide, and produces economic losses due to reduced milk production and the early culling of unproductive animals. *Staphylococcus aureus* is a major cause of contagious mastitis and the presence of this microorganism in dairy herds poses a risk to consumers, due to the production of a highly potent enterotoxin that is resistant to thermal treatments applied to milk. Mastitis is a major disease of dairy cattle requiring antibiotic therapy, however, any antibiotic used in cows by any route of administration may

result in residues in the milk, even in small concentrations. The *grace period* is the time after administration of the drug until the milk can be released for human consumption. The duration of this period depends on several factors such as the dose and treatment regime used, the route of administration, the milk production and the product formulation. The end of this period occurs when antibiotics residues in milk are below the maximum residue levels (MRL) established officially. For this reason it is so important daily monitoring for antibiotic residue detection using rapid tests and with results on the farm. The objective of this study was to evaluate the presence of antibiotic residues in raw milk samples, using the SNAP DUO Beta-Tetra ST® kit in dairy cows from 11 family-based properties in Rio de Janeiro State, Brazil. This kit is an enzyme immunoassay (ELISA), which detects the presence of beta-lactam and tetracycline residues in milk, a single enzyme-linked assay (detection level at or below 4 ppb, and 100 ppb or below respectively) without the need of heat or incubation, and the results can be read in 6 minutes. 125 milk samples were collected. First, tests were performed with the POOL (mixture of the four teats of milk from the cow) from each cow and in the case of a positive result; the test was conducted with the sample separately from each teat. As a result, we found 121 samples (POOL) negative for beta-lactam and 123 negative to tetracycline. As a positive result, there were 4 samples (POOL) positive for beta-lactam and 2 for tetracycline. From the positive results, each teat was individually tested and the results were: Four cows with 4 teats positive for beta-lactams, one cow with four positive teats to tetracycline and one cow with only 1 positive teat to tetracycline. Farmers confirmed all positive results obtained using the SNAP DUO Kits on raw cow milk samples, once all positive cows were treated with antibiotics few days before testing. The test can be performed in rural areas without the need for laboratory equipment, it is an innovative technology, fast results, reliable and specificity, and detection limits are compatible with the requirements of the market and international trade.

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## WORK-RELATED MRSA CC398 CARRIER STATUS HAD SERIOUS PSYCHOSOCIAL CONSEQUENCES TO A FARMER

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In Denmark the first MRSA CC398 related fatality occurred in 2012. Since then three additional deaths have been confirmed. Reported MRSA CC398 cases has increased from 643 in 2013 to 1276 in 2014. MRSA CC398 now represent 43% of the total reported MRSA cases. Of the cases, 89% were found in persons with direct contact to swine, or in their household. The interest from the public and the media has increased and MRSA CC398 is now on the political agenda. The fear of the bacteria in the public and the media has widely psychosocial consequences for farmers and their employees. The following describe one of these work-related cases. In March 2014 a 30 year old farmer was referred to the Department of Occupational Medicine Aalborg, Denmark. He had been in the industry the last 14 years. Within the last 5 years he had been colonized by MRSA CC398 and in March 2013 it was detected in an abscess after a minor surgical operation. The case was filed as "sick by MRSA" to the National Board of Industrial Injuries in Denmark. External eradication failed and through succesfull peroral eradication he was later found MRSA-negative. On four occasions, the farmer's pregnant girlfriend, without direct contact to swine, was found MRSA CC398 positive. External eradication failed and peroral eradication was postponed due to pregnancy. She was later tested MRSA-negative in 2014. When his girlfriend became

pregnant again the farmer feared transmitting MRSA CC398 to her and he developed stress related symptoms like insomnia, feelings of guilt, anxiousness and easy to tears. The symptoms led to sick leave and psychotherapy. He decided to terminate his career as a farmer. He described contradicting treatments in the health care system. On one hand he was informed that MRSA CC398 did not pose any risk neither towards his pregnant girlfriend nor to his child. On the other hand he experienced isolation, personal protection equipment and fearfull reactions from the medical staff. The National Board of Industrial Injuries sanctioned MRSA-infection as a work related condition, but declined the stress reaction as one. This case shows the consequences of occupational MRSA exposure towards farmers and to their families. It also indicates that MRSA CC398 can be difficult to eradicate despite no direct exposure. Finally it describes critical information sharing, the importance of knowledge of MRSA CC398, and socioeconomic consequences in MRSA-exposed occupations.

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## STUDIES ON THE INHIBITORY ACTIVITY ATTRIBUTES OF LOCALLY PRODUCED HONEY AGAINST MRSA

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This research aimed to evaluate whether (H2O2) was a responsible component for the antibacterial activity in diluted honey and to investigate the role of (H2O2) interaction with phenolic compounds in the inhibitory effect of honey. Eleven honey samples of locally produced honeys were tested against methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43330). Measurement of the antibacterial activity was performed by following the agar well diffusion method. Measurement of the antibacterial activity of the non-peroxide component of honey samples after treatment by catalase was also carried out adopting the agar well diffusion method. All honey samples at concentration (10%) failed to inhibit the growth of this organism. At concentrations (30%) and (50%), some of honey samples demonstrated slight inhibitory effect. Additionally, at concentration (70%), all honey samples presented inhibitory effect and some of them showed considerable effect. At concentration (100%), all of honey samples showed significant inhibitory effect. The majority of honey samples recorded a bacteriostatic effect against MRSA. The variation of the concentrations of honey samples was a considerable reason for the variation of the strength of the antibacterial activities of honey samples ($p= 0.05$). The non-peroxide component of honey samples after catalase treatment failed to inhibit the growth of tested organism, while few honey samples indicated inhibitory effect which was recorded as only bacteriostatic effect. This approved that eliminating H2O2 from honey indicated weakness of the inhibitory effect compared to the crude untreated honey samples between test concentrations (10%) to (50%). Studying the antibacterial activity of phenolic extract of honey samples was evaluated after extraction adopting the agar well diffusion method. Results approved considerable inhibitory effect of the phenolic extract of honey samples on the tested organism. In conclusion the current study investigated the inhibitory effect of peroxide component (presence of H2O2) and non-peroxide component (absence of H2O2) of locally produced honey against MRSA. Also, the results of this study approved that phenolic components in presence of H2O2 revealed strong inhibitory effects against the test organism similarly to the antibacterial activity of crude honey. However, when H2O2 was excluded, the extracted phenolic components had relatively negative effects on the strength of the antibacterial activity. Accordingly, this suggests that H2O2 was involved in the phenolic reaction to introduce the noticeable antibacterial activity of honey.

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### ANTIBIOTIC SUSEPTIBILITY PROFILE OF STAPH. AUREUS ISOLATED FROM POULTRY FARMS IN KANO STATE, NIGERIA

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*Staph. aureus* infection in poultry birds have been linked to the cause of increased morbidity and mortality in poultry management and treatment due to antibiotic resistance. This study aim to proffer better treatment option in the management of *Staph. aureus* infection from poultry farms in Kano State using standard microbiological methods. The result showed that out of the 1260 samples collected from 12 farms in Kano State, 600 were from cloacae, 600 from nostril and 60 were from poultry farm workers. Using conventional identification methods, 562 isolates showed presumptive characteristics of *Staph. aureus*. The use of STAPH Agglutination kit reduces the number of the isolates to 290 while further identification method using Microgen STAPH kit confirmed 33.8% (98) of the presumptive isolates to be *Staph. aureus.* 8.3% (5) of the confirmed *Staph. aureus* were from the nostrils of poultry farm works. The antibiotic susceptibility profile study showed that high percentage (71.4%) of the isolates were resistant to Oxytetracycline, Oxacillin and Ampicillin, 61.2% to Chloramphenicol, 55% to Erythromycin and 30.6% to Cefoxitin. Vancomycin showed high activity against 74.5% of the isolates, Augumentin 69.4%, Ciprofloxacin 64.3% and Gentamicin 60.2%. Three of the isolates were observed to be resistant to all the 12 antibiotics tested while only one isolate was susceptible to all the antibiotics tested. Statistical analysis showed that isolates from layers were more resistant than those from broiler and poultry farm workers at $p \leq 0.05$ level of significance. We conclude that the isolates from this study has varied antibiotic susceptibility pattern and that Vancomycin, Augumentin, Ciprofloxacin and Gentamicin could still be proficient in the management of *Staph. aureus* infection from poultry farm in Kano State, but surveillance and publicity should be carried out to curtail the wide spread of antibiotic resistance induced through irrational antibiotic misused. Key word: Poultry farm, antibiotics susceptibility profile, *Staph. aureus*, resistance.

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### STAPHYLOCOCCUS PSEUDINTERMEDIUS EFFLUX PUMP DETECTION AND CHARACTERIZATION

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Staphylococcus pseudintermedius is the most common cause of pyoderma in dogs and is associated with surgical-site and soft tissue infections in dogs and cats and occasionally other species, including human beings. The frequencies of methicillin and multi-drug resistance among S. pseudintermedius isolates have risen significantly over the last decade. Efflux pumps are often associated with broad resistance to both antimicrobials and sanitizing compounds. Plasmids characterized through genomic sequencing suggested that QacG and QacA may be prevalent in S. pseudintermedius. The purpose of this study was to screen a group of genetically diverse isolates collected from ten different regions of the United States for Qac genes as well as for efflux pump activity. The presence of Qac genes was determined by PCR, antimicrobial drug extrusion was estimated by a surrogate assay for ethidium bromide excretion and susceptibility to quaternary ammonium compounds was determined by broth dilution assay. A strong correlation was found between resistance to quaternary ammonium compounds and the presence of Qac plasmids. Three plasmids were identified that contained QacA or QacG genes and the plasmids shared homology with those found in Steptococcus pneumoniae, Staphylococcus haemolyticus, and *Staphylococcus aureus*. These results suggest that resistance to qua-

ternary ammonium compounds can reside on mobile genetic elements capable of being exchanged between different Gram-positive species. The results have implications for the effectiveness of these compounds in both therapy and hygiene.

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## PROTEIN A IN STAPHYLOCOCCUS PSEUDINTERMEDIUS

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Staphylococcus pseudintermedius is an opportunistic pathogen in dogs and the most frequent cause of canine pyoderma. Much of what is known today about *S.pseudintermedius* comes from comparative research with *Staphylococcus aureus*. Protein A, a highly potent virulence factor in *S.aureus* is encoded by the *spa* gene. *S.pseudintermedius* possesses genes analogous to *spa*, but their expression is unknown. If they are expressed, it is worth investigating if these proteins are functional. We hypothesize that *S.pseudintermedius* possesses both cell wall-associated Protein A and extracellular (secreted) Protein A. The purpose of this study was to screen isolates from various genetic backgrounds for the presence of the *spa* gene and quantitate its expression levels. We also wanted to determine the ability of surface-bound Protein A to bind to canine IgG and to measure the amount of secreted Protein A in culture supernatant. Our results suggest that isolates representing the major clonal populations in the United States and Europe bound significantly more IgG than isolates with other genetic backgrounds (sequence types). Many of these isolates were methicillin resistant (MRSP). Also, our results showed that canine IgG binds to Protein A on *S.pseudintermedius* via its Fc region and not via Fab. The expression profile of *spa* differed based on the sequence types. Extracellular Protein A was detected in culture supernatants and quantified. Taken together, our results suggest that *S.pseudintermedius* produces Protein A which may serve as a potential virulence factor for evading the host immune system.

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## METHICILLIN-RESISTANT STAPHYLOCOCCUS SPECIES FROM CANINE AND FELINE CYSTOCENTESIS SAMPLES

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Staphylococcus species are a common cause of canine and feline urinary tract infections (UTI). There has been a documented increase in the prevalence of methicillin resistant Staphylococcus species isolated from skin and soft tissue infections from companion animals. A retrospective analysis of urinary tract isolates was performed to determine typical antibiograms, and risk factors for infection. Between 2008 and 2014, Staphylococcus was isolated from 364 urine samples from animals that presented with signs of a UTI, or for routine screening. The data that follows was performed on the results obtained only from177 cystocentesis samples, to remove any potential confounding results due to contamination from normal flora. Thirty eight (21%) of the 177 isolates were methicillin resistant Staphylococcus (MRS). The majority (59%) were Staphylococcus pseudintermedius (23/38). S. schleiferi (18%, 7/38) was the second most common MRS species The remainder of the isolates were coagulase negative staphylococci (CoNS) and included: S. epidermidis, S. hominis, S. simulans and S. auricularis. No MRSA isolates were identified. The majority of the isolates (87%) were from dogs. Forty seven percent (18/38) of the isolates were also resistant to fluoroquinolone antimicrobials, 39% (15/38) were resistant to tetracycline and 37% (14/38) were resistant to trimethoprim-sulfamethoxazole. Nine isolates were co-resistant to all three of the aforementioned antimicrobial classes; however, all isolates were susceptible to

nitrofurantoin. Concurrent urinalysis was performed on 17 of the MRS cases and revealed an average specific gravity of 1.025 (moderately concentrated) and 14/17 (82%) of cases had hematuria and 11/17 (65%) were positive for protein on urine dipstick. Only 3/17 (18%) cases had associated crystalluria. Bacteria were visible on microscopic sediment examination in 15/17 (90%) of the cases, with the majority (78%) being subjectively classified within the highest tier of bacterial quantities. None of the evaluated potential risk factors (antibiotic use within the previous 6 months, owners within the veterinary field or a diagnosis of a chronic disease) predisposed patients to MRS infection. The most common comorbidity of MRS UTI patients was urolithiasis (9/38). Methicillin resistant infections can be very troublesome as clinical cases, therefore, understanding the epidemiology and resistance patterns of these organisms is important as methicillin resistance continues to rise.

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## THE PREVALENCE OF CHLORAMPHENICOL RESISTANCE IN CLINICAL ISOLATES OF STAPHYLOCOCCUS AND METHICILLIN-RESISTANT STAPHYLOCOCCUS FROM COMPANION ANIMALS THE UNITED STATES

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Chloramphenicol is a broad spectrum antimicrobial with good activity for Gram-positive and Gram-negative, aerobic and anaerobic bacteria. Bacterial resistance to chloramphenicol is most commonly associated with the presence of chloramphenicol acetyltransferases (CAT). Chloramphenicol analogs including the fluorinated derivative florfenicol have a similar spectrum of antimicrobial activity as chloramphenicol but the presence of the fluorine residue at C3 renders florfenicol resistant to inactivation by CAT enzymes. Consequently, chloramphenicol-resistant strains, in which resistance is exclusively based on the activity of CAT, are susceptible to florfenicol. Florfenicol is used solely in veterinary medicine and has recently been added to otic gel preparations as a new treatment for inflamed or infected canine ears. These gels are indicated for the treatment of otitis externa associated with susceptible strains of Staphylococcus pseudintermedius. Little is known about florfenicol resistance in Staphylococcus species. The aim of this study was to determine the prevalence of chloramphenicol resistance in staphylococci from companion animal infections, with emphasis on isolates obtained from otic infections. Data were available for 18,515 clinical isolates of Staphylococcus species from January 2003 to December 2014. Antibiotic susceptibility data were collected using a commercial broth microdilution panel and the results were interpreted using Clinical Laboratory Standards Institute (CLSI) guidelines. The annual prevalence of resistance to chloramphenicol and oxacillin was determined for each of year, in addition co-resistance to both of these antimicrobials was also determined. Additionally, there were 2558 staphylococcal isolates from otic infections and the prevalence of chloramphenicol, oxacillin and chloramphenicol/oxacillin resistance was determined for isolates from this source. Analysis of antibiotic susceptibility data from 18,515 Staphylococcus isolates showed that chloramphenicol resistance has increased steadily from 1.5% in 2003 to 11.7% in 2014. Oxacillin resistance has also increased during that time from 25.2% to 51.3%. Co-resistance ranged from 35.8% to 77.7%. 173/2558 (6.7%) staphylococcal isolates obtained from ear infections were resistant to chloramphenicol. 926/2558 (36.9%) were oxacillin resistant and co-resistance was observed in 81 isolates. There are no CLSI approved breakpoints for florfenicol against Staphylococcus spp. and because little is known about specific florfenicol resistance mechanisms in staphylococci, veterinary practitioners will most likely use susceptibility results obtained from testing chloramphenicol as a "group" drug to guide treatment with topi-

cal products that contain florfenicol. This may over or under estimate the prevalence of florfenicol resistance.

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## IN VITRO ANTIBACTERIAL ACTIVITY OF TRADITIONALLY USED MEDICINAL PLANTS AGAINST MULTI-DRUG RESISTANT (MDR) BACTERIA ISOLATED FROM CLINICAL CASES

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Acacia etbaica, Aloe vera and Mukia genus have been reported as they were used traditionally to treat diseases of humans and domestic animals by local communities in Tigray Region, Ethiopia. Methanol extracts of leaf of these plants were tested for their in-vitro antibacterial activity against multi drug resistant (MDR) *Staphylococcus aureus*, Escherichia coli and Salmonella using agar disc diffusion method. The Minimum Inhibitory Concentration (MIC) of the plant crude extract that showed good antibacterial effect was also determined using micro-dilution method in 96-well plates. Among the tested plants, Acacia etbaica showed significant antibacterial activity with mean zone of inhibition of 16.9(±0.80) mm, 14.91(±0.44) mm and 14.39(±0.17) mm in diameter at a concentration of 2000µg of plant extract per disc against MDR *Staphylococcus aureus*, Escherichia coli and Salmonella respectively. However, methanol extracts of the other tested plants did not show antibacterial activity against the tested bacteria. The MIC of the crude extracts of Acacia etbaica was determined to be 0.03mg/ml, 0.01mg/ml and 0.05mg/ml against *Staphylococcus aureus*, Escherichia coli and Salmonella respectively. The results suggest that the methanol extracts of Acacia etbaica could be a rich source of antibacterial compounds against resistant *Staphylococcus aureus*, Escherichia coli and Salmonella. The results also provide an indication of merit in the Acacia etbaica ethno-medicine use by the local communities.

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## LOW PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PIGS IN THE USA

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Prior to the recognition that pigs and other livestock species can be reservoirs of methicillin resistant *Staphylococcus aureus* (MRSA), S. aureus was considered a relatively unimportant organism in animals. Previous studies of MRSA in pigs in North America have variably reported predominance of ST398 or ST5 variants, and ST9 variants have also been detected. However, the dynamics and health implications of interspecies transmission of MRSA from pigs to humans are still incompletely understood. To address these questions, better understanding is required of the broader ecology of S. aureus in pigs, including MRSA. The goal of the current study was obtain more geographically representative information about the prevalence and diversity of S. aureus and MRSA in growing pigs on commercial farms in the USA. A cross sectional study was conducted on 36 farms, and one MRSA positive control farm, located in 11 states in the USA. Overall, 739 pig nasal swabs were collected, of which 558 (76%) were culture positive for S. aureus. Except the positive control farm, on which all 20 pigs tested MRSA positive, no MRSA were detected in any of the pigs. Among the 35 S. aureus positive farms there was considerable diversity found with 33 spa types detected within 4 MLST sequence types. The most prevalent spa types (sequence type) were t337 (ST9), t034 (ST398) and t002 (ST5) which collectively accounted for 59% of isolates. Antimicrobial and zinc susceptibility testing and PCR testing for enterotoxin genes (seA to seE) were performed on a subset of 130 isolates selected purposively to maximize the diversity by spa type and farm of origin. Antimicrobial resistance was most common to spectinomycin (100%), tetracycline (94%), clindamycin (75%) and penicillin (72%), and

a majority of isolates were resistant to 5 or more antibiotics (multidrug resistance SA, MDRSA). ST398 (t034) MRSA isolates from the positive control farm were positive for the czrC gene, while no MSSA isolates tested positive, although some showed phenotypic resistance to zinc. The association of the czrC gene, but not specific antimicrobial resistance patterns, with MRSA variants is aligned with other studies indicating that non-antimicrobial factors including zinc may have played a role in the emergence of livestock associated MRSA. Given the apparent absence of MRSA among the farms of unknown status, the 95% upper confidence limit for herd prevalence is less than 10%. The observation of a low prevalence of MRSA among swine isolates, but a high prevalence of MDRSA, is consistent with several recent studies of occupationally exposed groups in the USA.I Although staphylococcal enterotoxicosis is not uncommonly associated with pork products, no enterotoxin genes were detected in these isolates suggesting that post-harvest contamination could be the principal source of toxigenic strains involved in foodborne outbreaks.

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## IS THERE A LA-MRSA TRANSMISSION FROM HOSPITAL EMPLOYEES TO HOSPITAL ENVIRONMENT?

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The identified cases of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) clonal complex (CC) 398 in Denmark have increased over the past years as in many other countries. In Denmark this might be ascribed to a combination of increased infection rates among investigated farms and additional increased screening of all hospitalized patients. This presentation will describe a present Danish study of LA-MRSA among all employees in a hospital. The aim is to describe point prevalence of LA-MRSA carriage in this population as well as to investigate whether LA-MRSA is present in airborne dust samples from different locations in the hospital. Additionally, dust sampling will be carried out from 20 participating households with a farm relation, and 20 participating households without any farm relation. On the basis of DNA analyses of identified LA-MRSA, the exact match of each LA-MRSA could be followed in employees, hospital departments, selected homes and farms for any linkage. The study was initiated by the Department of Occupational Medicine, Aalborg University Hospital, Denmark as we have observed more patients with LA-MRSA and an increased stigmatizing of farmers and their households. The study has invited all the employees (approx 1350) from a Danish regional hospital in a rural and dense agricultural area of North Jutland to participate in the study. All participants were asked to fill in a questionnaire and have a swab collected from their anterior nares. All swabs will be analyzed anonymously at Statens Serum Institut, Denmark. Along with the data sampling among employees, the hospital airborne dust samples will be collected. The private and farm related dust sampling will also be managed anonymously. Half of the samples from the farm related households (n=10) will be selected due to positive LA-MRSA status among the employees and the other half due to negative LA-MRSA status among the employees. The same distribution will be used in the households without any farm relation. Hospitals in Denmark screen all inpatients, but unknown factors are the employee carriage status and its relevance. Is it a problem or are the established hygiene initiatives sufficient? At the same time we have to be aware that we still don't know anything about most ambulant patients and visitors at the hospital, and that

no hospital-acquired MRSA CC398 have been identified at the selected hospital. Data on the point prevalence of LA-MRSA carriage are expected to be ready for presentation at the time of the conference.

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### ASSESSMENT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM COW'S MILK IN THE BAHIRDAR AREA, NORTH ETHIOPIA

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Staphylococci are important opportunistic pathogens in most animal species. In recent years, methicillin-resistant *Staphylococcus aureus* (MRSA) have become a truly global challenge. MRSA is considered as a growing problem in human and veterinary medicine. Methicillin resistance has emerged as an important problem with significant concerns about animal and public health. **Objective:** To determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in samples of cow's milk obtained from Bahirdar area, Northern Ethiopia. **Hypothesis:** S.aureus is the most important caus¬ative agent of subclinical mastitis. **Methodology:** Quarter milk samples were aseptically taken from all lactating cows (CCP1-CCP5) and screened for the presence of S. aureus. Samples were plated directly and bacteriological analysis was performed by culture on blood-agar plates. Laboratory investigations and like Gram staining, oxidase, catalase, DNase, haemolysis and coagulase tests were employed for bacterial identification. A total of 486 *Staphylococcus aureus* isolates from bovine mastitis milk collected during 2012-2013 from cow's milk in the Bahirdar area Northern Ethiopia. All isolates will be subjected to CLSI disc diffusion susceptibility testing for 16-18 antimicrobials of importance to veterinary and public health. **Results:** All the milk samples were contaminated with S. aureus. A total of 76 S. aureus isolates were obtained during this study. The levels of contamination with S. aureus were higher in milk obtained from CCP1, CCP2, CCP3, CCP4 and CCP5 at Bahirdar area farms (16.0%, 28.1%, 30.0%, 15.0% and 9.2%) respectively. A large percentage of the S. aureus isolates were from CCP2 (28.1%) and CCP3 (30.0%). The antimicrobial resistance profiling of S. aureus observed to Penicillin G (77.1%), Ampicillin (65.3%), Amoxicillin-Clavulanic acid (21.4%), Ciprofloxacin (0%), Erythromycin (36.0%), Ceftriaxone (14.8%), Trimethoprime-Sulfamethoxazole (6.8%) Oxacillin (53.2%) and Vancomycin (29.9%) was observed. **Conclusion:** The proportion of isolates resistant to Ciprofloxacin, Trimethoprime-Sulfamethoxazole, Ceftriaxone, Amoxicillin-Clavulanic acid, Erythromycin and Vancomycin were low compared to Ampicillin, Penicillin G and Oxacillin. Therefore, this study demonstrates that high prevalence of MRSA in the cow's milk, emphasizing the need to improve sanitary conditions in the milking environment.

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### MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANT STAPH. AUREUS FROM POULTRY FARMS IN KANO STATE, NIGERIA

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The increasing rate of drug resistance associated with Methicillin resistant *Staph. aureus* is not only a problem in clinical sector but also in livestock disease treatment and management. This study was set out to evaluate the incidence of Methicillin resistant *Staph. aureus* from poultry farms among the birds and the farm workers and the likelyhood of cross-infection between the birds and the farm workers in Kano State, Nigeria, using standard microbiological and molecular methods. The results showed that out of the 1260 samples collected, 98 isolates were confirmed to be *Staph. aureus*. Using disc diffusion method, the antibiotics

susceptibility test results showed that 69.4% (68) of the isolates were susceptible to cefoxitin while 30.6% (30) were resistant. The antibiotics susceptibility profile of the resistant isolates showed that the isolates were 100% resistant to Ampicillin and Amoxicillin, 93.3% to Oxytetracycline, 90% to Chloramphenicol, 80% to Erythromycin, 76.7% to Oxacillin, 63.3% to Trimethroprim/Sulphamethozaxole, 30% to Ciprofloxacin and 26.7% to Gentamicin. The result also showed that 83.3% of the isolates had MARI of > 0.3, 16.7% had MARI of ≤ 0.3 while 93.3% were multidrug resistant. The molecular analysis showed that all the isolates were *Staph. aureus* of 800bp, 66.7% of the MDR isolates posses *MecA* gene (162bp), while 33.3% had *MecA* of 500bp. Further analysis showed that 3 of the seven housekeeping genes (*pta, gmk* and *yqil*) were also present in the MDR isolates at 43.3%, 20% and 16.7% respectively while 10% express spa typing. The results showed that there is a correlation between phenotypic cefoxitin resistance and carriage of *MecA* gene. The implication of these results in cross infection between the farm workers and the poultry birds and the danger of transfer of these resistant isolates to the community will be discussed. Key words: *Staph. aureus,* cefoxitin resistance, poultry farms, seven housekeeping genes, spa typing and MDR

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## ASSESSMENT OF METHODS TO QUANTIFY LIVESTOCK ASSOCIATED MRSA IN PIG HERDS

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A high prevalence of LA-MRSA, especially in the pig production, has been reported from many countries. However, there is a lack of knowledge about the actual MRSA loads on infected farms. It is highly relevant to assess the LA-MRSA level on animals and in the environment, to be able to study a potential connection between the level of LA-MRSA and the risk for human exposure, and to estimate the effect of intervention strategies. The present study was undertaken to assess methods to quantify LA-MRSA on animals and in the environment in pig herds. In addition, the role of flies as potential environmental carriers of MRSA was investigated. Sampling was conducted on two different occasions at a confirmed MRSA infected farm. Samples were collected in the farrowing section and the weaner section. Nasal samples from sows (n=5), piglets (n=5) and weaning pigs (n=18) were collected with dry flocked swabs. Additionally, piglets and weaning pigs were sampled behind one ear with a moistened flocked swab. The swab material was extracted in 1 mL liquid Amies medium. Samples were analyzed individually and quantified by direct plating from dilution $10^0$ and $10^{-1}$ onto a MRSA selective agar. Following incubation, colonies were counted, and counts expressed as cfu/swab for each sample. Airborne MRSA in the weaning section was quantified by calculation of cfu/m$^3$ of various air volumes, collected with two different sampling devices for comparison. One sampling directly onto a selective MRSA agar plate (Sampl'air Air sampler) and the other sampling onto a membrane filter (Sartorius, MD8 air sampler). Flies were sampled both individually and in pools of 5 and enriched prior to MRSA determination on MRSA selective agar. All tested animals (28/28) were found to be MRSA positive, with a nasal MRSA load ranging from $6.6\text{-}\times10^1$-$3.9\times10^4$ cfu/swab and skin load ranging from $1.1\times10^1$-$2.6\times10^5$ cfu/swab. Both air sampling devices were able to detect MRSA. A higher cfu/m$^3$ level was found with Sampl'air, range $5.8\times10^2$-$1.56\times10^3$, than with MD8, range

$4.1\times10^2$-$6.3\times10^2$, however MD8 gave a more stable detection level of MRSA from the different volumes. In sections with animal activity, flies treated individually and in pools, were found to carry MRSA, but in sections with no animal activity both individually treated flies and pooled samples were found MRSA negative. In conclusion: This study demonstrated that fast quantification of the animal MRSA load in pig herds is possible by direct plating of either nasal or skin swab samples. The MD8 air sampler provides fast environmental quantification and it was possible to detect MRSA in flies with animal contact demonstrating their potential as environmental MRSA carriers. Intensive sampling from both animals and environment can be used to estimate an overall farm MRSA level. Our results will now be used to study possible transmission routes and investigate the effect of different intervention strategies.

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## COMPARISON OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ADHERENCE ON TWO LIMB SALVAGE ENDOPROSTHETIC IMPLANTS FOR DOGS

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Osteosarcoma (OSA) is a malignant primary bone tumor in dogs that is locally aggressive and highly metastatic. Limb-salvage surgery has grown in popularity for management of OSA of the distal radius in dogs that suffer from severe osteoarthritis or neurologic disease. The most commonly used distal radius endoprosthesis is produced from solid stainless steel (SS) and has designated screw inlets for placement. A new endoprosthesis has been developed using a metal called tantalum (TA). This endoprosthesis has a porous and trabecular design, allowing for increased bone and soft tissue attachment and a more rapid recovery and bacterial adherence to TA has been shown to be lower than to SS. However, it is unclear if the porous design of the implant could have an impact on bacterial adherence and biofilm formation. The purpose of this study was to determine the bacterial adherence properties of both distal radius endoprostheses and understand the risk it may create for the development of SSI. Ten methicillin-resistant *Staphylococcus pseudintermedius* isolates from canine infections that were determined to be strong biofilm producers were used to inoculate both types of implants. Implants were rinsed to remove non-adherent bacteria, were placed in a sterile vessel with 400ml PBS and then sonicated in order to remove adhered bacteria from the implant. Serial dilutions of the resulting suspension were plated and median $\log_{10}$ colony forming unit (CFU) counts were completed the following day. Adherent bacteria were identified on all plates following inoculation, with median $\log_{10}$ counts ranging from 3.45 CFU/ml to 5.07 CFU/ml for SS (mean: 4.32 CFU/ml, median: 4.29 CFU/ml) and 4.59 CFU/ml to 6.36 CFU/ml for TA (mean: 5.20 CFU/ml, median: 4.93 CFU/ml). It was determined that bacterial adherence on the SS endoprosthesis was significantly less than on the TA endoprosthesis (P=0.009), despite previous reports of decreased bacterial adherence to TA. The porous design of the TA implant allows for superior bone and soft tissue attachment as well as ease of placement since screws can be placed through any part of the implant for maximum compatibility. Yet, the porosity may account for the increased bacterial adherence given previous data about inherently reduced bacterial adherence to TA. Whether the benefits could be outweighed by the drawbacks of increased bacterial adherence and biofilm formation when compared to the SS endoprosthesis is unknown. This study provides information about inherent differences in MRSP attachment to two canine limb-salvage endoprostheses and highlights the need to evaluate implant surface characteristics in addition to material properties.

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## EVALUATION OF THE IMPACT OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* BIOFILM FORMATION ON ANTIMICROBIAL SUSCEPTIBILITY

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Methicilin-resistant *Staphylococcus pseudintermedius* (MRSP) is an emerging cause of surgical site infections (SSI) in dogs. The ability of MRSP to produce a biofilm is associated with the bacteria's resistance to antibiotic treatment as well as the host's immune response. Understanding the antibiotic susceptibility of biofilm-embedded bacteria is therefore important when developing effective treatment plans. The objective of this study was to compare the minimum inhibitory concentration (MIC) of planktonic versus biofilm-embedded bacteria. Twenty seven MRSP and 27 MSSP isolates from dogs with clinical infection or colonization were tested. MICs of amikacin, cefazolin, enrofloxacin and gentamicin were detected for planktonic bacteria using agar dilution. Biofilm embedded bacteria MICs was determined through a broth microdilution assay after establishment of biofilm in microtitre plates. MICs were significantly higher in biofilm-embedded versus planktonic bacteria for all antimicrobials; amikacin (median MIC: biofilm - 2000ug/ml vs planktonic 3ug/ml, $P<0.0001$), cefazolin (1000ug/ml vs 0.5 ug/ml, $P<0.0001$ ), enrofloxacin (1000 ug/ml vs 0.25 ug/ml, $P<0.0001$) and gentamicin (1000 ug/ml vs 0.3 ug/ml, $P<0.001$). For all antimicrobials, there were significant differences in planktonic cell MICs of MRSP and MSSP (all $P<0.0001$) but those differences were lost when bacteria were established in biofilms (all $P=0.08$-$1.0$). Standard methods for determining MICs do not accurately reflect antimicrobial susceptibility in biofilm-embedded *S. pseudintermedius*. This must be considered when determining treatment regimens for infections that potentially involve biofilms, and further study of methods to control biofilm-associated infections is needed.

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*Bold number indicates presenter.*

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