Adjustment of pH of enrichment media might improve selective isolation of MRSA from pig samples

Cavaco, Lina; Agersø, Yvonne; Mordhorst, Hanne; Aarestrup, Frank Møller

Published in:
2nd ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

ASM CONFERENCES

2nd ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications

September 8-11, 2011 • Washington, DC

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

Printed in the United States of America
ISBN: 978-1555817251

ASM CONFERENCES

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The Conference Organizers and the American Society for Microbiology would like to acknowledge the following for their financial contributions to this conference:

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General Information

GENERAL SESSIONS

All general sessions will be held in the Blue Room at the Omni Shoreham. A name badge is required for entry into all sessions, meals, and social events. ASM staff will be available during the sessions.

POSTER SESSIONS

Poster boards are located in the Blue Room Foyer at the Omni Shoreham. Please check your assigned number in the abstract index. The same number is used for the presentation and board number. Odd-numbered posters will be presented in the A session on Friday, September 9, and even-numbered posters will be presented in the B session on Saturday, September 10. The posters are to be mounted by Friday morning and should be removed by 3:00 pm on Sunday. The poster area will be open for informal viewing throughout the conference.

CERTIFICATE OF ATTENDANCE

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

Note: Certificates of Attendance do not list session information.

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

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As noted in the program, designated conference meals and social events are included in the registration fee for conference participants. Registered participants may also register an accompanying guest (age 16 and older) to attend the welcome reception for an additional fee of $50. Guests are not permitted in the general sessions or poster sessions, other conference meals or coffee breaks. Guests must present their registration badge for entrance to the welcome reception. Non-registered guests are not permitted to attend any part of the conference or social events.

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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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Szilvia Vincze

Scientific Program

Thursday, September 8, 2011

2:00 - 8:00 pm	Registration and poster setup
6:00 - 7:30 pm	Welcome Reception

Friday, September 9, 2011

9:00 - 9:15 am	Welcome and Introductions
9:15 am - 12:00 noon	**Session 1: Food Animal Epidemiology**
9:15 - 9:55 am	MRSA in Livestock: Just Another *Staphylococcus aureus* for Animals? *Jaap Wagenaar, Utrecht University, Utrecht, Netherlands*
9:55 - 10:15 am	Characteristics of *Staphylococcus aureus* in Connecticut (CT) Swine Industry *Lynda U. Odofin, Yale University School of Public Health, New Haven, CT*
10:15 - 10:35 am	Evaluation of Anatomical Sampling Sites for Detection of Methicillin-resistant *Staphylococcus aureus* in Pigs *Larissa J. Pletinckx, Catholic University Leuven, Roeselare, Belgium*
10:35 - 11:00 am	Coffee break
11:00 - 11:20 am	Longitudinal Study for Livestock-associated MRSA: Determination of the Piglet Colonization Age, Effect of the Sow Status, and Genotype Presence *Marijke Verhegghe, Institute for Agricultural and Fisheries Research (ILVO), Melle, Belgium*

11:20 - 11:40 am	Presence of *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) in Retail Pork *Blake M. Hanson, University of Iowa, Iowa City, IA*
12:00 - 2:00 pm	Lunch and poster viewing
2:00 - 4:50 pm	**Session 2: Companion Animal Epidemiology**
2:00 - 2:40 pm	MRSA: Companion Animal Epidemiology *Anette Loeffler, Royal Veterinary College, London, UK*
2:40 - 3:00 pm	Transmission of MRSA between Companion Animals and Infected Human Patients Presenting to Outpatient Medical Care Facilities *Jorge Pinto Ferreira, North Carolina State University, Raleigh, NC*
3:00 - 3:20 pm	Staphylococcal Nasal Carriage in Healthy Humans and Pets in the Same Household: Potential Interspecies Transmission *Elena Gómez-Sanz, University of La Rioja, Logroño, Spain*
3:20 - 3:50 pm	Break
3:50 - 4:30 pm	Is MRSP ST71 Something More Than Just a *Staphylococcus pseudintermedius* Strain Containing *mecA*? *Luca Guardabassi, University of Copenhagen, Frederiksberg, Denmark*
4:30 - 4:50 pm	Multilocus Sequence Typing (MLST) for Characterization of Methicillin-resistant and Methicillin-susceptible Clones of *Staphylococcus pseudintermedius* *Stephen Kania, University of Tennessee, Knoxville, TN*
5:00 - 6:00 pm	**Poster Session A**

Saturday, September 10, 2011

9:00 am - 12:00 noon	**Session 3: Animal-Associated MRSA in Humans**
9:00 - 9:40 am	How Modelling can Add Value to Infectious Disease Research: Illustrated Examples for LA-MRSA *Katharina D. C. Stärk, Royal Veterinary College University of London, London, UK*
9:40 - 10:00 am	MRSA Harbouring a Novel *mecA* Homologue Emerging in Human and Bovine Populations in the UK and Denmark *Mark A. Holmes, University of Cambridge, Cambridge, UK*
10:00 - 10:20 am	Genomic Characterization of "Livestock-associated" ST398 Methicillin-resistant *Staphylococcus aureus*, Canada *George R. Golding, National Microbiology Laboratory, Winnipeg, MB, Canada*
10:20 - 10:50 am	Coffee break
10:50 - 11:10 am	The Distribution of Mobile Genetic Elements (MGEs) in MRSA CC398 is Associated with Both Host and Country *Alex J. McCarthy, St. George's University of London, London, UK*
11:10 - 11:30 am	Longitudinal Study of Methicillin-resistant *Staphylococcus pseudintermedius* in Households *Engeline van Duijkeren, Utrecht University, Utrecht, Netherlands*
11:30 - 11:50 am	Comparative in vivo Host-Specificity of Human- and Pig-associated *Staphylococcus aureus* Strains *Arshnee Moodley, University of Copenhagen, Frederiksberg, Denmark*
12:00 - 2:00 pm	Lunch and poster viewing

2:00 - 3:20 pm	**Session 4: Environment and Ecology of MRSA in Animals**
2:00 - 2:40 pm	Environment & Ecology of MRSA and Animals *Shawn Gibbs, University of Nebraska Medical Center, Omaha, NE*
2:40 - 3:00 pm	Staphylococci Present in Dutch Swine Farms Act as a Reservoir of SCC*mec* *Pawel Tulinski, Utrecht University, Utrecht, Netherlands*
3:00 - 3:20 pm	Detection of Methicillin-resistant *Staphylococcus* in Environmental Waters Near Industrial Swine Operations in Eastern North Carolina *Kevin Myers, University of North Carolina, Chapel Hill, NC*
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3:50 - 5:00 pm	**Session 5: Infection Control**
3:50 - 4:30 pm	Infection Control & Animal-Associated Methicillin-resistant Staphylococci *Maureen Anderson, University of Guelph, Guelph, ON, Canada*
4:30 - 4:50 pm	Use of Environmental Marking for Evaluation of Efficacy of Cleaning in a Veterinary Hospital *J. Scott Weese, University of Guelph, Guelph, ON, Canada*
4:50 - 5:10 pm	A Model for Infection Control Program in Equine Hospitals: Implementation, Evaluation of Effectiveness and Compliance *Ulrika G. Andersson, National Veterinary Institute, Uppsala, Sweden*
5:10 - 5:30 pm	Methicillin Resistant *Staphylococcus spp* in Commercial Pigs Used in Veterinary Student Training *Paul S. Morley, Colorado State University, Fort Collins, CO*
5:30 - 6:30 pm	**Poster Session B** (remove posters after this session)

Sunday, September 11, 2011

9:00 am - 12:10 pm	**Session 6: Molecular Epidemiology & Genomics of Animal-Associated MRSA**
9:00 - 9:40 am	*Staphylococcus aureus* ST398: What can the Genome Teach Us? *Ad C. Fluit, University Medical Center Utrecht, Utrecht, Netherlands*
9:40 - 10:00 am	Molecular Analysis of Methicillin-resistant *Staphylococcus aureus* (MRSA) Isolates from Food and Food Products of Poultry Origin *Andrea T. Feβler, Insitute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany*
10:00 - 10:20 am	Characterization of a Small Apramycin Resistance Plasmid from Porcine MRSA ST398 which Carries a Variant of the *apmA* Gene *Stefan Schwarz, Insitute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany*
10:20 - 10:50 am	Coffee break
10:50 - 11:10 am	The Origins and Evolution of MRSA ST398 Through the Lens of Genome Sequencing *Lance B. Price, Translational Genomics Research Institute (TGen), Flagstaff, AZ*
11:10 - 11:30 am	Genotype Diversity of *Staphylococcus aureus* on Belgian MRSA-positive Pig Farms *Stein Vandendriessche, Hôpital Erasme, ULB, Brussels, Belgium*
11:30 - 12:10 pm	Evolution of Molecular Microbiology of LA-MRSA: The SCC*mec* Cassettes in CC398 *Robert Skov, Statens Serum Institut, Copenhagen, Denmark*
12:15 - 12:45 pm	**Concluding Discussion**
12:45 - 2:30 pm	**Closing Reception/luncheon**

Speaker Abstracts

S1:1

MRSA IN LIVESTOCK: JUST ANOTHER *Staphylococcus aureus* FOR ANIMALS?

Jaap Wagenaar, DVM PhD
Dept Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands. j.wagenaar@uu.nl

Since 2004 MRSA emerged in animals, particularly in pigs and veal calves but also in chickens. This new variant, Livestock Associated MRSA (LA-MRSA) belongs in Europe and Northern America predominantly to clonal complex (CC)398 whereas in Asia ST9 seems to be dominant in pigs. In animals no MRSA-specific pathology is seen and there is no indication for increased numbers of clinical MRSA cases in pigs compared to clinical infections caused by MSSA. The presence of LA-MRSA is not an animal health problem but merely a public health problem. Based on Dutch surveillance data, it is assumed that the introduction of ST398 into the Netherlands has occurred recently (around 2004). Molecular data suggest a multiple introduction of the SCC*mec* element into MSSA. The use of antimicrobials in livestock is a risk factor for the presence of MRSA. However, other factors like hygiene and trade/animal contact are important for the spread of LA-MRSA.

Persons in direct contact with LA-MRSA positive animals have an increased risk of becoming MRSA positive. The risk of carriage is mainly related with the intensity of animal contact and with MRSA prevalence among animals on the farm. In contrast with its success in animals, it seemed that MRSA CC398 is a poor persistent colonizer in humans. MRSA ST398 can, however, cause serious (invasive) infections and outbreaks, although, only incidentally reported so far. The problem of LA-MRSA is currently restricted to occupationally exposed people like farmers and veterinarians.

Until now, the presence of LA-MRSA on meat products has been observed to be a minor risk factor for LA-MRSA in humans. However, the ongoing exposure of humans through food products needs attention in particular for immunocompromised people and people treated with antimicrobials.

LA-MRSA is widely spread in livestock and control measures to reduce or even eliminate LA-MRSA on farms will be difficult. As farm hygiene and antimicrobial use contributed to MRSA occurrence in animals, these two determinants should be incorporated into MRSA-control programmes in animal production.

Like any other microorganism, LA-MRSA is expected to be able to adapt to new hosts and may change over time in the potential to colonize and to produce toxins. Also, the current circulating clone CC398 may be replaced in Western countries by another clone or emerge in countries where this clone is currently low-prevalent. Ongoing MRSA surveillance in humans and animals is needed to detect changes in epidemiology and to implement effective control measures.

S1:2

CHARACTERISTICS OF *Staphylococcus aureus* IN CONNECTICUT (CT) SWINE INDUSTRY

L. U. Odofin[1], *B. Hanson*[2], *T. Smith*[2], *R. Heimer*[1];
[1]*Yale University School of Public Health, New Haven, CT,* [2]*The University of Iowa-College of Public Health, Coraville, IA.*

Background: This study explores the prevalence and characteristics of *Staphylococcus aureus* (*S.aureus*) in live pigs and their human handlers in a convenience sample of 35 farms in Connecticut (CT), where husbandry practices are clearly different from better-known **Concentrated Animal Feeding Operations (CAFOs),** with greater demand for organic products and less intensive rearing conditions.

Methods: Nasal samples were collected from 263 pigs and 9 humans on 35 farms during the

2010 rearing season. Samples were analyzed using established microbiology methods and resulting Methicillin sensitive (MSSA) and resistant (MRSA) isolates were typed by Pulsed-field gel electrophoresis (PFGE) and *spa* typing. PCR was used to detect the presence of Panton-Valentine Leukocidin (PVL) gene, a cytotoxin usually associated with CA-MRSA infection. A farm assessment form and questionnaire were used to obtain information about husbandry practices and human exposure risk respectively.
Results: Farms, swine and humans were colonized with *S. aureus* at a rate of 51% (18/35), 30% (85/259) and 22% (2/9) respectively whereas the overall MRSA rate was 11% (4/35) for farms, 2% (5/259) for swine and 22% (2/9) for humans. For MRSA, humans were colonized with USA 100 (t002) and 200 (t007) generally related to hospital-acquired (HA) MRSA whereas most swine strain was similar to the community acquired (CA) MRSA USA 300 (t008) strain. Positive humans had 2-3 known risk factors for HA-MRSA. The PVL gene was found in all MRSA swine isolates, the first time this gene has been seen in colonized pigs sampled on US farms. All MRSA isolates were resistant to erythromycin and all human (100%) and one pig (20%) isolates were resistant to clindamycin. For MSSA, the isolates belonged to four *spa* types: t337 (60%) t034 (15%), t4529 (15%) and t1166 (7%). The first two *spa* types have been previously associated with pig farming. A proportion of MSSA isolates were resistant to tetracycline (77%), erythromycin (59%) and clindamycin (13%). All strains belonging to *spa* types t337 and t4529 were resistant to tetracycline, while one isolate from each group had an intermediate resistance to doxycycline. Also, isolates belonging to *spa* types t337 and t4529 originated from farms that reported using antibiotics prophylactically. Resistance to gentamicin and doxycycline was seen only in swine fed processed waste (garbage)
Conclusion: This is the first study that sampled small swine enterprises (farms with <99 pigs) which differ from the CAFOs previously studied and the first to report live pig colonization with PVL+US 300, the classic human CA-MRSA strain, implying not only reverse zoonosis but that swine may be a competent reservoir. The other notable finding was the presence of the primary HA-MRSA strain, US 200 in both human and swine. Antibiotic susceptibility profiles of MSSA isolates showed resistance to antibiotics commonly used in human medicine.

S1:3

EVALUATION OF ANATOMICAL SAMPLING SITES FOR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN PIGS.

L. J. Pletinckx[1], *J. Dewulf*[2], *Y. De Bleecker*[3], *B. M. Goddeeris*[4], *I. De Man*[3];
[1]*Catholic University Leuven, Department of Biosystems, Division of Gene Technology - Catholic University College South-West-Flanders, Leuven - Roeselare, BELGIUM,* [2]*Ghent University, Faculty of Veterinary Medicine, Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Ghent, BELGIUM,* [3]*Catholic University College South-West-Flanders, Roeselare, BELGIUM,* [4]*Catholic University Leuven, Department of Biosystems, Division of Gene Technology, Heverlee, BELGIUM.*

Livestock-associated MRSA (LA-MRSA), with sequence type (ST) 398 has been reported since 2005 with increasing frequencies. This type has been isolated mainly from pigs but also from other livestock.
A first step in controlling the spread of MRSA ST398 in pig husbandry, is to develop an appropriate screening method for MRSA detection in pigs in which salt enrichment broth and chromogenic agar are optimized and the best anatomical sampling site is determined. The optimal salt enrichment broth and chromogenic medium were determined in a previous study (Pletinckx et al., 2009). The objective of this study was to identify the best single and multiple anatomical sampling site(s) for the detection of MRSA in pigs.
Samples were collected from 209 pigs, originating from 4 closed Belgian pig farms. On farm A, 29 pigs were sampled, while on farm B, C and D

60 pigs were sampled. From each pig, samples were obtained from 3 different anatomical sampling sites (anterior nares, skin behind both ears and perineum). All 627 swabs were enriched overnight in nutrient broth (Oxoid, Germany) supplemented with 7.5% NaCl. After 18-20 hours a loopful was plated onto ChromID MRSA (BioMérieux, France). Characteristic colonies were interpreted following the manufacturers instructions and further analyzed by multiplex-PCR for 16S *r*RNA, *mec*A and *nuc* gene.

In this study the overall MRSA prevalence of the investigated pigs for the 4 Belgian farms was 89.5% (187/209). For single-anatomical sampling sites, skin behind the ears (81.3%) was the most prevalent site of MRSA detection, followed by perineum (68.4 %) and anterior nares (64.1%). Logistic regression showed that the prevalence estimated from samples taken of skin behind the ears differed significantly from samples taken of anterior nares and perineum ($P<0.05$). When sampling anterior nares solely, 17.2% of the MRSA colonized pigs would have been missed in comparison to only sampling skin behind the ears. The true MRSA prevalence was better approximated when different anatomical sites were combined, there was no significant difference between the different combinations (nose and skin 87.1%, skin and perineum 85.2% and nose and perineum 82.8%). The relative sensitivity of skin behind the ears was remarkably higher than the sensitivities of perineum and anterior nares, namely 90.9%, 76.5% and 71.7%. The lower detection rate in the nares, might be explained by mucociliary clearance of the bacteria. Another explanation could be that skin behind ears and perineum, both external sampling sites, are not always colonized but perhaps transiently contaminated with MRSA present in the environment. The relative sensitivities of the multiple sampling sites were: nose and skin 97.3%, skin and perineum 95.2% and nose and perineum 92.5%. This data show that sampling of only the anterior nares underestimates the real pig MRSA prevalence.

Pletinckx et al., 2009

S1:4

LONGITUDINAL STUDY FOR LIVESTOCK-ASSOCIATED MRSA: DETERMINATION OF THE PIGLET COLONIZATION AGE, EFFECT OF THE SOW STATUS AND GENOTYPE PRESENCE

M. Verhegghe[1], *L. J. Pletinckx*[2], *M. Bekaert*[3], *F. Crombé*[4], *F. Haesebrouck*[5], *P. Butaye*[6], *M. Heyndrickx*[1], *G. Rasschaert*[1];
[1]Institute for Agricultural and Fisheries Research (ILVO), Melle, BELGIUM, [2]Catholic University College South-West Flanders-Departement HIVB (KATHO), Roeselare, BELGIUM, [3]Ghent University, Faculty of Sciences, Department of Applied Mathematics and computer science, Ghent, BELGIUM, [4]Department of Bacteriology and Immunology, Veterinary and Agrochemical Research Centre (VAR), Brussels, BELGIUM, [5]Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases, Ghent, BELGIUM, [6]Department of Bacteriology and Immunology, Veterinary and Agrochemical Research Centre (VAR)-Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases, Brussels-Ghent, BELGIUM.

A longitudinal study was performed to determine the colonization age of the piglets and the effect of the sow status at farrowing on the piglet status. Further, molecular typing was performed to detect the origin of the colonization and changes over time. Knowledge of these factors may help to develop or ameliorate intervention measures to reduce the colonization rates of pigs.

On four farrow-to-finish farms (A to D), nasal swabs were collected from 12 sows per farm and their offspring. Piglets were sampled at 10 different time points from farrowing till slaughter age, whereas the sows only on all samplings in the nursing unit. After overnight incubation (18-20h, 37°C) of the swabs, one loopfull was inoculated on MRSA-ID (Biomérieux). Suspect colonies were confirmed with a MRSA specific multiplex PCR. Statistical data analysis occurred with SAS®. A selection of sow and

piglet isolates was typed using multiple-locus variable-number tandem-repeat analysis (MLVA). From every MLVA type, isolates were further typed with *spa* typing and pulsed field gel electrophoresis using *BstZ*I.
Two trends were observed in the study. On farms A and B, MRSA was detected only occasionally in sows. The colonization rate in piglets increased remarkably at the end of the stay in the growing unit. On farms C and D, the MRSA colonization rate of sows and piglets was high from the beginning. In both situations, a decrease in colonization was observed towards slaughter age. The overall colonization age of the piglets was 17.8 days [11.5-25.6]. The average colonization age on farms A and B was 46.6 [41.3-52.1] and 24.0 [18.7-29.3], respectively, whereas it was 0.3 [0.22-0.47] and 3.1 [3.00-3.19] days on farms C and D, respectively. On farms A and B, only one dominant MLVA type was observed, whereas two or more MLVA types were observed on farms C and D. Sows did not always carry the same MLVA types as their offspring. Piglets of trend 1 farms (A and B) carried only one MLVA type during their life, whereas piglets of trend 2 farms (C and D) alternated between two dominant types.
In conclusion, the colonization age of the piglets differed amongst farms. It appears that on farms with a high prevalence of colonized sows, the infection age of the piglets is at or within days after farrowing, which is in contrast to farms with low sow colonization rates. Molecular typing revealed that there is a certain dominance of MRSA strains within the animals and within a group of animals over time.

S1:5

PREVALENCE OF STAPHYLOCOCCUS AUREUS AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN RETAIL PORK

*A. M. O'Brien[1], **B. M. Hanson**[1], S. A. Farina[1], J. Y. Wu[1], J. E. Simmering[1], S. E. Wardyn[1], M. E. Kulick[2], D. B. Wallinga[2], T. C. Smith[1];*
[1]University of Iowa, Iowa City, IA, [2]Institute for Agriculture and Trade Policy, Minneapolis, MN.

Background: This study aims to determine the prevalence, molecular characteristics and strain types of Staphylococcus aureus present on retail available pork samples in Iowa (IA).
Methods: Three hundred and ninety-five raw pork samples were collected from 36 retail food stores in Iowa, Minnesota, and New Jersey. Presumptive S. aureus isolates were confirmed through Gram stain, catalase test, tube coagulase test, and S. aureus latex agglutination assay. Methicillin resistance was assessed by penicillin binding protein (PBP2') agglutination assay and confirmed with mecA polymerase chain reaction (PCR). PCR was used to determine the presence of Panton-Valentine leukocidin (PVL), to perform Multi locus sequence typing (MLST), and to assign spa types.
Results: Staphylococcus aureus was isolated from 256 samples, giving an overall prevalence of 64.8%. Twenty-six of the isolates were resistant to methicillin, giving a prevalence of 6.6% for MRSA. S. aureus was isolated from 67.3% of conventional pork samples (202/300) and 56.8% of pork samples (54/95) labeled as "raised without antibiotics" and "raised without antibiotic growth promotants". 26.9% of MRSA isolates had spa types that were "livestock associated" ST398 while t002 and t008 accounted for 46.2%.
Conclusion: This study represents the largest sampling of raw meat products for MRSA contamination in the U.S. to date and confirms the presence of S. aureus on raw pork products in Iowa. Overall, the prevalence of MRSA on retail pork products was similar to what has been found in Canada, but is higher than previously identified in U.S. studies.

S2:1

MRSA: COMPANION ANIMAL EPIDEMIOLOGY

***A. Loeffler**;*
Royal Veterinary College, Hatfield, UNITED KINGDOM.

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be a major concern for human healthcare providers worldwide. In com-

panion animal medicine, MRSA infections are recognized as complex and important complications. However, an epidemic spread in pets and horses similar to that seen in human hospitals since the 1980s and amongst pigs since 2005, has not been recognized to date.

Risk factors for MRSA in companion animals mirror those described for people and include repeated courses of antibacterial therapy, invasive procedures and admission to veterinary clinics but most infections can still be treated successfully in animals provided underlying factors are addressed. In addition, MRSA carrier pets have been implicated as vectors and reservoir for infections and outbreaks in human patients and hospitals for the past two decades but screening studies from many countries have now shown that carriage in healthy animals is infrequent and typically associated with either an infected human or animal source or with contaminated environments.

Genetic typing has shown that the occurrence of MRSA in pets is driven by a spill-over from human hospitals, while equine-associated MRSA appears to have evolved separately, possibly from older hospital-associated MRSA strains. Individual cases of unusual, non-hospital associated strains have been seen in dogs, including infections and carriage of the pig-adapted MRSA ST398, but transmission from other hosts or vectors has seemed likely in all cases rather than the emergence of a truly pet-adapted lineage. However, as *S. aureus* continues to evolve and due to the often close contact between people and their animals, monitoring of genetic and resistance profile changes in animal-MRSA remains warranted in the interest of public health.

A still unexplained finding relates to the role that animals may play as risk factors for MRSA colonization in people. Unexpectedly high MRSA carriage frequencies had been identified referral veterinary hospital staff in Europe and North America soon after MRSA emerged as a veterinary pathogen. Subsequently, an occupational risk for MRSA carriage was documented in UK first opinion veterinary personnel. Similarly, dog ownership was a risk factor for community-associated MRSA carriage in a 2010 US study of predominantly university students. A better understanding of why animal contact facilitates *S. aureus* carriage and possibly selects for MRSA is urgently needed to help limit its spread.

S2:2

TRANSMISSION OF MRSA BETWEEN COMPANION ANIMALS AND INFECTED HUMAN PATIENTS PRESENTING TO OUTPATIENT MEDICAL CARE FACILITIES

J. P. Ferreira[1], *K. L. Anderson*[1], *M. T. Correa*[1], *R. Lyman*[1], *F. Ruffin*[2], *L. B. Reller*[2], *V. G. Fowler*[2];
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Transmission of MRSA Between Companion Animals and Infected Human Patients Presenting to Outpatient Medical Care Facilities

Jorge Pinto Ferreira[1,2], *Kevin L. Anderson*[1], *Maria T. Correa*[1], *Roberta Lyman*[1], *Felicia Ruffin*[2], *L. Barth Reller*[2]*and Vance G. Fowler, Jr.*[2]
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[2] *Duke University School of Medicine, Department of Infectious Diseases, Durham, NC, USA*
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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant pathogen in both human and veterinary medicine. The importance of companion animals as reservoirs of human infections is currently unknown. The companion animals of 49 MRSA-infected outpatients (cases) were screened for MRSA carriage, and their bacterial isolates were compared with those of the infected patients using Pulsed-Field

Gel Electrophoresis (PFGE). Rates of MRSA among the companion animals of MRSA-infected patients were compared to rates of MRSA among companion animals of pet guardians attending a "veterinary wellness clinic" (controls). MRSA was isolated from at least one companion animal in 4/49 (8.2%) households of MRSA-infected outpatients vs. none of the pets of the 50 uninfected human controls. Using PFGE, patient-pets MRSA isolates were identical for three pairs and discordant for one pair (suggested MRSA trans-infection p-value= 0.1175). These results suggest that companion animals of MRSA-infected patients can be culture-positive for MRSA, representing a potential source of infection or re-infection for humans. Further studies are required to better understand the epidemiology of MRSA human animal trans-infection.

S2:3

STAPHYLOCOCCAL NASAL CARRIAGE IN HEALTHY HUMANS AND PETS IN THE SAME HOUSEHOLD: POTENTIAL INTERSPECIES TRANSMISSION.

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Background: Staphylococci are common colonizers of the skin and mucosa of animals and humans. Close contact to pet animals has been described as a risk factor to acquire these bacteria. The objective of this study was to detect and identify the coagulase positive staphylococci (CoPS) and/or methicillin-resistant coagulase negative staphylococci (MRCoNS) nasal carriage rate of healthy humans and their pets, determine their antimicrobial resistance pattern, and to assess the potential staphylococcal interspecies transmission.
Methods: 44 unrelated pet-owning households were screened for CoPS and MRCoNS nasal carriage in La Rioja (Spain) from June-2009/ February-2011. Sixty-nine owners and 66 pets (54 dogs, 12 cats) were sampled. Following enrichment in nutrient broth samples were cultured on ORSAB and Mannitol-Salt-agar plates. Two colonies per selective plate were analysed. Isolates were identified by PCR, PCR-RFLP, and sequencing of *sodA* gene. Antimicrobial susceptibility to 16 antibiotics was determined by disc-diffusion. All methicillin-resistant (MR) isolates were tested for *mecA* gene by PCR. Isolates suspected of interspecies transmission were typed by *Sma*I-PFGE and, if CoPS, *spa*-type and MLST were determined.
Results: 33 of the 44 households (75%) were positive for CoPS and/or MRCoNS. Thirty-six owners of the 69 (52%) studied carried CoPS and/or MRCoNS with 42 isolates obtained: 24 *S. aureus*; 3 *S. pseudintermedius*, and 15 MRCoNS [*S. epidermidis* (14), *S. lentus* (1)]. Thirty-one of the 66 studied pets (47%) were positive for CoPS and/or MRCoNS with 36 isolates obtained: 10 *S. aureus*; 12 methicillin susceptible *S. pseudintermedius*, 2 methicillin resistant *S. pseudintermedius* (MRSP) and 12 MRCoNS [*S. epidermidis* (8), *S. lentus* (1) *S. pulvereri* (1), *S. vitulinus* (1), *S. cohnii* (1)]. Isolates were resistant to the following families of antimicrobial (no isolates CoPS/MRCoNS): βlactams (2/27), tetracyclines (11/3), macrolides-lincosamides (14/15), aminoglucosides (26/8), co-trimoxazol (4/4), mupirocin (0/7), chloramphenicol (5/1), and ciprofloxacin (3/3). All 27 MRCoNS and 2 MRSP of dog origin carried the *mecA* gene. MRSP were typed as t02-ST71 and t06-ST92. No MRSA was detected. In 16 households (36%) there were *mecA*-carrying strains. Identical strains by PFGE from both owners and their pets were identified in 7 households: 4 *S. aureus*: 1 t073-ST45, 1 t209-ST109, 1 t021-ST1654(new), 1 t159-ST121; 1 *S. pseudintermedius spa* no-typable-ST142(new); 1 MR *S. epidermidis*, and 1 MR *S. lentus.*
Conclusions: CoPS and/or MRCoNS are common colonizers of healthy humans and their pets. No MRSA was detected but MRSP was detected in 2 dogs, one being ST71. In 7 households (16%) both owner/s and pet/s carried indistinguishable strains what suggests interspecies transmission and emphasises the transference risk of these microorganisms between humans and their companion animals.

S2:4

IS MRSP ST71 SOMETHING MORE THAN JUST A *Staphylococcus pseudintermedius* STRAIN CONTAINING mecA?

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This talk will draw attention to some noteworthy differences between the methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) clone ST71 and the other members of the species. MRSP has emerged in small animals a long time after the acquisition of methicillin resistance by *S. aureus* in humans. The first MRSP isolates were reported in Brazil and in the USA approximately 40 years after the first appearance of methicillin-resistant *S. aureus* (MRSA) in the early 1960s. In Europe MRSP was first reported in 2006, when the epidemic clone ST71 made its first appearance. This clone is now widespread in Europe and its presence has also been documented in the USA and in Asia. The data currently available indicate that MRSP ST71 accounts for up to 20% of *S. pseudintermedius* isolates from canine diagnostic specimens in some European countries. The reasons for the rapid spread of this specific MRSP clone are presently unknown. Extensive antibiotic use may have favoured selection and spread of MRSP ST71, which is consistently resistant to the most common antimicrobial classes used in small animal practice. However, additional factors such as an increased ability to colonize, cause infection or survive in the hospital environment may have contributed to the evolutionary success of this lineage. Indeed, MRSP ST71 has several atypical genetic and epidemiological traits. In addition to *mecA*, it contains other antibiotic resistance determinants that are unusual in methicillin-susceptible *S. pseudintermedius* (MSSP), and is consistently associated with the presence of the *S. intermedius* exfoliative toxin (SIET) and of the PVL-related leukotoxin LukI. From an epidemiological standpoint, this MRSP clone has been recovered from the nasal cavity of small animal dermatologists at significantly higher frequency compared to MSSP, and its multi-locus sequence type appears to be extremely rare among MSSP isolates.

S2:5

MULTILOCUS SEQUENCE TYPING (MLST) FOR CHARACTERIZATION OF METHICILLIN- RESISTANT AND METHICILLIN- SUSCEPTIBLE CLONES OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

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Staphylococcus pseudintermedius, the bacterium commonly associated with canine pyoderma and wound infections, is a newly emerging methicillin-resistant pathogen. There is limited information about the population genetic structure within this species. Foundational studies of the *Staphylococcus intermedius* group examined allelic polymorphisms in four genes. In this study, four additional slowly evolving genes were identified from numerous candidates and used to develop a new multilocus sequence typing (MLST) scheme. The included loci are contained within adenylosuccinate synthetase (*purA),* formate dehydrogenase (*fdh),* perfringolysin O regulatory protein (*pfoR)* and sodium: sulfate symporter *(sar*) genes. It was hypothesized that additional loci would increase the discriminatory power of MLST typing of methicillin-resistant *S. pseudintermedius* (MRSP) isolates and provide a more detailed picture of *S.pseudintermedius* population genetics. The 8 locus MLST method was applied to genetically diverse *S. pseudintermedius* isolates. Most MRSP isolates were further subdivided into multiple STs. ST 71, the major MRSP lineage found in Europe, was not further differentiated. MRSP were distributed in all of the major genetic branches of *purA, fdh, pfoR* and *sar* genes. This study reveals methicillin resistance in diverse genetic backgrounds and a lack of clustering based upon host species of origin

or geographical region. The expanded MLST scheme provides an unambiguous method for identifying *S. pseudintermedius* clonal populations and its widespread use may give new insight into clonal spread, population genetics and evolution of the species.

S3:1

HOW MODELLING CAN ADD VALUE TO INFECTIOUS DISEASE RESEARCH: ILLUSTRATED EXAMPLES FOR LA-MRSA

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Infectious disease modelling can be applied to the study of antimicrobial resistance with exploratory, descriptive or predictive objectives. Particularly, models can address infection and/or intervention scenarios that would otherwise be impossible to assess due to time, logistical or ethical constraints. However, infectious disease models have also been criticised for providing unrealistic scenarios and hereby contributing to over- or under-estimation of risks. Another constraint is that these models require data and - if they are not available - assumptions have to be made.

There are a number of different model types that can be applied in the context of infectious disease: (1) statistical models and (2) mathematical models. Both types of models were used in the PILGRIM project (www.fp7-pilgrim.eu). The objective of this paper is to encourage collaboration between mircobiologists and modellers. We illustrate the strength of collaboration using model examples of MRSA colonisation and transmission.

In PILGRIM, experimental work was conducted by inoculating piglets with MRSA ST398 alone or combined with maternal staphylococcal flora. The antagonistic effect was first evaluated using statistical modelling (linear mixed-effect regression model). Results showed that the level of MRSA ST398 colonisation was unaffected by inoculation of maternal staphylococcal flora. Using a similar experimental design, growth equations were developed and fitted to the data using another statistical model (nonlinear regression model). Results indicated that MRSA ST398 rate of growth was not inhibited but promoted in the presence of antagonists, reaching greater levels of maximum carriage.

A stochastic meta-population model was developed to evaluate the rate at which MRSA ST398 can spread. All individuals of a theoretical community were classified according to their exposure to pigs. For various degrees of persistent MRSA ST398 carriage, we computed the net reproduction value (R_0) of MRSA in the meta-population and evaluated key determinants for MRSA spread. Results of this model showed that MRSA ST398 may have a low capacity of spreading in the community (R_0<1) and that a period of 4 to 6 months is sufficient for the community to be naturally cleared.

Building models is not a quick process and requires iterative fine-tuning, particularly if the system of interest is complex. Although models should be as simple as possible, complexity may be required to describe the underlying real life processes. The PILGRIM project illustrates how modelling can use input from lab- and field-based studies to answer complex control questions. However, infectious disease modelling is an inter-disciplinary activity that needs a common language, willingness to collaborate and trust. We promote this type of collaboration in the interest of increased research impact on infection prevention and control.

S3:2

MRSA HARBOURING A NOVEL MECA HOMOLOGUE EMERGING IN HUMAN AND BOVINE POPULATIONS IN THE UK AND DENMARK

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In the last three decades methicillin-resistant *Staphylococcus aureus* (MRSA) has become widespread in the human population. Recent reports of ST398 MRSA spreading from pigs to the human population have highlighted the potential for MRSA to emerge from animal reservoirs. The isolation of a *S. aureus* strain isolated from bovine milk (LGA251), showing resistance to beta-lactam antibiotics characteristic of MRSA, but testing negative for the *mecA* gene was investigated. Whole genome sequencing was employed to determine the genetic basis for the antibiotic resistance. A *mecA* homologue ($mecA_{LGA251}$) was discovered in the LGA251 genome carried in a novel staphylococcal cassette chromosome mec, designated SCCmec XI. Systematic screening of collections of *mecA*-negative bovine and human *S. aureus* isolates demonstrating antibiotic resistance patterns characteristic of MRSA was undertaken. The mecA homologue was found in 13 *S. aureus* from dairy cattle geographically dispersed throughout England. The isolates belonged to four different MLST lineages (CC130, CC705, CC1943 and ST425); *spa* type t843 (associated with CC130) was identified in 60% of bovine isolates. When human *mecA* negative MRSA were screened, the *mecA* homologue was identified in 12 isolates from Scotland, 15 from England and 25 from Denmark. The $mecA_{LGA251}$ gene was found in *S. aureus* from clinical infections including bacteraemia and wound infections, but most commonly from general screening samples. Among the human isolates the same clonal complexes were represented (except CC151), and t843 was also the most common *spa* type found. This novel *mecA* homologue is associated with resistance to beta-lactam antibiotics which is characteristic of MRSA. Current gold standard diagnostic techniques such as PCR (using existing primers for *mecA*), and slide agglutination tests using monoclonal antibodies targeting PBP 2a, fail to identify these resistant strains. Whilst routine culture and sensitivity testing will identify MRSA, these techniques are unable to distinguish $mecA_{LGA251}$ from conventional *mecA*. The strains of *S. aureus* encoding $mecA_{LGA251}$ described herein belong to clonal complexes which have previously been considered to be bovine lineages. This suggests that the human isolates have a bovine origin.

S3:3

GENOMIC CHARACTERIZATION OF "LIVESTOCK- ASSOCIATED" ST398 METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*, CANADA.

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Background. Despite reports of high colonization rates of ST398 "livestock- associated" MRSA (LA-MRSA) amongst pigs and pig farmers, the incidence of LA-MRSA in the general population in Canada appears to be rare in comparison to some European countries. In this study, the complete genome sequence of a Canadian representative LA-MRSA isolate (08 BA 02176) from a person was acquired and compared to a recently sequenced genome of a LA-MRSA isolate (S0385) from Europe to identify genetic traits that may explain differences in the success of these particular strains.
Methods. The 08 BA 02176 isolate was obtained from a human post-operative surgical site infection and sequenced using the 454 GS FLX system. Gaps between contigs were closed by conventional PCR and Sanger sequencing. Genome annotation was performed using an in house version of GenDB.

Results. As with S0385 and other characterized ST398 isolates, 08 BA 2176 lacked many virulence determinants present in other epidemic MRSA strain types including enterotoxin and exfoliative toxin genes. Of interest, both S0385 and 08 BA 2176 harboured SAP1-S0385, encoding two putative extracellular proteins with similarity to staphylococcal complement inhibitor and von Willebrand factor-binding protein, which have previously been implicated in ruminant host specificity. A few major differences between S0385 and 08 BA 2176 included the absence in 08 BA 2176 of all 3 plasmids, the 3 integrative conjugative elements (ICESa1A, ICESa1B, ICESa2), and phages phiSa2 and phiSa6. Unique to 08 BA 2176 was the identification of a novel phage located at the same insertion site as phiSa6; insertion of Tn*5406* into the chromosomal *att554* site, harbouring a variant of *vgaA* encoding an ABC transporter conferring resistance to pleuromutilins, lincosamides, and streptogramin A; and a unique SCC*mec*V subtype. The 08 BA 2176 SCC*mec*V element included a novel J3 region as well as a CRISPR array in the J1 region. PCR screening of a collection of ST398 isolates revealed an additional 5 Canadian (n=15) and only 1 international (n=30) isolate harbouring this CRISPR element.

Conclusions. As in Europe and North America, the lack of many virulence determinants in the ST398 lineage is likely contributing to the comparatively low incidence of observed infections in humans. Further work is required to delineate whether presence of this CRISPR element confers resistance to plasmids and phages, and might explain why 08 BA 2176 contains fewer antimicrobial resistance genes and phage-encoded virulence factors relative to S0385 and other epidemic MRSA strains.

S3:4

THE DISTRIBUTION OF MOBILE GENETIC ELEMENTS (MGES) IN MRSA CC398 IS ASSOCIATED WITH BOTH HOST AND COUNTRY.

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Methicillin-resistant Staphylococcus aureus (MRSA) clonal complex (CC)398 has emerged from pigs to cause human infections in Europe and North America. Identifying host specificity factors of pathogens is crucial for (i) developing therapeutics for pathogen detection and control (ii) understanding the specific host-pathogen interaction. We designed, validated and used a new 62-strain S. aureus microarray (SAM-62) to compare genomes of isolates from three geographical areas (Belgium, Denmark and The Netherlands) to understand how CC398 colonises different mammalian hosts. The core genomes of 44 pig isolates and 32 isolates from humans in contact with pigs did not vary; there was no difference in carriage of surface genes, secreted genes or their variants. However, mobile genetic element (MGE) distribution was highly variable. Phi3 bacteriophage and human specificity genes (chp, sak, scn) were found in invasive human but not pig isolates. SaPI5 and putative ruminant specificity gene variants (vwb, scn) were common but not pig specific. Virulence and resistance gene carriage was host-associated, but was country specific. We conclude MGE exchange is frequent in CC398 and greatest amongst populations in close contact. This feature may help determine epidemiological associations amongst isolates of the same lineage.

S3:5

LONGITUDINAL STUDY ON METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN HOUSEHOLDS

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Background: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is an emerging pathogen in dogs and has been found in Europe, Asia and North America. To date most studies are one-point prevalence studies and little is known about the dynamics of MRSP in dogs and their environment. In this longitudinal study MRSP colonization in dogs and the transmission of MRSP to humans, contact animals and the environment was investigated.
Methods: Dogs with a recent clinical MRSP infection were included. The index dogs (n=16), contact animals (n=7), owners (n=25) and environments (n=16) were sampled once a month for six months. Samples were taken from the nose, perineum and infection site (if present) of the index cases and contact animals, the nose of the owner, the sleeping place, the feeding place and one site not physically accessible to the animals and cultured using pre-enrichment. From each household the first and last MRSP isolates were selected for genotyping using multilocus sequence typing, pulsed field gel electrophoresis, *spa* typing and SCC*mec* typing.
Results: A total of 229 samples were taken from the index dogs and 61 (27%) were found MRSP-positive. Index cases were often found positive for prolonged periods of time, in 2 cases during all six samplings; 5 dogs were intermittently MRSP-positive, occasionally with 3 months between two MRSP-positive samplings, 7 dogs remained MRSP-negative after they tested negative for the first time and 2 dogs were only sampled once. Index cases showing clinical signs of infection could test MRSP-positive (38/63; 60%) or negative (25/63; 40%). MRSP-positive samples were found from contact animals (13/68; 19%) and the environment (48/236; 18%), most often in combination with a MRSP-positive index. In four households positive environmental samples were found while no animals or humans were tested MRSP positive, indicating that MRSP can survive in the environment for prolonged periods of time. A total of 140 nasal swabs were taken from humans and 5 (3.6%) were found MRSP-positive. Genotyping revealed that generally similar or indistinguishable MRSP isolates were found in patients, contact animals and environmental samples within the same household.
Conclusions: Dogs can be MRSP-positive for prolonged periods of time, even after repeated MRSP-negative cultures or in the absence of clinical infection. There is a high risk of transmission of MRSP to animals in close contact with MRSP patients. Humans were rarely MRSP-positive and never tested MRSP-positive more than once suggesting occasional contamination or rapid elimination of colonization of the owners.

S3:6

COMPARATIVE IN VIVO HOST-SPECIFICITY OF HUMAN- AND PIG-ASSOCIATED STAPHYLOCOCCUS AUREUS STRAINS

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Introduction: Some S. aureus lineages are able to colonize both humans and animals, indicating that these strains have escaped their host-specific boundaries. Clonal complex (CC) 398 and CC30 are among the two most common lineages found in pigs, and strains belonging to these CCs are also isolated from human infections. In contrast, USA300 (CC8) and EMRSA-15 (CC22) are typical human-associated strains and rarely isolated from pigs.
Objectives: To compare the host-specificity of human- and pig-associated strains in a pig colonization model
Methods: Three pregnant S. aureus-free sows were inoculated using a model recently described by our group (Moodley et al., 2010). Shortly before farrowing, each sow was inoculated intravaginally with one of the three

following strain mixes: 1) human (t034, ST398) and pig (t011, ST398) CC398; 2) human (EMRSA-16, t018, ST36) and pig (t1333, ST433) CC30; and 3) pig CC398 (t011, ST398), human CC30 (t018, ST36), human CC22 (EMRSA-15, t032, ST22) and human CC8 (USA300, t008, ST8). Nasal swabs were collected from piglets on days 0, 7, 14, 21 and 28 post farrowing, and swabs were cultured on plates containing combinations of oxacillin, streptomycin, ciprofloxacin, cadmium and/or mupirocin to enable detection of the different strains. Colonization was defined as five consecutive positive cultures over four weeks.
Results: The sow inoculated with CC398 developed acute necrotising and purulent endometritis, resulting in abortion of the piglets. Both the human and pig strains were cultured from necropsy samples from the reproductive organs and immunohistochemistry confirmed presence of S. aureus in the tissue sections. In the CC30-inoculated sow, all piglets were positive for the pig strain for the entire duration of the experiment, whereas the human strain was only detected at farrowing. In the third experiment, USA300 was the only strain consistently isolated from the nasal cavity of all piglets. EMRSA-15 was only present in the first two weeks after farrowing and the pig CC398 was only detected in one piglet on two separate sampling days. Human CC30 was not detected in any of the piglets.
Discussion and conclusion: The presence of both CC398 strains in the lesions of the first sow provides experimental evidence that this lineage may cause genital tract infections leading to abortion of the piglets. In the second experiment, the pig CC30 strain (ST433) was a better colonizer of the piglets compared to the human CC30 strain (ST36), indicating that these two lineages have evolved separately and adapted to different hosts despite belonging to the same CC. Surprisingly, USA 300 (ST8) outcompeted all other strains including the pig CC398 strain in third experiment. Although USA300 has never been isolated from healthy pigs, this result suggests that pigs can be colonized and may become a potential reservoir of this human epidemic clone.

S4:1

ENVIRONMENT & ECOLOGY OF MRSA AND ANIMALS

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In the environment, particularly when animals are involved, there are numerous potential sources and reservoirs for Methicillin-resistant *Staphylococcus aureus* (MRSA). There are several routes of exposure from MRSA sources and reservoirs to Humans and Animals. In order to determine the presence of MRSA a wide range of environmental sampling may need to be conducted; followed by interpretation of the results. Through utilization of the exposure assessment literature this presentation will conduct a brief review of these sources, reservoirs, and routes of exposure, as well as, an overview of several sampling techniques to assess exposure.

S4:2

STAPHYLOCOCCI PRESENT IN DUTCH SWINE FARMS ACT AS A RESERVOIR OF SCCmec.

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SCC*mec* is a mobile genetic element carrying the *mecA* gene, which causes methicillin resistance in staphylococcal species. It is believed that CNS act as reservoir for SCC*mec* exchange and for generation of new methicillin-resistant *Staphylococcus* spp. However, knowledge about the presence of SCC*mec* types in staphylococci on farms is limited to *S. aureus* isolated from clinical or environmental samples. MRSA ST398 is highly prevalent at Dutch swine farms. Determination of the SCC*mec* reservoir on swine farms is essential for better understanding of the ecology of MRSA ST398. Here we report

a pilot study focusing on swine farms which will allow us to elucidate if the same SCC*mec* type can be shared between staphylococcal species.
Ten Dutch swine farms with different antibiotic usage were investigated. Staphylococci were isolated from nasal swabs and dust samples using standard microbiological procedures. Methicillin-resistant staphylococci were identified by *mecA* PCR. Species identification was performed by MALDI-TOF. SCC*mec* typing was performed using Kondo-Multiplex PCR strategy.
Forty four isolates were analyzed which belonged to 10 different staphylococcal species. Species distribution and number of recovered *mecA*-positive staphylococci differed between farms. Farms with high antibiotic usage tended to have a relatively higher number of *mecA*-positive staphylococci. All recovered *S. aureus* belonged to ST398, with the dominant SCC*mec* types V and IVa. Type IVc as well as type III, VI and novel non-typeable elements were only found in CNS. *S. aureus* and *S. epidermidis* from one farm shared SCC*mec* type V. SCC*mec* type IVc was detected in several staphylococcal species isolated from one pig farm. These observations suggest horizontally transfer of SCC*mec* between staphylococci.
In conclusion, our study showed that a reservoir of SCC*mec* is present in staphylococcal flora on swine farms and may thereby create the possibility for SCC*mec* transfer to *S. aureus*. Longitudinal surveillance studies are needed to monitor the transfer to *S. aureus* of the novel SCC*mec* subtypes that are currently only found in CNS. Additionally, antibiotics usage and presence of *mecA*-positive staphylococcal seems to be linked, which should be further investigated.

S4:3

DETECTION OF ANTIBIOTIC-RESISTANT STAPHYLOCOCCUS IN ENVIRONMENTAL WATERS NEAR INDUSTRIAL SWINE OPERATIONS IN EASTERN NORTH CAROLINA

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Agriculture in the United States has shifted from small operations to larger industrial operations. The effects of this shift on water quality have not been well characterized and there are concerns about the safety of using sub-therapeutic antibiotics in animal feed. This study examined the presence of antibiotic resistant *Staphylococcus* in surface waters adjacent to swine lagoon waste spray fields. Surface water samples (n=183) were collected over the course of a year from public access waters in proximity to swine lagoon spray fields in eastern North Carolina. These samples were filtered onto 0.45µm mixed-cellulose esters membrane filters and incubated on CHROMagar™ MRSA (BD BBL™) media at 37°C for 18-24 hours. Colonies with morphologic characteristics of *Staphylococcus aureus* were counted, and up to ten of these colonies from each sample were selected and streaked onto CHROMagar™ Staph aureus (BD BBL™) plates for isolation. Following incubation, each colony was inoculated in 0.75mL of Brain Heart Infusion Broth with 15% glycerol, and stored at -80°C until further characterization. Of the 183 surface water samples analyzed, 92% of them were positive, with at least one colony with morphological characteristics of *S. aureus* that grew on CHROMagar MRSA plates. A fixed effects linear regression model (N=179) was used to determine a statistically significant ($p<0.05$) positive relationship (0.123, SE=0.060) between

$\log_{10}$ antibiotic resistant *Staphylococcus* concentrations (CFU/100mL) and inches of cumulative rainfall 48 hours before sample collection. There were 698 isolates saved from these water samples that will be further characterized using gene-specific PCR assays and sequence typing. A preliminary screening of 7% of these isolates revealed that 96% of isolates tested positive for the 16S gene of *Staphylococcus*, while none of the isolates have tested positive for the *clfA* or *mecA* genes that would be characteristic of MRSA. Further testing will be done to investigate the resistance of these isolates to various antibiotics and to assess the virulence of these strains. Results to date demonstrate the presence of antibiotic resistant *Staphylococcus* in surface waters in proximity to livestock operations.

S5:1

INFECTION CONTROL & ANIMAL-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCCCI

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Over the past few years, the need for better infection control practices in veterinary medicine, ways to assess infection control and to improve compliance have become more clear. There are many reasons for this, but perhaps the most imperative driving force for better infection control is the ongoing pandemic of antimicrobial resistant bacteria, with the dramatic emergence of some highly resistant pathogens, including MRSA and methicillin-resistant S. pseudintermedius (MRSP), and their impact on infection rates in companion animals. It has also been shown in numerous studies that veterinary personnel, whether they work with small companion animals, horses, swine or even cattle, may be at increased risk of colonization with MRSA, making precautions to protect both personnel and patients of great importance.
Consideration of basic infection control measures is essential in any situation. Even in people's homes, particularly when zoonotic pathogens such as MRSA are involved, infection control precautions must not be ignored. However, with regard to companion animals, infection control is arguably of greatest importance in veterinary clinics, which form community hubs where animals and people of all different kinds - and all different levels of health - meet and interact. A recent study of 101 companion animal clinics identified that not one of them had a recognized infection control program. Infection control practices are more likely to be underutilized under these circumstances, and their effectiveness cannot be evaluated without established policies and a formal infection control program, even if the program is very basic. Basic routine infection control practices are a critical factor in preventing the spread of infections. In particular, improving hand hygiene can have a significant impact on disease rates in hospitals. Despite the well-demonstrated importance of hand hygiene in human healthcare, there is a dearth of information in the same area in veterinary medicine. Objective assessments of hand hygiene compliance and quality in veterinary clinics are lacking, but are needed to assist in the design of infection control programs and educational information.
In human medicine, vast efforts have recently been undertaken to evaluate and improve infection control practices. Measuring compliance is a key aspect of improving these practices and protecting patients. This is highlighted by the recent implementation of the requirement for hospitals in various regions to publicly disclose their hand hygiene compliance data. Clearly, hand hygiene is considered to be of paramount importance in human medicine, and its importance should be no less in veterinary medicine.

S5:2

USE OF ENVIRONMENTAL MARKING FOR EVALUATION OF EFFICACY OF CLEANING IN A VETERINARY HOSPITAL

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Hospital-associated infection (HAI) is an inherent risk of hospitalization. HAIs cause a poorly defined but significant degree of morbidity and mortality amongst veterinary patients, particu-

larly with the emergence of methicillin-resistant staphylococci, and some (e.g. MRSA) may pose a risk to veterinary healthcare workers. The epidemic of methicillin resistant staphylococcal infections has highlighted the need for good infection control practices. One important aspect of this is environmental cleaning and disinfection, an area that has received little study. The objectives of this study were to use environmental marking to evaluate cleaning efficacy in a veterinary healthcare facility.

Water-based fluorescent dye was inoculated onto various surfaces in a small animal veterinary tertiary care referral facility and an associated but physically separate primary healthcare centre (PHC). The dye was not readily visible to the naked eye but was clearly identified with a handheld UV light. Various personnel and patient contact surfaces were inoculated and examined 24h later to see if dye had been removed.

284 sites have been examined to date, 263 (93%) in the referral facility and 21 (7%) in the PHC. Results varied with site types, with notable cleaning rates being 7.4% for computers/computer mice, 16% for consumable items, 19% for medical equipment, 22% for hand sanitizer and soap bottles, 28% for various human hand contact surfaces, 31% for miscellaneous sites, 33% for chairs, 62% for cage handles, 67% for sink/sink taps, 68% for counters, 71% for tables, and 75% for floors.

This study indicated some areas that could be improved upon. It was not meant to assess the overall prevalence of cleaned sites. Further, different surfaces have different expectations, and the necessity of regular cleaning varies between surfaces. Therefore the data should be examined at the site level as part of an assessment to determine whether the observed cleaning rates are acceptable. The low rate of cleaning of surfaces like walls and cabinets (0%) is probably of limited concern while the low rate of cleaning of surfaces such as consumable items (e.g. gauze packages, disinfectant bottles) and medical equipment (e.g. otoscopes, thermometers, fluid pumps) that are frequently handled and used in patient care is of greater concern.

This method was an easy and cost-effective means of evaluating cleaning practices. It identified reasonably high rates of cleaning of most patient contact surfaces however some surfaces were consistently not cleaned. This type of approach can identify areas for improvement, and for development and testing of interventions to improve cleaning efficacy. This method could be an effective routine or periodic surveillance tool, or as part of educational efforts directed towards veterinary hospital cleaning personnel.

S5:3

A MODEL FOR INFECTION CONTROL PROGRAM IN EQUINE HOSPITALS: IMPLEMENTATION, EVALUATION OF EFFECTIVENESS AND COMPLIANCE

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Background: The first outbreak of methicillin resistant *Staphylococcus aureus* (MRSA) 2008 at an equine clinic in Sweden demanded the need for infection control program. The aim of this project was to develop a model for infection control program including implementation of infection control protocol, evaluation of compliance and effectiveness of the given protocol. The ultimate goal was to decrease the spread of multi-resistant among horses and between horses and humans in equine hospitals. **Methods:** Five equine hospitals voluntarily joined the project. At each hospital 2 members of staff was chosen by the hospital management to be responsible for infection control program so called infection control practitioners. Before implementation of infection control protocol**,** the hospitals were asked to start to monitor the number of surgical site infections (SSI) for a given period. In addition horse owners were called a week after surgery to check wound status. All SSI was sampled and analysed using routine bacteriology including susceptibility testing. Infection control protocol was implemented and the infection control practitioners were asked to register staff compliance to the infection control protocol once a week by a given procedure. They were also asked to

monthly report data on sales of antimicrobials, consumption of alcohol-based hand sanitizers and sales of gloves to project. These data collections together with registration of SSI and compliance protocol were continued for a year. During this time, the project educated and informed the hospitals' staff on antimicrobial resistance and infection control. **Results:** Three hospitals could fulfil the requirements of the model. Preliminary results from one hospital during the first 6 months are shown. More data will be presented at the conference. About 10% SSI had occurred during this period of time. From 72 samples taken, betahemolytic streptococci were the most common finding followed by *Staphylococcus aureus*. Compliance to infections control protocol was high regarding short-sleeved working-clothes, but compliance to disinfection of hands before and after handling each patient and before and after gloving was poor. Also gloves were worn at many other occasions than stated in the protocol. Sales of gloves and consumption of alcohol-based hand sanitizers was increased markedly. **Conclusion:** Successful implementation was associated with supportive and enthusiastic infections control practitioners and management that could convey staff the importance of infections control. Routines regarding disinfection of hands and wearing gloves needs further communication. In addition, extraction of data from soft ware systems for patient records and sales figures have to be easy to facilitate objective surveillance of compliance to infection control program.

S5:4

METHICILLIN RESISTANT STAPHYLOCOCCUS SPP IN COMMERCIAL PIGS USED IN VETERINARY STUDENT TRAINING

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Purpose: Research groups have recently identified methicillin resistant Staphylococcus aureus (MRSA) as a common colonizing agent in pigs, and that people with frequent contact have increased risks for colonization and infection. MRSA has also been identified as one of the most common causes of outbreaks of nosocomial infections in veterinary hospitals. The Colorado State University College of Veterinary Medicine and Biomedical Sciences utilizes commercial pigs in veterinary student training. These pigs are a potential source of environmental contamination as well as nosocomial and zoonotic infections. Objectives of this study were to estimate the frequency of MRSA colonization in young pigs used in teaching laboratories and evaluate associated environmental contamination.
Methods: Environmental samples were collected twice weekly from the holding facility (n=28) and twice daily from the teaching laboratory (n=140) using a commercially available electrostatic dust collection wipe (Swiffer, Procter and Gamble). Nasal and rectal swabs were collected twice from each pig (n=100). All samples were cultured for the presence of methicillin resistant Staphylococcus spp.
Results: Overall, MRSA colonization was detected in 51% and 71% of pigs at the holding facility and teaching laboratory (respectively) or 87% at any time. The pigs arrived in two groups (one month apart) and the frequency of colonization was markedly different between groups. Cultures also demonstrated significant environmental contamination. Genotyping of isolates showed that most were Spa-type 2 (consistent with USA100).
Conclusions: This study demonstrates a previously unrecognized risk associated with the use of young pigs in training and research in both veterinary and human medicine. Colonization of these pigs does not preclude their use, rather it demonstrates the importance of biosecurity measures to control the associated risks.

S6:1

Staphylococcus aureus ST398: WHAT CAN THE GENOME LEARN US?

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Staphylococcus aureus belonging to sequence type (ST) 398 appears to be primarily to be a colonizing lineage of both humans and in particular animals and fatal infections in humans and pigs rare. Analysis of the first ST398 genome by our group provided at least some explanations. The accessory genome content differed considerably from other *S. aureus* genomes. Several virulence factor encoding genes such as those for enterotoxins and phage encoded toxins were lacking. However, a unique pathogenicity island was present which may help explain the host range. The isolate lacks a restriction modification system in one of the vSa islands. This may enhance the ability acquire novel mobile elements and thereby increase the pathogenicity of ST398 isolates. In fact isolates with Panton Valentine leukocidin (PVL) genes have been described. The lack of a restriction modification system may also enhance the ability to acquire antibiotic resistance genes. The sequenced isolate had a novel composite of a type V staphylococcal cassette chromosome *mec* (SCC*mec*), but other isolates carry a type IV SCC*mec*; an indication that methicillin resistance was acquired at least twice. The sequenced isolate carried two *tet* resistance determinants which explain the selection of ST398 after treatment of pigs with tetracycline derivatives. In addition, a zinc resistance gene (initially annotated as a copper resistance gene) was present in SCC*mec*, which may lead to selection under zinc pressure, a commonly used feed additive. Besides these resistance genes a variety of different antibiotic resistance genes have been reported in different isolates including several novel ones. Another interesting feature of the sequenced ST398 is the presence of two integrative conjugative elements (ICE). These are related to type IV secretion systems and we speculate that they are involved in the transfer of DNA to other bacteria. This would make these isolates an active source for SCC*mec*. Sequencing of 3 isolates of ST398 with a SCC*mec* type IV from different sources on the same farm showed no striking differences between them. However, differences between S0385 and these 3 isolates in some mobile elements, in particular SCC*mec* and phages, were present. In addition smaller differences were noted, including one affecting a global regulator that also affected downstream genes. Some of these differences were confirmed in metabolic assays. Control of ST398 will probably difficult to achieve due to its wide host range and variety of environments involved.
Summarizing, ST398 MRSA arose at least two times and rapidly spread across the world and appears to evolve at a rapid pace, but control will be difficult to achieve.

S6:2

MOLECULAR ANALYSIS OF METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) ISOLATES FROM FOOD AND FOOD PRODUCTS OF POULTRY ORIGIN

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Background: During recent years, MRSA have been detected in animals and food of animal origin. Since there is little information available about MRSA from food of poultry origin in Germany, the aim of the present study was to investigate MRSA isolates from poultry meat for their genetic background and their resistance and virulence properties.
Methods: In total, 32 MRSA isolates were obtained during a survey of 86 samples from fresh meat and meat products of poultry origin. All isolates were subjected to *spa*, SCC*mec* and *dru*

typing, as well as to two CC398-specific PCRs. For isolates negative in the CC398-specific PCRs, MLST was conducted. Susceptibility testing was performed by broth microdilution. Resistance and virulence genes were detected by a *S. aureus*-specific DNA microarray or specific PCRs.

Results: Twenty-eight of the MRSA isolates belonged to the clonal complex CC398, two isolates showed the multilocus sequence type ST9 and single isolates belonged to ST5 and CC5/ST1791. The CC398 isolates showed the *spa* types t011, t034, t899, t2346 and 6574, carried SCC*mec* elements of type IV and V, and the *dru* types dt2b, dt6j, dt6m, dt10a, dt10as, dt10at, dt10q, dt11a, dt11v and dt11ab. Both ST9 strains had *spa* type t1430, SCC*mec* type IV and *dru* type dt10a, whereas the ST5 and ST1791 strains exhibited *spa* type t002, carried SCC*mec* elements of type III and had *dru* type dt9v. Various resistance patterns were seen, with 30 isolates showing a multi-resistance phenotype with resistance against at least three classes of antimicrobial agents. The respective resistance genes *mecA*, *blaZ* [beta-lactams], *tet*(K), *tet*(L), *tet*(M) [tetracyclines], *dfrS1*, *dfrK* [trimethoprim], *erm*(A), *erm*(B), *erm*(C), *erm*(T) [macrolides/lincosamides/streptogramin B], *aacA-aphD* [gentamicin/kanamycin/tobramycin], *aadD* [kanamycin/neomycin], *apmA* [apramycin], *vga*(A), *vga*(C) [streptogramin A/lincosamides/pleuromutilins] were detected. The two ST9, the ST5 and the ST1791 isolates harboured the *egc* gene cluster for enterotoxin G, I, M, N, O and U genes, whereas the MRSA CC398 isolates did not carry any of the enterotoxin genes tested. Genes encoding the major virulence factors PVL, TSST-1 and exfoliative toxins were not detected.

Conclusions: The presence of MRSA in 37.2% of the analysed meat and meat products of poultry, including four enterotoxigenic strains, raised concerns. Further studies are needed to investigate possible hazards for human health and to identify the routes of transmission.

S6:3

CHARACTERIZATION OF A SMALL APRAMYCIN RESISTANCE PLASMID FROM PORCINE MRSA ST398 WHICH CARRIES A VARIANT OF THE *apmA* GENE

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Background: Apramycin is an aminocyclitol antibiotic which is exclusively used in veterinary medicine for the treatment of *Escherichia coli* infections in swine, cattle, sheep, poultry or rabbits. While apramycin resistance in Gram-negative bacteria is commonly associated with the acetyltransferase gene *aac(3)-IV*, a single apramycin resistance gene, *apmA*, has been identified recently on ca. 40-kb multi-resistance plasmids in bovine and porcine MRSA ST398 strains. The aim of this study was to characterize a small plasmid conferring apramycin resistance.

Methods: During a survey on the presence of apramycin resistance in staphylococci of animal origin, the porcine MRSA ST398 isolate E49 showed an apramycin MIC of >= 32 mg/L. Plasmids were prepared and transferred into *S. aureus* RN4220 with subsequent selection of the transformants on apramycin-containing media. The apramycin resistance plasmid pKKS49 was linearized by EcoRI and XbaI digestion, cloned into pBluescript II SK+ and sequenced completely.

Results: Plasmid pKKS49 had a size of 4809 bp and a GC content of 38.6%. Its sequence exhibited the presence of five reading frames for proteins of >100 amino acids (aa). The first reading frame coded for a potential plasmid replication protein of 216 aa. This Rep protein was distantly related to Rep proteins with the best match of 71% aa identity to one of a small *Staphylococcus hyicus* plasmid. Three in part overlapping reading frames for a 382-aa MobA protein, a 182-aa MobB protein and a 109-aa

MobC protein were detected. The MobA and MobC proteins showed 82% and 77% aa identity to MobA and MobC proteins of the aforementioned *S. hyicus* plasmid, respectively. In contrast, the MobB protein exhibited only 28% aa identity to a MobB protein of *Pediococcus pentosaceus*. The fifth reading frame coded for a 274-aa protein that differed by 12 aa from the same-sized ApmA protein of plasmid pAFS11 from bovine MRSA ST398. Analysis of the sequences flanking this *apmA* gene variant identified homology to plasmid pAFS11 for only 72 bp in the *apmA* upstream and for only 64 bp in the *apmA* downstream region.
Conclusions: The results of this study confirmed that *apmA*-like genes do not only occur on large multiresistance plasmids, such as pAFS11, but also on small resistance plasmids, such as pKKS49, in MRSA ST398. Such small plasmids may be mobilizable and able to recombine with larger plasmids, thereby enhancing the dissemination of the gene *apmA* coding for apramycin resistance.

S6:4

THE ORIGINS AND EVOLUTION OF MRSA ST398 THROUGH THE LENS OF GENOME SEQUENCING

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(no abstract available)

S6:5

GENOTYPE DIVERSITY OF *STAPHYLOCOCCUS AUREUS* ON BELGIAN MRSA-POSITIVE PIG FARMS

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Background. Numerous studies have shown the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs and farmers. We investigated the epidemiology of methicillin-susceptible *S. aureus* (MSSA) on MRSA ST398-positive pig farms, in order to assess the existence of a MSSA reservoir for the emergence of MRSA ST398 through acquisition of SCC*mec*.
Methods. On ten farms shown positive for MRSA ST398 in 2007, nasal swabs were collected from 200 pigs and from the farmer and relatives (total n = 25). MRSA and MSSA were confirmed by a 16S rRNA-*nuc*-*mecA* PCR. Susceptibility to 16 antimicrobials was tested by disk diffusion. All isolates were characterized by *spa*- and SCC*mec*-typing. MLST was performed on a subset of isolates.
Results. Twelve persons from eight farms carried MRSA (48%) and four persons from three farms carried MSSA (16%). All MRSA were assigned to ST398, while MSSA belonged to *spa* t337-ST9 (n = 1), t127-ST1 (n = 2) and t213 (n = 1). In total, 164 pigs (82%) from 10 farms carried MRSA and all were ST398. MSSA was detected in 56 pigs (28%) from 8 farms. These MSSA isolates represented 12 *spa*-types corresponding to four lineages: ST398 (n = 8), with *spa*-types t011, t034 and t571; ST9 (n = 28), with t337, t1430, t1939 and t2315; ST433 (n=11), a single locus variant of ST30, with t021, t318, t1298 and t3427; ST1 (n = 9), with t127. These lineages were detected on four, two, eight and two farms, respectively. SCC*mec*-typing showed the presence of *ccr*-complex type 2 and 5 in 50% of MSSA ST398 isolates and 36% of ST433 isolates. MSSA ST398 showed resistance to tetracyclines, trimethoprim, ciprofloxacin, macrolides and aminoglycosides. MSSA non-ST398 isolates showed considerable less resistance to the agents tested.
Conclusions. While all MRSA strains belonged to ST398, MSSA isolates showed a larger genotypic diversity. The fact that identical *spa*-types were found in MSSA from humans and animals suggests that transmission between animals and humans also occurs with MSSA. The detection of SCC*mec* remnants in MSSA of ST398 and

ST433 indicates these strains might have been involved in SCC*mec* recombination events. The multiresistant phenotype of *S. aureus* ST398 appears to be independent of methicillin resistance.

This study was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment (contract RF-6189).

S6:6

EVOLUTION OF MOLECULAR MICROBIOLOGY OF la-mrsa: THE *sccmec* CASETTES IN CC398

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Livestock, especially pigs, have recently been shown to constitute a zoonotic reservoir for a few subtypes of methicillin-resistant *S. aureus*. These has been named livestock associated MRSA (LA-MRSA). The majority of LA-MRSA belong to the by MLST clonal complex 398

LA-MRSA as an entity was first recognized in The Netherlands in 2004. Since then, it has been reported in pigs / other animals and humans in several European countries, in Asia and Canada and USA and South America

The SCC*mec* cassettes of ST398 are remarkably diverse given its short evolution.

Recently, the structure of SCCmec elements found at the International pig veterinarian conference in Copenhagen including two new elements IX and X and a variant of type V (Vc) (LI, AAC, 2011). These data will be presented as well as new data on whole genone sequencing of 78 ST398 isolates including 48 MRSA In this collection nine different SCC*mec* types and variants were identified among the 48 MRSA ST398 isolates, including II, IV, IVa, IVa*, mec class C, V, V*, VII-like, and three untypable. The most common was type V which was present in 63% (30/48) of the isolates. Independent phylogenetic analysis of the type V SCC*mec* cassette and the genomes of those strains that carried them revealed that the two phylogenies were inconsistent thus, suggesting that this SCC*mec* cassette has itself been introduced to the ST398 genome multiple times.

Eleven different spa types were identified among the 78 ST398 isolates, including t011, t034, t108, t567, t571, t899, t1451, t1793, t2876, t5462, and t5463. The two most common spa types t011 and t034 represented 67% of the isolates. While some spa types were more common within individual clusters, spa types were inconsistent with the overall ST398 phylogeny. Remarkably, resistance determinants towards heavy metals were found in many of the cassettes in addition to antibiotic resistance genes.

Poster Abstracts

1

RHODOMYRTONE FROM *RHODOMYRTUS TOMENTOSA* (AITON) HASSK.: A POTENTIAL CANDIDATE AS A NOVEL ANTIBACTERIAL AGENT TO CONTROL ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus is dangerous in animals for not only the potential effect on the infected animal's health, but also the case with which infections it causes can be transmitted to humans. The problem exists worldwide and is serious because the organism is highly contagious and difficult to treat. A large numbers of *S. aureus* strains have become resistant to most employed antibiotics. Many researchers have tried to implement an alternative approach to attend the infections. Among these, rhodomyrtone, a potent compound in acylphloroglucinol group, isolated from a plant species named *Rhodomyrtus tomentosa* has been recently reported as a challenge drug for the treatment of *S. aureus*. However, as the organisms often develop resistance to most forms of antibiotics. Therefore, the objective of this study was to further investigate the efficacy of rhodomyrtone on antibiotic-resistant mutants. Spontaneous mutation was performed by growing the bacteria on tryptic soya agar supplemented with 10 x MIC of different antibiotics and incubated at 37°C for 24 h. The antibiotics included benzalkonium chloride, erythromycin, gallidermin, gentamycin, penicillin, norfloxacin, rifampicin, tetracycline, and vancomycin. The colonies grew on agar with 10 x MIC antibiotic were transfered to tryptic soya broth and incubated at 37°C overnight. The cultures were then plated on TSA containing 10 x MIC of antibiotics and kept as spontaneous antibiotic-resistant mutants. Minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and bacterial growth were determined. The MIC and MBC range of the antibiotics against *S. aureus* were between 0.25-8 µg/ml. After spontaneous mutation, the results demonstrated that the MIC and MBC of rhodomyrtone on all antibiotic-resistant mutants were consistently the same at 0.5 µg/ml. The study suggests that rhodomyrtone is a promising agent for the treatment of antibiotic-resistant *Staphylococcus aureus* infections. Applications in veterinary care of household pets and farm animals, both as topical uses and internal drug administration are promising but need further studies.

2

METHICILLIN-RESISTANT STAPHYLOCOCCI FROM SWISS LIVESTOCK, CHICKEN CARCASSES, BULK TANK MILK, MINCED MEAT, AND CONTACT PERSONS

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Background: Methicillin-resistant staphylococci are increasingly reported in animals and humans as colonizing organisms and as pathogens causing infections. The *mec*A gene, which encodes methicillin-resistance, can be transmitted between bacterial species and is now distributed amongst both coagulase-positive and -negative staphylococci. The aim of this study was to assess the occurrence and characteristics of methicillin-resistant *S. aureus* (MRSA) and coagulase-negative staphylococci (MR-CNS) isolated along the meat and milk production line.

Methods: Samples collected at slaughter comprised nasal swabs from 716 pigs, 300 calves, 340 cattle, and 72 herd-wise pooled neck skin samples from chicken carcasses. Human nasal swabs originated from 148 pig farmers, 133 veterinarians, and 179 slaughterhouse employees.

Besides, 100 bulk tank milk (BTM) and 111 minced meat samples were tested. MRSA and MR-CNS were isolated on oxacillin-containing agar (Oxoid Brilliance MRSA Agar) after a two-step enrichment procedure (Mueller-Hinton broth with 6.5% NaCl; phenol red mannitol broth with aztreonam and cefoxitin). Methicillin resistance of isolates was confirmed by detection of *mec*A. MRSA isolates were characterized by MLST, *spa* typing, and SCC*mec* typing. MALDI-TOF MS was used for species identification of MR-CNS isolates. Additionally, antibiotic resistance profiles were determined by disk diffusion.

Results: MRSA were isolated from 10 (1.4%) pigs, 3 (1.0%) calves, 1 (0.3%) cattle, and 4 (3.0%) veterinarians. Sixteen of the 18 isolates belonged to sequence type (ST) 398, *spa* type t011 or t034, and SCC*mec* type IV or V. The other 2 isolates originated from a veterinarian (ST8, *spa* type t064, SCC*mec* type IV) and a young bull (ST1, *spa* type t127, SCC*mec* type IV). MR-CNS were detected in 48.2% of samples from livestock and chicken carcasses (from 36.3% in pigs to 72% in calves), in 62% of BTM samples, in 32.4% of minced meat samples, and in 49.4% of human samples (from 26.3% in slaughterhouse employees to 67.6% in pig farmers). The 414 selected MR-CNS isolates belonged to 7 different species (*S. sciuri*, 32.6%; *S. fleurettii*, 25.1%; *S. haemolyticus*, 17.4%; *S. epidermidis*, 14.5%, *S. lentus*, 9.2%; *S. warneri*, 0.7%; *S. cohnii*, 0.5%). *S. sciuri* and *S. fleurettii* predominated in livestock, BTM, and minced meat, whereas *S. epidermidis* and *S. haemolyticus* predominated in humans.

Conclusions: MRSA have entered Swiss farming operations, but to date occur at low numbers. On the other hand, a high prevalence of MR-CNS was found in livestock production. This is of concern in view of potential spread of *mec*A.

3

OBSERVATION OF PATIENT AND SURGEON PREOPERATIVE PREPARATION IN COMPANION ANIMAL CLINICS

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Infection control practices in veterinary clinics and hospitals are becoming increasingly important, with rising client expectations, growing concern about the spread of antimicrobial-resistant pathogens, and the potential for zoonotic transmission of disease. Surgical patients are at increased risk of developing infections, and can serve as sources of these pathogens for other animals and people with whom they have contact within and outside the clinic. Taking all reasonable precautions to reduce the risk of surgical site infections, beginning with preoperative preparation of the surgeon and patient, is therefore an important part of any infection control program. While guidelines are available for preoperative preparation procedures, there has been no objective investigation of compliance with these guidelines in veterinary practices. The objectives of this pilot study were to describe a range of preoperative hand scrub and surgical site preparation practices in veterinary clinics, and to describe any areas that require improvement across multiple clinics. Observation of preparation practices was performed in each of ten clinics over 9-14 days using 2-3 small wireless surveillance cameras. Data was coded for 148 surgical patients, and 31 surgeons performing a total of 190 hand scrubs. Patient hair removal was most commonly performed using clippers (117/138, 85%) and after induction in all but one animal. Steps in surgical site preparation ranged from 1-4. Contact time with soap ranged from 18-369s (mean 82s, median 53s), and with alcohol from 3-220s (mean 41s, median 30.5s). Application of alcohol or antiseptic using a "cleanest to dirtiest" pattern was infrequent (29/66 (44%) and 11/83 (13%), respectively). Non-sterile contact with the surgery site during the final preparation step in surgery was seen in 7/64 (11%) of cases.

Preoperative alcohol hand rub was used in 2/10 facilities, but soap and water hand scrub was most commonly used at all clinics. Proximal-to-distal scrubbing was noted in 95/142 (67%) of soap and water scrubs. Contact time during surgeon hand preparation ranged from 7-529s (mean 144s, median 124s) for soap and water and from 4-123s (mean 34s, median 25s) for alcohol-based hand rub. No significant changes in scrub times were identified over the course of the observation period.

Some preoperative preparation practices were fairly consistent between clinics in this study, while others varied considerably. Contact times with preparatory solutions were often far shorter than recommended, and the techniques with which they were applied were highly variable. The camera system used to perform this study did not appear to have a significant time-dependent effect on the behavior of participants, and could be useful for performing similar field-based observational studies in the future.

4

mecA GENE AND SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS IN *Staphylococcus* ISOLATED FROM MILK OF HEALTHY AND SICK COWS

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Mastitis is one of the most frequent diseases and the major cause of antimicrobial use in dairy cows. *S. aureus* is considered one of the main pathogens in clinical and subclinical mastitis. Coagulase-negative staphylococci (CNS) are more frequently isolated than *S. aureus*, causing primarily subclinical mastitis, with decreased of milk production and appear to be more resistant to antibiotics than *S. aureus*. In mastitis treatment, the resistance to methicillin, caused by *mec*A gene expression is of particular interest, because this mechanism confers resistance to almost all β-lactams. This resistance appears to be rarer in *S. aureus* than in CNS. This study aimed to determine a presence of *mec*A gene and the susceptibility of *Staphylococcus* isolated from milk of healthy cows (MHC) and sick cows (MSC) to various antimicrobial agents. Milk samples were collected in farms of Sao Paulo State, Brazil. The milk classification was done by California Mastitis Test (CMT) and somatic cell count (300 Somacount). The staphylococci isolation was on blood agar. Characteristic colonies were submitted to API Staph (BioMérieux). For the detection of *mec*A, DNA was purified using the commercial kit "MiniSpin" (GE Healtcare). The strains were tested for antimicrobial susceptibility according to CLSI (2011), using penicillin G, oxacillin, cefoxitin and others**.** Were tested 293 MSC and 80 were positive for *Staphylococcus*, with 66 strains of CNS and 14 *S. aureus*. Among 279 MHC, 63 were positive, with one strain of *S. aureus* and 62 of CNS. Among *S. aureus* isolated from MSC, only 1 was resistance to penicillin. To oxacillin, a major phenotypic marker of *mec*A gene, 18 among the 63 strains isolated from MHC and 12 of 80 from MSC were resistant (difference not statistically significant). Among the 30 resistant strains to this antibiotic, only 6 had the *mec*A gene, but none *S. aureus*, demonstrating that resistance can occur through other mechanisms. Considering all 143 strains, 14 had *mec*A gene, all CNS, none *S. aureus*. Among these 14, 2 were resistant to cefoxitin and 6 to oxacillin. By the results we can observe the presence of *Staphylococcus* sp, isolated from milk of cows with mastitis and healthy, resistant to several antibiotics. These data suggest that there may occur the dissemination of resistant strains among healthy animals, reinforcing the epidemiological importance of mastitis on milk production and animal health.

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN SWISS DOGS FROM 2007 TO 2011

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Background: *Staphylococcus pseudintermedius*, member of the *S. intermedius* group (SIG), is a commensal living on skin and mucosae of companion animals and the most common bacterial pathogen causing pyoderma and otitis in dogs. Recently, methicillin-resistant *S. pseudintermedius* (MRSP) have emerged in companion animals and have also been found colonizing people and causing human infections. The aim of this study was (i) to evaluate MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) for identification of *S. pseudintermedius* and (ii) to assess the occurrence and antibiotic susceptibility of MRSP in Swiss dogs over the past 5 years.
Material and methods: A set of *Staphylococcus* strains showing beta-hemolysis and coagulase production was selected for this study. The 110 randomly selected strains were obtained from dogs presented at a Swiss small animal clinic from 2007 to 2011 (8 to 26 isolates per year). Strains mainly originated from skin, wound, ear, and urinary tract infections. Species identification was performed by MALDI-TOF MS and results were verified by species-specific PCR. Amongst the strains identified as *S. pseudintermedius*, the occurrence of *mec*A encoding methicillin-resistance was evaluated by PCR and antibiotic susceptibility profiles of MRSP strains were determined using broth microdilution according to CLSI guidelines.
Results: Of the 110 strains analyzed, MALDI-TOF MS identified 106 (96.4%) isolates as *S. pseudintermedius* and 4 (3.6%) isolates as *S. aureus*. Other coagulase-positive *Staphylococcus* species, in particular *S. intermedius*, were not found. Species-specific PCR (detection of specific *nuc* for *S. pseudintermedius* and detection of *fem*B for *S. aureus*) confirmed the results obtained by MALDI-TOF MS. Of the 106 *S. pseudintermedius* strains, 49 (46.2%) harbored the *mec*A gene. The proportion of genetically methicillin-resistant *S. pseudintermedius* strains thereby accounted for 65.2% in 2007, for 62.5% in 2008, 61.5% in 2009, 20.8% in 2010, and 32.0% in 2011. In addition to beta-lactam resistance, the majority of MRSP strains (81.6-100%) were phenotypically resistant to clindamycin, enrofloxacin, erythromycin, gentamicin, tetracycline, and sulphamethoxazole/trimethoprim, whereas no strain was resistant to amikacin.
Conclusions: MALDI-TOF MS proved to be a fast and reliable tool for species identification and for differentiation between members of the SIG. Although no increase of MRSP was observed in strains isolated from clinical cases of dogs over the last 5 years, almost 50% of the *S. pseudintermedius* isolates were resistant to methicillin. The frequent finding of MRSP showing multiple resistances represents a challenge to therapy and handling of nosocomial infections in veterinary clinics.

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GENETIC ENVIRONMENT OF THE ERM(T) GENE IN METHICILLIN-RESISTANT AND -SUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* ST398 FROM ANIMALS AND HUMANS: PHYSICAL LINKAGE TO A FUNCTIONAL CADMIUM RESISTANCE OPERON

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Background: Recently, the macrolide/lincosamide resistance gene *erm*(T) has been

described for the first time in porcine and bovine MRSA ST398 isolates. The aim of the present study was to determine the location and the genetic environment of the *erm*(T) gene in MRSA and MSSA ST398 isolates of porcine and human origin.

Methods: Nine *erm*(T)-positive *S. aureus* ST398 strains (four porcine MRSA, two human MRSA, and three human MSSA) isolated in Spain were investigated. Protoplast transformation into *S. aureus* RN4220 was conducted and obtained transformants were investigated by susceptibility testing, PCRs of resistance genes and restriction analysis of the transferred plasmids. The *erm*(T)-carrying restriction fragments (cut with EcoR1 or BglII) were cloned and the *erm*(T)-flanking regions sequenced.

Results: The *erm*(T) gene was located on a plasmid in eight of the nine strains studied. Four different types of plasmids from four distinct strains could be differentiated on the basis of their sizes (ca. 6, 22, 25, and 32 kb), restriction patterns, and resistance genes. Plasmids were designated as pGS3912, pGS1902, pGS2940, and pGS2941. Plasmids pGS1902, pGS2940 and pGS2941 also mediated resistance to tetracycline via *tet*(L) gene, which was located immediately downstream *erm*(T), and pGS2940 also to trimetropim via *dfrK*, located between *tet*(L) and *erm*(T). Analysis of *erm*(T) region showed that in pGS3912, the *erm*(T) gene had a complete translational attenuator associated with its inducible expression. In the other three plasmids, the presence of deletions and mutations in their attenuators implicated a constitutive expression. Sequence analysis of plasmid pGS3912 [6.2 kb, from a human MSSA] and *erm*(T) flanking regions of pGS1902 [14 kb, from a porcine MRSA], pGS2940, and pGS2941 [16 kb and 9 kb, respectively, from human MRSA] showed that *erm*(T) gene of pGS3912 was flanked by two inverted copies of the insertion sequence IS*431* whereas the *erm*(T) flanking regions of pGS1902, pGS2940, and pGS2941 carried at least one copy of the recently described insertion sequence IS*Sau10*. The *erm*(T)-plasmids of pGS3912 and pGS1902 also carried a cadmium resistance operon with the genes *cadD* (coding for an energy-dependent cadmium efflux ATPase) and *cadC* (transcriptional regulatory protein) within the sequenced fragments. The comparative analysis of *S. aureus* RN4220 and the four *S. aureus* RN4220 transformed with such plasmids revealed 4- to 16-fold increases in cadmium MICs.**Conclusions:** This study showed that *erm*(T) is mostly plasmid-borne and often associated with the *tet*(L) gene. The finding of a functionally active cadmium resistance operon on an *erm*(T)-carrying plasmid is a novel observation. The co-localization of antimicrobial resistance genes and genes that confer resistance to inorganic ions facilitates their persistence, co-selection and dissemination

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CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* FROM BULK TANK MILK FROM MN DAIRY FARMS.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly recognized in livestock, particularly pigs. Few studies have investigated the prevalence of MRSA in dairy herds in the United States. The current study was undertaken to determine the prevalence of MRSA in Minnesota dairy herds. Farm prevalence of *S. aureus*, including MRSA, was estimated from bulk tank milk sampled in duplicate over 3 seasons from 50 dairy herds. All isolates were genotyped using spa, SCC*mec* typing, MLST, and PFGE. The bulk tank level prevalence of *S. aureus* and MRSA was 62% and 1.3%, respectively. Antibiotic susceptibility testing revealed that 17.2 % (16 of 93) of *S. aureus* isolates were multi-drug resistant (MDR), and confirmed the presence of 2 MRSA isolates. Seven isolates, including the two MRSA isolates, produced staphylococcal enterotoxins B, C, D and E on overnight culture in nutrient media. Of the 2

MRSA isolates, one had a composite genotype profile of - ST 5-USA 100- spa 2 type (ridom unknown) which has been reported among hospital associated-MRSA, while the second isolate carried - ST 8-USA300-t121 genotype commonly identified amongst community associated-MRSAs. These results suggest that MRSA genotypes associated with hospitals and community are present in MN bulk tank milk, albeit at a very low prevalence. Furthermore, the presence of enterotoxin genes and production of heat stable toxins by these isolates poses a potential food intoxication risk to humans. Large-scale studies on MRSA are required to delineate risks associated with emergence and/or spread of MRSA in dairy environments.

8

STUDIES ON METHICILLIN - RESISTANT STAPHYLOCOCCI ASSOCIATED WITH BOVINE MASTITIS AND THEIR ZOONOTIC IMPORTANCE

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A study was undertaken to understand the occurrence of Methicillin resistance in Staphylococci associated with bovine mastitis and their potential role in transmission to humans. A total of 158 milk samples were collected from cases of bovine mastitis and 126 nasal swabs from the animal handlers in and around Puducherry, South India. Using Staphylococci genus specific primers 96 bovine and 64 human isolates were confirmed as Staphylococci by PCR. Among the 96 isolates 22 were identified as coagulase-positive (CPS) and 74 were identified as coagulase-negative staphylococci(CONS), while 29 were identified as coagulase-positive and 35 were identified as coagulase-negative staphylococci among the 64 human isolates. The predominant species were Staphylococcus aureus and S.epidermidis. Based on the antibiogram pattern it could be concluded that chloramphenicol and enrofloxacin can be advocated for the control of Staphylococcal mastitis. Beta-lactamase production was detected in 45.45% and 28.38% of the coagulase-positive and negative staphylococci of bovine origin respectively whereas in human isolates 41.38% of the coagulase-positive and 31.43% of coagulase-negative staphylococci showed beta-lactamase activity. Among the Staphylococci isolated methicillin resistant gene (mec A) was detected by PCR in eight isolates of S. aureus and eleven isolates of CONS of bovine origin and in six isolates of S. aureus and seven isolates of CONS of human origin indicating that methicillin resistance is on raise among the bovine and human Staphylococcus isolates in this geographical region. The three bovine and human isolates carrying the mec A gene was taken up for further analysis for determining the genetic relatedness. Detailed analysis of mec A gene was conducted by amplifying a ~2kbp segment of mec A gene using four sets of primers. The amplified PCR products were sequenced and aligned and the analysis of the sequences revealed that the three MRS isolates from human and bovine taken up for detailed analysis had 90-100% homology with the mec A gene sequences available in GenBank. Pair wise BLAST analysis of mec A gene of human isolates obtained in this study had maximum identity (99-100%) with the bovine isolates. Multiple sequence alignment and phylogenetic analysis based on nucleotide and amino acid sequences, further confirmed the genetic relatedness among the isolates as well as between the isolates and GenBank entries. Both phenotypic and genotypic analysis carried out for the six MRS isolates (three bovine and three human) described in this study were indistinguishable and epidemiologically related, which may indicate the possible transmission of MRS between bovine and human.

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IS THERE AN EFFECT OF SOW WASHING ON THE LIVESTOCK-ASSOCIATED MRSA STATUS?

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Good hygiene management is necessary to reduce the load of pathogens on pig farms. One of the measures is washing the sows before entering the nursing unit. We have demonstrated that a higher sow colonization degree increases the piglet colonization degree. Therefore, the present study investigated the effect of sow washing on the MRSA status of skin and nose of the sow.
Sows were washed in the nursing unit as follows: first they were sprayed with water, soaped in with sow shampoo and finally rinsed with water. Forty-eight sows, originating from four different farms, were sampled before and after washing (once the sows were dry). A nasal swab and a skin sponge were taken at both occasions. The sponges were diluted ten times with salt-enriched Mueller Hinton Broth (Oxoid). Subsequently, swabs and dilutions were incubated overnight (18-20h, 37°C). The day after, one loopfull was plated onto MRSA-ID (bioMérieux). A MRSA specific multiplex PCR was used to confirm suspect colonies. *Spa* typing occurred on a small selection of isolates. Statistical analysis of the data occurred in SAS®. Before washing, MRSA was found in 24 skin and 19 nasal samples (27 animals). Sampling after washing revealed that 18 and 15 of the corresponding skin and nose samples remained positive. From the negative samples before washing, MRSA was detected after washing in 11 and 4 skin and nasal samples, respectively. The statistical analysis indicated that there was no effect of washing on the presence of MRSA on skin and nose level (GENMOD procedure, p=0.32 and p=1.00, respectively). Contrary, washing seemed to increase the colonization with MRSA. *Spa* typing revealed the presence of *spa* types t011 and t034, which are both associated with livestock-associated MRSA. Although hygiene management is used to diminish the load of pathogens on a farm, the present study indicates that sow washing before farrowing has no significant effect on the MRSA status of the sow at nose and skin level. There are even indications that the current washing method increases the MRSA load. Other strategies need to be developed to decrease this load after washing.

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GROWTH INHIBITION OF *STAPHYLOCOCCUS AUREUS* SUBSP. *AUREUS* BY GINSENOSIDE COMPOUND K

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Ginsenoside is a main active compound in the plant ginseng and it constitutes 3-5% of the total dry mass of a ginseng root. Compound K (C-K; CAS number 39262-14-1), which could be produced by deglycosylation of the major protopanaxadiol-type ginsenosides has shown an anti-tumor activity. We report here that C-K inhibited the growth of *Staphylococcus aureus* subsp. *aureus* (DSM 20231= type strain) with minimum inhibitory concentrations of 6 and 20

µg/ml, determined using an agar disk method and a liquid culture, respectively. C-K also inhibited the growth of other strains or species of *Staphylococcus* such as S. *warneri,* and *S. haemolyticus*. A further study on the molecular basis of inhibitory activities of C-K may lead a way to develop a new therapeutic antimicrobial to eradicate MRSA.

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN FINISHED PIGS IN TAIWAN

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Increasing prevalence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) raised attention recently. We collected piggery nasal samples from auction market and slaughterhouse in northern Taiwan to identify the existence of LA-MRSA. Forty-three percents of pigs were found to colonize MRSA and all were MLST ST9 and PVL negative. Based on PFGE results, five clusters of pulso-types were grouped for the 166 isolates while one was *Sma*I non-typeable. Twenty-eight percents of the pigs harbored more than one MRSA with the same PFGE profiles. However, similarity matrix of minimum spanning tree (MST) displayed genetic diversity among pigs. Most isolates were SCC*mec* type V (96%), few were type IV and one was non-typeable. Among *spa* typing, 92% isolates were t899. All isolates were susceptible to vancomycin and more than 90% of the isolates showed multiple drug resistances to clindamycin, gentamicin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole. The results showed endemic LA-MRSA prevalence in piggery farms in Taiwan, while the identified clones of ST9 were similar to nearby countries but differed to ST398 in European and North America.

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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* STRAINS FROM FOODSTUFF OF ANIMAL ORIGIN CONSUMED IN MIDDLE BLACK SEA REGION OF TURKEY.

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Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most important public health problems in many countries. In recent years, the existence of MRSA in foodstuff of animal origin and its transfer among farm animals, foodstuff of animal origin and human beings have been shown with molecular typing studies. The objective of this study was investigation of exis**tence, methicillin resistance** and clonal relationship of *Staphylococcus aureus* strains from foodstuff of animal origin consumed in Middle Black Sea region of Turkey. A total of **62** *Staphylococcus aureus* strains isolated from meat, milk and fishery products were included in the study. Identification and methicillin resistance were confirmed by PCR technique in which appropriate primers for nuc and mecA gene were used. Methicillin resistance was detected in 15 *Staphylococcus aureus* strains. Clonal relationship between **the** 15 MRSA strains was investigated by PFGE method. PFGE typing of the 15 MRSA strains yielded **6** PFGE patterns**. Pattern A and E were found to be dominant types in our study. Pattern E consisting of 7 strains was from fishery products. Pattern A consisting of 4 strains was from meat and fishery products. Patterns B, C, D and F were single**

isolates from milk, meat, meat and milk products, respectively.

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TWO NOVEL SYNTHETIC PEPTOIDS EXHIBIT RAPID *IN VITRO* KILLING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

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Objectives: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is of increasing concern due to its recent spread amongst dog populations worldwide. A very limited range of antibiotics are available for treatment of MRSP infections, and this creates an urgent need for the development of alternative antimicrobial drugs. As part of the Danish Centre for Antibiotic Research and Development (DanCARD), we investigated the *in vitro* antimicrobial activity of two novel synthetic peptoids against MRSP and methicillin-susceptible *S. pseudintermedius* (MSSP).
Methods: Two out of ten peptoids synthesized by our group (B1 and D2) were selected for the study based on preliminary data indicating excellent antibacterial activity and low haemolytic toxicity. The minimum inhibitory concentrations (MIC) of B1 and D2 were determined by broth micro-dilution using 50 *S. pseudintermedius* isolates of clinical origin. Ten of the isolates were MRSP representing distinct genotypes and 40 isolates were MSSP of unknown genetic background. Time kill kinetics of both peptoids were evaluated against one representative isolate at concentrations corresponding to 0*MIC, 1*MIC and 4*MIC.
Results: Both peptoids displayed almost identical MIC distributions (1.56 - 6.25 µM), and no difference was observed between MRSP and MSSP isolates. Time kill kinetic studies showed that both B1 and D2 had a rapid concentration-dependant bactericidal effect. B1 was the most effective with complete killing of bacteria after 30 minutes at 4*MIC and after 5 hours at 1*MIC. For D2, complete killing was obtained after 2 hours at 4*MIC.**Conclusion:** The study shows a rapid bactericidal effect of B1 and D2 on both MSSP and MRSP. The low concentrations required to obtain complete killing suggest that these two synthetic peptoids, especially B1, are excellent candidates for the development of new antimicrobial products for treatment of pyoderma. A series of *in vivo* studies have been scheduled to evaluate the clinical efficacy and toxicity of these peptoids in topical formulation.

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ISOLATION AND CHARACTERIZATION OF NOVEL LYTIC BACTERIOPHAGES AGAINST MULTI-DRUG RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS ST71 AND ST68

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Introduction: Staphylococcus pseudintermedius is a commensal of the dog skin and the most common bacterial pathogen associated with canine skin infections. The emergence and global spread of methicillin-resistant S. pseudintermedius (MRSP) resistant to all antibiotics licensed for systemic therapy poses a serious therapeutic challenge for veterinary practitioners. Therapeutic alternatives are urgently needed and bacteriophages (viruses that specifically target and kill bacteria) represent one of the possible alternatives that could be developed in the future.
Objectives: To isolate phage displaying lytic activity against MRSP and to characterize their host range and morphology.
Methods: Lytic phages were isolated from canine faecal samples using homogenization and various concentrations of NaCl. The lysis efficacy and host range of the seven phages was tested by performing the spot test on 11 MRSP belonging to the two major clonal MRSP lineages found in Europe (ST71, n=6) and North

America (ST68, n=5), and 44 methicillin-susceptible S. pseudintermedius (MSSP) isolated from clinical specimens. Transmission electron microscopy was used for presumptive identification of the phages.
Results: Seven virulent phages were isolated. All phages were able to lyse all ST71 isolates, while only 5 of the 7 phages were able to lyse all ST68 isolates. Variable lysis patterns were observed among the MSSP, with two phages showing the poorest lysis efficacy of 39% and 20%, respectively. Based on transmission electron microscopy, all phages were of a similar morphology and belong to the Siphoviridae family, which are characterized by having a long non-contractile tail and an isosahedral (isometric) capsid.
Discussion and conclusion: S. pseudintermedius lytic phages mainly belong to the Siphoviridae family, as previously shown for S. aureus. The phages described in this study are promising candidates for phage therapy of S. pseudintermedius skin infections. Further in vivo experiments are needed to determine their clinical efficiency.

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GENETIC RELATIONSHIP OF CLINICAL METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* (MRSP) ISOLATES FROM DOGS AND CATS IN SWEDEN

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Background: *Staphylococcus pseudintermedius* belong to the normal flora of dogs and cats, but it is also an opportunistic pathogen. In recent years an increase in occurrence of methicillin-resistant *S. pseudintermedius* (MRSP) has been noticed in Sweden, which has lead to a therapeutic challenge due to limited treatment options. However, the epidemiology of MRSP in Sweden is scarcely studied. The aim of the current study was to get a deeper insight in the genetic relationship of clinical MRSP isolates in Sweden.
Methods: Primary clinical isolates was characterised using Pulsed-Field Gel Electrophoresis (PFGE) with *Sma*I, *spa*-typing and SCC*mec*-typing. Furthermore, antimicrobial susceptibility testing was performed using VetMIC™ GP-mo microtitre plates, containing 12 antimicrobials belonging to 9 classes.
Results: The study contained isolates from 228 individuals, 216 from dogs and 12 from cats, isolated 2008-2010. The origin of isolation was mainly from wounds, skin, ears and **furuncles**. One PFGE cluster clearly dominated containing 96 % of the isolates when applying a cut-off of 80 %. In total were four PFGE identified in the study. The *spa*-types identified were t02, t06, t10, t29 and t35, t02 being the most common 93 % of the isolates. Five isolates was non-typable using *spa*-typing. All isolates with a defined *spa*-type were identified to carry SCC*mec* II-III, with two exceptions, one t10 and one t35 carrying non-typable SCC*mec*. Four isolates with non-typable *spa* carried SCCmec IV and one a non-typable SCC*mec* positive for the *ccr*C gene. One isolate was non-typable using PFGE, *spa*- and SCC*mec*-typing. The t02-SCC*mec* II-III, t06- SCC*mec* II-III and t29-SCC*mec* II-III corresponded to the dominating PFGE-cluster, with the exception of one t02-SCC*mec* II-III isolate. Forty-nine percent of the isolates was susceptible only to chloramphenicol, tetracycline and fusidic acid and 40 % only to tetracycline and fusidic acid.
Conclusions: One clonal lineage dominated Swedish MRSP clinical isolates and the majority was of *spa*-type t02 carrying SCC*mec* II-III. In addition, all isolates were descried has multi-resistant. This was in concordance with earlier studies describing that one linage of MRSP has disseminated in Europe and based on the results in the current study the same lineage is emerging in Sweden.

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DINAMICS OF MRSA ST398 NASAL CARRIAGE IN PIG FARMERS AND HOUSEHOLDS IN BEGIUM.

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Objective: Prevalence of LA-MRSA (ST398) has been widely reported on pigs and humans in contact with pigs. However, most of the studies report point prevalence data only. The objective of this longitudinal epidemiological study was to determine the persistence and the dynamic of MRSA nasal carriage and identify secondary transmissions to households.
Methods: A longitudinal study was carried out in 2 LA-MRSA positive farrow-to-finish farms in Belgium. Pig farmers, their households and their home environment were monitored over a 25 week period. Nasal swabs were taken to determine the MRSA presence at 8 selected sampling time-points. MRSA was isolated using standard laboratory methods and suspected colonies were identified by triplex PCR for mecA, spa and PVL genes. MRSA isolates were subsequently genotyped by SCCmec, spa-sequence typing and MLST.
Results: In total 164 samples were collected during the study period (n=20 pigs, n=80 humans and n=64 environmental). Of these, respectively 100% (20/20), 80% (65/80) and 51.5% (33/64), tested positive for MRSA. Farmers (n=2), their partners (n=2) and pigs from both farms (n=20) tested MRSA positive at the beginning of the study. Human and animal isolates had identical genotypic characteristics, spa-types (t011 and t2370), SCCmec types (IV, V or not typable) and none harboured PVL genes. All the MRSA isolates belonged to the ST398.
The longitudinal human study revealed that all the participants (n=10) had at least one MRSA-positive nasal sample over the study period. Farmers and their partners, who worked daily in the stables, tested continuously MRSA positive. Four of six household members were intermittent carriers, while the remaining two were continually MRSA positive despite not having daily exposure to pigs. MRSA, genetically identical to human and pig isolates, was recovered from the house environment of both farms. None of the participants developed an infection.
Conclusions: This study shows evidence of transmission of MRSA ST398 from pigs to farmers and the dynamic of nasal carriage over time. Nasal carriage was strongly associated with working in the stables. Farmers who were exposed daily to pigs were persistently healthy carriers, and household members were intermittent carriers even when not exposed to pigs, suggesting potential transmission or acquisition via direct contact among the households or indirect via the contaminated environment.

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MRSA CC398 IS TRANSMITTED BETWEEN FARMS BY PIG TRADE

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Introduction

Previous epidemiological investigations (Broens et al. 2010) have indicated that pig trade is an important factor for the spread of MRSA CC398 in pig farming. However the discriminatory power of the typing method used (*spa* typing) did not lead to conclusive associations. The objective of this study was to provide molecular evidence that farm-specific MRSA CC398 strains are transmitted vertically through the pig production chain by trade of MRSA-positive animals. For this purpose, MRSA isolates from farms linked by pig trade were typed using Pulsed-Field Gel Electrophoresis (PFGE). Our

hypothesis was that strains found in the recipient farms would also be present in the corresponding supplying farms.

Material and methods

Twenty-one MRSA CC398 isolates were selected from a previous epidemiological study in the Netherlands (Broens et al., 2010). The isolates originated from 14 farms organized in six chains. Each chain contained farms at two or three production levels including breeders, farrowers and finishers. All isolates were characterized by *spa* typing (Harmsen et al., 2003) and PFGE using a CC398-specific protocol (Bosch et al., 2010). GelCompar II (Applied Maths, Kortrijk, Belgium) was used for the PFGE cluster analysis (Unweighted Pair Group Method with Arithmetic Mean, UPGMA).

Results

PFGE profiles of MRSA CC398 isolates from farms situated at the bottom of the production pyramid (finishers) clustered together with the isolates from farms situated at upper levels in the production pyramid (farrowers and breeders) in four chains. In one chain, isolates showed undistinguishable PFGE profiles despite having three different *spa* types. Isolates originating from the other two chains did not cluster together.

Discussion

The association between the PFGE profiles of isolates belonging to the same chain confirmed that MRSA CC398 was transmitted by pig trading in at least four of the six chains studied. The lack of epidemiological relatedness between the PFGE profiles of isolates from the other two chains suggests that factors other than pig trade may play a role in the transmission of MRSA CC398 between farms e.g. use of additional pig suppliers that were not included in the study and the low number of isolates tested (one isolate per farm) could have hampered detection of multiple CC398 clones within each farm/chain. In conclusion this study provides conclusive molecular evidence that pig trading is an important source of transmission of MRSA CC398 in pig production. This finding has important implications for the development of intervention strategies for control and prevention of MRSA CC398 in pig farming.

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CHARACTERIZATION OF METHICILLIN-RESISTANT AND -SUSCEPTIBLE STAPHYLOCOCCI FROM JAPANESE RETAIL READY-TO-EAT RAW FISH

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Background:

Staphylococcal species occur as commensal colonizers of the skin and mucous membranes of different species of animals and humans; staphylococci are not part of the normal fish microflora. The presence of staphylococci on fish is an indication of (a) post-harvest contamination due to poor personnel hygiene, or (b) disease.

Material and methods:

Samples (n = 200) of ready-to-eat raw fish were collected from 25 retail grocery stores of five supermarket chains in Hiroshima (Japan). A 50 ml aliquot was added to an equal volume of enrichment broth (trypticase soy broth supplemented with 10% NaCl and 1% sodium pyruvate). After a 24 h incubation at 35°C, enrichment broth was streaked onto Baird-Parker agar, and plated in duplicate on BP and mannitol salt agar containing cefoxitin (4 µg/ml). All isolates were characterized by antimicrobial susceptibility testing for 19 antibiotics by disk diffusion method. PCR amplification of genes associated with resistance to macrolides, aminoglycosides, vancomycin, tetracycline, penicillin

and methicillin, was conducted for all isolated strains. All *S. aureus* isolates were characterized for the accessory gene regulator (*agr*) group and virulence genes. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was carried out for all methicillin-resistant isolates. For genotyping of methicillin-resistant *S. aureus* (MRSA) isolates, multilocus sequence typing (MLST) and single-locus DNA sequencing of the repeat region of the *Staphylococcus* protein A gene (*spa*) were performed.

Results:

A majority of the grocery stores surveyed (92%, 23/25) contained fish contaminated with *Staphylococcus* species. We recovered 175 *S. aureus* isolates from 174 (87%, 174/200) samples, with 170 isolates of methicillin susceptible staphylococci (MSSA). For the methicillin-resistant staphylococci, 10 isolates were obtained from 10 samples (5%, 10/200) collected from 10 shops (40%, 10/25) belonging to four supermarket chains. SCC*mec* typing revealed the presence of a type IV.1 SCC*mec* cassette in *S. warneri* isolates, a type II.1 SCC*mec* cassette in *S. haemolyticus* isolates and a cassette in MRSA isolates that could not be typed. The *agr* typing of *S. aureus* isolates revealed that *agr* type 1 was most prevalent (96.5%, 169/175) followed by type 2 (2.2%, 4/175) and type 3 (1.1%, 2/175). Virulence determinants were detected in 14.2% (25/175) of isolates. Molecular typing of both MRSA isolates by *Spa* sequencing and MLST identified t1767 and ST8, respectively.

Conclusion:

This is the first report to show MRSA and methicillin-resistant coagulase-negative staphylococci (MR-CoNS) isolated from retail ready-to-eat food in Japan. Our results showed that sashimi is a likely vehicle for transmission of multidrug resistant and enterotoxigenic staphylococci. Sashimi is typically not cooked prior to consumption; therefore, these pathogens present a major health hazard to humans.

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MOLECULAR ANALYSIS OF MRSA ISOLATES FROM CATTLE AND POULTRY DETECTED IN THE GE*RM*-VET PROGRAM 2008 AND 2009

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Background: The aim of this study was to characterize MRSA isolates from cattle and poultry obtained during 2008 and 2009 in the German national veterinary resistance monitoring program GE*RM*-Vet. In the GE*RM*-Vet program a variety of different bacterial species from different animals and various disease conditions is analyzed.

Methods: In total, 11 (1.5%) of 720 bovine and 2 (2.1%) of 96 avian *Staphylococcus aureus* isolates from the GE*RM*-Vet study periods 2008 and 2009 proved to be methicillin-resistant. These isolates were investigated for their *spa*, SCC*mec*, and *dru* types and were subjected to two CC398-specific PCRs. MLST was performed for non-CC398 isolates. Broth microdilution was conducted for 29 antimicrobial agents and the respective resistance - as well as virulence - genes were detected by a *S. aureus*-specific DNA microarray and additional specific PCR assays.

Results: Eleven isolates belonged to CC398 and single avian or bovine isolates to ST5 and ST9, respectively. The ST5 and ST9 isolates differed from the CC398 isolates in all typing parameters. The avian ST5 isolate exhibited *spa* type t002, SCC*mec* type III, and *dru* type dt9v and the bovine ST9 isolate *spa* type t1430, SCC*mec* type IV, and *dru* type dt10a, respectively. Among the CC398 isolates, the *spa* type t011 was predominant. It was seen in nine isolates, whereas the *spa* type t034 was detected in two

isolates. All CC398 isolates belonged to SCCmec type V. Ten of the eleven CC398 isolates showed *dru* type dt11a, whereas the novel *dru* type dt6r was identified in a single isolate. Susceptibility testing revealed seven resistance patterns with all isolates being resistant against beta-lactams and tetracyclines. The respective resistance genes *mecA*, *blaZ* [beta-lactams], *tet*(K), *tet*(L), *tet*(M) [tetracyclines], *erm*(A), *erm*(B) [macrolides/lincosamides/streptogramin B], *dfrK* [trimethoprim], *aacA-aphD*, *aadD*, *spc* [aminoglycosides/aminocyclitols] and *vga*(A) [pleuromutilins/lincosamides/streptogramin A] were detected.
All isolates were negative for PVL, TSST1 and exfoliative toxins. While all 11 CC398 isolates were negative for the enterotoxin genes tested, the ST5 and the ST9 isolates harboured the *egc* gene cluster for enterotoxin G, I, M, N, O and U genes.
Conclusions: MRSA isolates were found rarely in diseased cattle and poultry in the GE*RM*-Vet program during 2008 and 2009. The occurrence of ST5 (poultry) and ST9 (cattle) isolates may point towards an exchange of MRSA with humans and other animal species.

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MOLECULAR BASIS OF RIFAMPICIN RESISTANCE IN METHICILLIN-RESISTANT *Staphylococcus pseudintermedius* ISOLATES FROM DOGS

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Background: *Staphylococcus pseudintermedius* is the most frequent causative agent of canine pyoderma and is also associated with other infections. Methicillin-resistant *S. pseudintermedius* (MRSP) often display resistance to almost all classes of antimicrobials used in veterinary medicine. In the present study, we investigated the emergence of rifampicin-resistance in MRSP, the persistence of these isolates and identified the corresponding mutations in the *rpoB* gene.
Methods: Thirty-three MRSP isolates collected prior to and after rifampicin therapy from nine dogs at five Dutch veterinary hospitals were included in this study. These isolates were tested for resistance to rifampicin and other antimicrobial agents by broth dilution. The rifampicin-resistance determining region (RRDR) within the *rpoB* gene was amplified by PCR and the amplicons were sequenced. Pulsed-field gel electrophoresis (PFGE) served to determine the genetic relationships of the MRSP isolates.
Results: The rifampicin-resistant isolates had MIC values of 128 mg/L (n=1), 1024 mg/L (n=1) or >= 2048 mg/L (n=19), whereas the susceptible isolates (n=11) had MIC values of <= 0.002 - 0.015 mg/L and a single isolate had an MIC value of 2 mg/L. All isolates were multi-resistant with resistance to at least six classes of antimicrobial agents. In contrast to the rifampicin-susceptible isolates and the isolate with an MIC of 2 mg/L, all rifampicin-resistant MRSP isolates showed single or double mutations in the RRDR region of the *rpoB* gene. Nine different mutations that changed the amino acids at positions 508 + 509, 509 + 516, 513, 522, 526 or 531 were identified. PFGE analysis confirmed that the rifampicin-resistant MRSP isolates were indistinguishable from or closely related to the rifampicin-susceptible isolate(s) obtained from the same dog prior to rifampicin application. Isolates from two dogs were not typeable with *Sma*I while *Apa*I provided suitable PFGE patterns.
Conclusion: Therapy of MRSP with rifampicin results in the rapid emergence of rifampicin resistance and these isolates can still be isolated after months. As a consequence, rifampicin should only be used if unavoidable and therapy should be accompanied by continuous check for resistance development.

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CHARACTERIZATION OF MRSA ISOLATES FROM DAIRY CATTLE, OTHER ANIMALS AND HUMANS FROM DUTCH DAIRY FARMS

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Background: The aim of the present study was to characterize MRSA isolates from dairy cattle, as well as from other animals and humans living on dairy farms in the Netherlands for their genetic relationships and antimicrobial resistance properties.
Methods: In this study, 128 MRSA isolates from 26 dairy farms were investigated. The study population consisted of MRSA from dairy cattle (n=71), calves (n=7), pigs (n=16), dogs (n=2), a horse, a sheep, and humans (n=30). All isolates were subjected to two CC398-specific PCRs, *spa* typing, SCC*mec* typing and ApaI macrorestriction analysis. Susceptibility testing was performed for 29 different antimicrobial agents using broth microdilution.
Results: All 128 isolates tested belonged to the clonal complex CC398. Eight different *spa* types were identified: t011, t108, t034, t567, t1184, t1451, t2287 and t3934. SCC*mec* elements of types III, IV and V, as well as non-typeable ones were detected. In total, 26 different major macrorestriction patterns were observed, with two isolates being non-typeable using ApaI. Susceptibilty testing revealed 14 different resistance patterns.
Per farm analysis of the MRSA isolates revealed a wide variety of different scenarios. On some farms MRSA CC398 from dairy cattle, humans, pigs or sheep shared the same *spa* type, PFGE pattern and resistance pattern suggesting an interspecies exchange of the MRSA isolates. On several farms, different MRSA CC398 subtypes were identified. For example, the isolates found in a pig, a dog and humans were indistinguishable, but differed from another CC398 subtype found in dairy cattle, humans and pigs of that farm. Occasionally, MRSA CC398 obtained from the skin of dairy cattle differed in their characteristics from those isolated from milk samples from the same dairy farm. On other farms, MRSA CC398 subtypes isolated from calves and humans were indistinguishable, but different from those of the dairy cattle. These observations suggest a transfer of MRSA isolates between human and animal hosts.
Conclusions: The results of this study showed that similar MRSA CC398 isolates can be found in different animal species and humans on the same dairy farm. Occasionally, more than one MRSA CC398 subtype, which colonized different human and animal hosts, was identified on the same farm. These observations suggest the existence of different transmission routes within the farms and/or the occurrence of various routes by which different CC398 subtypes were imported into a farm.

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BIO-CHEMICAL PROPERTIES OF A *STAPHYLOCOCCUS AUREUS* SMALL COLONY VARIANT IN BOVINE MASTITIS

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Staphylococcus aureus small colony variants (SASCVs) are involved in *S.aureus* infections, most of which are recurrent or persistent. Hereby, we attempted to investigate the clinical importance of SASCVs in chronic bovine mastitis through the recovery of SASCVs from somatic cells (SCs) and to analyze the selected physio-biochemical traits of SASCVs. Definitely identificated by *nuc* gene detection and *16S rDNA* multiplex alignment, eight *S.aureus* and one SASCVs (S1 isolate) isolates were recovered from SCs lysates. The S1 isolate presented

a heterogeneity with one of the eight *S.aureus* (N3 isolate), and continuous passage showed a stable phenotype of S1. As for the growth curve of S1, adaptable phase and log phase was ambiguous and apparently delayed. S1 was negtive to reduce nitrate, produce coagulase and utilize mannose, mannite and N-acetylglucosamine into acid metabolites, but positive to produce arginine dehydrolase. Salt-tolerant test showed that the inhibitory effectiveness by NaCl on the growth of S1 was lower than that of N3, however, the growth was ceased after exposure to high-level NaCl (>85mg/ml). Hemolysis assay showed that the hemolytic ability of S1 was significantly reduced (P<0.05). Antimicrobial susceptibility tests showed that S1 had higher resistances than N3 to penicillin G, tetracycline, gentamicin and sulfadimethoxine and chloramphenicol. This study 1) was a development of a direct isolation method of bovine SASCVs without induction by gentamycin; 2) indicated an important pathogenic role of SASCVs in chronic mastitis; 2) provided novel research approaches to reveal the *S. aurues* persistent infectious mechanisms both in human and animals.

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COMPARATIVE GENOTYPIC CHARACTERISATION OF HUMAN AND ANIMAL ISOLATES OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ST398

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Objectives: The methicillin-resistant Staphylococcus aureus ST398 poses a significant risk to both farming industry as well as persons in contact with food-producing animals. In this study a panel of MRSA ST398 strains was subjected to comparative genotypic characterisation to identify features that may distinguish the lineage from other clonal complexes of S. aureus.

Methods: Sixty seven S. aureus strains belonging to nine clonal complexes were characterised and consisted of: ST398 (n=18, human and various animal), CC5 (n=4, human), CC8 (n=6, human and equine), CC15 (n=5, human), CC22 (n=9, human), CC30 (n=8, human), CC97 (n=8, cattle), CC130 (n=4, cattle), CC151 (n=5, cattle). The strains were selected from a larger panel to represent unique clonal types based on PFGE analysis. The presence of virulence and antimicrobial resistance genes was detected by Identibac MRSA array tube (version pm5.5). The strains were also screened by PCR for carriage of additional antimicrobial resistance genes (ermT, dfrK and tetL) and adhesin genes (clfA, clfB, fnbA, fnbB, fib, eno, ebpS, bbp, cna, sdrC, sdrD, sdrE, icaA, icaD and sasG).
Results: Strains belonging to ST398 were found to harbour no lineage-specific virulence or adhesion determinants but primarily genes found in all other analysed clonal complexes including: hl, hla, hld, hlgA, lukS, lukF, lukX, lukY, seX, seY, sarA, clfB, fnbA, icaA, icaD and eno. In addition the lineage also carried clfA, fnbB, fib, cna, sdrC and sdrE, all of which were also detected in at least one another clonal complex. ST398 strains displayed high prevalence of antimicrobial resistance genes, such as tetM (n=18), tetK (n=5), tetL (n=4), dfrK (n=10), aacA-aphD (n=9), aadD (n=4), ermC (n=9), ermT (n=4). This was comparable to equine MRSA CC8 strains, which were found to carry tetM, aacA-aphD and dfrA. However, the novel resistance genes ermT, dfrK and tetL were detected only among ST398 strains.
Conclusions: Comparative analysis revealed low content of virulence genes among MRSA ST398 strains. The apparent lack of additional enterotoxin and leukocidin genes distinguishes ST398 strains from other clonal complexes of both animal and human derived S. aureus strains. High frequency of antimicrobial resistance elements, including the carriage of tetM is a significant but not unique feature of MRSA ST398 strains. A distinguishing factor for ST398 is the presence of novel resistance genes, but the carriage frequency is variable among strains.

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SCREENING FOR METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN PIGS AND HORSES IN SWEDEN

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Background: So far, MRSA has not been widespread among animals in Sweden. The number of clinical cases in horses is limited and most of the isolates belong to *spa*-type t011. In screening studies of the pig population in Sweden (2006/07 and 2008), MRSA was not detected. The aim of the present study was to investigate the prevalence of MRSA in healthy fattening pigs and in horses in Sweden. **Methods:** In total, 191 pig herds were sampled at six abattoirs. From each herd five pigs were sampled with nasal swabs which were pooled at the laboratory. On admission to five animal hospitals, 284 randomly selected horses were sampled with nasal swabs. Pig samples were pre-enriched in Mueller-Hinton broth with 6.5% NaCl for 16-20 h in 37°C followed by selective enrichment in Trypton Soy Broth supplemented with cefoxitin 3.5 mg/L and aztreonam 75 mg/L for 16-20 h in 37°C. The horse samples were selectively enriched in Trypton Soy Broth supplemented with NaCl 4%, mannitol 1%, cefoxitin 3 mg/L and aztreonam 50 mg/L for 16-20 h in 37°C. For both pig and horse samples, 10 µL of the selective broth were spread on MRSA Brilliance agar (Oxoid) and bovine blood agar and incubated for 24-48 h in 37°C. Suspected colonies were confirmed with PCR for the *nuc*- and *mec*A-genes. The confirmed isolate was tested for antimicrobial susceptibility by determination of minimum inhibitory concentration (MIC) with a microdilution method using a VetMIC™ panel containing 12 antimicrobial substances. Genetic characterization was carried out using *spa*-typing, multilocus sequence typing (MLST) and detection of the panton valentine leukocidin (PVL) genes. **Results**: One of the 191 samples from pigs and none of the samples from horses were positive for MRSA. The pig isolate was, besides the beta-lactams, resistant to tetracycline, gentamicin, kanamycin and trimethoprim and belonged to *spa*-type t011, ST398 and was negative for the PVL genes. **Conclusions:** MRSA appears not to be widely spread within the pig population in Sweden. The isolate found belonged to the livestock associated type (CC398) that is commonly occurring in many European countries. The prevalence of MRSA carriers among horses in Sweden appears to be low.

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EVALUATION OF DIFFERENT CHROMOGENIC MEDIA FOR DETECTION OF MRSA ST398 IN POULTRY.

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Since 2005, livestock-associated MRSA (LA-MRSA) with clonal complex ST398 has emerged in a large number of animal species and is recorded worldwide. In this study broiler chickens were screened for MRSA ST398 with a screening method previously used for MRSA detection in pigs (Pletinckx et al., 2009). Because of a high number of false positives, different media were assessed in a subsequent study. Samples were collected from 250 broiler chickens from different anatomical sampling sites (nose, earlobe, skin beneath wing and cloaca) on 3 Belgian poultry-pig farms. All swabs were enriched overnight in Mueller Hinton broth (Oxoid) supplemented with 6.5% NaCl and inoculated the following day on ChromID MRSA

(BioMérieux). Characteristic colonies were interpreted following manufacturers instructions and further analyzed by multiplex-PCR for 16S *r*RNA, *mec*A and *nuc* gene.
Besides the low prevalence, 7.2% (18/250) of MRSA ST398 in the broilers, a high percentage of false positives, 19.7% (197/1000) was found, despite the fact that enrichment in high salt concentration broth and a chromogenic medium for MRSA detection was used. Especially when comparing to the number of false positives in pigs 3.1% (30/972) residing on the same farms (Pletinckx et al., 2010). A selection of 44 false positive isolates (proportional number of each poultry-pig farm) was further identified by sequencing and/or API (BioMérieux). The following microorganisms were found: 25 *Staph. sciuri*, 6 *Staph. lentus, 5 Staph. gallinarum, 5 Rothia nasimurium,* 1 *Staph. epidermidis,* 1 *Bacillus galactosidilyticus and 1 Enterococcus faecalis*. In a subsequent study on the same 3 farms was conducted, 50 broiler chickens were sampled both from nose shell and cloaca. All swab specimens were processed (specified above) and inoculated the following day on 3 chromogenic media: ChromID MRSA (BioMérieux), *Brilliance*MRSA (Oxoid) and MRSA*Select* (Bio-Rad).
ChromID MRSA (75/300) and MRSA*Select* (77/300) detected remarkably less true positive results than *Brilliance*MRSA (95/300). A substantial difference was noted in false positives between chromID MRSA (65/300), *Brilliance*MRSA (1/300) and MRSA*Select* (0/300). The differences in selective en elective components in the media may explain why some microorganisms give rise to false positives on one medium and not on another. Furthermore, *Brilliance*MRSA demonstrated the highest sensitivity (96.9%) compared to MRSA*Select* (78.5%) and chromID MRSA (76.5%). Regarding the relative specificity, MRSA*Select* (100.0%) and *Brilliance*MRSA (99.5%) showed the highest values compared to chromID MRSA (67.8%). *Brilliance*MRSA outperformed MRSA*Select* and chromID MRSA for the detection of MRSA in broilers. This study shows that the MRSA screening method may possibly be host-dependent, this is probably related to species-specific microflora.

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ADJUSTMENT OF PH OF ENRICHMENT MEDIA MIGHT IMPROVE SELECTIVE ISOLATION OF MRSA FROM PIG SAMPLES

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Methicillin resistant *Staphylococcus aureus* (MRSA) have emerged in livestock in several countries worldwide in recent years. MRSA may colonise in low numbers which makes both epidemiological studies and the implementation of control programmes difficult.
Methods for selective isolation of MRSA from animal samples have been developed. However, obtaining sufficient sensitivity has been a challenge. *Staphylococcus aureus* is normally found on the skin, surviving and growing under extreme conditions: dry environment with high salt and low pH. In the selective isolation so far used high salt concentrations has been the main selection. We hypothesized that also pH adjustment could be used for selection of this species. In this study we compared the growth of MRSA and back ground flora in enrichment media at several different combinations of salt and pH. Background flora isolates were obtained from pig swabs. Initially a total of seven strains, including: two MRSA, two enterococci, two CNS one *Aerococcus viridans* and one *Proteus* spp. strains, were tested for growth in Mueller Hinton II broth with pH ranging from 4 to 5.5 and salt addition of 4% to 7%. In the next step, these strains were tested for growth in 12 different combinations of salt (5%- 6.5%) and pH (4.5-5.5). For assessment of the growth of *S. aureus* in pH adjusted media, further 14 MRSA and 13 MSSA were tested in a similar way.
In a preliminary study using reference strains (data not shown) it was observed that pH ≤5.5 as well as salt concentrations ≥4% did allow growth of *S. aureus* but was inhibiting the growth of enterococci. Subsequent growth ex-

periments with isolates from background flora showed an inhibitory effect of pH below 5 for *Aerococcus* spp and enterococci, whereas less effect was observed on CNS and *Proteus* spp. In the assays using different combinations of pH and salt, the pH showed, in general, a larger effect than the salt concentration on growth. MRSA and MSSA strains were partially inhibited by pH below 4.5 and grew with a moderate growth rate at pH 5.5 with lower salt concentration (5%). The growth of enterococci strains was completely inhibited by pH ≤5.5 at any salt concentrations tested (5- 6,5%), whereas the growth of *Proteus* spp. was only inhibited totally at pH of 4.5, but the growth rate could be reduced combining pH at 5 or 5.5 with high salt concentrations.

In conclusion, pH adjustment of enrichment media might improve sensitivity of methods for detection of *S. aureus* by reducing background flora growth. Moreover, the combination of pH adjustment with reduction of the currently used salt enrichment concentration migth increase the sensitivity of the detection of MRSA. For screening purposes it will still be necessary to have further steps for the selective enrichment and isolation of MRSA. Further studies are underway to evaluate the value of this under field conditions.

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GENETIC ENVIRONMENT AND LOCALIZATION OF lnu(A), lnu(B) AND vga(A) GENES OF MRSA AND OTHER STAPHYLOCOCCI FROM ANIMAL AND HUMAN ORIGIN.

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Lincomycin-resistance/erythromycin-susceptible phenotype (LINR-ERYS) is very unusual and it has recently been detected in some MRSA ST398 strains. The objective of this work was to determine the localization and the genetic environments of lnu(A), lnu(B) and vga(A) genes in MRSA and other staphylococci from animal and human origin presenting LINR-ERYS phenotype.

Methods. Eleven Staphylococcus spp strains (8 from human, 2 from pig and 1 from cat origin) were included in this study: 8 S. aureus (6 MRSA and 2 MSSA), 2 S. epidermidis (1 MRSE and 1 MSSE) and 1 S. sciuri (MRSC). Antibiotic susceptibility testing was performed by agar-dilution and disk-diffusion. The presence of resistance genes was studied by PCR. MLST, spa- and agr-typing was performed by PCR and sequencing in all S. aureus strains. SCCmec-typing was carried out by Multiplex PCR in all methicillin-resistant strains. Plasmid or chromosomal gene localizations were determined by S1-PFGE or DNA plasmid extraction and I-CeuI-PFGE hybridization. Genetic environments were studied by inverse PCR and sequencing or by PCR-mapping and sequencing based on the previously reported structures included in GenBank.

Results. Five MRSA strains belonged to ST398 (spa-types: t011 or t108) and were typed as SCCmecV and agrI. The remaining MRSA was ST8-t008-SCCmecIV-agrI. Two MSSA strains were ST9-t337-agrII. Two strains (1 MRSA and 1 MRSC) presented the lnu(A) gene in a 2.3 kb plasmid which was identical to pLNU1 of S. chromogenes. lnu(B) gene was identified in three strains and was embedded in the chromosome in two MSSA strains and in a 500 kb plasmid in one MRSA strain. All three strains presented the same upstream genetic environment of the lnu(B) gene which was identical to pEF418 of Enterococcus faecalis. vga(A) gene was detected in six strains (4 MRSA, 1 MRSE and 1 MSSE) and in all of them it was located in large sized plasmids from 200 to 350Kb. Two MRSA strains presented vga(A) gene in a 240 kb plasmid and the same environment as pVGA of S. aureus. The MRSE strain harbored the vga(A) gene in a 250 kb plasmid with a upstream and downstream genetic context very similar to sequences included in pSE-12228-06

of S. epidermidis. One MRSA strain presented a 350 kb plasmid harboring the vga(A) gene with a new environment which seems to be a mosaic-like structure with reorganized sequences from plasmids of different staphylococci (pSK3, pSK6, SAP018A, SAP104B, SAP108B, pSHaeC and pIP1630).
Conclusion. LINR-ERYS phenotype seems to be related to animal clonal lineages as ST398 and ST9. lnu(B) and vga(A) genes were frequently detected in large sized plasmids. lnu(B) has also been detected in chromosome and it is the first detection of this gene in Staphylococcus. These data suggest the resistance gene transfer among bacterial genera and species.

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METICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) *SPA* TYPE t127 IN PIGS AND HUMANS IN FINLAND

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A survey for the presence of MRSA in pig farms in Finland was conducted between September 2009 and August 2010. The survey included 59 finishing farms and 36 farrowing farms. Isolates belonging to clonal complex (CC) 1 were found from five finishing farms and were all of *spa* type t127. In 2010, MRSA *spa* type t127 was isolated also from a farm with sow udder dermatitis; this farm was not in the survey. The six pig farms with MRSA t127 findings are scattered around the southern part of the country. Human findings of MRSA t127 are known to be community-associated in Finland. Between 2009 and 2010, nationwide MRSA-surveillance identified t127 isolate from 31 persons (1 % of all), in different parts of the country. The purpose of the present study was to determine the antimicrobial resistance profile of porcine MRSA *spa* type t127 isolates, and to compare the genetic background of porcine and human t127 isolates in Finland. The antibiotic resistance profiles were determined by using the broth microdilution method, and the genetic background by pulsed-field gel electrophoresis (PFGE) subtyping. The porcine isolates all shared a resistance to tetracycline and were susceptible to all other antimicrobials tested. The isolates were identical in PFGE profile, which differed from those of the human strains. The results suggest that the Finnish porcine and human isolates do not share a recent common origin. Instead, there is a distinct porcine MRSA CC1 clone present in pig farms in Finland.

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METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN A PIG HERD IN IRELAND

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Colonisation of pigs with livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), first identified in pigs and their handlers in the Netherlands in 2004, is now known to occur in many countries worldwide. MRSA was not detected in pigs in Ireland in an EU baseline study in 2008 or in a recent Irish study of pigs at slaughter. Pigs may be colonised by methicillin-susceptible *S. aureus* and *mecA* + coagulase negative staphylococci (CNS). As the *mecA* gene is present on a mobile genetic element, the staphylococcal chromosomal cassette (SCC*mec*), there is concern that transfer of this element from *mecA* + CNS could potentially lead to the emergence of MRSA within the Irish pig population.

As a part of a study investigating the prevalence of *S. aureus* at each production stage in a large pig herd, nasal swabs were collected from 537 pigs. The samples were processed using conventional microbiological techniques. One of the finisher pigs was identified as MRSA-positive. Further investigations at the farm failed to uncover additional MRSA isolates.
Antimicrobial susceptibility testing was carried out using the broth micro dilution (Trek Sensititre®). This isolate was resistant to kanamycin, gentamicin, ampicillin, streptomycin, tetracycline, sulphonamide, trimethoprim, and cefoxitin. The isolate was also typed using pulsed-field gel electrophoresis (PFGE) and compared to MRSA strains previously found in animals in Ireland. This comparison showed that the isolate had a similar pattern to equine MRSA isolates. DNA microarray analysis using the StaphType Kit (Alere, Germany) revealed that the pig isolate was similar to USA500 and belonged to CC8 and SCC*mec* type IV. The microarray also revealed the presence of genes encoding resistance to beta-lactams (*blaZ*), aminoglycosides (*aacA-aphD*), tetracycline (*tet*(M)), trimethoprim (*dfrA*) and fosfomycin (*fosB*) as well as the enterotoxin genes *sea, seb, sek and seq* and the immune evasion cluster genes *sak* and *scn*.
This is the first report of CC8-MRSA-IV in a pig herd in Ireland. This strain has been identified previously in horses and their handlers in the USA, Canada, Ireland and Germany. The isolate appears to be an incidental finding that is likely to have originated from an equine source and was possibly transferred to the pigs via their human handlers.

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FIRST REPORT OF RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* (MRSP) CLUSTERS IN SMALL ANIMALS AND SEALS IN IRELAND

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Staphylococcus pseudintermedius an important cause of pyoderma and soft tissue infections in small animals. A collection of 14 multi-drug resistant MRSP isolates, recovered from clinical specimens from animals over a 2-year period (2008/2009) were characterised in this study. Wound specimens (canine $n = 6$, seal n = 5) and environmental specimens ($n = 3$) were subjected to conventional microbiology methods. The canine wound and the environmental samples originated from a small animal practice whilst the seal samples were from patients in a seal sanctuary. Biochemical species identification was carried out using the API ID32 Staph kit. Antimicrobial resistance typing, including resistance to oxacillin, was carried out and oxacillin resistance was confirmed using a *mecA* PCR assay. Pulsed Filed Gel Electrophoresis (PFGE) was carried out on this cluster of isolates, using NCTC 8325 *Staph. aureus* and HH1 *Staphylococcus pseudintermedius* as controls. All isolates underwent DNA microarray analysis using the StaphType Kit (Alere Germany).
The isolates exhibited an identical multi-drug resistance pattern which had not been observed or previously described in *Staphylococcus pseudintermedius* isolates in Ireland. The PCR assay confirmed the presence of the *mecA* gene and the isolates were identified as being clonally related using PFGE. DNA microarray analysis identified the presence of SCC*mec* V in all isolates, as well as genes encoding resistance to beta-lactams (*blaZ*), aminoglycosides (*aacA-aphD* and *aphA3*), tetracycline (*tet(M)*), macrolides, lincosamides and streptogramin B compounds (*erm*(B)) and streptothriocine (*sat*). Some isolates also harboured genes encoding resistance to lincomycin (*ln*A), 13/14 isolates) and quaternary ammonium compounds (*qacC*, 4/14 isolates).
This study revealed cluster outbreaks of MRSP occurring in two geographically separate locations with no commonality factor being determined. The emergence of MRSP as a pathogen in Ireland is of considerable concern for both animals and veterinarians. There is also the potential for colonisation of humans. The isolates in this study do not appear to be related to the

European MRSP epidemic clone ST71 SCCmec II-III and SCCmec IV but may be related to MRSP ST68 SCCmec V which has been isolated in North America in dogs. Further analysis involving MLST and SPA typing would be advantageous in determining whether these isolates are uniquely indigenous to Ireland or are closely related to the North American strain.

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PREVALENCE AND MOLECULAR CHARACTERIZATION OF STAPHYLOCOCCI OBTAINED FROM HEALTHY DOGS IN NIGERIA: HIGH RATE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS SCIURI* AND FIRST DESCRIPTION OF *S. PSEUDINTERMEDIUS* IN AFRICA

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Background: Recent reports on staphylococci in dogs have shown that *S. pseudintermedius* is the most common staphylococcal species that colonizes these animals. However, there is no data on the prevalence of staphylococci in healthy dogs in Africa. The objective was to determine the occurrence of methicillin resistant staphylococci (MRS) in healthy Nigerian dogs and to characterize the recovered isolates. **Methods:** Groin swabs from 109 clinically healthy dogs were sampled. Dogs were from households (20), markets (50), and dogs presented to the University of Nigeria Veterinary Teaching Hospital (39) in Nsukka. One sample per dog was cultured on Mannitol Salt agar (+10 mg/L cloxacillin) and up to three colonies/sample were analysed. Isolates were tested for coagulase production and haemolytic activity. Identification was conducted by PCR, PCR-RFLP, and sequencing of *sodA* gene. Susceptibility testing to 16 antimicrobials was carried out by disc-diffusion. Antimicrobial resistance genes and the presence of virulence genes were tested by PCR. All coagulase positive staphylococci (CoPS) were characterized by *spa*- and MLST.
Results: Twenty-two isolates were obtained. They came from 18 different dogs (17% of animals tested). Species identified were as follows (number of isolates): *S. sciuri subsp. rodentium* (12), *S. lentus* (3), *S. aureus* (2), *S. haemolyticus* (2), *S. pseudintermedius* (1), *S. simulans* (1), and *S. lugdunensis* (1). Strains were resistant to the following families of antimicrobials (number of isolates): β-lactams (16), tetracyclines (17), aminoglucosides (15), co-trimoxazol (13), macrolides-lincosamides (11), and chloramphenicol (7). Resistance to methicillin was detected in sixteen coagulase negative staphylococci (CoNS), all carrying the *mecA* gene. Other antimicrobial genes detected were as follows (no isolates): *tet*(K) (15), *tet*(M) (10), *tet*(L) (1), *aacA-aphD* (14), *aphA3* (3), *str* (1), *dfr*(A) (3), *dfr*(D) (1), *dfr*(G) (4), *dfr*(K) (3), *erm*(B) (10), *lnu*(A) (6), cat_{pC223} (1), cat_{pC221} (6). The following virulence genes were harbored by both *S. aureus* strains: *lukED, eta, hla, hld,* and *hlg-v*, whereas those found in *S. pseudintermedius* were *lukSF-I, siet, si-ent,* sec_{canine}, *sel.* One of the MR-CoNS harboured *tst* gene and another one *eta* toxin gene. Both *S. aureus* strains were t084-ST15 and the *S. pseudintermedius* isolate was *spa* no-typable and showed a new ST, named ST141.**Conclusions**: Staphylococci are common colonizers of healthy dog in Nigeria (17%) with a major species detected being *S. sciuri subsp. rodentium* (55% of isolates recovered). Methicillin resistance was highly common among our CoNS studied (84%), most of them being multidrug-resistant. Although CoPS were susceptible to most antimicrobials tested they harboured a high rate of virulence properties. This is the first report on the prevalence and molecular characterization of staphylococci in dogs in Africa.

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METHICILLIN RESISTANT *S. PSEUDINTERMEDIUS* - AN EMERGING NOSOCOMIAL PATHOGEN IN HUMANS HOSPITALS?

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We report for the first time methicillin resistant *Staphylococcus pseudintermedius* (MRSP) infection in 2 human patients in a Swedish university hospital. *S. pseudintermedius* is coagulase-positive bacteria and is the major cause of canine pyoderma. In 2006, the first cases of MRSP were confirmed in Sweden and in 2008, methicillin coagulase positive staphylococci were made notifiable in veterinary medicine. Since 2006 the number of MRSP has increased and in 2010, 100 cases were diagnosed. The majority of MRSP in Sweden belong to a single clonal lineage corresponding to ST71-t02-II-III European clone. Sporadic human cases caused by *S. pseudintermedius* ; bacteremia, brain abscess, endocarditis, otitis, masteioditis and pneumoniae have been reported.Pet owners and veterinary staff have shown to carry the same strains of *S. pseudintermedius* as their pets, especially when the dogs have suffered from pyoderma. Infection with MRSP is rarely reported and colonization among owners of infected dogs is described as uncommon and transient. However, this case reports isolation of MRSP from two patients with clinical infections whom have not been in contact with dogs. Two elderly male patients with multiple risk-factors for cardiovascular disease including diabetes were admitted to hospital with erythemas and ulcers in March 2011. Wounds were sampled and analysed by routine bacteriology including susceptibility testing. *S. pseudintermedius* positive for *mecA* gene was detected. The MRSP strain was resistant to all antimicrobials tested except chloramphenicol, fusidic acid, linezolid, rifampicin, tetracycline and vancomycin. Both the strains belonged to s*pa*-type t02 and carried SCC*mec* II-III. One of the strains was typed using PFGE and had the similar pattern as the dominating canine cluster. One of the patients was owner of cats, but none had known contact with dogs. Staff members, who owned dogs that had taken care of one of the patient was screened for MRSP (samples taken from nose and wounds if present); none were positive. The two patients attended the same ward within a few days of each other but not at the same time. This raises the question if the pathogen has been acquired through human-to-human contact in hospital setting.

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SUSPECTED HORSE-TO-HUMAN TRANSMISSION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ST398

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Introduction: In the Netherlands, MRSA of the livestock-associated ST398 predominate in horses. Here we describe the suspected transmission of MRSA of ST398 between horses and humans.

Case 1: A 16-year old girl had an infected wound on her foot which did not respond to empirical treatment with clindamycin and ciprofloxacin. An MRSA resistant to clindamycin, ciprofloxacin, erythromycin, gentamicin, kanamycin, tetracycline, trimethoprim/sulfonamide and susceptible to rifampicin and fusidic acid was isolated**.** The girl did not have a history of foreign hospital admission, nor contact with pigs or calves, but had intensive contact with a foal. An MRSA with an identical susceptibility pattern was isolated from the nares of the

girl's healthy foal. The foal had been hospitalized due to a wound infection at a horse clinic two month earlier, but no samples were taken from the wound at that time. After eradication therapy, samples taken from the girl were MRSA-negative and the wound had healed. The isolates from the girl and the horse were further investigated by *mec*A PCR, ST398-specific PCR, *spa*-typing and PFGE using *SmaI* and *Cfr9I* as restriction enzymes. Both isolates belonged to MLST ST398, *spa*-type t011, were non-typeable by PFGE using *SmaI* and had indistinguishable PFGE patterns using *Cfr9I*.
Case 2: A patient was hospitalized and treated with meropenem because of a urosepsis with an ESBL-producing *E. coli*. Two weeks later an MRSA resistant to tetracyclines, ciprofloxacin, gentamicin, cotrimoxazole and susceptible to erythromycin, clindamycin, rifampicin and fusidic acid was cultured from a urine sample. The isolate had *spa*-type t588 (ST398), which is rare in humans. Personnel at the hospital were screened and a nurse was found MRSA-positive. MRSA with an identical susceptibility pattern was isolated from the nares of her husband, child, dog and horse. The isolates were further characterized by PCR targeting the *fem*A gene and the *nuc* gene, *mec*A PCR, ST398-specific PCR, *spa*-typing and PFGE using *Cfr9I* as restriction enzyme. All isolates were *mec*A positive, belonged to ST398, and had indistinguishable or similar PFGE patterns. All human isolates had *spa*-type t588 (08-16-02-24-25), the isolates from the horse and dog had the closely related *spa*-type t4628 (08-02-24-25).
Conclusions: Human infections with MRSA ST398 are rare in the region where the girl and nurse live. We suspect that the foal was the source of the clinical infection of the girl and a horse was suspected of being the source of the colonization of the nurse. It is likely that transmission occurred from nurse to patient at the hospital. The isolate from the horse and the dog probably lost repeat 16 resulting in *spa*-type t4628 instead of t588.

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METHICILLIN-RESISTANT NON-STAPHYLOCOCCUS AUREUS STAPHYLOCOCCI (MRNAS) IN BELGIAN VEAL CALVES

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Background & aims In the past years, methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 has been detected in a variety of farm-associated animals. Recently, it has been reported that MRSA ST398 is also widespread in Belgian veal calf farming. Another recent study demonstrated the existence of a reservoir for methicillin resistance in non-*S. aureus* staphylococci (NAS) in pigs. In the present study, we investigated the prevalence, species distribution and staphylococcal cassette chromosome *mec* (SCC*mec*)-types of methicillin-resistant NAS (MRNAS) from Belgian veal calves.
Material & methods On 15 Belgian farms exclusively breeding veal calves, nasal swabs were collected from 10 randomly chosen calves. MRNAS were confirmed by a 16S rRNA-*mecA*-*nuc* triplex PCR, identified by tDNA intergenic spacer PCR or *rpoB* sequencing and investigated with a SCC*mec*-typing PCR.
Results A total of 60 MRNAS were isolated. The farm-level prevalence of MRNAS was 100%, while the prevalence of MRNAS on the animal-level was 33.3%. Per farm the number of MRNAS varied between 1 and 13, and the number of MRNAS-positive animals varied between 1 and 8. Two MRNAS could not yet be identified; the other 58 belonged to five species *S. sciuri* (n = 21), *S. lentus* (n = 21), *S. epider-*

midis (n = 11), *S. haemolyticus* (n = 4) and *S. equorum* (n = 1). SCC*mec*-typing showed the predominance of cassettes of type III (n = 31) and IIIA (n = 8). Also some cassettes of type IV (n = 3) and type V (n = 4) were detected. Five cassettes were non-typeable and seven could not yet be assigned.

Conclusions MRNAS appear to be widespread on veal calf farms. The majority of MRNAS belonged to the *S. sciuri* species group. These species naturally occur in (farm) animals and are in general the most frequently detected MRNAS in animals. As most MRNAS carried SCC*mec* types that are seldom detected in MRSA ST398, their role as reservoir for SCC*mec* for MRSA ST398 is unclear.

This study was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment (contract RF-6189).

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INSIGHTS INTO EXTENDED HOST SPECTRUM GENOTYPES (EHSG) AMONG CLINICAL *S. AUREUS* OF HUMAN AND CANINE ORIGIN

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Introduction: *S. aureus* is able to cause a wide range of infectious diseases in various animal species. In the past, host specialization was assumed for the majority of *S. aureus* strains and these were denominated as ecovars according to their hosts. Nevertheless, some genetic lineages do not possess an obvious host tropism, since they infect a broad range of different hosts. These genetic lineages are representatives of extended host spectrum genotypes (EHSG), which seem to harbour the potential for a facilitated cross-species transmission. Since the relationship between humans and dogs has changed, and dogs live in the household nearly as family members nowadays, there might be an elevated risk for interspecies transmission, especially regarding EHSG- *S. aureus*. To gain more knowledge about EHSG of human and canine origin we conducted a broad comparative molecular analysis with regard to the genetic composition of clinical *S. aureus* strains from both hosts, including mecA-positive (MRSA) and negative (MSSA) strains.

Material and Methods: Initially, 96 canine *S. aureus* isolates of various geographic origins underwent *spa*-typing, pulsed field gel electrophoresis (PFGE), microarray hybridisation (MH) of 170 genes and multilocus sequence typing (MLST: for selected isolates). For further comparative analysis, clinical human strains were included, which were selected according to commonly observed *spa*-types associated with the canine strain collection.

Results: Among the investigated canine isolates the vast majority of detected genotypes had also been previously associated with human infections. Thirty representative canine isolates were selected for MLST, 27 of them yielded well-known STs associated with human strains, and three strains were single-locus variants of ST6, ST72 and ST30, respectively. Comparative analysis of typing results from human and canine isolates for the clonal complexes CC5, CC22, CC398, CC15 and CC30 by applying UPGMA clustering based on the Dice coefficient for PFGE and the Pearson correlation for MH using BioNumerics software (Applied Maths NS) revealed that the majority of *S. aureus* strains isolated from dogs showed PFGE- and MH-typing pattern which cluster with the human isolates.

Conclusion: Transmission of EHSG should be considered especially in cases of recurrent MRSA- infection in both, human or canine patients. Further analyses including potential functional differences and (highly) mobile genetic elements are necessary to investigate factors involved in different modes of strong or weak host tropism.

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Detection of a *fexA* gene variant that confers resistance to chloramphenicol, but not to florfenicol in canine *Staphylococcus pseudintermedius*

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Background: In staphylococci, phenicol resistance is mediated either by *cat* genes, which confer resistance to non-fluorinated phenicols (e.g. chloramphenicol) or any of the two genes *fexA* (coding for an efflux pump) or *cfr* (coding for a RNA methyltransferase) which mediate combined resistance to fluorinated (e.g. florfenicol) and non-fluorinated phenicols. The objective of this study was to determine the chloramphenicol and/or florfenicol susceptibility in *S. aureus* and *S. pseudintermedius* obtained from healthy dogs in Spain and to identify the phenicol resistance genes.
Methods: A total of 75 coagulase-positive staphylococci (44 *S. pseudintermedius* and 31 *S. aureus*) isolated from healthy dogs were investigated. Susceptibility to chloramphenicol and florfenicol was determined by disk diffusion and/or broth dilution tests. The phenicol-resistant isolates were tested by PCR for the presence of the *cat* genes cat_{pC221}, cat_{pC223}, cat_{pS194}, as well as *fexA* and *cfr*. The presence of the *fexA* gene as part of Tn*558* was investigated by PCR. The chromosomal/plasmid location of Tn*558*, as well as its specific integration site were determined by plasmid preparation, specific PCRs, and sequencing. The complete *fexA* gene was amplified and ligated to pCR® 2.1-TOPO vector. Transformation into *E. coli* Hb101 was conducted to test the functionality of the *fexA* gene in Gram-negative hosts.
Results: Ten (23%) chloramphenicol-resistant, florfenicol-susceptible *S. pseudintermedius* were identified whereas none of the *S. aureus* studied was resistant to chloramphenicol or florfenicol. Nine strains harbored the cat_{pC221} gene whereas the remaining *S. pseudintemedius* strain carried the *fexA* gene and exhibited a MIC to chloramphenicol of 64 mg/L and florfenicol of 2 mg/L. The *fexA* gene was part of Tn*558*, which was integrated within the chromosomal *radC* gene. Circular forms of Tn*558* were not detected. Sequence analysis revealed 99% similarity to the prototype FexA protein of *S. lentus* with four amino acid changes: G33A, A37V, L68I and I132V. The *fexA* gene conferred only chloramphenicol resistance in *E. coli* Hb101 host, whereas the florfenicol MICs remained unchanged.
Conclusions: Chloramphenicol resistance was commonly seen among the *S. pseudintermedius* (23%) but not among the *S. aureus* (0%) of our test population. This study presents the first description of a *fexA* gene in *S. pseudintermedius* and the first description of such gene conferring only resistance to chloramphenicol. The amino acids substituted in this FexA variant might be implicated in the inability to export florfenicol by a yet unknown mechanism.

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Carriage of methicillin-resistant Staphylococcus aureus among workers and household members at livestock farms in North Carolina

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Concerns are rising about the potential carriage of livestock-associated MRSA (LA-MRSA) among workers at livestock production facilities. In order to address this research need, an ongoing study is comparing MRSA prevalence among industrial hog farm workers to MRSA prevalence among organic antibiotic-free swine

farmers in North Carolina. MRSA prevalence is currently being assessed in 100 consenting antibiotic-free livestock farm workers and household members as well as 100 industrial livestock farm workers and household members, via a short questionnaire and nasal swabs. Questionnaires establish occupational, environmental, and healthcare exposures to MRSA, while nasal swabs are analyzed to determine actual MRSA carriage. Nasal swabs are incubated overnight in a pre-enrichment broth, then incubated overnight in an enrichment broth containing 10 mg/L aztreonam and 3.5 mg/L cefoxitin. Colonies with characteristic S. aureus morphology are saved for further molecular characterization. Currently, 2/19 swabs collected from industrial farm workers have tested positive for MRSA, as have 4/13 swabs from associated household members. Further characterization is planned to determine if positive samples are LA-MRSA. 0/7 swabs collected from antibiotic-free farmers have tested positive for MRSA. MRSA-positive individuals have been detected 100% of the time using CHROMagar™ Staph aureus (BD BBL™) media and 67% of the time using Baird-Parker media. All MRSA-positive isolates are being prepared for whole genome sequence typing, as are select false positive isolates and select methicillin-resistant non S. aureus isolates.

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GNOTOBIOTIC PIGLETS PROVIDE A MODEL FOR THE STUDY OF *STAPHYLOCOCCUS AUREUS* MRSA ST398 COLONISATION AND INTERACTION WITH OTHER STAPHYLOCOCCI *IN VIVO*.

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The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in pigs and its spread to other domestic animals and man has highlighted the need for models facilitating investigation of its processes of colonisation and interaction with other bacteria and the development of methods for its control. This study developed a gnotobiotic pig model to investigate MRSA ST398 colonisation of neonatal piglets, its pathogenicity and its interaction with coagulase negative staphylococci (CNS: *Staphylococcus warneri, xylosus* and *chromogenes*) isolated from the maternal vulvar flora, the first organisms to which piglets are normally exposed. Three groups of germ-free two-week-old piglets held in isolators were atraumatically inoculated on the skin with 1) MRSA only, 2) with MRSA followed 2 days later by CNS and 3) with CNS followed 2 days later by MRSA, respectively. Animals were swabbed periodically over a period of 23 days from the nasal mucosae, the skin behind the ears, and from the sacrum, and bacterial counts made on MRSA selective media (mannitol salt agar with 2 µg/ml oxacillin). Multilevel linear regression models were used to analyse bacterial growth. Inoculation of MRSA on piglet skin resulted in spontaneous colonisation without creation of skin lesions indicating that ST398 has a low pathogenic potential in gnotobiotic piglets. Bacterial populations increased similarly for both MRSA and CNS until day 32 demonstrating that early colonisation by MRSA was unaffected by prior or subsequent CNS inoculation. However, final MRSA counts on day 37 significantly decreased in the presence of CNS suggesting a possible late effect of bacterial interference. This work shows that gnotobiotic piglets can provide a reproducible model suitable for studies of MRSA colonisation and interaction with other bacteria. Further studies of bacterial interference in the control of MRSA ST398 in piglets are warranted.

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Occurrence and Clonal-Spread of Methicillin-Resistant *Staphylococcus pseudintermedius* in Dogs in the Netherlands

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Emergence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in companion animals has recently been described in different countries. A retrospective analysis of the data containing all clinical samples analyzed by the Veterinary Microbiological Diagnostic Center (VMDC) of Utrecht University, the Netherlands, that harbored methicillin-susceptible and methicillin-resistant *Staphylococcus pseudintermedius* was performed. From January 2006 to January 2010 clinical samples of approximately 850 veterinary clinics were analyzed and MRSP was isolated from clinical samples obtained from 84 clinics (9.9%). A remarkable increase in the percentage of MRSP isolates on the total number of *S. pseudintermedius* isolates, was observed during the study period; the percentage increased from 0.78% in 2006 to 9.2% in 2009. The majority of MRSP was isolated from (surgical) wound infections (n=57; 28%), from otitis externa (n=44; 21%), and from pyoderma (n=44; 21%).To gain insight in transmission and possible clonal distribution of MRSP in Dutch veterinary clinics 61 canine isolates, submitted by 25 clinics between 2007 and 2009 were analyzed; 55.7% of the isolates belonged to multilocus sequence typing (MLST) ST71, with pulsed-field gelelectrophoresis (PFGE) pattern J, *spa* type t002 and staphylococcal cassette chromosome *mec* (SCC*mec*) element II/III. MRSP with this genotype, designated ST71-J-t002-II/III, were obtained from 20 of the 25 veterinary clinics. Ten isolates belonged to the less prevalent ST29, ST100, ST106 and ST111. However, a strain with the rare type ST106-Uno *spa* type and a variant SCC*mec* element was obtained from two dogs that visited the same referral clinic within eight days, and suggests that transmission of the same MRSP strain within veterinary clinics occurs. This study shows that the multidrug-resistant MRSP-lineage ST71, which is predominant in Europe, is also widespread among Dutch veterinary clinics, and that for detailed study on transmission-routes further differentiation of this clone is needed.

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Methicillin-Resistant *Staphylococcus aureus* in Dairy Cows and Their Calves

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Livestock-associated MRSA (LA-MRSA) with sequence type (ST) 398 has been recovered from different geographic regions and has been associated with a large number of animal species. In cattle, this specific MRSA clone is mostly related to veal calves with a prevalence of 9.7% (Graveland et al., 2010). Recently this clone was also described in milk from dairy

cows with mastitis, with a prevalence at farm level between 3.9% and 7.4% (Vanderhaeghen et al., 2010). In this study, the MRSA prevalence in healthy dairy cows and their calves was determined together with the best single and multiple anatomical sampling site(s) for the detection of MRSA in cattle.
Samples were collected from 44 dairy cows and 23 calves, originating from 2 Belgian cow-pig farms. On farm A, 14 dairy cows and 8 calves were sampled. On farm B, 30 dairy cows and 15 calves were sampled. From each cow and calf, samples were obtained from different anatomical sampling sites (anterior nares, udder skin and perineum). Sampled cows were not known to have clinical mastitis. Sampling was performed by using a swab inserted into Mueller Hinton broth (Oxoid, Germany) supplemented with 6.5% NaCl. All 201 swabs were enriched overnight and after 18-20 hours a loopful was plated onto ChromID MRSA (BioMérieux, France). Characteristic colonies were interpreted following the manufacturers instructions and further analyzed by multiplex-PCR for 16S *r*RNA, *mec*A and *nuc* gene. MRSA were further characterized by *spa*-typing.
On farm A and B, 7.1% (1/14) and 83.3% (25/30) of the dairy cows were MRSA positive, while the percentage in calves was 12.5% (1/8) and 13.3% (2/15). The udder skin was the most positive site of MRSA detection in cattle with 26.9% (18/67), followed by perineum and anterior nares with both 22.4 % (15/67). The relative sensitivity of MRSA isolation for the udder skin (62.1%) was 10% higher than the perineum (51.7%) and anterior nares (51.7%). The true MRSA prevalence was better approximated when different anatomical sites were combined. Sampling of the udder skin and anterior nares appeared to be the most sensitive combination with a relative sensitivity of 89.7%, other combinations had a sensitivity of 79.3%. *Spa*-typing on a selection of strains showed that they belonged to *spa*-type t011 and t034, which are both associated to MRSA ST398.
This data show that LA-MRSA can be detected in nasal, skin and perineum samples of healthy cattle. When only one site is sampled for MRSA detection this should be the udder skin and if combining sampling sites, samples should be taken from nares and udder skin, the latter increasing the detection rate of MRSA in dairy cows and their calves. Also a farm dependent MRSA prevalence was observed. As this strain might be involved in clinical and subclinical mastitis, this situation is most worrisome and urges for a thorough surveillance on colonization of milking cows with LA-MRSA.

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IN VITRO SUSCEPTIBILITY OF CANINE STAPHYLOCOCCUS PSEUDINTERMEDIUS AND STAPHYLOCOCCUS AUREUS TO MICONAZOLE

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Staphylococcus pseudintermedius and to a lesser degree S. aureus are the most common causes of skin and ear infections in dogs. Treatment is increasingly problematic because of the tremendous increase in methicillin-resistant strains. There has been resurgence in interest in topical therapy (e.g. chlorhexidine, topical antimicrobials) for superficial infections. Fusidic acid and mupirocin are sometimes used but concerns about resistance and the role of these drugs in human medicine have lead to scrutiny of their use in animals. Miconazole, an antifungal that has been previously shown to inhibit S. aureus, does not have the same importance for human medicine and might be another topical option. The objective of this study was to assess miconazole susceptibility of MRSA, MRSP and MSSP.
96 MRSP, 69 MRSA and 37 MSSP from dogs were tested. Agar dilution was used to determine the minimum inhibitory concentration. Wilcoxon or Kruskal-Wallis tests were used for 2- and 3-way comparisons, respectively.
For MRSA, the MIC range, MIC50 and MIC 90 were 1-8 ug/ml, 4 ug/ml and 4 ug/ml. For MRSP, the corresponding data were 1-8, 4, and 4 ug/ml, while for MSSP they were 1-4, 2 and

2 ug/ml. There was a significant difference between MIC between MRSA, MRSP and MSSP (P<0.001), with MRSA > MRSP > MSSP. To assess repeatability, 35 isolates were tested in duplicate, with 29 (83%) returning the same MIC and the remaining 6 differing only by 1 dilution. To account for the potential influence of inter-run variation accounting for the significant differences between types, a set of MRSA and MSSP isolates were tested together on the same sets of plates. With that subset, the significant differences reported above were maintained. There are no established breakpoints for miconazole and staphylococci, however the MIC levels reported here are well below concentrations achievable with topical therapy (e.g. 2% commercial products equal 20000 ug/ml). The narrow spectrum of activity, safety, ready availability and low priority in human medicine make miconazole a potentially attractive treatment option for superficial MRSA and MRSP infections. Clinical study is required to determine whether miconazole may be an effective treatment of superficial MRSA and MRSP infections.

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THE EMERGENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS IN WESTERN CANADA

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Introduction: Methicillin resistant Staphylococcus pseudintermedius (MRSP) has recently and rapidly emerged as a cause of infection in dogs. In Western Canada there is little published data regarding the incidence or antimicrobial susceptibility profiles of MRSP. The purpose of this study was to address these gaps in our knowledge. Methods: Culture records from dogs from January 2006 through April 2011 from Saskatchewan and Alberta from two diagnostic laboratories (Prairie Diagnostic Services and Antech Diagnostics) were surveyed, and MRSP records were retrospectively analyzed. As much of these data predated the recognition of S. pseudintermedius by some diagnosticians, any isolates identified as either S. pseudintermedius or S. intermedius were considered to be S. pseudintermedius. The techniques now accepted for detecting MRSP (oxacillin vs. cefoxitin screening and current clinical breakpoints) were not used throughout the study period; some isolates were tested using cefoxitin disks. Isolates were considered to be methicillin resistant if they were categorized as methicillin resistant in the record, or if they were resistant to amoxicillin/clavulanate, ampicillin and penicillin. Varying cephalosporin resistance profiles were found. As cephalosporin (cefoxitin) resistance is known to be insensitive for detecting mecA among S. pseudintermedius, these data were not considered for MRSP categorization. Changes in the annual incidence of MRSP were evaluated by poisson regression and categorical susceptibility data (susceptible or resistant) were evaluated by logistic regression. Results: A total of 36 MRSP were identified and were most commonly isolated from dermatitis/otitis (n=9), surgical site infections (n=9), wounds (n=5) and urinary tract infections (n=3). The overall incidence of MRSP increased significantly (P = 0.043) from 2008, when the first case was identified, through 2011. The incidence of gentamicin resistance increased significantly (P = 0.02), while the frequency of enrofloxacin, tetracycline, chloramphenicol, trimethoprim/sulfamethoxazole, clindamycin and erythromycin did not change significantly (P ≥ 0.053). Discussion: The incidence of MRSP is increasing in Saskatchewan and Alberta and resistance to non-β-lactam antimicrobials is also becoming more common. Western Canadian practitioners should be aware of MRSP, as it has been reported as a cause of community associated infections and may be encountered in general practice. As these data were collected retrospectively, this study may underestimate the true incidence of MRSP. Prospective investigations using generally accepted laboratory techniques are required to better address the emergence of this organism.

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COMPARISON OF TWO ISOLATION METHODS FOR THE DETECTION OF METHICILIN RESTISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN CHICKENS

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Comparison of two isolation methods for the detection of MRSA in chickens
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The prevalence of Methicilin Resistant *Staphylococcus aureus* (MRSA) in pigs is well recognized. But, little is known about the prevalence in other animals. In order to obtain comparable prevalence results all over Europe, the European Food Safety Authority (EFSA) proposes a standardized protocol for the isolation of MRSA from dust samples. However, this protocol was estimated not to be very sensitive. In this study, we determined the prevalence of MRSA in chickens using the EFSA standardized protocol and an alternative protocol. A total of 132 pools of 20 nasal swabs were investigated. In the first MRSA isolation method, the sample was enriched in Mueller-Hinton (MH) broth with 6,5% NaCl. A loopfull (10µl) is then plated on MRSA-ID (Biomérieux) and incubated for 24 and 48 hours. In the second method (EFSA method), an additional enrichment in cefoxitin supplemented Tryptic Soy Broth (TSB) was included. Identification of suspected colonies was done by triplex PCR. MRSA strains were further characterized by a spa-typing and an MLST.
Using the method with two enrichments, no samples were found positive (0%, CI 3-5%), while with the other method, three samples were proven to contain MRSA (0.25%, CI, 0-7%). To date, one of the strains has been identified as spa type t037, which is usually present in humans. Further typing and testing is ongoing.
Although the differences between the two methods are not statistically significant, the most sensitive isolation method is preferred. It is important to detect as much positive farms as possible in a low prevalence environment to avoid the further spread to other farms.
This study shows that MRSA is present in chickens but unlike pigs, chickens have a low prevalence. The absence of positive in the EFSA protocol while three MRSA has been found with the other method shows that the use of TSB in this population may be too selective for the detection of MRSA.

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LACK OF MRSP DETECTION IN HEALTHY DOGS ATTENDING A DANISH REFERRAL HOSPITAL

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Introduction: Methicillin-resistant *S. pseudintermedius* (MRSP) is an emerging threat to pet animals due to its characteristic multidrug resistance profile. The current knowledge of the frequency of MRSP in the dog population is limited to few countries. Prior to this study, no information was available about the frequency of MRSP healthy dog carriers in Denmark.
Objectives: The objectives of this study were i) to estimate the prevalence of MRSP and methicillin-susceptible *S. pseudintermedius* (MSSP) among healthy dogs in the area of Copenhagen, and ii) to compare the carriage rates at different body sites.
Methods: A total 476 swabs were collected from perineum, groin, mouth and nasal vestibulum of 119 healthy dogs attending the Copenhagen University small animal hospital between October and December 2010. The dogs were defined as healthy and included in the study on the basis of veterinary records (no skin or wound infection and no antibiotic therapy in last 6 months) and clinical examination. Each swab was enriched in Mueller Hinton

Broth with 6.5% NaCl and subcultured on both blood agar and MRSA brilliance agar (Oxoid, Deutschland GmbH, Wesel, Germany). The species of presumptive *S. pseudintermedius* colonies from blood agar was confirmed by *nuc* PCR. Any light blue/blue colonies growing on Brilliance agar were subjected to *mecA* PCR for confirmation of methicillin resistance.

Results: Despite the use of a selective isolation method, no MRSP-positive dogs were detected. MSSP were isolated from 82 (69%) of the 119 healthy dogs sampled. The perineum (66%) and mouth (65%) were the most frequent colonization sites followed by nasal vestibulum (26.9%) and groin (23%). Most dog carriers were positive at only one body site (39/119; 33%). Carriage at two (25/119; 21%), three (13/129; 11%) and four body sites (5/129; 4%) was less frequent. Among dogs carrying *S. pseudintermedius* at multiple sites, positive culture of perineum and mouth samples was the most frequent combination and accounted for 90% (74/82) of the dogs.

Discussion/conclusion: The study indicates that the prevalence of MRSP is very low in the Copenhagen area. This finding may explain why MRSP is rarely isolated from canine clinical at our diagnostic laboratory. Differently from *S. aureus* in humans, the perineum and the mouth are the body sites most frequently colonized with *S. pseudintermedius* in dogs. This information is highly relevant for the design of MRSP screening studies in dogs. Based on our data, both sampling sites should be analyzed since the combination of the two samples increase the sensitivity of the screening by 25% and 26% in comparison to perineum or mouth samples alone, respectively.

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LONGITUDINAL INVESTIGATION OF MRSA IN PIGS FROM BIRTH TO SLAUGHTER AND ENVIRONMENTAL SURVEILLANCE IN A SLAUGHTERHOUSE

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MRSA is endemic in pigs internationally and is associated with significant public health concerns. While point prevalence data provide useful information, longitudinal study and study during slaughter and processing are important to help elucidate the epidemiology of this pathogen.

Nasal swabs were collected fro 30 piglets from 3 sows at an antibiotic-free commercial swine farm on days 1, 6, 20, 41, 74, 108, 150 and 188 of life. When shipped for slaughter, samples were also collected from pigs, carcasses and the slaughterhouse environment. A separate, broader environmental sampling study was also performed.

MRSA was isolated on one or more occasions from 24 (80%) of pigs, with individual pigs having 0-5 positive samples. There was a significant difference in prevalence over time ($P<0.0001$), with a peak prevalence (68%) on day 41. 36/45 (80%) isolates were spa t034, an ST398-associated spa type that was found at least once in all positive pigs. The 2nd most common was t064, an ST8 human epidemic clone that is also found in horses and has been found in pigs and pork in Canada. Most (56%) pigs with more than 1 positive sample harboured t034 each time, but 44% had t034 and one other type.

At the time of slaughter, MRSA was not detected from the nasal swabs of any of the 26 pigs that survived to slaughter, or 10 holding area floor and wall samples, but was found on 1/26 (3.9%) of bleeding area carcasses, 8/26 (31%) bleeding area nasal swabs, 0/5 scald water samples, 15 (54%) post scald nasal swabs, 0/26 polisher blades, 0/20 post-polish/pre-evisceration carcasses and 0/26 post-evisceration carcasses. t034 predominated, accounting for 78% (18/23)

of isolates, with t064 accounting for 8.7%.
In the 2nd environmental study, MRSA was only isolated from 3/140 (2.1%) samples; 1/20 holding area samples, 1/20 bleeding area carcasses, 1/20 cold room carcasses and 0 of holding area manure samples, post bleeding area carcasses, post-evisceration carcasses, polisher blades and scald water.
The high overall prevalence with a peak at 41 days is consistent with other evidence of the impact of age on MRSA colonization. While t034 predominated, the presence of a small percentage of other strains, including a human epidemic clone, is consistent with the diversity of MRSA that has been reported previously in Canada. While MRSA was common in pigs overall, the nasal prevalence at the time of slaughter was low. However, the high prevalence in bleeding area nasal swabs and post-scald nasal swabs was unexpected and suggests that nasal swabs may underestimate the prevalence of MRSA at slaughter. The very high rate in post scald nasal swabs could be the result of flushing of MRSA from deeper sites (e.g. pharynx), something that requires further study. It was encouraging, however, that MRSA was not found on carcasses and equipment later in processing. The slaughterhouse environment does not appear to be a high-risk source for MRSA contamination.

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IDENTIFICATION OF METHICILLIN-RESISTANT S. AUREUS (MRSA) OF ANIMAL ORIGIN USING BACTERIOPHAGE AMPLIFICATION AND A LATERAL-FLOW IMMUNOASSAY

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Background: Methicillin-resistant S. aureus (MRSA) has emerged as an important health hazard for animals. Rapid laboratory confirmation is essential for appropriate clinical management and to allow appropriate control measures for minimizing nosocomial and zoonotic transmission risks. Recently, bacteriophage amplification has been used as the basis for a commercial test designed to rapidly identify MRSA in human clinical specimens. The purpose of this study was to evaluate the ability of a similar prototype test to identify animal-source MRSA and methicillin-susceptible (MS) Staphylococcus-aureus (SA) and to evaluate the test's reactivity with relevant non-SA staphylococcal isolates.
Methods: Bacteriophage specific for SA were included in 2 broth media solutions, 1 that also contained cefoxitin to select for methicillin-resistant (MR) bacterial strains. A variety of genetically characterized staphylococcal isolates of animal origin were selected from an archival bank, and standardized concentrations were inoculated into the broth solutions. A lateral-flow immunoassay was used to detect phage that were amplified in overnight cultures of broth solutions.
Results: Thirty-six MRSA isolates belonging to 7 major genetic types (USA 100, USA 200, USA 300, USA 500, USA 600, USA 700, and CC398) were evaluated. Thirty-two MRSA were correctly classified; 4/15 CC398 isolates were misclassified. Eleven of 15 MSSA isolates were correctly classified, as were 25 of 25 other non-SA staphylococcal isolates (including MR S. pseudintermedius, MS S. pseudintermedius, MR S. hyicus, MR S. schleiferi, and coagulase-negative Staphylococcus spp).
Conclusions: This prototype rapid assay correctly classified a high proportion of widely diverse MRSA, MSSA, and other non-SA staphylococcal strains of animal-origin. However the failure to detect some CC398 isolates might be problematic for some regions and animal species. Modification of the bacteriophage mixture may help overcome this misidentification and requires further study. This inexpensive, rapid method has promise for practical applications requiring identification of RSA colonization and infection in animals.

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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATES FROM DOGS AND ENVIRONMENTAL SURFACES IN A VETERINARY TEACHING HOSPITAL

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In order to design effective prevention and control programs it is important to understand the molecular epidemiology and ecology of occupational and nosocomial pathogens present in the veterinary environment, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA). The active MRSA surveillance program at The Ohio State University Veterinary Medical Center (OSU-VMC) has generated a total of 161 MRSA isolates of which 71 were from canines at admission and 90 from human and animal contact surfaces. We have hypothesized that by phenotypically and genotypically characterizing MRSA isolates obtained at the OSU-VMC, we will better understand the ecology of MRSA strains and identify possible sources of this pathogen. All 161 isolates were phenotypically characterized by antimicrobial susceptibility testing, from which 116 unique isolates were selected for further genetic analysis. Forty canine and seventy-six environmental isolates were genotypically analyzed by Staphylococcal Chromosomal Cassette *mec* (SCC*mec*) typing and Pulsed Field Gel Electrophoresis (PFGE) to establish clonal relatedness. Ninety-one percent (106/116) of the fully characterized isolates were multi-drug resistant (≥3 classes of antibiotics in addition to resistance to beta-lactams). Among the canine isolates, the PFGE results showed little diversity (3 clusters with 16 pulsotypes (PTs) and 2 individual PTs); where 85% (34/40) carried the SCC*mec* type II and 88.2% (30/34) belonged to cluster 1, with USA100 as the predominant type. Also, no cross-colonization was observed between nasal and skin lesions in the same animal. Similarly, heterogeneous colonization by multiple MRSA strains in the same anatomical location on the animal was not observed. In the environment, little diversity was again observed (3 clusters with 31 PTs and 2 individual pulsotypes) where 77.6% (59/76) carried the SCC*mec* type II and 100% (59/59) belonged to cluster 3. We observed that some PTs survived 2-3 consecutives months on the same surfaces. Also, in a giving month unique pulsotypes were found in different sections of the hospital, from both animal and human contact surfaces. Our results suggest that there is little diversity of MRSA strains circulating in dogs at arrival and in the environment at the OSU-VMC, where the majority of strains were HA-MRSA strains. There is clearly movement of MRSA strains in the hospital environment, with some PTs persisting on surfaces for long periods of time. This data is being used to develop and revise biosecurity and biocontainment protocols to decrease hospital personnel occupational exposure to MRSA, in addition of protecting patients from this serious nosocomial pathogen.

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OCCURRENCE AND CHARACTERISTICS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATES FROM PETS IN CHINA

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Staphylococcus pseudintermedius is one of the most common opportunistic pathogens causing a variety of infections in dogs and cats. Recently, methicillin-resistant *S. pseudintermedius* (MRSP) has been increasingly isolated from pets and represents a challenge for therapy. Until now, little is known about MRSP from pets in China. The aim of this study was to investigate the prevalence and molecular characteristics of MRSP in South China. A total of 113 *S. pseudintermedius* isolates were recovered from dogs (n=95) and cats (n=18) in 8 pet hospitals in Guangdong Province of China between 2008

and 2009. Minimum inhibitory concentrations (MICs) of 16 antibiotics were determined by agar dilution methods and disk diffusion methods. Multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) fragment profiling were performed on MRSP isolates. Fifty *S. pseudintermedius* proved to be MRSP by oxacillin MICs of >4mg/L and carried *mecA* gene. Most of the MRSP strains (>80%) were multidrug resistant to erythromycin, clindamycin, trimethoprim-sulfamethoxazole, tetracycline, and ciprofloxacin. None of the MRSP yielded resistance against linezolid, vancomycin, quinupristin/dalfopristin, and teicoplanin. Seventeen different multilocus sequence types were identified. ST4 (n = 7), ST5 (n = 7), and ST95 (n = 6) were the dominant sequence types, followed by ST104 (n = 4), ST29 (n = 4), ST6 (n = 2) and ST133 (n = 2). In addition, three new sequence types (ST134, ST135, and ST137) were identified. PFGE analysis revealed 21 different patterns. According to PCR-based staphylococcal chromosome cassette *mec* (SCC*mec*) typing, SCC*mec*V was the most prevalent type (n = 25). The remaining isolates belonged to SCC*mec*VII (n = 10), II-III (n = 6) , III (n = 2) and non typeable(n = 7). In conclusion, high genetic diversity among MRSP isolates from pets in China was observed which is differed from those occurred in European and American.

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PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCAL PYODERMA IN DOGS AND PERSISTENCE OF COLONIZATION AFTER CLINICAL RESOLUTION OF DISEASE

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Methicillin-resistant staphylococci are critical pathogens in veterinary dermatology, yet longitudinal study or evaluation of the impact of routine antimicrobial therapy on emergence or resolution of resistance are lacking. In this study, skin, nasal and rectal swabs were collected from dogs with clinical and cytological evidence of pyoderma at the time of referral and after clinical resolution.

179 dogs were enrolled. 70 (40%) had MRSP pyoderma while 3 (1.7%) MR-*S. schleiferi coagulans* (MRSScoag) and 3 (1.7%) had MRSA. At presentation, MRSP was isolated from the nose or rectum of 59 (34%) dogs, 51/70 (73%) with MRSP pyoderma and 8/103 (7.8%) without (P<0.0001). MRSA was detected in nasal or rectal swabs of 1/3 (33%) dogs with MRSA pyoderma versus 10/170 (5.9%) others (P=0.18). MRSScoag was isolated from nasal or rectal swabs of 7 dogs (3.9%). There was no association between prior antibiotic or corticosteroid use and MRSP, MRSA or MRSScoag infection or colonization (individually or combined), however there was a non-significant (P=0.087) association between prior corticosteroid or cyclosporine use and decreased likelihood of MRSP infection.

102 dogs were tested 3-15 (mean 6.6) weeks after clinical resolution. Of those, MRSP was isolated from 36 (35%) skin samples, and 36 (35%) nasal or rectal sites. Of dogs initially diagnosed with MRSP pyoderma, MRSP was isolated at follow-up from skin of 19/42 (45%) and nasal/rectal sites of 20 (48%). Of the dogs that did not have MRSP pyoderma initially, MRSP was isolated from the skin of 17 (28%) and the prevalence of MRSP at the nose or rectum increased from 7.8% to 27% (P=0.002). There was no difference in prevalence of skin or nasal/rectal MRSP in dogs that initially had MRSP versus non-MRSP pyoderma (all P>0.09). There were significant differences in isolation rates with MRSA Chromogenic agar versus mannitol salt agar with oxacillin (MSA-OX). Overall, 207/224 (92%) MRSP positives were positive using MSA-OX compared to 67 (30%) with Chromogenic agar (P<0.0001). Similar results were present for MRSScoag (19/20 on MSA-OX vs 2/20 on Chromogenic agar, P<0.0001). For MRSA, Chromogenic agar was

superior, identifying 12/17 (71%) versus 9/17 (53%)(*P*=0.029).
Overall, 61 MR-tube coagulase negative staphylococci (MR-CoNS) were identified, including *S. epidermidis* (31%), *S. haemolyticus* (16%), *S. schleiferi schleiferi* (11%) and *S. lugdunensis* (11%). *S. epidermidis* was the most common MR-CoNS at all three body sites.
The high prevalence of methicillin-resistance amongst pyoderma cases was concerning. Persistence of MRSP on the skin and carriage sites is common after resolution of MRSP pyoderma and may be a reason for the apparently rapid increase in population prevalence of MRSP. The dramatic post-treatment colonization rates are of significant concern and the source(s) of infection require study.

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NASAL CARRIAGE RATES OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCUS PSEUDINTERMEDIUS IN VETERINARY STAFF AND DOG OWNERS IN POLAND

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Introduction: Various studies have indicated that people working or living in contact with animals are categories at risk for carriage of methicillin-resistant S. aureus (MRSA) and methicillin-resistant S. pseudintermedius (MRSP). This is the first study investigating the occurrence of these bacteria in veterinary staff and dog owners in Poland.
Objective: The primaryobjective of this study was to determine the nasal carriage rates of MRSA and MRSP in veterinary practitioners and dog owners in Poland. The carriage rates of methicillin-susceptible strains belonging to these species were additionally investigated as a secondary objective.
Methods: Nasal swabs were collected from 144 volunteers recruited at the small animal hospital of Warsaw University of Life Sciences. The study population included 74 small animal veterinarians, 14 technical staff members, 41 practicing students and 11 dog owners. Swabs were enriched in Mueller Hinton Broth with 6.5% NaCl for 24 h and 10µl of the enrichment culture were streaked on Columbia agar supplemented with 5% sheep blood. Species identification was done by standard phenotypic testing followed by nuc PCR. MRSA and MRSP were identified by PCR detection of the methicillin resistance mecA gene. S. aureus isolates were further characterized by a lineage-specific PCR for identification of the livestock-associated MRSA CC398.
Results: A total of 41 (28.5%) individuals carried S. aureus and seven (4.9%) were carriers of S. pseudintermedius. No other coagulase-positive species were identified. The positive individuals carried a single coagulase-positive species with the only exception of one individual who harboured both S. aureus and S. pseudintermedius. MRSA and MRSP carriage was observed in five (3.5%) and two (1.4%) of the individuals tested. All MRSA and MRSP carriers were veterinary workers. None of the MRSA belonged to CC398.
Discussion and conclusion: The carriage rate of S. aureus was in line with the expected frequency among healthy individuals in the community in Poland. Although it was lower compared to S. aureus, the observed S. pseudintermedius carriage rate supports the notion that this species can be transmitted at low frequency from small animals to veterinary workers and students. MRSA and MRSP would be expected to occur at markedly lower frequencies in the nasal cavity of healthy humans. Our results corroborate similar findings by previous studies conducted in other countries and confirm that veterinary workers, including both veterinarians and technical staff, have a higher risk to carry MRSA or MRSP compared to other people.

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SWINE MRSA ISOLATES FORM ROBUST BIOFILMS

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization of livestock animals is common and prevalence rates for pigs have been reported to be as high as 49%. Measures to prevent, control, or eliminate MRSA in swine is of considerable public health concern. Bacterial colonization of both biological and non-biological surfaces followed by survival or persistence is often linked to the development of attached microbial communities known as biofilms. Community-associated methicillin-resistant *S. aureus* (CA-MRSA) USA300 strains have recently emerged, causing epidemic outbreaks in humans. These USA300 isolates have been shown to form robust biofilms. Therefore, one hypothesis to explain high prevalence of MRSA in swine herds is the ability of these organisms to exist as biofilms.
Methods: To investigate the ability of MRSA swine isolates to form biofilms, a microtiter crystal violet assay was used to quantify biofilm formation by several swine and human isolates, including USA300. The contribution of known biofilm matrix components, polysaccharides, proteins and extracellular DNA (eDNA), was tested in all strains as well.
Results: All MRSA swine isolates formed robust biofilms similarly to human clinical isolates, including USA300. The addition of Dispersin B had no inhibitory effect on swine MRSA isolates when added at the initiation of biofilm growth or after pre-established mature biofilms formed. In contrast, the addition of proteinase K inhibited biofilm formation in all strains when added at the initiation of biofilm growth and after pre-established mature biofilms formed. Addition of DNase I at the initiation of biofilm growth inhibited biofilm formation in all strains, albeit with varied degrees of reduction. DNAse I treatment of pre-established mature biofilms failed to disrupt biofilm biomass in all swine MRSA isolates.
Conclusion: Swine MRSA isolates form robust biofilms and the biofilm matrix produced by these isolates is significantly composed of proteins and not polysaccharides or polysaccharide intercellular adhesion (PIA). These findings parallel reports for other MRSA isolates, including USA300. Additionally, eDNA is a component of the biofilm matrix, however the contribution of eDNA remains unclear given that not all pre-established mature biofilms produced by swine MRSA isolates were disrupted after DNase I treatment. Collectively, these findings provide a critical first step in designing strategies to control or eliminate MRSA in swine herds.

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IDENTIFICATION OF LIVESTOCK-ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (LA-MRSA) IN COMMUNITY HOSPITALS IN SOUTHERN ONTARIO, CANADA

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Background: Reports of LA-MRSA in hospitalized individuals in Canada have been rare. The majority of human data on MRSA are obtained from tertiary-care facilities, located in metropolitan areas that may not be representative of community hospitals. To determine if LA-MRSA strains are present in community hospitals two studies were conducted in parallel. In study #1 the objective was to analyze, molecularly, MRSA patient isolates collected prospectively over a year. In study #2 the objectives were to determine the prevalence of MRSA in the hospital environment and compare MRSA strains between patients and the environment. Methods: Three community hospitals located in southern Ontario were enrolled. For study #1, patient specimens from MRSA infections

and colonization were collected prospectively for a year. Only 1 MRSA isolate was collected per patient. For study #2, surfaces located in the general environment and patient rooms in the medical and surgical wards were sampled once a week for four consecutive weeks using sterile electrostatic cloths. Patient MRSA isolates were also obtained from the hospital's diagnostic laboratories during the study period. For both studies, selective enrichment culture for MRSA was performed and isolates were typed using ApaI PFGE and spa typing. All LA-MRSA isolates were investigated for susceptibility to tiamulin. Results: To date, 344 patient MRSA isolates have been analyzed for study #1. Only one (0.3%) isolate, from a colonized patient, was identified as the ST398-associated spa type 539/t034. MRSA was isolated from 13.1% (114/870) of environmental surfaces. Thirteen (11.4%) of these isolates, from two hospitals, were spa type 539/t034. No patient (n=43) was identified with this spa type during the environmental sampling period. For all LA-MRSA isolates collected, three different PFGE patterns were identified. For tiamulin, one isolate had a MIC >32 ug/ml while the MIC for the remaining isolates was 1 ug/ml. Conclusions: LA-MRSA has not been previously identified in the hospital environment in North America and the relatively high prevalence of environmental contamination with LA-MRSA was surprising given that reports of human infection in Canada are uncommon and the rarity of this strain in humans in this study. These hospitals serve rural communities where pig farming is present, which may increase the likelihood of LA-MRSA exposure. The discordance between patient and environmental MRSA strains suggests unidentified reservoirs such as hospital staff, visitors, or unscreened patients. Despite environmental contamination, nosocomial transmission of LA-MRSA was not identified. The majority of LA-MRSA strains were susceptible to tiamulin, in contrast to a recent report of common resistance in pig ST398 from Canada. Further surveillance is required for a better understanding of the epidemiology and microbiology of LA-MRSA strains in hospitals.

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LONGITUDINAL STUDY OF VETERINARY STUDENTS FOR ACQUISITION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ASSOCIATED WITH EXPOSURE TO PORK PRODUCTION FACILITIES

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Introduction: Methicillin-resistant *S. aureus* (MRSA) has been recently documented in U.S. swine and swine workers. Some of the recovered isolates belong to the group of livestock-associated MRSAs typified by sequence type 398. Transmission studies have primarily focused on long-term exposure to livestock. This study focuses on short-term exposures that veterinary students encountered during visits to pork production facilities on their senior year. A similar level of exposure could be expected with veterinarians involved in swine practice and this study provides insight into the level of occupational risk for LA-MRSA that swine veterinarians may encounter. **Objectives:** 1) Determine the rate of MRSA acquisition and longevity of carriage in uncolonized students exposed to pork production facilities during the two week rotation, and 2) characterize the MRSA isolates from pork production facilities and assess their relatedness to human isolates. **Methods:** Veterinary students participating in clinical swine rotations at Iowa State University were voluntarily enrolled. Student nasal swabs were collected at start of the rotation, pre- and post- visits to pork production facilities, non-visit days, and after completion of swine rotation. Five pig nasal swabs and 5 environmental sponge samples were collected during visits to pork production facilities during rotation. Samples were processed with enrichment in broth (10g tryptone/L, 75g NaCl/L, 10g mannitol/L and 2.5g yeast extract/L and incubated at 35º C for 24 hrs) and streaked onto chromogenic media (BioRad MRSASelect) and incubated for 24-48 hrs at 35º C. Suspect colonies were

streaked for purity on Sheep's blood agar and further characterized with biochemical testing, oxacillin screen with disc diffusion, PBP 2a latex testing, *mec*A and PVL PCR, and spa typing. **Results:** Thirty (30) veterinary students were enrolled in study from May-November 2010. Forty (40) pork production facilities were visited during same period. MRSA was detected in 30% (12/40) pork facilities and appeared to significantly cluster within particular production flows. MRSA was detected in students 22% (6/27) of the time following exposure to MRSA positive pork facility. Students found to be MRSA positive initially following visit were negative for MRSA within 24 hours post visit. Spa types found in pork facilities (t002, t034, t548) closely matched those recovered from students (t002, t034, t548, t1107, t126) with few exceptions. **Conclusions:** Veterinary students' visits to pork production facilities closely resemble exposure that veterinarians would experience during normal practice situations. Based on *spa* typing, MRSA in contaminated pork production facilities can be recovered in students following visitation. However, the duration of carriage in students was very brief and most likely represents contamination of nasal passages rather than colonization.

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TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* SEQUENCE TYPE 398 ISOLATES WITH A NEW OPTICAL MAPPING TECHNIQUE

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Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) has posed a considerable health threat for decades. In 2003, a new livestock-associated MRSA (LA-MRSA) was identified in the Netherlands and has emerged in many other countries. Typing of LA-MRSA isolates is notoriously difficult. Multi-locus sequence typing (MLST) provides hardly any resolution, nor does staphylococcal protein A (spa) typing and multiple-locus variable-number of tandem repeat analysis (MLVA) which both yield 2 predominant types. This makes it hard to further differentiate CC398 isolates, especially in transmission events. Pulsed-field gel electrophoresis (PFGE) can now also be performed using a neoschizomer of SmaI, namely Cfr9I. Recently, a high resolution microbial whole genome analysis named optical mapping was introduced. In this study we assessed the capability of optical mapping to better differentiate these LA-MRSA isolates.
Methods: A total of 69 CC398 isolates were typed by optical mapping and PFGE. This collection consisted of multiple isolates obtained from 17 veterinarians and their household members. Prior to this experiment, 6 CC398 isolates were analyzed by optical mapping. These 6 isolates consisted of 3 pairs. Pair 1 comprised isolates from a mother and her child that most likely was infected by the mother. Pair 2 consisted of isolates from a pig and a pig holder and pair 3 was made up of isolates from a pig holder and one of his family members. All pairs have been typed with spa-typing and PFGE using Cfr9I. The cells of the staphylococcal cultures were embedded in low melting point agarose to obtain high molecular weight DNA. After lysis and proteinase K treatment, the plugs were melted and the agarose was digested using ß-agarase to release the DNA. Using the OpGen Argus™ system, the DNA molecules were stretched and digested by the restriction enzyme XbaI in a micro fluids cell. Subsequently, the resulting fragments were sized and assembled to a genomic restriction map by the system.
Results: Both isolates of pair 1 were carrying spa-type t108. All isolates of pair 2 and 3 had spa-type t011. PFGE performed with Cfr9I distinguished the 3 pairs with a 100% similarity between the isolates of a pair. Pairs 1 and 2 showed a high similarity with each other and pair 3 was clearly distinct. Optical mapping of these pairs corroborated the PFGE results and grouped the pairs with a maximum of 0.2% difference between the members of a pair. Pair 1

and 2 were nearly identical and only displayed a 0.4% difference. The difference between pair 3 and pairs and 1 and 2 was 3.7%.
Conclusion: Until now PFGE using Cfr9I was the best method to differentiate LA-MRSA isolates. This new optical mapping technique proved to be an even better and more robust technique for differentiating LA-MRSA. Furthermore, additional information such as SCCmec type, presence of pathogenicity islands and phages can be easily obtained using optical mapping.

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EVALUATION OF DIRECT REPEAT UNIT (*dru*) TYPING FOR CHARACTERIZATION OF METHICILLIN-RESISTANT *Staphylococcus pseudintemedius* (MRSP)

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Background: MRSP has emerged in a remarkable manner as an important problem in dogs and cats. The pathogen can be found internationally at increasingly high numbers, and limited molecular epidemiological information is available. An important aspect of molecular epidemiology is the availability of a reproducible, objective, standardized, efficient, and cost-effective typing tool. For MRSP isolates MLST, PFGE, *spa* and SCC*mec* typing have been used, each with advantages and limitations. Only recently, we successfully applied *dru* typing, which uses the direct repeat unit (*dru*) adjacent to IS*431* in the SCC*mec* cassette, in an international collection of well-characterized MRSP isolates. In the presented study we used *dru* typing for a collection of MRSP isolates from North America.
Methods: This study was performed with 115 independent MRSP isolates, 52 from the U.S.A. and 63 from Canada, all collected from dogs and cats. For all isolates the *dru* region was amplified by PCR, sequenced and analyzed. In addition, 28 isolates were also subjected to MLST and *spa* typing. The discriminatory index of *dru* typing was calculated.
Results: All isolates could be successfully *dru* typed. The *dru* types dt9a and dt11a were most common, they were detected in 42 (36.5 %) or 37 (32.2 %) isolates, respectively. The *dru* type dt10h was identified in 15 (13.0%) isolates, dt11af in nine (7.8%) isolates, dt10a and dt6r in two (1.7%) each and dt8f, dt11b as well as dt11o in single MRSP isolates. The remaining 5 isolates had individual novel *dru* types. This corresponded to a discriminatory index of D = 0.745 which means that two randomly selected isolates from this test population could be assigned to different *dru* types with a probability of 74.5%. Among the 28 isolates that were further typed, five *dru* types, five MLST types and four *spa* types were identified in different combinations. The combinations ST68-t06-dt11a (n = 9), ST71-t06-dt9a (n = 8) and ST71-t02-dt9a (n = 5) were seen most frequently. A comparison of *dru* and *spa* typing showed that *spa* type t06 was present in isolates with *dru* types dt9a (n = 9), dt10h (n = 1) and dt11a (n = 12), whereas *spa* type t02 was seen in isolates with the *dru* type dt9a (n = 8) and dt8f (n=1).
Conclusion: The data of this study confirmed that there is a good agreement between *dru* typing results and the results of other characteristics of MRSP isolates. Occasionally, *dru* typing was even able to further discriminate between isolates that shared the same *spa* type. Moreover, the results of this study underline that *dru* typing is a useful tool for MRSP typing, being an objective, standardized, sequence-based method that is relatively cost-effective and easy to perform.

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EVALUATION OF AN ACTIVE SURVEILLANCE SYSTEM FOR METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) COLONIZATION IN HORSES AT A VETERINARY TEACHING HOSPITAL

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Introduction: MRSA is an important problem in hospitalized horses and a zoonotic risk to veterinary personnel. Active surveillance for colonized horses is used by some facilities to allow for identification of carriers and use of enhanced infection control practices. This study evaluated an active surveillance system in-place at a veterinary teaching hospital.
Methods: MRSA screening of nasal swabs is performed in horses admitted to the Ontario Veterinary College (OVC) on admission, every 7 days in hospital, and on discharge. Data from June 1, 2009 to April 30, 2011 were analysed. Cases were classified as community-associated (CA), community onset-hospital associated (CO-HA), hospital-associated (HA) or indeterminate (IN) using standard definitions.
Results: During the study period 2234 horses were admitted. MRSA was isolated from 80 samples during 50 visits by 40 horses (first positive visits: CA-19; CO-HA-3; HA-17; IN-1; range 0-7 per month). Overall, 2.9% of samples from 3.0% of horses were positive, with monthly sample and horse ranges of 0-9.8% and 0-8.6%, respectively). The prevalence at admission (CA and CO-HA combined) was 1.7% (monthly range 0-7.1%). No seasonal variation was identified. Three horses had MRSA infections at admission [eye (CA), leg wound (CA), umbilicus (CO-HA)] and all were colonized. No HA clinical MRSA infections were identified. Spa typing was available for 20 of the isolates from different horses; 18 (90%) were Canadian epidemic MRSA-5, an ST8 clone that predominates in horses in Canada.There was a mean lag of 5.2 d from sample collection to reporting of results (range 2-9 d). Results for first-time positive animals were available for 7 (18%) prior to discharge. However 11 (28%) colonized horses were later re-admitted, 7 still positive. Within 6 months of the first positive result, 5 owners of MRSA-positive horses brought additional horses to the OVC (range 1-4). Three horses from one of these owners were also colonized.
Discussion: The prevalence of MRSA colonization varied throughout the study period with temporal variations, however the 1.7% admission prevalence is similar to previous reports from this facility. The time delay associated with sample processing hampers the effectiveness of screening as most horses have been discharged by the time results are known. However, using screening results to identify subsequent high risk cases (e.g. previously positive, from a farm with a colonized horse) can allow for use of enhanced infection control practices in those cases while awaiting test results. Ultimately, rapid testing is required to optimize MRSA screening in horses.

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THE CHALLEGES OF ZOONOTIC INFECTION FROM MRSA: ENVIRONMENTAL SURFACE INFLUENCES & CONTROL STRATEGIES

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Infectious diseases are transferred by classic physical and biological means and impact the morbidity and mortality of humans and animals. Such transfers are both static and dynamic processes and involve source diseased host or sites, vectors, and susceptible host. The cycle of wound exudation, nasal discharge, fecal matter, urine, or other fomites that transfer to surfaces and then to susceptible hosts at dose levels that manifest disease is well understood and a clear risk factor for disease transfer. This scenario sets up as a classic example of the epidemiology of disease transmission. Environmental surfaces are unquestionably part of the cycle and demand use of traditional infection control practices. No place is this risk and demand for control practices more evident than in the transfer of MRSA from animals to humans, zoonosis,

and vice versa, humanosis. MRSA is the "poster child" of drug resistant microbes that have left the healthcare environment and moved into the community. Transfer in transit vehicles, at wrestling matches, on the football field, the locker rooms, and now clearly from pets, performance animals, and domestic food source animals have all been shown and often involve environmental surfaces. The literature is now replete with reports of MRSA strains that have arisen from the animal biome and transferred to humans. Successful strategies to control environmental surfaces as vector transfer sites thus reducing microbial dose and the available transfer sites has been done and demonstrates strategies that can have utility in the control of zoonotic MRSA. No complete protection from zoonotic transfer of MRSA can be accomplished without dealing with the environmental surfaces of the animal-human interface. This is part of a prevention strategy wherein prevention is the single most important thing we can do to reduce the risks associated with drug resistant pathogens. This paper reports on anti-MRSA active technologies that have been successfully used in healthcare and commercial buildings environments. Trials in equine facilities have also shown these strategies to be effective at reducing the dose of environmentally sourced bacteria and fungi reducing their dose and using the environmental surfaces as active antimicrobial sites. The paper provides the attendee insights into the epidemiological cycle of zoonotic MRSA and defensive strategies and tools to deal with any potential environmental sources.

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GENETIC DIVERSITY OF *Staphylococcus pseudintermedius* ISOLATED FROM HEALTY DOGS IN COPENHAGEN, DENMARK

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Introduction: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has recently emerged as an important animal health problem associated with the rapid spread of specific clones (ST71 in Europe and ST68 in the USA) displaying resistance to virtually all antimicrobial agents available in small animal practice. However, little information is available on the diversity of the species, and even less about the frequency of ST71 and ST68 in the methicillin-susceptible population.
Objectives: This study was undertaken to elucidate the diversity of *S. pseudintermedius* isolated from healthy dogs in the area of Copenhagen. As secondary objectives, we investigated the genetic relatedness of isolates from different body sites of the same dog and the occurrence of ST71 and ST68 among methicillin-susceptible isolates.
Methods: A selection of 58 methicillin-susceptible *S. pseudintermedius* (MSSP) isolates previously isolated from the nasal and mouth cavities, groin and perineum of 18 dogs colonized at three or four body sites (Paul et al. 2011) were typed by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST). SmaI-PFGE was done according to a modified version of the protocol described by Murchan *et al*. (2003). Ten strains, including five from the predominant PFGE cluster and five from the other major clusters, were analysed by the current MLST scheme (Bannoher et al. 2007).
Results: Using a 80% similarity cutoff, PFGE analysis revealed the presence of 10 clusters among the 58 isolates tested. One major group (cluster A) included 23 isolates from 11 dogs. Six dogs were carriers of identical or genetically related strains at different body sites. The remains 12 dogs carried at least two genetically unrelated strains. MLST analysis of 10 isolates resulted in 10 distinct sequence types. Differences in one or more loci were observed even among the five isolates belonging to cluster A. None of the allelic profiles corresponded to ST71 or others lineages previously associated with methicillin resistance.
Discussion/Conclusion: The majority of healthy dogs carried multiple strains displaying unrelated PFGE types. This is in contrast with

the common patterns of *S. aureus* colonization in human carriers, which are normally colonized with a single strain. PFGE and MLST data indicate that the *S. pseudintemedius* population is extremely heterogeneous within individual carriers as well as in the local dog population. The MRSP lineage ST71, which is responsible for the vast majority of MRSP infections in Europe and in Denmark, was not detected. If confirmed by similar studies in other countries, this finding would suggest that the rapid spread of MRSP ST71 is not attributable to the acquisition of *mecA* by a prevalent MSSP lineage in the dog population.

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NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* IN DAIRY AND BEEF COWS

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Background & aims Methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 has been isolated from a variety of farm-associated animals. Recent studies have also shown the potential of MRSA ST398 to cause mastitis in dairy cattle and found it to be ubiquitously present in Belgian veal calf farms. In this study, we investigated the nasal carriage of *S. aureus* in dairy and beef cows originating from single-bred farms.
Material & methods On 10 Belgian farms exclusively breeding dairy cattle and 10 farms exclusively breeding beef cattle, nasal swabs were collected from 10 randomly chosen cows with a minimum age of two years. Suspected *S. aureus* isolates were investigated with a 16S rRNA-*mecA*-*nuc* triplex PCR, allowing for distinguishing MRSA and methicillin-susceptible *S. aureus* (MSSA). All isolates were characterised by *spa*-typing.
Results On dairy farms, no MRSA was detected. Twenty-six MSSA isolates were cultured from 25 cows (25%) originating from eight farms, with an in-herd prevalence of 10-70%. *spa*-typing of 24 MSSA isolates showed them to belong to five different *spa*-types: t529 (n = 13), t1403 (n = 8) and single isolates of t190, t937 and t1247. On beef farms, three MRSA isolates were detected on two farms (20%), from respectively one (10%) and two (20%) cows. These were shown to be *spa*-type t011, associated with ST398. Also 19 MSSA were detected, from 17 beef cows (17%) on eight farms, with an in-herd prevalence of 10-50%. *spa*-typing has not yet been performed for 4 strains; the other were t267 (n = 4), t1403 (n = 3), t164 (n = 3), t2802 (n = 3) and single isolates of t529 and t1736.
Conclusions While MRSA ST398 has previously been shown to be capable of causing mastitis in dairy cows, no nasal carriage of MRSA ST398 by dairy cattle could be illustrated in this study. A variety of other *S. aureus* strains were detected, some of which have previously been associated with mastitis. The finding of MRSA ST398 in beef cows was unexpected, especially seen the fact that the investigated farms were single bred farms.
This study was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment (contract RF-6189).

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SURVEILLANCE FOR METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN DIAGNOSTIC SAMPLES SUBMITTED TO A VETERINARY DIAGNOSTIC LABORATORY IN THE MIDWEST UNITED STATES

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Background: Reports of livestock associated methicillin-resistant *Staphylococcus aureus*

(LA-MRSA) have received considerable attention. Strains of LA-MRSA have been found in cattle, chickens, and swine. This has led to concern about possible transmission of LA-MRSA in foods of animal origin. A veterinary diagnostic laboratory has access to a variety of animal specimens and serves as a good collection point to conduct surveillance for LA-MRSA. **Objectives:** The objective of this study was to investigate bovine milk samples, poultry environmental samples, and porcine oral fluids for the presence of MRSA. **Methods:** *Staphylococcus* isolates were recovered from mastitic milk samples, poultry environmental drag swabs, and porcine oral fluids submitted to the Iowa State University Veterinary Diagnostic Laboratory from June 2009 to May 2011. Bacterial identification was accomplished by evaluation of colony morphology and hemolytic pattern (single zone, wide zone Beta, or double zone) and biochemical tests (coagulase, maltose, lactose, trehalose, Voges-Proskauer). Methicillin-resistance was determined by screening isolates for sensitivity to oxacillin and activity of *mecA* with PBP 2a latex agglutination test. **Results:** Three hundred and fifteen (315) staphylococcal isolates were identified from the bovine milk samples. Of these, 96 were found to be resistant to oxacillin but only 7 were positive by PBP 2a test. Two hundred and fifty one (251) porcine oral fluids were tested. Of these, 19 were found to be oxacillin resistant and positive by PBP 2a test. Three hundred and sixty three (363) poultry swabs were tested. Of these, 4 were found to be resistant to oxacillin, but no samples were positive by PBP 2a test. Therefore the MRSA positive percentage for milk staphylococcal isolates was 2.2%, porcine oral fluids was 7.6%, and poultry environmental swabs was 0%. **Discussion:** These findings suggest that MRSA is found more commonly in porcine environments versus poultry environments or bovine milk. Further work includes determining the *spa* types found in the various samples, investigation of the antimicrobial susceptibility profiles of the MRSA isolates and evaluation of oral fluids as a useful matrix for surveillance of swine facilities for MRSA.

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MRSA IN IOWA

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Livestock farmers are an emerging group at risk of colonization with methicillin-resistant *Staphylococcus aureus* (MRSA). In a previous study conducted by our group, almost half of swine workers and pigs were found to be colonized with MRSA on farms in Iowa and Illinois. Livestock-associated MRSA has been documented in an increasing number of countries across Europe, Asia, and North America, although most studies have examined a relatively small number of farm workers in cross-sectional studies and have frequently been conducted on the farm site. A large-scale, longitudinal study of swine workers examining carriage of and infection with antibiotic-resistant *S. aureus* has not been conducted in the United States, but is of vital importance, as the epidemiology of MRSA is rapidly changing.

We have launched a prospective study of rural Iowans, examining 1) individuals enrolled in the Agricultural Health Study, focusing on those who raise swine and 2) a matched population-based group lacking exposure to livestock. In both of these groups, we will examine both colonization and infection with *S. aureus*. We hypothesize that individuals working in close proximity to livestock and poultry will be at risk of occupational exposure to MRSA, and that farmers in contact with livestock (and swine in particular) will be more likely to be colonized with "swine-associated" *S. aureus* strains than individuals lacking such exposures. We hypothesize that our population-based rural cohort and our cohort of farmers lacking animal exposure will have a lower prevalence of MRSA colonization than our cohort of swine farmers, and that they will be less likely to be colonized with "swine-associated" strains.

To date, we have enrolled 359 adults and 17 children in 10 counties throughout the state. 25.35% (91/359) of adults and 11.76% (2/17)

were positive for *S. aureus*. 77 *S. aureus* isolates have been tested for antibiotic susceptibility; 14.29% (11/77) were resistant to methicillin. 42.86% (33/77) were resistant to erythromycin, 24.68% (19/77) isolates were resistant to tetracycline, 15.58% (12/77) were resistant to clindamycin, and 11.69% (9/77) were resistant to levofloxacin. Molecular testing and data analysis are pending.

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POST-HOSPITAL DISCHARGE PROCEDURE SPECIFIC SURGICAL SITE INFECTION (SSI) SURVEILLANCE IN SMALL ANIMAL PATIENTS.

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Surgical site infections (SSIs) are an inherent risk of any surgical procedure and their implications have been increasing concurrent with the international epidemic of methicillin-resistant (MR) staphylococcal infections. Little is known about SSI rates and risk factors in companion animals, and available information is of variable quality. Long-term, prospective, active post-hospital discharge SSI surveillance programs in veterinary medicine are lacking. The objectives of this study are 1) to describe the incidence of SSI in all animals undergoing surgical procedures at the Ontario Veterinary College Health Sciences Centre (OVCHSC) over a 1-yr period, 2) to describe and compare procedure-specific SSI rates and 3) to identify risk factors for development of SSI

All dogs and cats undergoing surgical procedures at the OVSHSC were enrolled beginning Sept 1, 2010. According to standard definitions by the Centers for Disease Control and Prevention (CDC), active follow-up through telephone conversations with owners was performed 30 days after surgery, with 1 yr follow-up scheduled for cases with surgical implants. A standardized questionnaire was administered to detect and characterize SSIs.

30d follow-up has been completed on 400 patients, 381 dogs and 19 cats. SSIs were identified in 4 (1%) animals, all dogs. Of these 3 (75%) were categorized as superficial SSIs and 1 (25%) was categorized as a deep SSI. SSIs were identified in 2/187 (1.1%) orthopedic, 2/119 (1.7%) soft tissue (excluding oncology) 0/30 gastrointestinal and 0/64 tumour removal/resection procedures. Among orthopedic procedures, the SSI rate for tibial plateau leveling osteotomy (TPLO), a procedure anecdotally associated with a high rate of MRSP infections in many facilities, was 1/35 (2.9%). Of the confirmed SSI cases, only 2 (50%) were documented in the medical records of the patient, illustrating a potential underestimation of SSI rates from medical record-based studies. Cultures were submitted for 2 cases and both were methicillin-resistant staphylococci; 1 MRSP (TPLO) and 1 MRSA (lateral collateral ligament repair of the elbow).

This study has identified a low but not inconsequential SSI rate in a tertiary care veterinary hospital. Proper baseline data are important to monitor changes in rates, to identify risk factors and to develop and test interventions to reduce the occurrence of SSIs. While the overall rate was low, MR-staph accounted for 50% of identified SSIs and 100% of SSIs that were investigated through culture. The variable and sometimes high rates of MR-staphylococcal (especially MRSP) SSI in facilities internationally, the morbidity and mortality associated with those infections and concerns about antimicrobial use in animals indicate that ongoing active surveillance should be considered part of an SSI (and MRSP) control program.

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BIOFILM FORMATION BY METHICILLIN RESISTANT AND METHICILLIN SUSCEPTIBLE STAPHYLOCOCCUS PSEUDINTERMEDIUS

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Biofilms are complex communities of microorganisms embedded in an extracellular matrix of hydrated extrapolymeric substances (primarily

carbohydrates) and attached to biological or non-biological surfaces. Biofilm-associated bacteria behave differently than their planktonic counterparts, with different growth rates and resistance to antimicrobials, thereby creating difficulties for elimination of infections. Biofilm production can be an important virulence factor, particularly in device-associated and chronic infections. This is of particular concern in companion animals with surgical implant associated infections and infections of invasive devices (e.g. intravenous or urinary catheters). Despite the potential importance of biofilm formation in MRSP infections, there has been limited investigation.

30 epidemiologically unrelated MRSP and 30 MSSP recovered from dogs were included. Two assays were used, a quantitative microtitre plate assay that is considered the Gold Standard for in vitro biofilm production, and evaluation of colony appearance on Congo Red agar (CRA), a test that assesses slime production.

23/30 (77%) of MRSP isolates were classified as slime-producers based on colour change on CRA, as were 24/30 (80%) MSSP (P=1.0). With the microtitre plate assay, 25/29 (86%) tested MRSP isolates were classified as biofilm producers compared to 17/30 (57%) MSSP (P=0.02). 20 MRSP and 12 MSSP isolates were positive by both methods (P=0.037). No MRSP and 3 (15%) MSSP were negative by both methods (P=0.23). Agreement between the two assays was poor (Kappa -0.06, SE 0.12). Most of the disagreement was due to positive CRA results (particularly with MSSP) in isolates that were negative on the microtitre assay.

Biofilm production was common in vitro by the studied *S. pseudintermedius* isolates, particularly MRSP. The significant difference in biofilm formation with the microtitre assay suggests that biofilm formation may be an important virulence factor for MRSP. The ability to produce biofilms could be an important virulence factor that has allowed a limited number of MRSP clones to spread widely and rapidly, playing a role in the recent and dramatic emergence of this pathogen. Poor agreement between microtitre and CRA results has been reported for other organisms, and positive results on CRA indicate slime-production, not necessarily biofilm formation. Further study of biofilm production of MRSP and the role of biofilm in the pathogenesis of MRSP infection is required. In vitro study should use the microtitre assay, not CRA, because of the poor agreement between these two tests. Further phenotypic and genotypic study of biofilm production by *S. pseudintermedius*, particularly MRSP, is required.

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EVALUATION OF *mec*A AND PVL GENES IN SAMPLES OF BULK TANK MILK FROM DAIRY FARMS IN SÃO PAULO STATE, BRAZIL

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The first isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from animals occurred in milk from mastitic cows in 1972. Since then, MRSA has been reported in many diverse species, and its role as a humanosis or zoonosis has been evaluated. It has been found that MRSA strains can contain genes that encode the panton valentine leukocidin toxin (PVL) and this toxin has been shown to be responsible for severe clinical symptoms in infections with MRSA. The aim of this work was to evaluate MRSA and PVL toxin occurrence in strains isolated from bulk tank milk obtained from dairy farms in São Paulo state, Brazil. Milk samples from 71 farms were collected to select those that were positive for *Staphylococcus aureus* by microbiological cultivation. New milk samples from the 29 selected dairy farms were collected and cultivated in Baird-Parker agar for *Staphylococcus aureus* isolation and identification by coagulase, morphological characteristics and biochemical tests: fermentation of trehalose, maltose and mannitol. Two

or three strains of *Staphylococcus aureus* from each farm were selected considering differences in their morphology, totaling 63 samples, which were analyzed. The DNA of 63 *Staphylococcus aureus* was submitted for PCR amplification using primers for detection of the *mecA* gene and PVL toxin genes. Positive and negative controls were used to validate genotyping tests. PCR amplification with all 63 *Staphylococcus aureus* was carried out with species-specific primers as extraction and identification control. All isolates were negative for PVL toxin gene and 4 (6.4%) strains were positive for the *mecA* gene, with two strains from the same farm. Forty-three samples were tested for cefoxitin and oxacillin sensitivity by disk diffusion and 42 (97.7%) strains were sensitive to both antimicrobials, including two strains positive for the *mecA* gene. One sample that was *mecA* gene negative was oxacillin-sensitive, cefoxitin-resistant and positive for beta-lactamase by nitrocefin test. These results show that in this study three (10.3%) farms presented MRSA, and PVL genes were found in none of the farms. These farms, therefore, can play a role in spreading MRSA strains to humans, consequently further studies are needed considering the importance of milk in human nutrition, as many times it is consumed raw or used in producing dairy products.
Financial support: Postdoctoral fellowship - FAPESP n. 2010/17222-8

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MOLECULAR CHARACTERIZATION OF METHICILLIN RESISTANCE IN STAPHYLOCOCCI ISOLATED FROM ANIMALS AT THREE TEACHING HOSPITALS

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Infections due to methicillin-resistant Staphylococcus aureus (MRSA) and other staphylococcal species are increasingly being recognized as important and serious causes of disease in animals and humans. An important question and goal of this study is whether the staphylococcal cassette chromosome (SCC) and mec gene responsible for methicillin resistance in humans is present in animal isolates, and if so, the type. Methicillin-resistant Staphylococcus species were provided by 3 veterinary hospitals and consisted of MRSA isolates from 9 canines, 4 equines, 5 porcines, 1 feline, and 1 primate, S. epidermis from 3 canines, S. haemolyticus from 2 canines, S. intermedius group from 22 canines, S. schleiferi from 6 canines, 2 unidentified Staphylococcus species from a dog and a goat, and 6 staphylococcal species from unknown companion animals. Isolates were screened with BBL CHROMagar MRSAII (BD, Franklin Lakes, NJ), and the presence of the gene encoding for penicillin binding protein 2a (mec) was determined by real-time polymerase chain reaction (qPCR). Isolates were typed for SCCmec gene classification by multiplex PCR. OF 20 MRSA isolates thus far tested 18 had typical mauve colonies on BBL CHROMagar MRSAII agar, while one isolate had white colonies and the other isolate blue colonies. Colonies of methicillin-resistant staphylococcal species that were not S. aureus ranged in color from white, to pale pink, to dark purple. qPCR detected the presence of mec in all of 22 methicillin-resistant isolates thus far tested, including 11 isolates of MRSA, 2 isolates of S. haemolyticus, 6 isolates of S. pseudintermedius, and 3 isolates of S. schleiferi. Twenty-five methicillin-resistant isolates have thus far been typed by multiplex PCR. Of 8 MRSA isolates tested 3 were SCCmec type II, 1 was SCCmec type IVa, and 4 did not fall into one of the 5 SCCmec types. Of 12 S. intermedius group isolates tested 3 were SCCmec III, 2 were SCCmec V, and 7 were not categorized into one of the 5 SCCmec types. Of 4 S. epidermidis strains tested, 2 were SCCmec type II, 1 was SCCmec type III, and 1 was SCCmec type IVd. Therefore, preliminary testing indicated that methicillin-resistant

staphylococci from animals may contain SCCmec genes typical of human hospital-acquired strains, community-acquired strains, or a non-SCCmec mechanism of resistance.

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PREVALENCE AND ANTI-MRSA DRUGS SUSCEPTIBILITY OF OXACILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RAW MEAT AND BUTCHERS IN EASTERN NIGERIA

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BACKGROUND
During the past two decades, Methicillin-resistant *Staphylococcus aureus* (MRSA) has gained global attention as human pathogens. MRSA has been defined as *Staph aureus* testing Oxacillin resistant, or positive for molecular testing for mecA and PBP2a. Recently, there have been increasing reports of MRSA in animals, and possible transfer to humans. This raises the need for constant MRSA surveillance on animals and animal products, as well as their drugs susceptibility pattern. Information in this regard is lacking in Anambra State, Eastern Nigeria - hence the need for this study.
MATERIALS AND METHODS
Nnewi is the second most important commercial city of Anambra State of Nigeria, with a busy meat market. A total of 32 raw meat (23 cow and 9 goat) samples, displayed for sale, were collected from randomly selected butchers' meat stands in the main market; nasal samples were collected from fifteen of the butchers, and two water samples from containers for meat dressing were also collected. The samples were screened for Oxacillin resistant *Staph. aureus*. Isolation media used included Oxacillin resistance screening agar (Oxoid, England) and 6.5% NaCl broth for enrichment. Isolates were confirmed to be *Staph aureus*, using Staphaurex test kit (Remel, UK). Minimum Inhibitory Concentration (MIC) of Oxacillin, Linezolid, and Vancomycin was determined for the isolates, using MIC evaluator strips (Oxoid, England); Teicoplanin paper discs (Oxoid, England) were also used. Susceptibility test procedures and interpretative criteria were by CLSI standards.
RESULTS
Of the 32 meat samples, 26(81%) yielded Oxacillin resistant *Staph.* aureus. The isolation rate of Oxacillin resistant *Staph. aureus* from the butchers was 27%; one of two water containers yielded the organism. Of 31 isolates tested, 28 (90%) were Vancomycin susceptible and 3 were Vancomycin intermediate (VISA), No Linezolid and Teicoplanin resistance was encountered. Nine (29%) of the Oxacillin resistant isolates showed very high Oxacillin MICs ($\geq$128 µg/ml). Oxacillin MIC values did not affect the MIC values of other drugs.
DISCUSSION/CONCLUSION
The results showed that raw meat sold in this market constitute a significant public health hazard, and alerts of possibility of similar hazards in other parts of the world. Indiscriminate use of antimicrobial drugs should be avoided in animals and humans alike, as this encourages spread of resistance genes. That the isolates were susceptible to the mainstay therapies of MRSA seems to be encouraging, although the presence of some "VISA" could be of concern. Based on this report, it is recommended that regular microbiological inspections of raw meat be carried out by approved Government agencies, so as to prevent possible hazards to the human community.

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SCC*MEC* TYPE VI METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN RETAIL MEAT

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Staphylococcal cassette chromosome (SCC) *mec* type VI methicillin resistant *Staphylococcus aureus* (MRSA) have been associated with community and healthcare settings, however

they have not been reported in the United States. We conducted a survey of retail beef, pork and poultry meats and found that 1-5% of 1040 samples contained MRSA. Further characterization of the MRSA isolates (n = 32) revealed that SCC*mec* type VI (44%, 14/32) predominated, followed by type IIIB (22%, 7/32), type II (1%, 3/32) and 22% (7/32) were non-typeable. Also, 86% (12/14) of the type VI isolates were positive for Panton-Valentine leukocidin (PVL). Clonal analysis has revealed that most of the type VI isolates (71%, 10/14) are USA 300, which is commonly associated with community-acquired (CA) MRSA. Most of these USA 300 isolates were from ground turkey (60%, 6/10) and pork chops (30%, 3/10). Furthermore, two of the type VI isolates appear to be USA 800, a pediatric clone, and both were obtained from pork chops. One type VI isolate did not match any USA strain type and was detected in pork chops, whereas another isolate was non-typeable (NT) and detected in ground beef. Our results suggest the SCC*mec* type VI isolates detected are of human origin. However, further genotyping is needed to better assess the original source of these isolates. To our knowledge, this is the first report of SCC*mec* type VI in the United States.

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METHICILLIN-RESISTANT COAGULASE POSITIVE STAPHYLOCOCCUS SPECIES COLONIZATION OF PET DOGS AND CATS

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Methicillin-resistant *Staphylococcus* (MRS) infection and colonization are emerging issues in veterinary medicine. Anecdotal information indicates that the incidence of pet animal MRS infections has markedly increased, as has the prevalence of methicillin-resistance amongst staphylococci from dogs and cats. However, the prevalence of colonization of pet animals with MRS organisms has only received sporadic study. The purpose of the study presented here was to determine the proportion of dogs and cats presented to Purdue University Veterinary Teaching Hospital (PUVTH) colonized with coagulase-positive MRS (*S. aureus* or MRSA, *S. pseudintermedius* or MRSP, *S. schleiferi* subsp. *coagulans* or MRSS), and to identify risk factors for colonization. Hypotheses tested included 1) Prevalence of colonization of pet animals with MRS exceeds 5%; 2) Sampling nares only is sufficient to detect MRS carriage in dogs & cats, 3) Pets recently treated with antibiotics; frequently boarding or attending dog parks; or pets with exposure to people working in veterinary or human healthcare are more likely to be colonized with MRS than pets without those exposures.
All dogs and cats presented to PUVTH between November 9, 2009 and December 9, 2010 were eligible for enrollment. Owners provided informed consent to swab nares and rectum of pets for culture, and completed a questionnaire. Swabs were collected within 24 hours of presentation to PUVTH, and held at 4 degrees C until cultured. Enrichment broth culture was performed followed by inoculation onto MRSA Chromogenic agar and mannitol salt agar with oxacillin. Colonies were tested for PBP2a via latex agglutination test, and *Staphylococcus* species was verified by polymerase chain reaction testing. Data were analyzed by logistic regression, with significance level at $P<0.05$.
100 (15.5%) of 643 animals were colonized with MRS; including 87 (16.6%) of 523 dogs and 13 (10.8%) of 120 cats.
Table 1. MRS Colonization of dogs and cats
Dogs
Nasal MRSA 22; rectal MRSA 12
Nasal MRSP 22; rectal MRSP 34
Nasal MRSS 7; rectal MRSS 3
Cats
Nasal MRSA 5; rectal MRSA 2
Nasal MRSP 3; rectal MRSP 3
Nasal MRSS 0; rectal MRSS 1
Table 2 MRS colonization of dogs by site
Nasal MRSA 15; rectal MRSA 6; nasal + rectal MRSA 7
Nasal MRSP 17; rectal MRSP 28; nasal + rectal

MRSP 5
Nasal MRSS 6; rectal MRSS 2; nasal + rectal MRSS 1
Purebred dogs were more likely than mixed breed dogs to be colonized with MRS (P=0.044). Exposure to healthcare workers, boarding facilities, dog parks, or antibiotic treatment within 30 days of sampling were not significant risk factors for MRS colonization (P>0.05 for each). The proportion of animals colonized with MRS (15.5%) was higher than reported previously. Colonization of pet dogs with MRSP (45/543; 8.6%) was more common than colonization with MRSA (27/523; 5.2%) or MRSS (8/523; 1.5%). Sampling only one site (nares or rectum) is not sufficient for optimal detection of MRS colonization in dogs.

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ISOLATION AND ANTIMICROBIAL RESISTANCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS FROM OKLAHOMA RETAIL POULTRY MEATS

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Staphylococcus aureus is one of the main causative agents of life threatening infections both in humans and animals. Recent studies in both Europe and the United States have revealed a high prevalence of *Staphylococcus aureus* including MRSA strains from retail meats particularly pork. Despite these studies, very few surveys are available that investigates the prevalence of *Staphylococcus aureus* in retail meats such as poultry. In the present study, a total of 167 retail poultry meat samples (114 chicken and 53 turkey) were purchased from several grocery stores in Tulsa, Oklahoma. *Staphylococcus aureus* strains were isolated from the retail meats by enrichment and plating on Baird-Parker Agar medium. six prospective colonies were kept for each sample and subjected to PCR confirmation. The prevalence of *Staphylococcus aureus* was 83% (95/114) in the chicken samples, and 85% (45/53) in the turkey samples. Three chicken samples and one turkey sample was positive for MRSA using *mecA* PCR. Antimicrobial susceptibility to 16 different antibiotics was tested for 140 *Staphylococcus aureus* and 24 MRSA isolates. In the chicken and turkey isolates, resistances were respectively common to ampicillin (91%, 100%), penicillin (62%, 97%), azithromycin (50%, 65%), ampicillin, Tetracyclin (48%, 99%), erythromycin (47%, 65%), Kanamycin (39%, 35%), Doxycyclin (38%, 92%), oxacillin with 2% NaCl (35%, 85%), cefoxitin with 2% NaCl (33%, 56%), clindamycin (30%, 8%), ciprofloxacin (24%, 48%), vancomycin (21%, 49%), Gentamycin (18%, 39%), refampin (9%, 24%), and chloremphenicol (3%, 8%). In conclusion, *Staphylococcus aureus* is highly prevalent in poultry retail meats sold in Oklahoma with 2.5% being MRSA. Multidrug resistance is not only prevalent in the MRSA isolates but also in many *Staphylococcus aureus* poultry strains with a relatively higher incidence in those isolated from turkey than chicken meats.

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RARE OCCURENCE OF MRSA ST130 ,XI, CONTAINING A *MEC* HOMOLOGUE IN INFECTIONS IN HUMANS IN GERMANY

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Objective: *Staphylococcus aureus/MRSA* ST130 was reported so far from cattle in UK and in Denmark (1,2) . These MRSA contain the *mec* homologue *mec* LGA251. Here we report the characterization of MRSA ST130 isolates from infections in humans.
Methods: MRSA isolates ST130 were obtained from 11 patients treated in different German hospitals (10 wound infections needing surgical intervention, 1 colonization) from. Typing by means *spa*- typing und MLST, and microbroth assay for AST were performed as described previously (3). For detection of sets of resistance- and virulence associated genes the microarray platform array-mate “Staphtype” from Alere was used. Primers for the detection of *mec*LGA251 gene alone or for both, mecA

and mecLGA251 were deduced from the whole genome nucleotide sequence of *S.aureus* LGA 251 Sanger centre databank).

Results: ST130 is rare so far among MRSA isolated from humans (11 among 12691 isolates from 2006 until now). The 11 isolates investigated exhibited MLST ST130, they contained SCC*mec*XI, *spa*- types were t843, t1736, and t1773. All of the isolates were phenotypically resistant to oxacillin and to oxacillin/sulbactam , and two in addition to ciprofloxacin. PCR for *mec*LGA251 known for MRSA ST130 from UK was positive. The isolates were negative for *sak, chp,* and *scn,* and as expected positive for *hla,* untruncated *hlb,* and *hld,* they furthermore contained *edin*B, *aur, slp*A, *slp*B, *slpE.* From genes coding for surface and cell wall associated products the *ica*-operon, *cap*8, *clf*A, *clf*B, *ebp*S, *fnb*A, *fnb*B, *sdr*C were detected but not *cna.* The isolates were positive for a number of *set* determinants and negative for enterotoxin genes and *tst,* as well as for *eta,* and *etb. agr*-type was III. Chromogenic agar plates from three different producers detected MRSA ST130 only insufficiently.

Discussion: the *mec*LGA251 gene and the pattern of virulence associated genes widely correspond to MRSA ST130 already reported. Transmission from animal sources and a zoonotic potential with respect to infections in humans seem likely. The lack of the innate immune evasion operon is typical for most of *S.aureus* isolates of primarily animal origin, probably other enzymes (*aur*) take over this function. For the interdisciplinary surveillance of further spread proper AST and confirmation by PCR for the alternative *mec* gene are important.

This work was supported by grant 01KI1014G, BMBF, VetMedStaph

References:

1. Shore, A., et al. Antimicrob Agents Chemother, 2011, June, ahead of print
2. Garcia-Alvarez, I. et al. Lancet Infect Dis. 2011, ahead of print
3. Witte,W. Et al. Emerging Infect Dis.2007;13: 255 – 258

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ABSENCE OF LIVESTOCK ASSOCIATED MRSA FROM PIGS RAISED IN ALTERNATIVE SYSTEMS AND FROM INDIVIDUAL SMALL HOLDINGS

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Objective: Colonization of pigs with LA-MRSA ST398 and of humans exposed to them has been reported from conventional pig farms so far. Typical for conventional farms are high density of pigs, slatted floors, antibiotic usage over the whole period of fattening, and high importation rate from farms specialized for reproduction. Alternative farms lack these attributes.

Methods: Nasal swabs were taken from at least 5 pigs each raised in alternative farms in Northern Germany und from humans working with these pigs. Swabs were inoculated onto Chrom-agar MRSA and in parallel onto blood agar plates. In case of demonstration of colonies suspicious for MRSA confirmation performed by PCR for *mec*A and subsequent typing (*spa*-typing, MLST, SCC*mec*).

Results: None from 178 animals investigated was positive for MRSA and only 1 from 89 humans exposed to pigs. This person carried LA-MRSA ST398, V and was previously employed in a conventional farm. Neither susceptible *S.aureus* nor MRSA were detected in nasal swabs from 120 wild boars.

Discussion: As already reported for conventional farms MRSA LA-ST398 were found in 82,4% in pigs from 47 farms in the same geographical area in which the study on alternative farms was performed and from 80% of humans exposed to pigs (1). The investigation of wild boars indicates that *S. aureus* is most probably not a natural colonizer of pigs; it was not detected in nasal swabs from pigs before introduction of industrial pig farming (2). According to our results colonization of pigs by LA-MRSA

ST398 is largely influenced by the production system.

1. Cuny C, et al. PloS One.2009 ;4(8) :e6800
2. Pulverer G ,Entel HJ, 1967, Zblk.Bakteriol Orig.202(3) :344-51.

This work was supported by grant 01KI1014G, BMBF, VetMedStaph

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STAPHYLOCOCCUS AUREUS AND MRSA IN THAWING LIQUID OF BROILER CHICKEN CARCASSES AND THEIR RELATION TO CLONAL LINEAGES FROM HUMANS

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Objective: As known from the origin of zoonotic enteric pathogens broiler chicken carcasses can be a substantial source for infections in humans.
Here we report the finding of *S. aureus*/ MRSA various clonal linages in thawing liquid of chicken broilers which are also known from infections in humans.
Methods: Thawing liquid from broiler chicken purchased in supermarkets (119 samples from 5 different producers) were investigated for contamination by direct plating on sheep blood agar and in parallel onto Chrom-agar MRSA. Isolates were subjected to typing by means of *spa*-Typing, MLST and genotyping by use of the "Alere"-microarray, antibiotic susceptibility testing (microbroth MIC) and in case of MRSA genotyping of SCC*mec*-elements

Results:

- LA-MRSA ST398 was found in 41 from 119 samples. The isolates presented *spa*-types t011, t034, t324, t571, t2576, contained SCC*mec*-element V and the pattern of virulence associated gens typical for this clonal linage. Resistance phenotypes were: PEN, OXA, ERY, CLI, TET, (GEN).
- *S. aureus*/ MSSA ST12 were present in 7 from these samples. The isolates extributed spa-type t160 and were phenotypically resistant to PEN only or susceptible. The pattern of virulence associated gens were *ent*A/ *ent*B, *clf*A/ *clf*B, *can*, fubA, *slp*A, *slp*B, *aur, sdr*C but not *sak*, *scu* and *chp*
- *S. aureus* ST 5 were detected in 7 from 119 samples. The exhibited spa-Type t002 and different resistance phenotypes including CIP, ERY, CLI.

The pattern of virulence associated gens typical for this clonal linage.
Conclusion: The proportion of ST398 among HA-MRSA in Germany is 41%; among community MRSA even 17%. Professional exposition and/or familial association is not always documented. MLST ST12 was represented by 2 isolates among 198 *S. aureus* (MSSA) from infections in humans typed by the Reference Center in 2011.
Thus contaminated broiler chicken carcasses can be a source of acquisition of LA-MRSA ST398 and for *S. aureus* with less pronounced host specificity able to cause relevant infections in humans.
This work was supported by grant 01KI1014G, BMBF, VetMedStaph

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