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

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*Indicates Committee Liaison for this Conference.

### ASM CONFERENCES MISSION

To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 700 people.

To encourage student and postdoctoral participation.

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

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

Principal Organizer:
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Ontario Veterinary College/University of Guelph

ESCMID Liaison:
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Radboud University Medical Centre

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Tim Nuttall, BSc, BVSc, PhD, CertVD, CBiol,
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Mireille Wulf, MD
PAMM Laboratory for Medical Microbiology

ACKNOWLEDGMENTS

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

Department for Environment, Food and Rural Affairs (DEFRA)
National Pork Board

UK Cooperative Partner:
Bella Moss Foundation
GENERAL INFORMATION

GENERAL SESSIONS
All general sessions will be held in the New Connaught Rooms in the Covent Garden district of central London. A name badge is required for entry into all sessions. In consideration of other participants, no children are permitted in the sessions.

MEALS
The conference registration includes the welcome reception, 3 lunches, 3 breakfasts, coffee and tea breaks, the networking reception and the conference dinner. A name badge is required for all meals. Guests may purchase tickets for meals, space permitting, from the ASM staff.

POSTER SESSIONS
Poster boards are located in the Drawing and Edinburgh rooms at the New Connaught Rooms. Please check your assigned numer and letter in the abstract index. The number is the board number, the letter represents whether you are assigned to present in the A session on Wednesday or B session on Thursday. If you are assigned to session A you are to put up your poster starting at 8:00 am on Wednesday and remove it by the end of the last session. If you are assigned to session B you are to put up your poster starting at 8:00 am on Thursday and remove it by the end of the last session. Each poster is allotted a board face. Please check your assigned number in the abstract index and mount your poster on the board space bearing that number. You are to stand at your poster during the session (A, or B) noted next to your poster number.

SOCIAL EVENTS
Welcome Reception, Tuesday, September 22, 5:30 pm – 7:00 pm, in the Drawing and Edinburgh rooms
Welcome to Covent Garden, London. It is famous for its shops, street performers, bars, restaurants, theatres and the Royal Opera House. Covent Garden is an Italian-style piazza packed with restaurants, bars and fashionable boutiques. Surrounded by Theatreland, in the heart of London’s West End, the area is recognized as the capital’s premier entertainment and leisure destination. We hope you enjoy the meeting and the location.

Networking Reception, Thursday, September 24, 7:00 pm – 8:00 pm, in the Drawing and Edinburgh Rooms

Conference Dinner, Thursday, September 24, 8:00 pm - 9:30 pm, in the Grand Hall

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

Carmen Arriola  Meghan Davis  Francesca Latronico  Joseph Rubin
Thijs Bosch  Meredith Faires  Wei-Ying Lee  Li Song
E. Broens  Elena Gomez  Urszula Lipinska  Becky Valentine
Monika Chlebowicz  Blake Hanson  Carmen Lozano  Briditte van Cleef
Natacha Couto  Abby Harper  Thomas Maddox  Annelies Van den Eede
**TUESDAY, SEPTEMBER 22**

3:00 pm – 7:00 pm  Registration  
Grand Hall Foyer

5:30 pm – 7:00 pm  Welcome Reception  
Drawing and Edinburgh

**WEDNESDAY, SEPTEMBER 23**

9:00 am - 9:15 am  Welcome and Introductions  
Grand Hall

9:15 am – 12:00 pm  **Session I: Companion Animals**

Moderators: Andy Hillier, The Ohio State University, USA  
Susan Dawson, University of Liverpool, UK

9:15 am - 9:55 am  Questions and Controversies with MRSA in Companion Animals  
**Keynote:** J Scott Weese, Ontario Veterinary College, University of Guelph, Guelph, Canada

9:55 am - 10:15 am  Clonal Spread of Methicillin-resistant *Staphylococcus pseudintermedius*  
Kristina Kadlec, Institute of Farm Animal Genetics FLI, Germany

10:15 am - 10:35 am  Methicillin-resistant *Staphylococcus pseudintermedius*: An Emerging Companion Animal Health Problem  
U. Gronlund Andersson, National Veterinary Institute, Sweden

10:35 am - 11:00 am  Break

11:00 am – 11:20 am  Methicillin-resistant *Staphylococcus aureus* in Horses and Horse Personnel: An Outbreak Investigation  
Engeline van Duijkeren, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, The Netherlands

11:20 am - 11:40 am  Prevalence and Profile of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Positive Dogs: Results of a Year Long Active Surveillance  
Armando E. Hoet, The Ohio State University, USA

11:40 am - 12:00 pm  Comparison of Methicillin-resistant and Methicillin-susceptible *Staphylococcus aureus* Infections in Dogs and Cats  
Meredith C. Faires, Ontario Veterinary College, University of Guelph, Guelph, Canada

12:00 pm - 2:00 pm  **Lunch and Poster Session A**  
Drawing and Edinburgh Rooms
Session II: Food Animal Epidemiology

Moderators: J. McClure, University of Prince Edward Island, Charlottetown, Canada
Tara Smith, University of Iowa, USA

2:00 pm - 2:20 pm
spa Type Distribution of Staphylococcus aureus Isolated from Pigs, Poultry and Cattle in Denmark Between 1952 and 2007
Henrik Hasman, DTU - National Food Institute, Denmark

2:20 pm - 2:40 pm
MRSA ST9 and a Single Locus Variant of ST9 in Commercial Pig Farming in China
Jaap A. Wagenaar, Central Veterinary Institute, The Netherlands

2:40 pm - 3:00 pm
Transmission of MRSA ST398 during Transport of Pigs from Farm to Slaughterhouse and during Time Spent in Lairages at the Slaughterhouse
E. M. Broens, Group of Quantitative Veterinary Epidemiology, Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands

3:00 pm - 3:20 pm
Prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) in Organic and Confinement Swine Operations in the Midwestern United States
Abby L. Harper, University of Iowa, USA

3:20 pm - 3:50 pm
Break

3:50 pm - 4:10 pm
Prevalence of Methicillin-resistant Staphylococcus aureus in Pig Carcasses in Hong Kong
Maureen V. Boost, The Hong Kong Polytechnic University, China

4:10 pm - 4:30 pm
Colonization and Persistence of MRSA Sequence Type 8 (ST8) on a Pig Farm
Marianne Sunde, National Veterinary Institute, Norway

4:30 pm - 4:50 pm
Methicillin-resistant Staphylococcus aureus (MRSA) in Livestock Animals and Foods of Animal Origin in Switzerland
Helen Huber, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland

THURSDAY, SEPTEMBER 24

8:30 am – 12:00 pm
Session III: Public Health

Moderators: Jeff Bender, University of Minnesota, USA
Maureen Boost, Hong Kong Polytechnic University, China
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<tr>
<td>8:30 am - 9:10 am</td>
<td>Carriage of MRSA Related to Animal Husbandry: Who is at Risk?</td>
<td>Keynote: Jan Kluytmans, VU Medical Center, The Netherlands</td>
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<tr>
<td>9:10 am - 9:50 am</td>
<td>Intrahousehold Transmission of Methicillin-resistant Staphylococci</td>
<td>Keynote: Engeline van Duijkeren, Utrecht University, The Netherlands</td>
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<tr>
<td>9:50 am - 10:20 am</td>
<td>Break</td>
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<tr>
<td>10:20 am - 10:40 am</td>
<td>High Prevalence of MRSA in Slaughterhouse workers in Contact with Live Pigs</td>
<td>Brigitte A. van Cleef, RIVM, The Netherlands</td>
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<td>10:40 am - 11:00 am</td>
<td>The Development in Human Cases of CC398 MRSA in Denmark</td>
<td>Jesper Larsen, Robert Skov; Statens Serum Institute, Denmark</td>
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<tr>
<td>11:00 am - 11:20 am</td>
<td>Transmission of MRSA CC398 to Humans Exposed to Colonized Pigs and Transmission to Nonexposed Humans</td>
<td>Wolfgang Witte, Robert Koch Institute, Germany</td>
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<tr>
<td>11:20 am - 11:40 am</td>
<td>Infections and Colonisation with Methicillin-resistant Staphylococcus aureus ST398 versus other MRSA in an area with a High Density of Pig Farms</td>
<td>Mireille Wulf, PAMM Laboratory, The Netherlands</td>
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<td>11:40 am - 12:00 pm</td>
<td>Prevalence of MRSA in Dutch Broiler Slaughterhouses</td>
<td>Mick N. Mulders, RIVM, The Netherlands</td>
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<td>12:00 pm - 2:00 pm</td>
<td>Lunch and Poster Session B</td>
<td>Drawing and Edinburgh Rooms</td>
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<td>Session III: Public Health - cont.</td>
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<tr>
<td>2:00 pm – 2:20 pm</td>
<td>Biosecurity Challenges and Infection Control Practices at Veterinary Teaching Hospitals</td>
<td>Paul S. Morley, Colorado State University, USA</td>
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<td>2:20 pm – 2:40 pm</td>
<td>Whole Genome Microarray Analysis of Meticillin-resistant Staphylococcus aureus Isolated from Pets and their In-Contact Humans</td>
<td>Anette Loeffler, Royal Veterinary College, UK</td>
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<td>2:40 pm – 3:00 pm</td>
<td>Characterization of Methicillin-resistant Staphylococcus (MRS) spp. Isolated from Animal Hospitals in Korea</td>
<td>Yong Ho Park, Seoul National University College of Veterinary Medicine, Republic of Korea</td>
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| 3:00 pm – 3:20 pm | A Year Long Environmental Surveillance of Methicillin-resistant Staphylococcus aureus (MRSA) on Human and Animal contact Surfaces in a Small Animal Hospital  
Armando E. Hoet, The Ohio State University, USA |
| 3:20 pm – 3:40 pm | Break                                                                  |
| 3:40 pm - 5:00 pm | **Breakout Discussion Groups**                                        |
|               | Grand Hall and Balmoral Room                                          |
|               | 1) Transmission of MRSA between Humans and Companion Animals          |
|               |   a. Brandi Limbago, USA                                              |
|               |   b. David Lloyd, UK                                                  |
|               | 2) Foodborne Risks of MRSA                                            |
|               |   c. Jaap Wagenaar, The Netherlands                                   |
|               |   d. Raj Mody, USA                                                    |
|               | 3) Control of Livestock-Associated MRSA                               |
|               |   Moderator: Marine Hallin, ULB-Hopital Erasme                        |
|               |   e. Marc Struelens, Belgium                                          |
|               |   f. Robert Skov, Denmark                                             |
|               | 4) MRSP                                                               |
|               |   g. Stefan Schwarz, Germany                                          |
|               |   h. Linda Frank, USA                                                 |
| 7:00 pm – 8:00 pm | **Networking Reception**                                              |
|               | Drawing and Edinburgh Rooms                                           |
| 8:00 pm - 9:30 pm | **Conference Dinner**                                                 |
|               | Grand Hall                                                            |

**FRIDAY, SEPTEMBER 25**

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<tr>
<td>8:30 am – 5:00 pm</td>
<td><strong>Session IV: Diagnosis, Typing and Antimicrobial Resistance</strong></td>
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<td>Moderators: Michael Mulvey, National Microbiology Lab., Canada</td>
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<td>Stephen Kania, University of Tennessee, USA</td>
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<td>8:30 am - 9:10 am</td>
<td>Typing Methods for <em>Staphylococcus pseudintermedius</em></td>
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<td><strong>Keynote:</strong> Arshnee Moodley, University of Copenhagen, Denmark</td>
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<tr>
<td>9:10 am - 9:30 am</td>
<td>The use of Raman Spectroscopy in Typing Methicillin-resitant</td>
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<td><em>Staphylococcus aureus</em> Including MLST ST398</td>
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<td></td>
<td>Mireille Wulf, PAMM Laboratory, The Netherlands</td>
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9:30 am - 9:50 am  
Non-Cfr-mediated Pleuromutilin Resistance in a Porcine MRSA ST398 Isolate Conferred by the Novel ABC Transporter Vga(C)  
Kristina Kadlec, Institute of Farm Animal Genetics FLI, Germany

9:50 am - 10:10 am  
PFGE Variation within the Methicillin Resistant Staphylococcus aureus ST398 Clonal Lineage using Restriction Enzyme Cfr9I  
Thijs Bosch, National Institute for Public Health and the Environment, The Netherlands

10:10 am - 10:40 am  
Break

10:40 am - 11:00 am  
New CLSI Guidelines for S. pseudintermedius Susceptibility Testing  
Stefan Schwarz, Institute of Farm Animal Genetics (FLI), Germany

11:00 am - 11:20 am  
Evaluation of Different Salt Concentrations and Three Different Chromogenic Media for Detection of Methicillin-resistant Staphylococcus aureus in Pigs  
Larissa J. Pletinckx, Catholic University College South-West-Flanders, Belgium

11:20 am - 11:40 am  
Molecular Characterization of the Livestock-Associated MRSA ST398 Clonal Lineage  
Xander Huijsdens, National Institute for Public Health and the Environment, The Netherlands

11:40 am - 12:00 pm  
Association between Resistance to Zinc and Methicillin-resistance in Staphylococcus aureus-Results of a Comparative Study of MRSA and MSSA CC398 Strains Isolated from Pigs in Denmark  
Lina M. Cavaco, National Food Institute- DTU, Denmark

12:00 pm - 2:00 pm  
Lunch

2:00 pm – 5:00 pm  
**Session V: Molecular Biology**

Moderators: U. Gronlund Andersson, National Veterinary Institute, Sweden  
Kristina Kadlec, Institute of Farm Animal Genetics, Germany

2:00 pm - 2:40 pm  
SCCmec in MRSA and MRSP  
**Keynote:** Vincent Perreten, University of Berne, Switzerland

2:40 pm - 3:00 pm  
The Complete Genome Sequence of a Bovine Associated Methicillin-resistant Staphylococcus aureus Isolate  
Matthew T. Holden, The Wellcome Trust Sanger Institute, United Kingdom
3:00 pm - 3:20 pm  Molecular Characterization of Antimicrobial Resistance Genes and Virulence Genes by Using Microarrays in Representative ST398 MRSA Isolates from Pigs in France
Frederic Laurent, French National Reference Centre for Staphylococci - Hospices Civils de Lyon, France

3:20 pm - 3:40 pm  Break

3:40 pm - 4:20 pm  Investigations of S. aureus Host Specificity and Evolution with Whole Genome Multi-strain Microarrays
Keynote: Jodi Lindsay, St. George’s University of London, United Kingdom

4:20 pm - 4:40 pm  Staphylococcal Cassette Chromosome (SCCmec): Evidence of Recent Transfer From Staphylococcus aureus to Staphylococcus pseudintermedius
Stephen A. Kania, University of Tennessee, USA

4:40 pm - 5:00 pm  Identification of Two Novel SCCmec Elements Carried by MRSA CC398 Strains Isolated from Veterinarians
Robert Skov, Statens Serum Institut, Copenhagen, Denmark

5:00 pm - 5:10 pm  Closing announcements
S1:1
CLONAL SPREAD OF METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS

K. Kadlec1, S. Schwarz2, A. Moodley1, S. Kania1, L. Frank1, D. A. Bemis3, A. Franco4, A. Battisti5, J. Wagenaaar1, E. van Duijkeren5, J. S. Weese6, R. Fitzgerald7, A. Rossano8, V. Perreten4, L. Guardabassi2, 1Institute of Farm Animal Genetics, FLI, Neustadt-Mariensee, GERMANY, 2Faculty of Life Sciences, University of Copenhagen, Copenhagen, DENMARK, 3University of Tennessee, Knoxville, TN, 4Instituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, ITALY, 5Utrecht University, Utrecht, NETHERLANDS, 6University of Guelph, Guelph, ON, CANADA, 7University of Edinburgh, Edinburgh, UNITED KINGDOM, 8University of Berne, Berne, SWITZERLAND

Background: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has recently emerged in companion animals, preferentially in dogs. Currently, little is known about the epidemiological relationships of MRSP isolates from distinct geographical areas. The objective of the present study was to investigate the genetic diversity of this new emerging veterinary pathogen by typing a large collection of isolates originating from nine countries.

Methods: In total, 117 epidemiologically unrelated MRSP isolates were collected from dogs (n=103), cats (n=12), a fox (n=1) and a hoopoe (n=1) from seven European countries, the U.S.A. and Canada during 2004 and 2008. The isolates were analysed by spa typing and MLST protocols developed for this species. The SCCmec types were determined by PCR assays and classified according to the current *S. aureus* nomenclature.

Results: Seven different spa types were identified: t02 (n=80), t06 (n=18), t03 (n=2), t05 (n=2), t07 (n=2), t021 (n=1), and t022 (n=1). The remaining isolates were non-typeable. MLST revealed 14 different types: ST71 (n=87), ST68 (n=13), ST58 (n=3), ST106 (n=2), single isolates of ST5, ST73, ST110, ST111 - ST116, and ST118 as well as two non-typeable isolates. SCCmec types II-III was present in most isolates (n=90). The remaining isolates contained SCCmec types I.VI (n=2), V (n=15), and VII (n=4), whereas six isolates were not typeable (1: ccrA/B2 with mec type B or 2: ccrA/I1 with mec type A). Of the 16 isolates collected in the U.S.A., 12 exhibited spa type t06 and MLST type ST68 in association with the SCCmec type V. In contrast, only three European isolates belonged to spa type t06 together with the SCCmec type II-III and the MLST types ST71 (n=2) or ST114 (n=1). A single isolate from Europe (Germany) exhibited SCCmec V, t021 and ST115. Among the European isolates, SCCmec II-III was frequently found associated with spa type t02 and MLST type ST71 (73 of 92 isolates). One isolate from the U.S.A. was identified with the same characteristics. Among the isolates from Canada (n=9), one isolate for each major type was identified. Three isolates were non-typeable by spa typing, but exhibited ST58 with SCCmec VII, two isolates showed t06, ST71 and SCCmec II-III, and the remaining two isolates were also non-typeable by spa typing and showed ST100 with SCCmec V or ST113 with a non-typeable SCCmec (1).

Conclusions: The recent spread of MRSP in Europe is largely due to the emergence of a single genetic lineage (t02, ST71, SCCmec II-III). In the U.S.A. another genetic lineage (t06, ST68, SCCmec V) appears to be predominant. The results indicate that MRSP spreads clonally and that the emergence of methicillin resistance in this species is due to multiple acquisi-

S1:2
METHICILLIN-RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS: AN EMERGING COMPANION ANIMAL HEALTH PROBLEM

U. Grönlund Andersson1, M. Finn1, K. Kadlec2, S. Schwarz2, A. Moodley1, S. Kania1, L. Frank1, D. A. Bemis3, A. Franco4, A. Battisti5, J. Wagenaaar1, E. van Duijkeren5, J. S. Weese6, R. Fitzgerald7, V. Perreten4, L. Guardabassi2, C. Greko3, 1National Veterinary Institute, Uppsala, SWEDEN, 2Institute of Farm Animal Genetics, FLI, Neustadt-Mariensee, GERMANY, 3Faculty of Life Sciences, University of Copenhagen, Copenhagen, DENMARK, 4University of Tennessee, Knoxville, TN, 5Instituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, ITALY, 6Utrecht University, Utrecht, NETHERLANDS, 7University of Guelph, Guelph, ON, CANADA, 8University of Edinburgh, Edinburgh, UNITED KINGDOM, 9University of Berne, Berne, SWITZERLAND

Objectives: Methicillin resistance has recently emerged in *Staphylococcus pseudintermedius*, a commensal living on the skin and mucosa of a wide range of animals, mainly dogs, and the most common bacterial pathogen associated with pyoderma, otitis and wound infections in dogs. In this study, minimum inhibitory concentrations (MICs) of 21 antimicrobials and resistance gene profiles were determined in a large collection of methicillin-resistant *S. pseudintermedius* (MRSP) isolates originating from dogs in nine countries.

Methods: We analysed 103 epidemiologically unrelated MRSP isolates from dogs, including 79 isolates from various clinical conditions, 22 were of non-clinical and two of unclear origin, collected between 2004 and 2008 in Canada, Denmark, France, Germany, Italy, Sweden, Switzerland, the Netherlands, and the USA. Antimicrobial susceptibility was determined by broth microdilution using VetMIC™ or by gradient diffusion, Etest®.

Results: In addition to β-lactam resistance, non-susceptibility was observed to ciprofloxacin (88%), clindamycin (88%), erythromycin (89%), gentamicin (81%), kanamycin (93%), streptomycin (89%) or trimethoprim (91%). Sixty percent of the isolates showed non-susceptibility to all these substances. Non-susceptibility to tetracycline and chloramphenicol was observed for 71 and 57% of the isolates, respectively. Most of the isolates were susceptible to fusidic acid (88%) and all isolates were susceptible to linezolid, quinupristin/dalfopristin and vancomycin. The most frequently detected non-β-lactam resistance genes were ermA(B) coding for macrolide/lincosamide resistance (89%), aac(6’)-Ie-aph(2’)-Ia for gentamicin resistance (88%), aph(3’)-IIa for kanamycin resistance (90%), ant(6’)-Ia for streptomycin resistance (90%) and dfrG for trimethoprim resistance (91%). The most common susceptibility pattern among European MRSP differed from that of isolates from North America with the latter isolates mostly being susceptible to chloramphenicol.

Conclusion: The multi-resistance profile of MRSP strains spreading in Europe and North America typically includes resistance to all oral antimicrobials routinely used for treatment of infections in dogs. Most of the drugs to which these bacteria remain susceptible are not authorised for animals and are used as last-resort drugs in human medicine. Their use in veterinary medicine is therefore controversial. Infections with such MRSP strains represent a serious therapeutic challenge that is not likely to be solved by new antimicrobials for animals. Joint efforts...
are therefore urgently needed to understand the risk factors for emergence and spread of these multi-resistant pathogens in dogs, and to develop adequate preventive measures to control this important animal health problem.

S1:3
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN HORSES AND HORSE PERSONNEL: AN OUTBREAK INVESTIGATION

E. van Duijkeren1, M. Moleman2, M. Sloet van Oldruitenborgh-Oosterbaan2, J. Muls3, A. Troelstra4, A. Fluitt5, W. van Wamel6, H. de Neeling6, D. Houwers7, J. Wagenaar8; 1Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht, NETHERLANDS, 2Department of Equine Medicine, Faculty of Veterinary Medicine, Utrecht, NETHERLANDS, 3Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht, NETHERLANDS, 4Department of Medical Microbiology, University Medical Center Utrecht, Utrecht University, Utrecht, NETHERLANDS, 5Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center, Rotterdam, NETHERLANDS, 6National Institute of Public Health and the Environment, Laboratory for Infectious Diseases and Screening, Bilthoven, NETHERLANDS

Objective: The emergence of two clusters of MRSA infections at an equine veterinary teaching hospital and the subsequent demand for outbreak management and MRSA control measures, prompted us to perform a study on colonization rates of horses and horse personnel, the possible occurrence of nosocomial transmission and the degree of contamination of the hospital’s interior. Methods: In 2006/2007 and 2008 several horses which had undergone surgery at the same equine clinic had (wound)infections with MRSA. The clinic was suspected to be the source of the infections and therefore environmental samples were taken and personnel were sampled. In addition, samples from the nares of 259 horses just before entering the clinic (n=149) were taken and cultured for MRSA. The samples from the horses present at the clinic were taken at 5 moments with a one week interval. All MRSA isolates were spa-typed and susceptibility of the isolates was determined using an agar diffusion method. Results: During the first outbreak, MRSA isolates of the rare spa-type t011 were cultured from 7 horses and 4/61 personnel which indicated zoonotic transmission. After intervention by cleaning and disinfection of the facilities the outbreak stopped. However, another outbreak occurred in 2008, where different equine MRSA isolates were found, i.e., spa-type t011 (n=12), t2123 (n=4), and t064 (n=1). This time, 16/170 personnel were found to be positive for MRSA with spa-type t011 (n=11) and t2123 (n=5), which were also the most prevalent types in horses at the hospital. Personnel in close contact with horses were more often MRSA-positive (15/106) than those without (1/64). Screening of horses upon admission to the hospital showed that 9.3% were MRSA-positive predominantly with spa-type t011. Weekly cross sectional sampling of all hospitalized horses for 5 weeks showed that 42% of the horses were MRSA-positive at least once, again predominantly with spa-type t011, which suggests that nosocomial transmission took place. Fifty-three% of the wipes of the interior of the hospital were MRSA-positive and all were spa-type t011. Positive samples included those from canteens, students’ and staff members’ rooms, which demonstrates that dissemination into the environment readily occurs and that humans also play a part in spreading the organism. All isolates appeared to be resistant against beta-lactams, tetracycline, gentamicin and kanamycin. Conclusion: Our results show that nosocomial transmission occurs in equine clinics and suggests that personnel play a role in the transmission.

S1:4
PREVALENCE AND PROFILE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) POSITIVE DOGS: RESULTS OF A YEAR LONG ACTIVE SURVEILLANCE

A. E. Hoet, J. Van-Balen, A. Reed, R. Nava-Hoet, S. Bateman, A. Hillier, J. Dyce, W. Gebreyes, T. Wittum; The Ohio State University, Columbus, OH

Methicillin Resistant Staphylococcus aureus (MRSA) infections in dogs have become a growing concern in the field of veterinary medicine. Zoonotic and nosocomial outbreaks in veterinary hospitals affecting humans and animals have been increasingly reported in the last few years. However, the prevalence of this pathogen in incoming dogs and their epidemiological profile is largely unknown, thus our goal was to establish a baseline prevalence of MRSA-positive dogs in a large veterinary teaching hospital (VTH) through active random sampling of incoming dogs, as well as to identify potential risk factors in MRSA positive dogs. Therefore, each month samples were randomly collected from 36 dogs arriving to targeted sections of the hospital. An epidemiological survey was given which obtained information about the dog’s medical history, living conditions, and diet. Swabs were taken from the anterior nares, ears, perianal area, and if present, any skin lesions. These samples were screened using standard pre-enrichment, isolation, and identification procedures to determine the presence of MRSA. In a 12 month period, 6.6% (26/394) of dogs arriving in all five sections tested MRSA-positive, of which 4 out of these 26 MRSA positive dogs (15.4%) were “healthy” dogs, and thus asymptomatic carriers. Nineteen (73.1%) of the positive dogs carried MRSA in the nares, 4 (15.4%) in the perianal region, 3 (11.5%) in the ears, and of the 12 that had skin lesions, 50% carried MRSA (6/12). Nineteen percent of dogs carried MRSA in 2 sites (5/26), and 3.8% carried MRSA in 3 sites (1/26). Mix infections of MRSA, MSSA, and other Methicillin resistant coagulase positive Staphylococcus aureus (MSSA), such as MR S. intermedius, were detected in several individual (26.9%), 7/26. Most of the MRSA isolates (25/26) were multidrug resistant to several classes of antimicrobial, including quinolones and aminoglycosides. Previous antimicrobial usage was not significantly associated with the detection of MRSA in these incoming dogs; however, the owner’s occupation appears to be a risk factor for the presence of MRSA. Analysis of other 40 epidemiological variables is on going. In conclusion, our results clearly indicate that MRSA is highly prevalent in dogs arriving at OSU-VTH, with a similar prevalence compared to humans arriving in hospitals in the USA. The presence of MRSA in incoming canine patients is of concern due to the potential nosocomial and zoonotic transmission of MRSA to patients and personnel at the veterinary hospital.
Methicillin-Resistant Staphylococci in Animals

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) is emerging as an important pathogen in companion animals, but there has been minimal objective comparison of MRSA infections with those caused by methicillin-susceptible Staphylococcus aureus (MSSA) infections. The objectives of this study were to compare the infection types, clinical outcomes, and determine the risk factors associated with MRSA, compared to MSSA infections, in dogs and cats. Methods: A retrospective case-control study was conducted at 3 veterinary referral hospitals. An MRSA infection was identified and was matched by species, veterinary referral hospital, and date of admission to 2 MSSA controls: the MSSA infection immediately preceding and following the MRSA case. A questionnaire was used to collect information from the medical record of all cases and controls. Data were collected concerning signalment, medical and surgical history, infection, and clinical outcome. Outcomes were defined as animals having an MRSA or MSSA infection. Analyses were performed using exact logistic regression. Due to the sample size, a multivariable model could not be constructed due to concerns of model stability and issues associated with over-fitting the model. Consequently, only univariable models were constructed. Results: A total of 46 MRSA cases and 92 MSSA controls were enrolled consisting of 120 (86.9%) dogs and 18 (13.1%) cats. The largest proportion of MRSA and MSSA infections were located on the skin (58.7% (27/46) and 64.4% (58/90), respectively. The majority of animals with MRSA (93.3%, 42/45) and MSSA (91.1%, 81/89) infections were discharged from the hospital. Antimicrobial administration (OR = 3.55, 95% CI: 1.22-11.94; P = 0.014) and fluoroquinolone administration (OR = 3.77; 95% CI: 1.03-17.03; P=0.037) were significantly associated with the infection of MRSA and MSSA infections were located on the skin (58.7% (27/46) and 64.4% (58/90), respectively. The majority of animals with MRSA (93.3%, 42/45) and MSSA (91.1%, 81/89) infections were discharged from the hospital. Antimicrobial administration (OR = 3.55, 95% CI: 1.22-11.94; P = 0.014) and fluoroquinolone administration (OR = 3.77; 95% CI: 1.03-17.03; P=0.037) were significantly associated with the development of an MRSA infection. Conclusions: This study is the first to objectively compare MRSA and MSSA infections in dogs and cats and identify risk factors for the development of an MRSA infection. The identification of antimicrobials, specifically fluoroquinolones, as risk factors for MRSA infection supports the need for prudent use of antimicrobials in pets. The high survival rate indicates that MRSA is largely a treatable infection, likely because of the high frequency of non-invasive infections. While no difference in outcome for MRSA and MSSA infections was observed, further study of invasive infections is warranted.

spa type distribution of Staphylococcus aureus isolated from pigs, poultry and cattle in Denmark between 1952 and 2007

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spa type distribution of Staphylococcus aureus isolated from pigs, poultry and cattle in Denmark between 1952 and 2007. H. Hasman, A. Moodley, L. Guardabassi, M. Stegger R. L. Skov and F. M. Aarestrup. Most MRSA isolated from pigs, veal calves and most recently poultry in Europe belongs to distinct spa types (t011, t034 and t108) associated with ST398/CC398. However, the spa distribution of methicillin susceptible S. aureus isolates from these animal reservoirs has not been studied previously. Therefore it is not known, if ST398 is the dominating type among production animals. In this study, a total of 296 unbiased S. aureus isolates from infections and colonization of pigs, cattle and poultry isolated between 1993 and 2007 in Denmark were analyzed by spa and multi-locus sequence typing (MLST) and compared to each other as well as to the spa type distribution of bovine mastitis isolates from the mid-1950ties and human bacteremia isolates. All isolates were methicillin susceptible and little overlap in spa types was seen between isolates from the three animal reservoirs of recent dates where as the spa type distribution of the bovine mastitis isolates from the 1950ties and recent years showed considerable overlap. Most of the porcine isolates had the spa types t034 (CC398), t1333 (CC30) and t337 (CC9), while the bovine isolates mainly had spa types t518 (CC50), t524 (CC97) and t529 (CC151). None of the spa types found in porcine and bovine isolates are common among human blood isolates in Denmark. On the contrary, almost all of the poultry isolates (96%) belonged to CC5 (spa types t002 and less frequently t306), which is a relatively common among human blood isolates in this country. This study confirms the host-specificity of S. aureus types and also clearly shows that CC398 is a classic pig clone also before it acquired the mecA gene.

MRSA ST9 and a Single Locus Variant of ST9 in Commercial Pig Farming in China

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Methicillin resistant Staphylococcus aureus (MRSA) with Sequence Type (ST) 398 has been found in pig production in several
A new strain of methicillin resistant *Staphylococcus aureus* (MRSA ST398) was found in pigs and people in contact with pigs recently. A study on Dutch slaughterhouses tested 81% of farms and 39% of pigs positive for MRSA ST398. Another study on Dutch pig farms reported prevalences of 39% at farm level and 11% at pig level. For *Salmonella* spp. it is reported that short-term exposure to a contaminated environment, such as transport lorries and lairages in slaughterhouses, is sufficient to result in *Salmonella* positive pigs. The discrepancy between prevalences for MRSA ST398 found on slaughterhouses and on farms might be explained by the same feature. The objective of this study was to evaluate the possibility of negative tested pigs becoming MRSA ST398 positive during transport from farm to slaughterhouse and/or during their stay in lairages.

Four batches of 30 slaughter pigs, from 4 MRSA negative farms, were selected. Pigs were delivered to 3 different commercial slaughter houses. A nasal swab was taken from the pigs just before loading for transport, at arrival at the slaughterhouse and just after stunning. After transport and after time spent in lairages, 3-5 wipes were taken from the environment to test for MRSA ST398. Microbiological analysis was done on all samples. Confirmation of MRSA suspected colonies was done by multiplex PCR. *spa* types and antimicrobial susceptibilities are to be determined. On all farms, all pigs tested negative at the moment of loading before transport. Transport lasted from 2 to 5 hours. In 2 out of 4 batches, 17% respectively 26% of the pigs tested positive after transport. For these 2 batches, 1 out of 5 environmental wipes taken from the lorry was positive as well. Pigs that stayed in contaminated lorries had a higher chance of being MRSA positive after transport than pigs that stayed in lorries that tested negative for MRSA (OR=21.7 [3.4-∞]; *P*=.0002). Subsequently, the pigs spent from 1.5 to 11 hours in the lairages before entering the slaughter process. After this period, in 3 out of 4 lairages, MRSA was found in 1 to 4 environmental wipes. Finally, in all slaughter batches MRSA was found in 7% to 100% of the tested pigs. Again, pigs that stayed in a contaminated lairage had a higher chance of being MRSA positive than pigs that stayed in lairages that tested negative for MRSA (OR=48.0 [10.6-452.2]; *P*<.0001). These results demonstrate that transmission of MRSA takes place in the short-term period of transport to the slaughterhouse and during the time spent in lairages. For reducing the introduction of MRSA in the food production chain, assembly of pigs from various sources on transport lorries and in lairage facilities seems to be an important factor. Further research is needed to elucidate the route of transmission and factors affecting it. The possible health hazard for slaughterhouse personnel and consumers of pork needs further investigation.
farms (7 confinement, 6 organic/antibiotic free) in Iowa and Illinois. To date, no MRSA has been found on organic farms in Iowa. Nasal swabs were taken from 312 swine. Overall MRSA prevalence in swine was found to be 13% (39/312). MRSA prevalence in confinement swine was 23% (39/168). Overall, MRSA was found on 4/13 farms (31%). In addition to swine, nasal and pharyngeal swabs were taken from humans working on swine farms. MRSA prevalence in humans was 37% (26/71). All of the colonized humans were employed at confinement operations; humans working in confinement had a prevalence of 58% (26/45). These results suggest a significant number of U.S. swine may be colonized with MRSA, adding to the concern about domestic animal species as a reservoir of this bacterium. Furthermore, occupational exposure to these colonized pigs may spread the bacteria from the farm to the community via a high number of colonized swine workers. Additional studies are ongoing to examine the carriage rates of MRSA in rural Iowa.

S2:5 PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PIG CARCASSES IN HONG KONG

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) a major pathogen in both the hospital and community has been reported to colonize pigs in both Europe and North America. In the Netherlands, 11% of pigs were nasally colonized, whilst 39% of carcasses were MRSA-contaminated. In Canada, almost 25% of pigs and 45% of farms had MRSA colonization. In USA, there is a report of 36% colonization of adult pigs at one facility. There have been limited reports of MRSA in pigs from Asia with MRSA being isolated in samples from slaughterhouses in Taiwan and from one pig snout in China, but sampling in Korea did not yield MRSA. Pigs have been implicated as the source of MRSA infections in humans and increased MRSA nasal colonization in pig-farmers has been reported. A pilot study of slaughtered pigs had revealed MRSA colonization. This study aimed to determine the prevalence of MRSA nasal colonization of carcasses in Hong Kong.

Method: Swabs were collected from the nares of 260 pigs from local markets which receive carcasses after slaughter. Specimens were cultured within 4 hours on selective agar for MRSA, and mannitol salt agar with oxacillin, following enrichment in 5% NaCl brain-heart infusion broth. Colonies with staphylococcal morphology were identified and susceptibility testing performed. Presence of the mecA gene was confirmed in isolates exhibiting resistance to either oxacillin or cefoxitin, and characterized by SCCmec typing. Results: Of 260 pig snouts sampled, 87 (33.5%) were colonized with MRSA and overall 106 MRSA strains were isolated. All MRSA isolates were multi-drug resistant, harboured the mecA gene, and carried SCCmec Type IV (88.7%) or V (11.3%). All isolates were resistant to clindamycin, and there was a high level of resistance to tetracycline (95%), erythromycin (86%), ciprofloxacin (73%), chloramphenicol (68%), cotrimoxazole (43%), and quinopristin/dalfopristin (38%). Resistance to linezolid, fusidic acid, and rifampicin was present in 5%, 4%, and 4% of isolates respectively. Conclusion: 1. MRSA colonization levels of pigs were high and similar to those reported in slaughtered pigs in the Netherlands. 2. MRSA isolates were resistant to a broader range of antibiotics than previously described for MRSA ST398 from European studies. A preliminary study had shown that the MRSA isolates from pig carcasses in Hong Kong belonged to a different MRSA lineage (ST9) (see abstract by Guardabassi et al). 3. High levels of porcine MRSA colonization indicate that pigs may represent an important reservoir of MRSA and suggests a need for increased infection control measures and colonization surveillance of personnel exposed to pigs.

S2:6 COLONIZATION AND PERSEVANCE OF MRSA SEQUENCE TYPE 8 (ST8) ON A PIG FARM

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In 2008, the EU-initiated baseline survey on the prevalence of Salmonella spp. in herds of breeding pigs in the European countries also included a survey of MRSA prevalence in the same holdings. These investigations were initiated as recent reports had documented high prevalence of ST398 MRSA among pigs in several European countries. Standardized sampling protocols and laboratory methods were used and the material investigated was dust samples from the herds as instructed by EFSA. In Norway only one MRSA positive pig farm was found. Genotyping using multi-locus-sequence-typing (MLST) showed that the isolate belonged to sequence type 8 (ST8). The spa-type was t008 (spa-repeats: 11-19-12-21-34-24-32-25). This is a rather common MRSA variant among humans in Norway, and a possible human source was suspected. Subsequent MRSA screening of family members on the farm showed that two persons were MRSA positive, carrying the same MRSA variant as detected from the holding. Treatment to eradicate MRSA carriage in the humans was initiated, but the risk of MRSA re-contamination from the pigs was considered as a possible threat. However, MRSA colonization of the pigs was uncertain as the samples investigated were dust samples, and not samples from the animals. Knowledge about colonization rate and persistence of human MRSA variants among animals is very limited. To gain more knowledge about the particular MRSA variant and its eventual distribution among the pigs, all animals (n=346) were screened for MRSA by testing swabs from the nostrils. The laboratory method was the same as used in the baseline surveys. The pigs were held in two separate houses, dust samples representing dust from all pens in both houses were also screened for MRSA. MRSA was detected from one sample, containing five pooled swabs from five fattening pigs housed together in one pen. MRSA was not detected from other pigs in the same pen (four animals). Dust samples from the house where MRSA positive pigs were held were positive. The dust samples from the other building housing MRSA negative animals were negative. Positive pigs were out-ranged and washing and disinfecting was carried out. Three months later a second follow-up testing was performed. Dust samples representing dust from all pens in the herd were tested, all...
S2:7  
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN LIVESTOCK ANIMALS AND FOODS OF ANIMAL ORIGIN IN SWITZERLAND

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Introduction: Along with hospital-associated and community-associated MRSA, livestock-associated (la) MRSA are of increasing importance worldwide. Up to now, only few and incomplete data are available in Switzerland. The aim of this study was therefore to assess the spread of la-MRSA in the meat and milk production line throughout Switzerland and to further characterize isolated strains. Such data form the basis for further measures within the veterinary public health tasks.

Materials and methods: Livestock samples were obtained at slaughter: nasal swabs from calves (n=150), cows (n=270) and pigs (n=550) as well as neck skin samples from carcases of 60 chicken flocks. Moreover, 190 raw milk cheese and 150 minced meat samples were collected. After a two-step enrichment procedure in Mueller-Hinton broth supplemented with 6.5% NaCl (24 h at 37°C) and phenol red mannitol broth supplemented with aztreonam and cefoxitin (24 h at 37°C), these samples were plated on Oxoid Brilliance MRSA Agar (24 h at 37°C). In addition, 125 S. aureus strains isolated from cattle with mastitis were directly streaked on Oxoid Brilliance MRSA Agar (24 h at 37°C). In addition, 125 S. aureus strains isolated from cattle with mastitis were directly streaked on Oxoid Brilliance MRSA Agar. Presumptive positive colonies were tested by PCR for the presence of the mecA gene and confirmed as S. aureus by 23S rRNA PCR. Isolated MRSA were genotyped by SCCmec-typing, spa-typing, MLST-typing and PFGE (using Smal and EagI). Furthermore, strains were tested by PCR for presence of pvl encoding for Panton-Valentine leukocidin and spa to sed encoding for staphylococcal enterotoxins A to D. Results: MRSA were only detected in two (1.3%) calves, two (0.4%) pigs, one (0.4%) cow, and three (2.4%) mastitis milk samples. Up to now, further characterization data are available from five of the eight MRSA strains. Of the five strains (two from calves, three from mastitis), three belonged to sequence type (ST) 398 (two from calves, one from mastitis), one to ST8 (mastitis milk), and one to ST352 (mastitis milk). The two MRSA from calves belonged to SCCmec type V, tested negative for PVL and SE. Of the three mastitis strains, one (ST8) belonged to SCCmec type II and two were non-typeable by SCCmec. Moreover, two (ST8, ST352) tested positive for pvl, and one for spa.

Significance: The current study confirms a favorable situation of MRSA distribution in livestock animals and foods of animal origin in Switzerland. Nevertheless, la-MRSA were found in low prevalence in pigs, calves, a cow, and mastitis milk samples. Therefore, future changes have to be carefully monitored and a risk-based surveillance system should be established.

S3:1  
HIGH PREVALENCE OF MRSA IN SLAUGHTERHOUSE WORKERS IN CONTACT WITH LIVE PIGS

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Introduction: Methicillin resistant Staphylococcus aureus (MRSA) has emerged as a zoonosis with an extensive reservoir in pigs and veal calves. These MRSA strains belong to the Clonal Complex 398 (CC398), determined by multi-locus sequence typing (MLST). In The Netherlands, persons working on pig and veal farms have a high prevalence of MRSA (30%), compared to the background prevalence (<0.1%). Also, considerable prevalences of MRSA in different categories of raw meat have been found (overall 11%). The objective of this study was to determine the prevalence of MRSA-CC398 in pig slaughterhouse workers, and to determine the presence of MRSA in the different sections of the slaughterhouse. Methods: In a cross-sectional survey, employees from 3 commercial pig slaughterhouses in The Netherlands were tested. They were divided into 3 categories: (1) contact with live pigs, (2) dead pigs or (3) other. Data on personal characteristics (i.e. amount of hours per week working in the slaughterhouse, presence of livestock at home) were collected. Environmental samples of surfaces in different sections of the slaughterhouse were collected at the beginning and end of the day. MRSA was determined in all samples using standard protocols, and MRSA was confirmed with a multiplex PCR for the S. aureus specific DNA-fragment, the Panton Valentine Leucocidin (PVL) genes and the mecA gene. Staphylococcal protein A (spa)-typing was performed and antimicrobial susceptibility testing was conducted. Results: In total, 249 subjects entered the study (response about 50%). The general MRSA-prevalence was 5.6% (14/249). MRSA was found exclusively in persons working with live pigs (14/93=15.1%). No MRSA was detected in persons working with dead pigs (n=127) and persons working in other sections of the slaughterhouse (n=29). Multivariate analysis showed that working with live pigs is a significant risk factor for acquiring MRSA (OR 57.1 [3.4-969.4]; P<0.0001). Furthermore, 25% (30/118) of the environmental samples were MRSA-positive. At the beginning of the working day, all positive samples (10/59) were located in the lairages. However, at the end of the working day, MRSA was found throughout the slaughterhouse: lairages (11/59), dirty area (5/59) and clean area (3/59). All spa-types found belong to CC398. Conclusions: 1) Livestock-associated MRSA is found in pig slaughterhouse workers at high percentages compared to the background prevalence in The Netherlands. 2) Working with live pigs is an important risk factor for acquiring MRSA. This corresponds with current “search and destroy” guidelines, that requires - among others - persons in contact with live pigs to be screened for MRSA on admission to a hospital. 3) MRSA is predominantly present...
on surfaces in the slaughterhouse where live pigs reside, but spreads throughout the slaughterhouse during the production process.

S3:2
THE DEVELOPMENT IN HUMAN CASES OF CC398 MRSA IN DENMARK

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates belonging to clonal complex 398 (CC398) have emerged worldwide over the last few years in livestock, particularly pigs, and in people working with colonized livestock. Denmark is a large producer of pigs with a production of ~25 mill/year. Isolates of CC398 from human clinical cases have been found in Denmark since 2003. The aim of this study was to describe the development of human cases of MRSA CC398 in Denmark. **Material and Methods:** In Denmark, all MRSA and most *Staphylococcus aureus* isolates from blood have been referred to the Staphylococcus reference laboratory at Statens Serum Institut (SSI) for typing since 1988. Since November 2006, MRSA has been a notifiable disease, prior to that clinical and epidemiological information was obtained consecutively by requesting discharge summaries from hospitals or notes from outpatient clinics and GPs for each case at the time of initial diagnosis. MRSA isolates belonging to spa types associated with CC398 were included in this study. All isolates were spa typed and susceptibility tested, and a selection of 52 isolates was SCCmec typed by the method described by Kondo et al.

**Results:** In the period 2003 - 2008, MRSA CC398 were detected in 109 persons (both colonised only and patients with infections). The annual number of cases was 1, 6, 14, 9, 14, and 64. The large increase in 2008 was partly due to screening projects. The vast majority of index cases had professional contact with pigs and the geographic density of MRSA CC398 infections correlated with the density of pig farming. A total of 35 cases had an infection at the time of MRSA detection. However, more cases of infections are likely to have occurred as the clinical status has only been recorded at the time of initial finding of a CC398 isolate in a patient. Superficial skin and soft tissue infections were most frequently encountered but two cases of severe infections have been observed; one arthritis followed by multi-organ failure, and one pneumonia in a newborn. t034 was the most common spa type (101 cases (94%) followed by t011 (n=4), t108 (n=2) and t1793 (n=1). Six isolates have been PVL positive, four with spa type t034, one with spa type t011, and one with spa type t108. The four PVL positive t034 isolates have been found in two families who both had adopted children from China. SCCmec type V was the most common, present in 36 of the 52 isolates tested, but in seven isolates type IV was detected. Nine isolates could not be classified. Most of the isolates were tetracycline resistant (93%), and 51% were erythromycin resistant. **Conclusion:** This study shows that MRSA CC398 is increasingly causing infections in humans in Denmark, primarily skin and soft tissue infections. The epidemiology seems different compared to Holland as t034 is the dominating type. Nearly all isolates with established connection to livestock is tetracycline resistant.

S3:3
TRANSMISSION OF MRSA CC398 TO HUMANS EXPOSED TO COLONIZED PIGS AND TRANSMISSION TO NONEXPOSED HUMANS

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**Objective:** MRSA CC398 is widely disseminated as nasal colonizer among industrially raised pigs and frequent in nasal swabs from humans directly exposed to pigs. It is also able to cause infections in humans. Knowledge on further transmission to nonexposed humans is an important prerequisite for assessing the potential risk for humans. We addressed this question by study on nasal colonization. **Methods:** The study was performed on 47 farms with MRSA colonized pigs and 229 humans (113 exposed, 116 nonexposed) working and living on these farms. Furthermore colonization of veterinarians and of their family members was investigated. For addressing dissemination beyond farms nasal swabs from 462 pupils attending a secondary school in a high density pig farming area were investigated. Nasal swabs were streaked onto chromagar MRSA as selective agar. MRSA detected were typed by means of spa-typing and PCR-typing of SCCmec elements. For antibiotic susceptibility testing MIC was performed by microbroth assay. **Results:** From 113 humans exposed 97 (86%) revealed as nasal carriers; from their 116 nonexposed family members 5 were positive (4.3%). According to multivariate regression analysis the risk for acquisition of MRSA is 138 times greater for exposed humans than for nonexposed ones; antibiotic treatment before sampling was without influence. Among veterinarians attending pig farms (n = 49) nasal colonization was found in 22 (45%) of them, In 15 families of veterinarians with 18 exposed humans 4 from 44 family members (9%) revealed as colonized. Among 462 pupils in only 3 of them MRSA CC398 was detected (3 from living on pig farms). **Conclusions:** As already observed in studies in other European countries nasal colonization of humans exposed to pigs is frequent, and there is also transmission at low frequencies to humans living in contact. Spread beyond farms is obviously rare.

S3:4
INFECTIONS AND COLONISATION WITH METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ST398 VERSUS OTHER MRSA IN AN AREA WITH A HIGH DENSITY OF PIG FARMS

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The south-east of the Netherlands is an area with a large density of pig farms. After the implementation of the screening of people in contact with pigs and veil calves for MRSA, the average number of newly identified carriers increased from 16/year between July 2002- July 2006 to 148 between July 2006-Dec 2008, a 930% increase of which 81% (108/132) was due to ST398. The majority came from targeted screening (98/year, 98/132 = 74%) but 7% was due to unexpected cases. Infections of ST398 occurred mainly in pre-existing wounds, varying from post-operative wound infections in patients with
and without contact with livestock to sepsis to post-trauma osteomyelitis. However, there were significantly less skin and soft tissue infections due to ST398 than to other MRSA. There was an overrepresentation of spa type t567 in the clinical isolates, which is also a spa-type more prevalent in our region than in the rest of the Netherlands. Data on resistance, showed more multi-resistant isolates (>4 classes of antibiotic) in ST398 than in other isolates.

S3:5
PREVALENCE OF MRSA IN DUTCH BROILER SLAUGHTERHOUSES

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Introduction: Since 2003, a new type of MRSA (CC398) has emerged in Dutch hospitals and has been found particularly in persons having contact with pigs or veal calves. This so-called livestock-associated (LA-) MRSA was found to be highly prevalent in pigs, veal calves and those persons in contact with these animals. While, in a recent Dutch survey on MRSA in raw meat products one quarter of the samples of chicken was found positive, data on the occurrence of LA-MRSA in poultry are scarce. Materials & method: By order of a Dutch Ministry (VWS), and in close collaboration with the Association of Dutch Poultry Processing Industries, the RIVM conducted a study on MRSA in broiler slaughterhouses during Q1+2 of 2009. The aim of the study was to estimate the prevalence of MRSA in Dutch flocks of broilers, to determine the degree of MRSA contamination in the different compartments of the slaughterhouse, and to estimate the risk of MRSA carriage among personnel. Personnel participated on a strictly voluntary basis. Informed consent was obtained prior to sampling personnel. At each slaughterhouse, five different Dutch flocks (slaughter batches) were sampled. From each flock 10 broilers were sampled just after stunning by taking pharyngeal swabs, and additionally at three slaughterhouses swabs were taken from 5 transport containers per flock. Both at the beginning and at the end of a working day, 20 environmental samples were taken from different compartments including all phases of the production process. Results: Preliminary results from 5 slaughterhouses are presented. MRSA was detected in environmental samples from all 5 slaughterhouses, and it was observed that contamination of the different compartments mostly occurred during the production process. In 6 of the 25 flocks sampled, MRSA was detected in the pharyngeal swabs with 1-10 chickens positive per flock. Totally, MRSA was isolated from 22 (9%) of 255 broilers and from 7 (9%) of 76 containers examined. By combining these results, MRSA was detected in 8 (32%) of the 25 flocks examined. Of the total 706 employees, 444 agreed to be sampled. Of these, 23 were MRSA positive. Of 114 persons having contact with live animals, 18% were positive; compared to 1% of 200 persons only having contact with dead animals. Of the 121 administrative and technical personnel 3% were positive. The following spa types were found, all livestock-associated: t002, t011, t034, t1430 and t1456. Conclusion: The prevalence of LA-MRSA in Dutch broiler flocks is around 32% and slaughtering of flocks leads to contamination of the slaughterhouse compartments during a slaughter day. Slaughterhouse workers having direct contact with live animals have a significantly increased probability of MRSA carriage (5.2% vs estimated 0.1% background rate).

S3:6
BIOSECURITY CHALLENGES AND INFECTION CONTROL PRACTICES AT VETERINARY TEACHING HOSPITALS

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Objective: To characterize biosecurity challenges and infection control practices at veterinary teaching hospitals located at institutions accredited by the AVMA. Design: Cross-sectional survey. Population: 50 biosecurity experts at 38 veterinary teaching hospitals. Procedures: Data were collected in telephone interviews regarding the occurrence of outbreaks of nosocomial infections, as well as policies for hygiene, surveillance, patient contact, education, and awareness. Respondents were also asked their opinion regarding the rigor of their programs. Results: Despite the infrequent use of systematic surveillance methods, 31 of 38 (82%) hospitals reported identifying outbreaks of nosocomial infection during the 5 years prior to the interview. Seventeen (45%) reported >1 outbreak, 22 (58%) had restricted patient admissions to aid mitigation, and 12 (32%) had completely closed sections of the facility to control disease spread. The most common cause of outbreaks was Salmonella (65%, 20/31), followed by MRSA (42%, 13/31). Nineteen (50%) hospitals reported that zoonotic infections had occurred during the 2 years prior to the interview. Only 16 (42%) hospitals required personnel to complete a biosecurity training program, but 20 of the 50 (40%) respondents indicated that they believed their hospitals ranked among the top 10% in regard to rigor of infection control efforts. Conclusions and Clinical Relevance: Results suggested that outbreaks of nosocomial infections are common in both large and small animal hospitals, and there appeared to be differences among infection control programs at these institutions. Despite the relative frequency of outbreaks and the consequences of these events, education and training in methods to mitigate these events were limited. Perceptions of experts regarding program rigor appeared to be skewed, possibly because of a lack of published data characterizing programs at other institutions. Results may provide a stimulus for hospital administrators to better optimize biosecurity and infection control programs at their hospitals and thereby optimize patient care.
S3:7
WHOLE GENOME MICROARRAY ANALYSIS OF METICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM PETS AND THEIR IN-CONTACT HUMANS

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Most meticillin-resistant Staphylococcus aureus (MRSA) isolated from dogs and cats in the UK belong to one of two dominant hospital-associated lineages, EMRSA-15 (CC22) and EMRSA-16 (CC36), but little is known about genetic variation within the same MRSA lineage. This study explored whether host-specific resistance or virulence genes occur in MRSA. Six MRSA CC22 from canine and feline infections identified by a veterinary laboratory were compared with six nasal carriage MRSA CC22 from their respective in-contact people, either veterinary staff or pet owner, by whole genome microarray analysis (BuG@S, Wellcome Trust). Genetic characteristics identified as potentially associated with animal hosts were investigated by PCR screening of additional isolates. There was no significant variation between the six pairs in the core variable genes and the majority of mobile genetic elements (MGE) except for two regions within the plasmid genomes. Both were present in 4/6 human MRSA but absent from the remaining human and all animal isolates. PCR analysis of an additional 63 animal and 33 human MRSA for the presence of two plasmid-associated target genes showed that both were more frequent in MRSA from humans (P=0.012 and P=0.002, respectively). The plasmid is currently being sequenced to determine its exact genetic content. These findings indicate that animal-specific genetic variation can occur within the same MRSA lineage, similar to findings from a comparison of bovine and human S. aureus isolates where only a limited group of MGE showed host-specificity. This highlights that different selection pressures may occur in animals and further monitoring is warranted. Funding: This study was funded by the UK Department for Environment, Food & Rural Affairs (DEFRA), project number OD2019.

S3:8
CHARACTERIZATION OF METICILLIN-RESISTANT STAPHYLOCOCCUS (MRS) SPP. ISOLATED FROM ANIMAL HOSPITALS IN KOREA

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Staphylococcus spp. is recognized as a major infectious pathogen, which can be the reservoir for a possible transmission of meticillin-resistance genes in animals and human. In order to set a baseline data on the risk assessment of Staphylococcus spp. in animal hospitals, we searched for the prevalence and characterization of MRS isolated from animals, staffs and environment in animal hospitals in Korea. Staphylococcus spp. was isolated from a total number of 529 samples, where 271 samples were obtained from 54 animals, 170 samples from 77 hospital staffs and 88 samples from hospital environment in five animal hospitals. Staphylococcus spp. was isolated by various biochemical tests and confirmed by Vitek and PCR with Staphylococcus spp.-specific primers (S. aureus (SA), S. epidermidis (SE), S. intermedius (SI)). To analyze the susceptibility of the isolates to 16 antimicrobials, disk diffusion test and the MIC test were performed. The mecA gene was detected to confirm meticillin-resistance Staphylococci. Epidemiological characterization and genetic relatedness were determined by SCCmec typing, MLST (Multi-locus sequence typing), PFGE (Pulsed field gel electrophoresis), Staphylococcal enterotoxins (SEs) production and panto valentine leukocidin (pvl) toxin. The characterization of 379 Staphylococcus isolates revealed 91 (24%) of the isolates to be meticillin-resistant, 3 MRSA (0.8%), 49 MRSE (12.9%) and 39 MRSI (10.3%), respectively. Among them, 50 (31.3%) isolates were from hospital staffs, 31 (19.5%) from animals and 10 (16.7%) from hospital environment. The antimicrobial result showed high resistance to ß-lactam antibiotics penicillin (96%), ampicillin (95%) and oxacillin (65%) followed by erythromycin and tetracycline. The epidemiological characterization and genetic relatedness have determined that MRSA was SCCmec type IV, the sequence type (ST) 72 belongs to CC8 and produced SEI, SEG, SEM, SEN and SEO but no pvl toxin. In case of MRSE, type II and IV SCCmec were classified and 14 sequence types ST 20, ST 5, ST 2 and ST 64 (Single variant locus (SVL) and Double variant locus (DVL)) with high frequency. On the basis of the eBurst, 86% belonged to CC2 and 14% to ST 64 singleton. SCCmec type IV and V were determined from MRSI and SCCmec type V isolates from staffs, animal and hospital environment and PFGE showed the same pattern. Most of the MRS isolates was found as the community associated type IV and V. However, MRSA isolates was low and most of them was MRSE from human (67.7%) and MRSI from animal (74%). These MRSE isolates had high genetic diversity especially the SLV and DLV of ST 64 with a unique sequence type. In case of MRSI an identical genetic characteristic was found in human, animal and hospital environment. This result showed the possible transmission of MRS and MRS-related genetic properties could be occurred in animal hospital.

S3:9
A YEAR LONG ENVIRONMENTAL SURVEILLANCE OF METICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ON HUMAN AND ANIMAL CONTACT SURFACES IN A SMALL ANIMAL HOSPITAL

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Methicillin-Resistant Staphylococcus aureus (MRSA) is emerging as a nosocomial and zoonotic pathogen in veterinary settings with importance in both animal and human health. Even though several studies have shown the environment as a potential source of this pathogen, there is very little research on environmental MRSA prevalence in veterinary hospitals. Therefore, the present study was performed to monitor the prevalence of MRSA on human and animal surfaces in a veterinary teaching hospital over a year long period. Twenty-two different human and canine contact surfaces in four target surveillance sections of the hospital were sampled using swabs and Swiffers®. In total, 47 samples per month were collected for a 12 month period. Standard pre-enrichment, isolation, and identification
procedures were used to detect the presence of MRSA. Seventy-eight out of 569 (13.7%) collected samples tested positive for MRSA, detecting a greater prevalence on the canine contact surfaces (43/290, 14.8%) than in human contact surfaces (35/279, 12.5%), however this difference was not statistically significant. The human contact surfaces with the greatest number of MRSA positive results were doors, computers, IV pumps, and drawer handles. The animal contact surfaces with the highest prevalence were carts, examination tables, floors, and water bowls. A subset of 12 MRSA isolates were confirmed to carry the SCC-type II, which is generally associated with HA-MRSA strains. The largest prevalence of MRSA in the environment was detected during the summer months, with a 29.8% peak in June. Over 80% of the MRSA isolates detected in the environment were resistant to multiple classes on antimicrobials. In conclusion, it was found that MRSA can be present on both human and canine contact surfaces across the different sections. These results are of importance because have help to identify “hot spots” and the development of specific prevention and control strategies for MRSA and other nosocomial pathogens in the veterinary hospital environment.

**S4:1**

**THE USE OF RAMAN SPECTROSCOPY IN TYPING METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS INCLUDING MLST ST398**

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**Objective:** The Netherlands has an active search and destroy policy for methicillin resistant Staphylococcus aureus (MRSA). In order to maintain this policy, a reliable and quick typing method for isolates is essential. The objective of this study was to see if Raman spectroscopy provides such a method for normal- and livestock-associated MRSA. **Methods:** Between 2002 and 2007 a total of 433 MRSA positive subjects were identified. Of these a total of 392 isolates were analysed using Raman spectroscopy. Spectroscopic fingerprints were obtained using a dedicated Raman spectrometer, requiring approx. 40 seconds per sample. Cluster analysis on these fingerprints was performed using the pair wise correlations as a distance measure in combination with Ward’s cluster algorithm. Results were compared with PFGE cluster typing results obtained from the national reference library and with epidemiological data. **Results:** Of the 403 isolates analysed, 157 were non-typable by PFGE and belonged to ST398. The remaining 246 represented a total of 51 different PFGE types. Raman typing resulted in a total of 86 Raman clusters. Analyses of 20 clusters of epidemiologically linked isolates (n=154) showed that 128 (82%) had identical PFGE clusters. Raman typing of the same isolates showed that 126 (81%) had corresponding Raman clusters. Of the 28 mismatches, 13 (8.4%) isolates had both a different Raman and PFGE of the outbreak strain. Analyses of ST398 isolates showed that in one outbreak with 10 cases, all had identical Raman types, in one case of possible transmission, 2 Raman clusters were seen. An overall analyses of the PFGE non-typable isolates in our collection (n=157) resulted in 24 different Raman clusters. **Conclusions:** Raman spectroscopy is a reproducible method for epidemiological typing of all MRSA including ST398. The results obtained with this technique are comparable to PFGE and in good concordance with the epidemiological data.

**S4:2**

**PIG-ASSOCIATED METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATES WITH SPA TYPE T899 BELONG TO TWO UNRELATED CLONAL TYPES**

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Livestock, especially pigs, has recently been identified as a reservoir of methicillin resistant Staphylococcus aureus (MRSA) as well as methicillin sensitive *S. aureus* (MSSA). In Europe, the rapid emergence of pig-associated MRSA is evident. The MRSA isolates belong to clonal complex (CC)398 according to multi locus sequence typing (MLST), the predominant type being sequence type (ST)398, whereas MSSA isolates mainly belong to either CC398 or the unrelated ST9 clonal complex (CC9). MRSA CC398 consist of twelve MLST types and a range of closely related spa types (e.g., t011, t034, t108, and t1793), whereas t899 has predominantly been found among pig-associated MSSA ST9. However, a recent report showed that MRSA CC398 isolates of *spa* type t899 emerged in the Dutch pig population in 2006. Subsequently, a cluster of MRSA isolates with *spa* type t899 was identified among Italian delegates at the International Pig Veterinary Society (IPVS) congress in Copenhagen, Denmark. In this study, we performed MLST on this collection as well as on the first Danish MRSA t899 isolate identified through the Danish national surveillance of MRSA in April 2009. All MRSA t899 isolates belonged to ST398 (allelic profile 3-35-19-2-20-26-39), and not ST9 (allelic profile 3-3-1-1-1-1-10).

The sequence identity between the *spa* genes in unrelated sequence types might reflect the result of horizontal gene transfer of *spa* genes, which in the case of ST398 and ST9 might have happened in the pig reservoir. Sequence analysis of *spa* types associated with CC398 and CC9 indicates that t899 is more closely related to *spa* sequences carried by CC9 isolates than those carried by CC398 isolates, which suggests that t899 originated in the ST9 complex and that CC398 has acquired t899 from ST9. However, further studies are required to better understand the molecular evolution of these clonal types. The present study has revealed important limitations of *spa* typing in epidemiological studies of pig-associated MRSA and has emphasised the usefulness of MLST for identifying clonal types of this emerging zoonosis.

**S4:3**

**PFGE VARIATION WITHIN THE METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ST398 CLONAL LINEAGE USING RESTRICTION ENZYME CFR9I.**

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**Introduction:** Since 2003, a new reservoir for methicillin resistant *Staphylococcus aureus* (MRSA) was identified in pigs and
other farm animals. Most of the livestock related MRSA strains share the same Multi Locus Sequence Type (MLST), ST398. Throughout Europe, Canada and the United States, ST398 has been found in association with animal husbandry, indicating a worldwide spread of this clonal lineage. With conventional Pulsed Field Gel Electrophoresis (PFGE), no DNA fragments could be generated for ST398 isolates due to DNA methylation of the \( Smal \) restriction sites. Recently, the restriction enzyme \( CfrI \), a neoschizomer of \( Smal \), was shown to be insensitive to this DNA-methylation leading to macrorestriction patterns for previously non-typeable (NT\(_{\text{smad}}\))-MRSA isolates. In this study, we optimized PFGE with \( CfrI \) and assessed its usefulness for the characterization of NT\(_{\text{smad}}\)-MRSA isolates from diverse ecological backgrounds. **Material and Methods:** The National Institute for Public Health and the Environment (RIVM) serves as the Dutch National MRSA reference center. All primary MRSA isolates, one per patient, are sent to the RIVM for epidemiological typing. We selected 95 NT\(_{\text{smad}}\)-MRSA to study the genetic diversity among NT\(_{\text{smad}}\)-MRSA in the Netherlands: 60 NT\(_{\text{smad}}\)-MRSA isolates of the two most prevalent spa-types (t011 (n=30) and t108 (n=30)) and 35 ST398 isolates with distinct spa-types. **Results:** All PFGE patterns of the NT\(_{\text{smad}}\)-MRSA were compared with a database consisting of more than 4000 isolates representing over 700 different PFGE-types. The National database. The 30 t011 isolates revealed 20 different PFGE types. The minimal similarity between the patterns was 60%. The 30 t108 isolates revealed 9 different PFGE types with a minimal similarity of 82%. The minimal similarity of the 60 NT\(_{\text{smad}}\)-MRSA isolates was 68%. The 35 ST398 isolates with distinct spa-types revealed 32 different PFGE types with a minimal similarity of 55%. **Conclusions:** Our \( CfrI \) PFGE protocol generated highly informative banding patterns. Comparison of the PFGE results of ST398 isolates with other typeable MRSA isolates showed no match with any known PFGE type in the national database. The 30 t011 isolates revealed 20 different PFGE types. The fact that these are a large number of distinct, yet related PFGE types among ST398 isolates, may indicate that the ST398 clonal lineage is still adapting to gain a larger host range.

**S4:4**

**EVALUATION OF DIFFERENT SALT CONCENTRATIONS AND THREE DIFFERENT CHROMOGENIC MEDIA FOR DETECTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PIGS**


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The performance of chromogenic media for detection of MRSA in humans, has been evaluated in previous studies. On the contrary, no comparative studies have been made for detection of MRSA in pigs. The purpose of this study was to evaluate three selective chromogenic media: BrillianceMRSA (Oxoid), MRSASelect (Bio-Rad) and MRSA-ID (bioMérieux) for detection of MRSA in pigs. This was done after optimizing the enrichment method in Nutrient Broth (Oxoid) (NB) supplemented with 2.5%, 5% or 7.5% NaCl. A total of 103 samples were taken from: piglets (15), weaned piglets (15), fattening pigs (42), sows (15), environmental specimens (wall, floor, air)(10), farmers (2), samplers (3) and a puppy (1). Swabs of pigs were taken from the anterior nares, skin behind the ear and perineum. Samples were enriched in NB containing 2.5%, 5% or 7.5% NaCl. After 24 h, the enrichments in 2.5% and 7.5% NaCl were inoculated on the 3 media. The enrichment in 5% NaCl was only inoculated on MRSA-ID. Suspect colonies, with characteristic growth, were confirmed by multiplex-PCR for 16S rRNA, mecA and nucA (1). After enrichment in 2.5% NaCl, 54 samples showed characteristic colonies on BrillianceMRSA, 57 on MRSASelect and 41 on MRSA-ID. After multiplex-PCR analysis, 40 (74%), 43 (75%) and 39 (95%) samples were found true positive. The sensitivity was 87%, 90% and 87% and the specificity was 75%, 75% and 97% respectively. BrillianceMRSA, MRSASelect and MRSA-ID. After enrichment in 5% NaCl, 48 samples showed characteristic colonies on BrillianceMRSA, 60 on MRSASelect and 56 on MRSA-ID. After multiplex-PCR analysis, 33 (83%), 51 (85%) and 55 (98%) samples were found true positive. The sensitivity was 59%, 89% and 95% and the specificity was 85%, 80% and 98% on respectively BrillianceMRSA, MRSASelect and MRSA-ID. The difference in sensitivity of the 3 chromogenic media, after enrichment in 2.5% NaCl was marginally significant. The specificity was however highest for MRSA-ID. After enrichment in 7.5% NaCl, the sensitivity and specificity of MRSA-ID was highest followed by BrillianceMRSA and MRSASelect. Several studies, using bacterial and hospital patients collection, showed that MRSA-ID and MRSASelect have excellent sensitivity and specificity, when compared to other media (2, 3, 4). In conclusion, the highest sensitivity and specificity in detecting MRSA in pigs was obtained by enrichment in high salt concentration and subsequent screening on MRSA-ID.

**REFERENCES:**


**S4:5**

**MOLECULAR CHARACTERIZATION OF THE LIVESTOCK-ASSOCIATED MRSA ST398 CLONAL LINEAGE**


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**S4:6**

**ASSOCIATION BETWEEN RESISTANCE TO ZINC AND METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS—RESULTS OF A COMPARATIVE STUDY OF MRSA AND MSSA CC398 STRAINS ISOLATED FROM PIGS IN DENMARK**

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Methicillin resistant *Staphylococcus aureus* (MRSA) of clonal complex 398 (CC398) were first identified in 2003 in the Netherlands. Since then, this clone has been found associated with pigs and other food animal species and as a cause of colonization and infection in humans in several countries. However, the origin of CC398 and the factors contributing to its success are yet to be explained. In this study, MRSA and MSSA strains isolated from pigs in Denmark were compared and characterized, to find factors that could have favoured the selection of MRSA CC398 in the Danish pig farms. A total of 31 MRSA and 60 MSSA were typed by spa typing and antimicrobial susceptibility to penicillin, tetracycline and erythromycin. MRSA were additionally subjected to SCCmec typing. In addition, all isolates were tested for susceptibility to zinc chloride by agar dilution. All MRSA isolates belonged to spa types previously assigned to CC398 (t011, t034, t108), and carried either SCCmec type V (28) and either III or V in three isolates and either II or IV in one isolate. The MSSA were more diverse and belonged to spa types assigned to several clonal complexes (CC5, CC9, CC30, CC97 and CC398). Resistance to tetracycline was observed in all 31 MRSA and 30 MSSA belonging to CC398 and penicillin and erythromycin resistance was observed among 100% and 42% of the MRSA and 80% and 43% of the MSSA of this clonal complex, whereas lower prevalences of resistance were found in other CC-types. Resistance to zinc (MIC 4-12mM) was observed in 74% (N=23/31) of MRSA CC398, whereas all MSSA were susceptible to zinc chloride (MIC 0.5–2 mM). It has previously been suggested that the use of antimicrobials and particularly tetracycline, might be related to the emergence of MRSA CC398. However, this association was indicated by observations of MRSA isolates. Our observations showed that also the MSSA belonging to CC398 displayed tetracycline resistance, indicating that tetracycline resistance is not exclusive of MRSA. Interestingly, a very strong association between methicillin resistance and reduced susceptibility to zinc (p<0.001, OR 40.388) was observed. Zinc compounds are used very frequently in feed for production animals as a feed additive or for control of diarrhoea. Thus, our finding of a strong association between zinc susceptibility and MRSA CC398 could indicate that the use of zinc has contributed to the emergence in Denmark of MRSA CC398 and/or to its further spread. In conclusion, antimicrobial susceptibility patterns were similar among MRSA and MSSA belonging to CC398 except in the case of zinc susceptibility. The finding of an association of zinc resistance and methicillin resistance, might give some interesting perspectives. Future studies on strains from diverse origins, and data from experimental and epidemiological studies will help to understand the selection and spread of CC398 and help to establish adequate control measures.

**S5:1**

**THE COMPLETE GENOME SEQUENCE OF A BOVINE ASSOCIATED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATE**

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Genome sequencing has provided an unprecedented insight into the genetics and biology of Staphylococcus aureus. We have sequenced the genome of a bovine associated methicillin-resistant S. aureus (MRSA) recently identified in a transmission study investigating the spread of bovine mastitis in UK dairy herds. The isolate sequenced belonged to a novel sequence type, ST425, and exhibited resistance to oxacillin (MIC of 16 mg/L) and cefoxitin (MIC of 32 mg/L). Although the isolate presented a resistant phenotype for β-lactam antibiotics, screening for the penicillin-binding protein 2a (PBP2a) using the latex agglutination test, and for the PBP2a gene (mecA) by PCR and Southern hybridization proved negative. Whole genome sequencing and comparative genomic analysis was used to investigate the genetic basis of the observed methicillin resistance. The genome contained a novel staphylococcal cassette...
Objectives: The aim of the study was to analyze diversity of virulence genes as well as antimicrobial resistance genes in ST398 MRSA strains isolated from French fattening pigs at slaughterhouses and in farms by using a new microarray approach. Methods: Forty-five strains, representatives (MST, spa-types, geographical areas) of the isolates collected from French fattening pigs at slaughterhouses or in farms, were included in the present study. DNA microarray, based on the ArrayStrip\textsuperscript{TM} platform (CLONDIAG, GmbH, Jena, Germany) was used for genetic characterization of all strains. This array carries covalently 334 immobilized probes to detect a large number of genes related to antibiotics resistance, exotoxin production, enzymes secretion and other virulence factors. After DNA extraction, linear amplification (one single primer per gene) and hybridization of labelled PCR product, spot recognition as well as geometric layout is performed automatically based on scans of the array and advanced image processing with automated machine (CLONDIAG). The data obtained were also compared to those from ST398 MSSA isolates from infected patients. Results: Our data indicated a high level of diversity in antimicrobial resistance genes including *erm, ssa, vbg, aac(6')-aph(2''), ant(4')-Ia, dfrA, tet, cat, fexA, qac* genes in animal ST398 MRSA. Conversely, toxin profiles (including 19 toxin) genes were homogeneous and marked by the absence of any toxin except *sed* (n=1) and *seg* (n=1), Alpha- and delta-Hemolysin were constantly positive. The study of the other virulence factors especially MSCRAMMs, revealed the same high rate of homogeneity. The human ST398 MSSA isolates, used as comparators, revealed slight different features with homogeneous negative results for most of antimicrobial resistance genes as well as for most of virulence factors. Nevertheless we noticed that all human ST398 MSSA harbour *clp* gene coding for CHIP virulence factor that protects *S. aureus* from innate immune defense systems and that one human ST398 MSSA harboured PVL gene and was responsible for necrotizing pneumonia. These two genes were never detected in animal ST398 MRSA. Conclusion: The present study is the first one to propose an extensive molecular screening of the presence of virulence factor genes and antimicrobial susceptibility genes in a large collection of animal ST398 MRSA in comparison with human ST398 MSSA. Our results highlight the question of the molecular diversity of the ST398 isolates and the adaptation of ST398 clones to their environments and their hosts.

**STAPYLOCOCCAL CASSETTE CHROMOSOME (SCCmec): EVIDENCE OF RECENT TRANSFER FROM STAPHYLOCOCCUS AUREUS TO STAPHYLOCOCCUS PSEUDINTERMEDIUS**

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Methicillin resistance in staphylococci is associated with expression of the *mecA* gene contained in the staphylococcal cassette chromosome mec (SCCmec). The mechanism of horizontal transfer of this large mobile element is poorly understood. An SCCmec was detected in a clonal population of MLST 68, methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) that is predominant in the southeastern United States. Initial screening with the multiplex PCR assay described by Zhang et al. indicated that it was a type V cassette. Sequence analysis of approximately 27,000 bases showed that the MRSP cassette is nearly identical to one named V\textsubscript{P} and tentatively designated SCCmec type VII occurring in methicillin-resistant *Staphylococcus aureus* (MRSA). This is the first report of SCCmec cassettes of such high sequence homologies occurring in both MRSA and MRSP. This finding has important implications for the epidemiology of methicillin resistance in both staphylococcal species and may help elucidate the mechanism of SCCmec transfer in these pathogens.

**IDENTIFICATION OF TWO NOVEL SCCmec ELEMENTS CARRIED BY MRSA CC398 STRAINS ISOLATED FROM VETERINARIANS**

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Background: Livestock, especially pigs, have recently been shown to constitute a reservoir for methicillin resistant S. aureus (LA-MRSA) of clonal complex CC398 which has spread to humans, in particular to people in close contact with colonized livestock. Methods: SCCmec elements carried by 31 ST398 MRSA strains isolated from the participants at an international conference on pig health conference in Copenhagen, 2006 were SCCmec typed using the multiplex PCR by Kondo et al. Two strains were untypeable by this method PC-DK1 from a Thai participant and PC-DK6 from a Canadian participant. The full nucleotide sequences of the SCCmec-elements were determined by assembling sequences obtained from fosmid.
libraries. Results: Using the multiplex PCR by Kondo et al., SC-Cmec element type V was found in 24 isolates with one being a composite element (5&5C2), type IVa in five isolates, and two isolates were untypeable. Determination of the nucleotide sequence of the two untypeable strains, showed that the SCCmec cassette of PC-DK1 is 43.7 kb in length and carries a novel combination of a class C2 mec gene complex and a type 1 ccr gene complex. We suggest that the cassette is designated as SCCmec type IX. The SCCmec cassette of PC-DK6 is 50.8 kb in length and carries a novel ccr combination ccrA1 and ccrB6 and class C1 mec. We suggest that the novel ccr combination is designated ccr7 and the cassette is designated as SCCmec type X. Both of these novel SCCmec cassettes carried clusters of genes encoding heavy metal resistance, e.g., copper, arsenate, and cadmium. The significance of carriage of these genes within the SCCmec cassettes is under investigation. Conclusions: Two novel SCCmec cassettes in CC398 isolated from veterinarians are described. The cassettes are larger than SC-Cmec cassettes carried by extant community associated MRSA (i.e. type IV and V). The presence of multiple genes encoding heavy metal resistance is remarkable.
**1A**

**CHANGES IN SUSCEPTIBILITY TO ANTIBIOTICS STAPHYLOCOCCUS AUREUS UNDER EFFECT OF DISSOLVED OZONE**

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Aims: To investigate the in vitro change in sensitivity to antibiotics of clinical strains to Staphylococcus aureus under the influence of dissolved ozone (pO3). Methods: 20 strains of Staphylococcus aureus resistant to the antibiotics of penicillin series were evaluated. Suspension was prepared from the culture of each strain in ratio 1-2x10⁸ CFU/ ml, which was treated with dissolved ozone (pO3 2 mg / ml) during from 5 to 20 min. Then strain crops were treated by pO3 and inoculated on Mueller-Hinton agar containing strain resistant antibiotics. Then inoculation of the crops of the same strain not treated by pO3 on agar with a disk of the same antibiotic have taken as control serians. After a day of incubation with temperature 37°C was produced record of results according to change in the diameter of the zone of growth inhibition from different discs.

Results: In 17 cases out of 20 of inoculation of Staphylococcus aureus strains a growth inhibition area from disc increased from 3.8 to 24.5 ± 7.3 mm. In the control strains change in the area of growth inhibition was not recorded. Conclusion: possibility of restoring sensitivity to the antibiotic-resistant strains of Staphylococcus aureus after treatment with dissolved ozone.

**2B**

**INVESTIGATION ON METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PIG AND COW FARMS IN CHINA**

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Methicillin-resistant Staphylococcus aureus (MRSA) are a global health concern which is resistant to most antibiotics has become more prevalent among animals in Sweden, Denmark and Netherlands. However, there was insufficient information to investigate the MRSA in food animal in China. 189 Staphylococcus aureus isolates from tonsil of healthy pig were sampled from two farms and 129 Staphylococcus aureus were isolated from milk of two cow farms in China in 2007. Antimicrobial susceptibility testing was performed using 15 antimicrobials by disk-diffusion. The result indicated that the Staphylococcus aureus from healthy pigs and milk, their percentage of resistant to penicillin were 96.3% and 68.3%; to cefazolin were 3.7% and 0; to cefoxitin were 7.4% and 7.3%; to gentamicin were 32.1% and 4.9%; to clindamycin were 96.4% and 12.2%; to tetracycline were 89.3% and 9.8%; to sulfisoxazole were 96.4% and 97.6%; to ciprofloxacin were 57.1% and 4.9%; to Azithromycin were 89.3% and 29.3%; to vancomycin were 17.9% and 0; to meticillin were 25.0% and 7.3%. 91.7% strains with TEM gene and no mecA detected in cefoxitin-resistant and/or methicillin-resistant strains by PCR amplification. There was more severe antimicrobial resistance (AR) in pigs, which represented multi-AR with 7-10 of 14 drugs and not evident multi-AR from MRSA which Methicillin resistant mechanism was unaware. This study implied AR and multi-AR are correlation to clinical drug usage which were more excessive in pig farms than in cow farms.

**3A**

**PREVALENCE OF MRSA AMONG DANISH PARTICIPANTS AT A CONFERENCE FOR DANISH PIG PRODUCERS**

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Background: In 2008, the presence of MRSA CC398 has been confirmed in several Danish pig herds. All participants at the yearly conference for Danish pig producers, were offered a test for MRSA, to gain an estimate of the occurrence of MRSA among persons working in the Danish Pig Production. A total of 1945 persons working within various fields of pig production (pig producers, veterinarians, researchers etc.) participated in the conference. Materials and Methods: Participation was on a strictly voluntary basis. Nasal sampling was performed by swabbing both nostrils. Prior to sampling a standardized questionnaire on profession (farmer, veterinarian, consultant or other), weekly hours of contact with pigs, working within sow units, finisher units or both, previous hospitalisation and visits to pig units abroad was completed. All swabs were screened for MRSA using incubation in enrichment broth followed by plating on chromogenic agar. MRSA was confirmed by PCR, typed by sequencing the staphylococcal protein A (spa) gene and susceptibility tested. Results: Questionnaires and samples were obtained from 791 persons, of whom 22 were excluded (13 were foreign visitors and 9 did not complete the questionnaire). The remaining 769 persons comprised 487 farmers, 109 agricultural consultants, 25 veterinary practitioners and 150 persons working in other fields. In total, 19 (2.5 %) of the sampled persons were tested positive for MRSA CC398 (15 (3.1%) farmers; 2 (1.8 %) consultants and 2 (1.4 %) persons working within other fields). No veterinarians were MRSA-positive. Of the 19 CC398 isolates, 3 belonged to spa-type t011, the remaining 16 to spa-type t034. Two persons, both farmers, carried other MRSA-types (t032 and t044). All MRSA CC398 positive persons had weekly contact with pigs. However, the duration of contact with pigs was not statistically associated with the MRSA CC398 carrier status. Persons working only in finisher units were at a significantly lower risk of carrying MRSA CC398 compared with persons working in sow units.

Conclusion: The results confirmed that MRSA CC398 carriage is more frequent among persons working within the Danish pig industry than in the population in general. Working in sow units increased the risk of being an MRSA CC398 carrier. These findings support previous reports, indicating that intensive and regular exposure to pigs increases the risk of humans getting colonized with CC398.
Objective: MRSA CC398 is widely disseminated as nasal colonizer of pigs, of other livestock and humans professionally exposed to colonized animals. Here we report on its emergence as the cause of deep seated infections of skin and soft tissue in humans. Methods: MRSA from skin and soft tissue infections in humans were sent to the author's laboratory as the German national reference centre for further characterization. The isolates were typed by means of spa-typing, SCCmec typing and microbroth MIC assay for antibiotic susceptibility. Attribution of spa-types to clonal complexes was performed by means of the BURP algorithm. Results: Among 127 MRSA isolates originating from abscesses and furuncles 5.2% were assigned to CC398. This corresponded to the prevalence of caMRSA (PVL +) ST1, 3.7%; ST5, 3%; ST22, 3%; ST59, 2.2%; and ST30, 5.2%. Most frequent were PVL pos. caMRSA ST8, 41.7% and ST80, 35%. There was no association with a particular spa-type (spa t011, t034, t0521). The isolates were resistant to beta-lactams, erythromycin, clindamycin, fusidic acid and partly also to cotrimoxazole, and susceptible to linezolid, rifampicin, fusidic acid, fosfomycin, tigecyclin and daptomycin. Infections caused by CC398 needed surgical treatment. All 7 humans affected had been exposed to colonized pigs as farmers or as veterinarians. Conclusions: MRSA CC398 is able to cause deep seated infections in humans with professional exposure to livestock comparable to PVL-positive caMRSA.

Resistance of Staphylococcus aureus strains to different antibiotics is most often caused by the presence of mobile genetic elements carrying resistance genes. The best example is the Staphylococcal Chromosomal Cassette mec (SCCmec). This cassette carries the mecA gene for penicillin-binding protein 2a, which confers resistance to β-lactam antibiotics, such as methicillin. We have characterized a novel SCCmec cassette from community-acquired MRSA and MSSA strains that were isolated from a mother and her daughter who suffered from pneumonia and umbilicus infections, respectively. Both the MRSA and MSSA strains are Panton-Valentine leukocidin-positive and their DNA cannot be digested with SmaI for Pulsed Field Gel Electrophoresis. MLST-typing showed that these strains belong to the ST398 type, and spa typing revealed the type t034. This type of S. aureus lineage has been found on pigs in the Netherlands, but the PVL positive genotype and resistance to tetracycline may point towards another origin. Interestingly, the MSSA strains still contain remnants of the SCCmec cassette. PCR amplification of the SCCmec cassette from the MRSA and MSSA strains yielded fragments of ~26 kb and ~11 kb in size, respectively. Sequence analysis and comparisons with known SCCmec cassettes revealed a type VII-like SCCmec cassette, which was named SCCmecUMCG. Sequence comparisons suggest that SCCmecUMCG may have resulted from recombination events.
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Cassette and was resistant to β-lactam antibiotics, erythromycin, for an MRSA isolate of sequence type ST225 and PVL negative, carried a SCC type V cassette and was resistant to β-lactam antibiotics, erythromycin, clindamycin, and enrofloxacin. The dog had regular contact to the dog owner's 85-year-old mother-in-law who lived in the same household and received nursing care at home because of an infected decubital ulcer on her foot. An MRSA isolate, indistinguishable from that of the dog, was isolated from the decubital ulcer of the dog owner's mother-in-law.

**Conclusions:** These two case reports show that MRSA strains of different sequence types are readily transferred between humans and pets living in the same household. The most likely route of transmission of the MRSA ST398 isolate in case 1 was from pigs to the veterinarian and subsequently from the veterinarian to his dog. In case 2, the mother-in-law appeared to be the source of the MRSA ST225 isolate. MRSA ST225 isolates have frequently been found in humans, either as colonizers or as cause of infection. They represented the second most frequently detected MRSA type among humans in Germany in 2007 and 2008 whereas they have not been detected so far in dogs.

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**7A**

TWO CASES OF TRANSMISSION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES BETWEEN HUMANS AND DOGS

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**Background:** During Sep 2007 - Jan 2008, dogs and cats admitted to the Small Animal Clinic of the University of Veterinary Medicine Hannover, Germany, were screened for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA). Swabs were taken from the nose and the pharyngeal region as well as from the perineum of dogs and cats before they entered the clinic. A questionnaire for background information on the sampled pet and the pet owner was completed on a voluntary basis. Of the 803 dogs and 117 cats sampled, three dogs were positive for MRSA. The MRSA isolates of the two cases for which sufficient background data were available were subjected to molecular analysis. **Methods:** The MRSA isolates were characterized by multilocus sequence typing (MLST), spa typing, as well as macrorestriction analysis with Smal and ApaI. Moreover, the isolates were subjected to PCR-directed SCCmec typing and detection of the PVL toxin genes lukS-PV and lukF-PV. The antimicrobial susceptibility status was determined by broth microdilution according to CLSI standards. **Results:** In case 1, a 6-month-old, healthy dog carried an MRSA isolate of multilocus sequence type ST398 and spa type t034 which was non-typeable by Smal but typeable by ApaI. The strain was PVL negative, carried a SCCmec type V cassette and was resistant to β-lactam antibiotics, erythromycin, clindamycin, and tetracycline. The dog owner, a specialist veterinarian in swine diseases, showed nasal colonization by a strain which showed the same characteristics and an indistinguishable ApaI macrorestriction pattern. In case 2, an 11-year-old dog was positive for an MRSA isolate of sequence type ST225 and spa type t014. The isolate was PVL negative, harboured a SCCmec type II cassette and was resistant to β-lactam antibiotics, erythromycin, clindamycin, and enrofloxacin. The dog had regular contact to the dog owner's 85-year-old mother-in-law who lived in the same household and received nursing care at home because of an infected decubital ulcer on her foot. An MRSA isolate, indistinguishable from that of the dog, was isolated from the decubital ulcer of the dog owner's mother-in-law.

**Conclusions:** These two case reports show that MRSA strains of different sequence types are readily transferred between humans and pets living in the same household. The most likely route of transmission of the MRSA ST398 isolate in case 1 was from pigs to the veterinarian and subsequently from the veterinarian to his dog. In case 2, the mother-in-law appeared to be the source of the MRSA ST225 isolate. MRSA ST225 isolates have frequently been found in humans, either as colonizers or as cause of infection. They represented the second most frequently detected MRSA type among humans in Germany in 2007 and 2008 whereas they have not been detected so far in dogs.

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**8B**

COMPARISON OF FINGERPRINTING METHODS FOR TYPING MRSA ST398

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Animal-associated methicillin-resistant *Staphylococcus aureus* (AA-MRSA) strains of clonal complex (CC)398 are not typeable using pulsed-field gel electrophoresis (PFGE) with Smal digestion, the gold standard for (MR)SA typing. For this reason, until now, sequence-based typing methods, such as multilocus sequence typing (MLST) and spa typing, have been used for typing AA-MRSA. Although these methods have the advantage of reliability and good inter-laboratory reproducibility, their discriminatory power is weak and, consequently, less useful for short-term epidemiological studies. This study evaluates various fingerprinting methods such as multilocus variable number tandem repeat assay (MLVA) and PFGE with restriction enzymes *Bst* II and *Apa* I. With each method, the MRSA ST398 isolates were characterized previously by multilocus sequence typing (MLST) and spa typing, and staphylococcal cassette chromosome *mec* typing (SCCmec). Typeability and discriminatory power were analyzed and concordance between the methods was determined. All MRSA ST398 isolates were typeable by MLVA and PFGE using *Bst*II, *Sac*II and *Apa*I. With each method, the MRSA ST398 isolates formed a separate group from two non-ST398 MRSA strains. PFGE, performed with any of the three restriction enzymes, had the most discriminatory power (D between 0.88-0.92), followed by MLVA (D=0.81), spa typing (D=0.74) and SCCmec typing (D=0.51). For this data set, the discriminatory index of PFGE with the restriction enzymes *Bst*II and *Apa*I was as high as the D1 of all three restriction enzymes together (D=0.97). Combining PFGE (using *Bst*II and *Apa*I) with spa typing had the most discriminatory power (D=0.98). MLVA showed the
9A PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ST398 ON BELGIAN PIG FARMS AND PIG FARMS WITH OTHER LIVESTOCK

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Recent studies indicate that methicillin-resistant Staphylococcus aureus (MRSA) ST398 is frequently found in livestock animals, especially pigs. In humans, MRSA ST398 is less frequently found, most of the time in humans who have contact with livestock animals. Epidemiological questions arise about its transmission within farms, between farms and from farms to the population. The present study is part of a larger project during which epidemiological routes will be studied on pig farms. Thirty farms (from which ten with only pigs, ten with pigs and poultry and ten with pigs and cattle) were screened for MRSA presence. Samples were taken from pigs (12-13 weeks old), poultry (5 weeks old) and cattle (6-12 months old). From pigs and cattle, a swab of the nares was taken of 10 animals. From poultry, one swab was taken from each chicken (10 in total) from the nose, ear and cloaca. Farmers were asked to give a nose swab on voluntary basis. The swab was brought into salt-enriched nutrient broth. After 24 hours of incubation, a loopful was plated onto a chromogenic selective medium for MRSA. Suspected colonies were confirmed as meticillin-resistant staphylococci (MRSA) by using a multiplex PCR for the presence of mecA, nuc and a S. aureus specific signature sequence of -16S rDNA after another 24 hours of incubation. Currently, the MRSA isolates are further typed by Pulse Field Gel Electrophoresis (PFGE). From each farm MRSA isolates of different kinds of animals and - if present - a sample of the farmer, were selected. Results demonstrate that all the pig farms with exclusively pigs were positive for MRSA. Eight out of ten pig-poultry farms were MRSA positive for the pigs. Of these eight MRSA positive farms in pigs, MRSA was isolated from the poultry in only one farm. None of the pig-poultry farms was MRSA positive for poultry and MRSA free for pigs. MRSA was isolated from pigs in eight of the ten pig-cattle farms. Of those eight MRSA positive farms, four farms were positive for MRSA in cattle. On one pig-cattle farm MRSA was isolated from cattle, but not from the pigs. PFGE will show if MRSA isolates of the various livestock animals and if possible the farmer are the same pulsotype. The results will be available at the conference. As demonstrated in other studies, the prevalence of MRSA in pigs is high on all types of farms. Interestingly, the prevalence of MRSA in other livestock, especially poultry is much lower. Maybe the prevalence of MRSA in poultry is flock dependent and therefore low.

10B PREVALENCE AND ANTIMICROBIAL RESISTANCE OF METICILLIN-RESISTANT STAPHYLOCOCCI OBTAINED FROM HORSES IN GREAT BRITAIN

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Background: Nasal carriage of meticillin-resistant staphylococci, including meticillin-resistant Staphylococcus aureus (MRSA), has been demonstrated in horses. The prevalence of carriage in the general horse population of the United Kingdom has yet to be established. Hypothesis: The prevalence of MRSA carriage is low in horses from the community in the UK. Methods: A cross-sectional study was conducted with nasal swab samples collected from 677 horses seen on routine visits by 65 randomly selected veterinary practices. Following enrichment in nutrient broth containing 6% NaCl, samples were cultured for staphylococci on mannitol-salt agar and screened for meticillin-resistant staphylococci (MR) on oxacillin-resistance screening agar. Isolates obtained were Gram-stained for morphology and subjected to catalase, staphylase (Pro-Lab, UK) and coagulase tests. Isolates confirmed as meticillin-resistant by antimicrobial disc diffusion were screened against a further ten antimicrobials in accordance with British Society for Antimicrobial Chemotherapy guidelines. Staphylase-positive isolates were confirmed as S. aureus by femA and mec PCR assays, and all meticillin-resistant isolates were subjected to mecA PCR. SCCmec typing was carried out via multiplex PCR on all MRSA isolates recovered and a selection of coagulase-negative staphyloccoci. MRSA isolates were subjected to spa typing. Results: In total, 942 staphylococcal isolates were obtained from the nasal samples of 617 of the horses. The prevalence of MRSA carriage was 31.2% (215 horses). Only four of these horses (0.6%) carried MRSA, with the remainder carrying meticillin-resistant coagulase-negative staphylococci (MR-CNS). Multidrug-resistance (to three or more antimicrobial classes) was identified in 78.4% of the MR isolates; with high prevalences of resistance to minocycline (31.0% of isolates), co-trimoxazole (29.2%), tetracycline (22.6%) and gentamicin (19.3%). The four MRSA isolates had differing multidrug-resistant profiles, all carried a SCCmec type IV cassette; two were spa type t604, one type 451 and the fourth unidentified. SCCmec types I and II have been detected in the MR-CNS isolates. API Staph™ (Bio Merieux, France) identification of thirty MR-CNS isolates has detected four species: S. zylopus, S. equorum, S. sciuri and S.
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also provides evidence suggesting that MRSA colonization in animals can occur between people and animals. Furthermore, this study demonstrates that interspecies transmission of human epidemic clones is possible.

This study shows the impressive ability of MRSA to colonize many animal species for extended periods. While colonization was present in one dolphin, as has been reported in many animal species; however, long-term (15 months) colonization was present in one dolphin. This study shows the impressive ability of MRSA to colonize various animal species and provides further evidence suggesting that interspecies transmission of human epidemic clones can occur between people and animals. Furthermore, this study also provides evidence suggesting that MRSA colonization in many animal species can be transient and that application of good infection control and hygiene measures may be critical control tools for the management of MRSA in animals.

12B MICROBIOLOGICAL CHARACTERIZATION OF STAPHYLOCOCCUS PSEUDINTERMEDIUS ISOLATES FROM DOGS

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Background: In dogs, Staphylococcus intermedius has traditionally been regarded as the predominant pathogenic Staphylococcus species and a leading cause of skin and soft tissue infections. However, it has been recently reported that most isolates identified conventionally as S. intermedius are truly the related species S. pseudintermedius. The objectives of this study were to determine the prevalence of S. pseudintermedius among isolates from infections from dogs that have been classified, phenotypically, as S. intermedius and determine the prevalence of selected virulence factors and methicillin-resistance of S. pseudintermedius isolates. Methods: Isolates from various infections in dogs that were phenotypically identified as S. intermedius were collected. Isolates were molecularly identified by sequence analysis of the sodA gene. For all isolates identified as S. pseudintermedius, genes for exfoliative toxins A (ETA) and B (ETB), S. intermedius exfoliative toxin (SIET), toxic shock syndrome toxin-1 (TSST-1), Panton-Valentine leukocidin (PVL) toxin, and methicillin-resistance (mecA) were investigated. Results: 102 isolates phenotypically identified as S. intermedius, were analyzed. 88/102 (86.3%) were molecularly identified as S. pseudintermedius. None were identified as S. intermedius. The SIET gene was detected in 84% (91/102) of S. pseudintermedius isolates. Genes for ETA, ETB, TSST-1, and PVL were not detected. The mecA gene was identified in 15.9% (14/88) isolates. 11/14 (78.6%) methicillin-resistant strains were phenotypically resistant to oxacillin and produced PBP2a. However, none were identified as resistant to cefoxitin. Conclusions: The re-classification of a large proportion of S. intermedius isolates as S. pseudintermedius provides additional support to the hypothesis that S. pseudintermedius is the predominant pathogenic Staphylococcus species in dogs. The SIET gene was common and its role in disease requires further study. The low rate of cefoxitin-resistance but high rate of oxacillin-resistance in methicillin-resistant strains is opposite to that reported for S. aureus and must be considered when developing testing regimens for methicillin-resistant S. pseudintermedius.
13A
HIGH METHICILLIN AND MULTIDRUG RESISTANCE RATES IN FRENCH STAPHYLOCOCCUS PSEUDINTERMEDIUS

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Background: S. pseudintermedius is one of the main skin-and-ear pathogen found in companion animals. Recent reports indicate that, in addition to being increasingly resistant to methicillin, it also frequently displays multidrug resistance patterns (Weese et al., Vet Microbiol 2009, in press). Moreover, the epidemiological importance of this bacteria is enhanced by its serious pathogenicity in animals and its potential transmissibility to humans. This study is the first one assessing the prevalence of resistance in S. pseudintermedius in France. Methods: In total, 145 S. pseudintermedius were isolated from sick cats and dogs between October 2007 and March 2009. They were characterised according to standard methods and the species was confirmed by amplification and restriction analysis of the pta gene (Bannoehr et al., J Clin Microbiol 2009, 47:469-471). Antibiotic resistance was determined by disk diffusion according to the recommendations of the Antibiogram Committee of the French Society for Microbiology. Penicillin and methicillin resistance genes (blaZ and mecA) were detected by PCR. Biofilm formation was assessed in 96-well plates. Results: Overall, 11 isolates (7.6%) contained the mecA gene and were considered as methicillin-resistant S. pseudintermedius (MRSP). Three were resistant to cefoxitin, 5 intermediate and 3 sensitive. All presented an associated multidrug-resistance pattern with the KTG phenotype and resistances to penicillin, enrofloxacin, lincomycin, erythromycin, spiramycin and tetracycline (all but one). Frequent and multiple resistances were also observed in non-MRSP, since 80% were resistant to at least one family of antibiotics and 67.6% to at least two. High prevalence of resistance was detected to penicillin (60% of phenotypic resistance, 89% of blaZ carriage), tetracycline (50.3%) and the macrolides (47.6%). No resistance was found to fusidic acid, pristinamycin, florfenicol, vancomycin and teicoplanin. The capacity to form biofilms was detected in 62% of the isolates, but did not correlate with a specific antibiotic pattern. Conclusion: Our results indicate that S. pseudintermedius can be considered as a serious pathogen of dogs and cats in France. Eleven MRSP were identified and multidrug patterns were observed for both MRSP and non-MRSP. Moreover, two thirds of the bacteria, mainly isolated from skin-and-ear infections, were capable of growing in biofilms. This might phenotypically increase their intrinsic resistance and thus threaten even more the success of antibiotic therapy. In the future, surveys on S. pseudintermedius (resistance and typing) combined with studies on transmissibility to humans are mandatory to assess the pathogenic and zoonotic capacities of these bacteria.

14B
METHICILLIN-RESISTANCE AND PREVALENCE OF ANTIBiotic RESISTANCE IN BOVINE MASTITIS IN FRANCE

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Background: Staphylococcus spp. are known to cause about one third of bovine mastitis, infections that cause major economic loss worldwide. Since cows might be a source of contamination either by milk drinking or by direct contact, we performed a survey on antibiotic resistance on bovine mastitis representative of the French cattle population. Methods: In total, 199 Staphylococcus spp. were isolated from bovine mastitis between the end of 2007 and the end of 2008. They were all characterised according to standard methods (catalase, coagulase, API20Staph). Antibiotic resistance was determined by disk diffusion according to the recommendations of the Antibiogram Committee of the French Society for Microbiology. PCR were performed to detect the mecA gene and a set of virulence-associated enterotoxin genes. Biofilm formation was assessed in 96-well plates. Results: Overall, 139 (70%) isolates were characterized as S. aureus and 60 as coagulase-negative staphylococci (CoNS). Only three strains were resistant to cefoxitin and the presence of the mecA gene was confirmed in one S. aureus (MRSA associated with resistances to kanamycin and tobramycin) and one CoNS (MRS associated with kanamycin, gentamycin, tobramycin, lincomycin, macrolides and marbofloxacin). The prevalence of resistances in non-MRS(A) strains was low, with 33.7% of resistances to penicillin, 12% to erythromycin, 10.5% to tetracycline and 7.5% to lincomycin. The prevalence of virulence genes and the ability to form biofilms was assessed on a subset of 30 S. aureus and 30 CoNS. At least one enterotoxin gene was detected in 18 isolates and the MRS(A) strain contained 7 of them. Besides, four CoNS could grow in biofilms. Conclusion: The prevalence of MRS isolated from French bovine mastitis is very low (1%), as well as the resistance rates to other families of antibiotics. Since staphylococcal mastitis are known to poorly respond to antibiotic treatment, we investigated the virulence of a subset of strains. The results indicate first that the capacity of forming biofilms was rare (4/60, 6.6%) and might not generate phenotypic resistance but, second, that 30% (18/60) of the isolates presented at least one virulence-related gene. Whether the presence of virulence-associated genes could explain the pathogenicity of such isolates remains to be determined. Yet, our results suggest that cattle mastitis do not constitute a high risk of transmission of resistance to humans, considering the overall low prevalence of antibiotic resistance.

15A
CARRIAGE OF METHICILLIN RESISTANT STAPHYLOCOCCUS PSEUDINTERMEDIUS IN DOGS

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Infections with methicillin resistant Staphylococcus pseudintermedius (MRSP) in dogs are increasing. It is not known if dogs continue to carry this organism following treatment or whether this same organism is the source of subsequent infections. The purpose of this study was to obtain cultures from carriage sites in dogs with active and previous MRSP infections and compare the carriage isolates to those isolated from active infection. Dogs were enrolled in the study if they had active infection with suspect methicillin resistant Staphylococcus sp. or if they had a history of infection with MRSP. Carriage cultures were obtained from nasal, oral, and anal mucosa in that order using a single sterile culturette. Staphylococcus isolates were identified
using conventional biochemical tests and antimicrobial susceptibility tests were performed by the Kirby Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Pulse field gel electrophoresis (PFGE) was performed on MRSP isolates in order to compare carriage organisms to those isolated from active infections. Twenty-six carriage cultures were obtained from 17 dogs with either a history of MRSP infection (10 dogs) or active infection (7 dogs). Six dogs (8/26 carriage cultures) had MRSP cultured from carriage sites. Of 7 dogs with active skin infection at the time of carriage culture, only 1 had MRSP isolated from both carriage and lesional sites. In 5/10 dogs without active infection, MRSP was isolated from carriage sites 1 to 5 months after the last active infection. PFGE showed greater than 96% homology among carriage organisms and pathogens in 5 dogs. For the 6th dog, an MRSP isolate from one active infection episode was different than the other carriage and pathogenic MRSP isolates and thus showed a 66.67% homology between clusters. In addition, the majority of the isolates showed >93% homology among the dogs. Results of this study show that dogs can continue to carry the same or very similar MRSP clones without active infection. Funding: Center of Excellence and Companion Animal Fund, University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee, USA

16B METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS: CORRELATION BETWEEN NASAL CARRIAGE AND ENVIRONMENTAL SAMPLES AT U.S. HORSE AND CATTLE FARMS

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Introduction: Increasing concern about livestock as a source and reservoir of methicillin-resistant Staphylococcus aureus (MRSA) is based primarily on reports from the European Union and Canada. In order to determine dissemination of MRSA in the farm environment and to confirm the origin of MRSA isolates, we have undertaken a study to evaluate correlation between MRSA isolates obtained from environmental sources and nasal swabs of horses and cattle at several farms in the Mid-Atlantic region of the United States. Materials and Methods: Nasal swabs from 13 racehorses, 26 pleasure horses, and 26 beef cattle from three farms were obtained. Environmental samples were taken from fencing or barn surfaces with sterilized dry Swiffer™ cloths. Samples were taken to the lab within four hours of sampling, and microbiologic analysis was conducted with a double-enrichment protocol and culturing on MRSA Select™ agar. Positive colonies were confirmed by PCR. Results of nasal swabs from individual animals and wipe samples taken at each animal’s local environment were compared via correlation analysis. Location of environmental sampling was determined by proximity to where an animal was housed. Results: On the pleasure horse farm and the cattle farm, none of the samples (nasal and environmental) were positive. On the racehorse farm, 8/13 (61%) nasal and 5/7 (71%) environmental samples were positive. Fourteen unique animal-environment pairs were available for analysis, seven from the negative farms and seven from the positive farm. Of these pairs, 11/14 (78%) were correlated (both negative or both positive), giving a chi-square value of 4.38 (p=0.04). In the three animal-environment pairs that were discordant, one was environment-positive and animal-negative, and two were environment-negative and animal-positive. Discussion: We observed significantly positive correlations between nasal carriage of MRSA in animals and in isolates from wipes of their local environments. Discordant samples may be related to persistence of MRSA in the environment that lasts longer than persistence of nasal carriage, to potential differences in sensitivity of nasal and environmental testing methods, or to cleaning or weathering of the environmental surface.

17A METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ST398 AND ST217 IN A VEAL AND A WILD BOAR FOOD SAMPLE RESPECTIVELY, IN SPAIN

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Recently, a new “livestock-associated” methicillin-resistant Staphylococcus aureus (MRSA) of clonal lineage ST398, has been reported at an increasing frequency, especially in Northern and Central Europe, and it has also been detected in America and Asia. Very few data exist about the prevalence MRSA ST398 in food samples. A previous work of our group detected MRSA ST398 in a food sample of pork origin and ST125 in two samples of rabbit and chicken origin (Lozano et al., 2009 5th Med-Vet-Net). The objective of our work was to determine the prevalence of MRSA in food samples from veal and wild animal origin, and to characterize the MRSA isolates. Methods: 66 raw food samples of animal origin [46 veal and 20 wild animal (8 game bird, 4 wild boar, 4 deer and 4 hare)] were obtained in 2008 in La Rioja (Spain) and were inoculated on ORS-AB plates (OXOID) for MRSA recovery. MRSA identification was confirmed by nuc and mecA genes PCR. The susceptibility to 16 antibiotics was determined by the disk diffusion method. MLST, SCCmec, spa and agrotyping, and the presence of antibiotic resistance genes [tet(K), tet(L), tet(M), ermA, ermB, ermC, msr(A), ant(4)’(4’), aph(3’)] and Panton-Valentine leucocidin (PVL) genes was performed by PCR. Amino acid changes in GrlA and GyrA proteins were also investigated. Results: Two MRSA strains were detected in this study, one among the 46 studied veal samples (2.2%) and the other among the 20 wild animal samples (5%). The strain from the veal sample showed resistance to tetracycline, erythromycin, clindamycin, tobramycin, kanamycin and ciprofloxacin, in addition to beta-lactams, and was typed as ST398-spa-t1197-SCCmecV-agrI, and carried the tet(K), tet(L), tet(M), erm(C), msr(A), ant(4)’-Ia and aph(3’) resistance genes. The MRSA strain from wild boar origin showed only resistance to beta-lactams and ciprofloxacin and was typed as ST217-spa-t0322-SCCmecVa-agrL. Both MRSA strains presented the amino acid changes Ser80Phe in GrlA and Ser84Leu in GyrA and were PVL negative. Conclusion: Although the prevalence of MRSA in food is low, it can represent a threat for humans. MRSA of different Sequence Types are detected in food samples of animal origin, and contamination of animal or human origin could be implicated. To our knowledge, this is the first study concerning the prevalence of MRSA in wild animals in Spain.
18B
MRSA ST398 IN SWITZERLAND: NOT ONLY COLONIZATION OF PIGS AND PIG WORKERS BUT ALSO COW MASTITIS

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Background: MRSA ST398 has been increasingly described primarily as a colonizer of pigs and pig workers. Curiously, human carriage of MRSA ST398 was epidemiologically linked to contact with veal calves even though, to our knowledge, this strain has been reported from cattle only once (cow mastitis). It is likely that ST398 has been associated with pigs since long time as MSSA and recent acquisition of SCCmec made this genotype more conspicuous. On the other hand, acquisition of SCCmec could have made it more capable of colonizing humans. To discern between these alternatives, more information on diversity, prevalence and host specificity of MSSA ST398 is needed.

Methods: The sampling was conducted in Western Switzerland in 2008 and 2009. S. aureus isolates from the following sources were analyzed: nasal swab isolates from 120 pigs and 35 pig workers; 430 cow mastitis isolates; 50 nasal swab isolates from cow farmers. The isolates were genotyped by AFLP and spa-typing, and selected isolates also by MLST. MRSA were confirmed by PCR. The type of SCCmec was determined with multiplex PCR. Results: MRSA ST398 was initially found on 4 farms: 2 pig farms (3 pigs and 1 pig worker); 1 cow farm without pigs (1 cow); 1 mixed cow-pig farm (2 farmers, 1 pig, 2 cows). Subsequently, mixed farm was investigated more thoroughly: all the cows, including those with low somatic cell counts (SCC) were screened. This resulted in the isolation of MRSA from 1 more farmer, 6 out of 6 pigs, and 5 out of 68 cows. 3 cows did not present any mastitis symptoms (low SCC). Among pig nasal isolates 42 % were MSSA ST398. 50% of S. aureus-positive pig workers were colonized with MSSA ST398. Conversely, none of the cow farmers had MRSA or MSSA ST398 with the exception of people working on the mixed pig-cow farm. MSSA ST398 was not found among cow mastitis isolates; however, apart from the mixed pig-cow farm with MRSA, cows with low SCC were not screened. All the isolates from a single farm were always identical to each other, but slightly different strains were present on different farms. Spa types t011, t034, and two new related types were found. MRSA contained SCCmec with ccr A2 and C (Type IV and V). Simultaneous colonization with isogenic MSSA and MRSA (identical AFLP patterns) was found in pigs and pig workers. Conclusions: MRSA ST 398 is not only capable of pig and human colonization but it can also successfully infect cows. The detection of MRSA ST398 in cow mastitis might be hindered by the fact that this strain was associated with low SCC. As suggested previously, MSSA ST398 frequently colonizes pigs and can be easily transmitted to pig workers. Simultaneous co-colonization with isogenic MSSA and MRSA suggests the possibility of local loss or gain of SCCmec. Relatively high diversity combined with low prevalence indicates that MRSA ST398 is emerging in Switzerland by multiple ways.

19A
HIGH-FREQUENCY OF CLINDAMYCIN ASSOCIATED RESISTANCE AMONG MRSA CC398 FROM BREEDING SWINE HERDS IN PORTUGAL

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Recent evidence of a community-acquired methicillin-resistant Staphylococcus aureus case of invasive infection caused by MRSA ST398 multiresistant strain of swine origin in a pig-farm worker in Cremona, Italy, reinforces the emerging problem of animal MRSA as a human occupational health hazard (Pan et al., 2009). The aim of this study was to characterize the antimicrobial susceptibility of MRSA strains isolated during the Portuguese European Union baseline study for the detection of MRSA in breeding pigs. Dust swabs were taken from 171 randomly selected breeding and production pig farms from different regions of Portugal. MRSA isolates were identified by polymerase chain reaction (PCR) for the meca gene (http://www.ccr-lar.eu), subjected to staphylococcal protein A (spa) (http://www.seqnet.org/) typing, and a new variant underwent multilocus sequence typing (MLST) (http://www.mlst.net). Isolates were also tested for the lukF/lukS genes encoding Panton-Valentine leukocidin (PVL). Antimicrobial susceptibilities were determined by minimal inhibitory concentration with DADE MicroScan® panels, and interpreted according to CLSI guidelines M31-A2 and M100-S17. Dust swabs from 21 herds tested positive for MRSA. The MRSA isolates from Portuguese herds were all identified as CC398 with spa types t108 (n=12), t011 (n=7), and t1255 (n=1), except for a newly identified ST398 spa variant named t4854. None of the MRSA isolates carried the PVL genes. Susceptibility testing revealed resistance to tetracycline in all MRSA isolates. Seven isolates of the 21 were resistant to clindamycin only and 6 both to clindamycin and erythromycin. Eight isolates were resistant to trimethoprim/sulfamethoxazole. Two strains were gentamicin resistant. One strain was resistant to all fluoroquinolones tested (ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin), and also chloramphenicol. All isolates were susceptible to mupirocin, fosfomycin, fusidic acid, rifampin, nitrofurantoin, linezolid, quinupristin/dalfopristin, teicoplanin and vancomycin. The occupational hazard for livestock associated MRSA colonization through the intensity of pig contact has been confirmed. Recent evidence-based guidelines for the prophylaxis and treatment of human MRSA skin and soft tissue infections (Gould et al., 2009) include the use in monotherapy or eradication therapy of tetracyclines, clindamycin and trimethoprim/sulfamethoxazole. The high frequency of resistance found in this study towards these antimicrobials in CC398 MRSA of animal origin is important. Further studies are required to elucidate the mechanism of resistance towards clindamycin with macrolide susceptibility in these isolates. The emergence of associated resistance in MRSA animal isolates is a concerning fact for the pig industry, compromising antimicrobial therapy of possible cases.
Methicillin-Resistant Staphylococci in Animals

20B
DISCOVERY AND CHARACTERIZATION OF A NOVEL BOVINE ASSOCIATED
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATE

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During a project evaluating the use of network analysis as a tool to predict the spread of bovine mastitis between UK dairy herds, milk samples were collected from 45 dairy farms in the west of England. As part of this project the mastitis pathogen, Staphylococcus aureus was isolated from the majority of the farms and strain typed using two techniques, multi-locus sequence typing (MLST) and random amplified polymorphic DNA (RAPD)-polymerase chain reaction (PCR). A collection of 176 different S. aureus isolates was screened for antimicrobial susceptibility using the disc diffusion method established by the British Society for Antimicrobial Chemotherapy (BSAC) for identification of methicillin resistant S. aureus (MRSA). The screening initially identified two S. aureus isolates from a single farm that demonstrated resistance to oxacillin and cefoxitin. While both isolates shared the same MLST sequence type (ST 425), they belonged to two different RAPD strain groups. After the initial discovery of the antibiotic resistant phenotype, a PCR assay was performed to look for the presence of mecA. This assay yielded a negative result. Subsequently additional phenotypic and genotypic tests were performed to investigate the characteristics of these bovine S. aureus isolates. Both test isolates presented oxacillin and cefoxitin MICs over the standard thresholds for resistance and they grew on chromogenic MRSA-screening agar (MRSA ID agar). They were, however, susceptible to a number of antimicrobial agents other than β-lactam antibiotics, as determined by disc diffusion testing. A negative result was obtained for detection of the PBP2a using a rapid latex agglutination test. In addition, Southern hybridization was performed as a more sensitive test to identify the presence of mecA and no evidence of this gene was found in the two bovine isolates. Both test isolates generated positive results for the Panton-Valentine leukocidin (PVL) using an established real-time PCR assay. Genome sequencing of one of the isolates revealed the presence of a novel staphylococcal cassette chromosome mec (SCCmec), carrying a divergent mecA homologue. A new PCR protocol has been developed and validated using the new SCCmec sequence to identify both existing mecA types and the divergent mecA. It is of concern that well-established PCR detection techniques failed to reveal the existence of the mecA gene present in this strain. Further investigations are underway to establish the distribution of this new mecA type and search for further variants of SCCmec and mecA within farm animal populations.

21A
FIRST DESCRIPTION OF AN MRSA SKIN INFECTION IN A DOG AND ATTENDING VETERINARIAN IN PORTUGAL

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Methicillin-resistant Staphylococcus aureus (MRSA) have been increasingly reported in veterinary medicine. We hereby report a case of an MRSA skin infection associated with hepatocutaneous syndrome (HS) in a geriatric female dog, as well as the colonization of one of the attending veterinarians. An eleven-year-old female Labrador Retriever presented five years ago at a veterinary hospital in the Lisbon area with a chronic right forelimb pododermitis and footpad hyperkeratosis. Leishmaniosis was diagnosed. Two episodes of acute onset hepatitis developed during leishmaniosis treatment with allopurinol and levamisol. Cutaneous lesions became exudative, ulcerative and purulent. Several courses of antimicrobial therapy allowed temporary remission. The infection extended to the other paws and anogenital skin. A fistulae swab was cultured. S. aureus was isolated in predominant culture along with Enterobacter cloacae. Antimicrobial susceptibilities were determined by the disk diffusion method and by minimal inhibitory concentration with DADE MicroScan® panels and interpreted according to CLSI guidelines M31-A3 and M100-S17. MRSA nasal carriage by the attending vet was evaluated three weeks after exposure. The two MRSA isolates were identified by PCR for the mecA gene, subjected to staphylococcal protein A typing, staphylococcal cassette chromosome mec typing, and underwent multilocus sequence typing. Isolates were also tested for the lukF/lukS genes encoding Panton-Valentine leukocidin (PVL). The animal and human MRSA isolates were identified as ST22, spa type t032 and SCCmec IV. None of the MRSA isolates carried the PVL genes. The isolates were also resistant to fluoroquinolones (enrofloxacin, marbofloxacin, ciprofloxacin, levofloxacin) and susceptible to aminoglycosides (gentamicin, tobramycin, netilmicine, amikacine), chloramphenicol, trimethoprim-sulfa-methoxazole, and vancomycin. The E. cloaca was resistant to amoxicillin, amoxicillin-clavulanate, cephalexin, cefuroxime and tetracycline. Treatment with gentamicin 5 mg/kg IM sid was carried out for five days. Although therapy was performed the cutaneous infection aggravated and clinical condition worsened. The owner asked for euthanasia. MRSA are an important cause of human nosocomial infections in Portugal. It seems that this dog’s MRSA infection may be community-acquired, possibly through the owner or transmitted through the environment. The severity of the footpads skin lesions, associated with the HS and the previous antimicrobial usage may have predisposed to the MRSA infection. To our knowledge, this is the first report of the epidemic clone EMRSA-15 in a dog in Portugal.
NASAL CARRIAGE OF METHICILLIN-RESISTANT COAGULASE-POSITIVE STAPHYLOCOCCI AMONG CATS AND DOGS HOSPITALIZED IN A VETERINARY TEACHING HOSPITAL IN PORTUGAL

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Many authors have emphasized the importance of methicillin-resistant staphylococci carriage in companion animals, as these bacteria are emerging as a significant problem in veterinary medicine, including both animal and public health standpoints. The objective of this study was to investigate the frequency of methicillin-resistant S. aureus (MRSA) and methicillin-resistant S. pseudointermedius (MRSP) carriage in a random sample of 40 cats and 146 dogs hospitalized in the Faculty of Veterinary Medicine Teaching Hospital, Lisbon. Nasal swabs were collected at the time of patient admission to the hospital and inoculated in 3 ml of an enrichment broth (Weese et al., 2006). After overnight incubation, 10 μL of bacterial suspension were inoculated in Columbia 5% blood sheep agar plates. Colonies were identified as coagulase-positive staphylococci based on colony morphology, ability to cause hemolysis, Gram staining, positive catalase test, positive tube coagulase test and BBL Crystal™ typing system. MRSA and MRSP carriage was screened by plating enrichment cultures on a selective medium, Chrom MRSA ID (bioMérieux, La Balme Les Grottes, France) and suspected colonies were confirmed by PCR identification of mecA (http://www.crl-ar.eu). Presumptive S. aureus isolates were confirmed by PCR amplification of the nuc gene and discrimination between S. pseudointermedius and S. intermedius was done by restriction fragment length polymorphism (RFLP) analysis of pta. All isolates were tested by PCR for the presence of lukF/pa1K genes encoding Panton-Valentine leukocidin (PVL). MRSA was only found in two cats (5%) and one dog (0.6%). Nine dogs carried MRSP (6%), whereas none of the cats was found to be positive. All isolates were PVL-negative. The results indicate that the relative risk of MRSA and MRSP carriage might vary between the two host species, with MRSP being more frequent among dogs and MRSA among cats. The observed MRSA carriage rates were not surprising considering the high prevalence of MRSA in humans in Portugal. As sampling was performed immediately after admission to the hospital, the observed MRSP carriage rate is likely to reflect the prevalence among dogs in the community. MRSP were first described in Europe in 2006 and the relatively high MRSP carriage rate observed in dogs suggest that these bacteria are quickly spreading in the canine population of this country.

DINAMIC OF MRSA ST398 NASAL COLONIZATION AFTER MUPIROCIN TREATMENT IN A PIG-ASSOCIATED FAMILY PREVIOUSLY COLONIZED BY MRSA ST398, IN SPAIN

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An skin infection caused by both MRSA ST398 and ST1 isolates was previously reported in the daughter of a pig farmer in Spain and all the four family members were colonized by ST398 and/or ST1 variants [patient daughter (ST398-t108), mother (ST398- t108 and ST1-t127), father (ST398-t108), and brother (ST398 -t011)]. (Aspiroz et al., ESCMID-2009). This family lived very close to the pig farm in which the father and the mother worked. The objective of our study was to analyze the dynamic of MRSA colonization in the four family members after a decontamination treatment with mupirocin (1 dose/12 h during 7 days). Methods. Eight nasal swab samples were taken to each of the four family members at different moments after mupirocin decontamination treatment: 1) immediately after treatment; 2) 48 h; 3) four days; 4) one week; 5) three weeks; 6) two months; 7) three months; and 8) eight months. Nasal swabs were inoculated into ORSA agar (Oxoid) blood agar and CNA plates for MRSA recovery. The presence of nuc and mexA genes were determined by PCR and the antibiotic susceptibility test was carried out by VITEK 2 system (BioMérieux) and disc diffusion agar. MLST, SCCmec, spa and agr-typing was performed by PCR, and the presence of genes encoding Panton-Valentine leucocidin (PVL) and antibiotic resistance mechanisms was analyzed by PCR. Results. Nasal swabs of all family members of samples 1 to 4 (up to one week after mupirocin decontamination) were negative for MRSA. Nevertheless, MRSA was detected in samples 5 to 8 in the mother (ST398-t011, ST398-t1255, ST398-t1197, ST1-t127), and in the father (ST398-t108, ST398-t1255). In case of the daughter, samples 5 and 7 were negative for MRSA, but samples 6 and 8 were positive for MRSA (ST1-t127). The brother was negative in all 8 samples for MRSA. All ST398 isolates were typed as SCCmecV-agrI and ST1 isolates as SCCmecI-agrII, being all MRSA negative for PVL. All ST1-t127 isolates contained tet(K), tet(L), erm(A, ermB, ermC), mecA, aph(2’)-accl(), ant(4’)-la, and aph(3’)-Ia, and the amino acid changes Ser80Phe and Ser84Leu in GrlA and GyrA, respectively. Some ST398-t011 and ST398-t1197 isolates also presented a multiresistant phenotype to tetracycline, macrolides, aminoglycosides and sulfamethoxazole-trimethoprim. Conclusion. The efficacy of nasal MRSA decolonization in persons with very close contact with pigs (as workers in a pig farm) is low, and MRSA ST398 and/or ST1 recolonization occurs after several weeks of decolonization. The efficacy seems to be higher in persons with sporadic contact with animals or with contact with MRSA colonized humans. MRSA isolates involved in recolonization belong to different spa types of ST398. The potential transmission of ST1 from animals to humans could also be suggested in this study.
The objective of this work was to determine the prevalence colonization by MRSA among adult swine and piglets at slaughter in Spain, and to characterize the recovered isolates.

**Methods:** Nasal swabs (NS) were taken from 53 adult swine and 53 piglets at two different slaughterhouses in La Rioja region (Spain) between September 2008 and March 2009, coming from 6 different farms (2 farms: adult swine; 3 farms: piglets; 1 farm: adult swine+piglets). Simultaneous skin swabs (SS) were also taken from the 53 piglets. Samples were inoculated into Brain-Heart-Infusion broth supplemented with 6.5% NaCl, incubated 37°C for 24h, and then streaked on ORSAB agar plates (37°C for 24-48 h). One MRSA isolate per sample (or up to three if they presented different antibiotic resistance profiles) was selected for further studies. MRSA identification was confirmed by detection of *mec* and *ermA* genes by PCR. Susceptibility for 16 antibiotics was tested by disk-diffusion agar. MLST, SCCmeC, and spa-typing was performed by PCR and sequencing. The presence of *ermA, ermB, ermA, ermA, msrB, linA, tetK, tetL, tetM, tetO, ant(4′)-Ia, aph(3′)-III, aac(6′)-le-aph(2′), ant(6′), ant(3′)-(9) and dfrA genes as well as Panton Valentine leukocidin (* lukF/lukS*) genes were tested by PCR.

**Results:** MRSA was detected in 11 of 53 adult swine (20.7%) coming from three tested farms and 14 MRSA isolates were obtained (11 isolates ST398 and 3 non-ST398). The following *spa* types were identified (number of isolates): t011 (5), t108 (6), and t3992 (3, which presented a new sequence type, ST1379). MRSA recovered from adult swine were typed as SCCmeCA (5 isolates) or SCCmeCV (9). Isolates SCCmeCV showed resistance to more antibiotics than those SCCmeCA. All SCCmeCV isolates harbored the *aac(6′)-le-aph(2′)* gene. On the other hand, MRSA was detected in 26 of 53 piglets analyzed (49%) obtained from three of the four tested farms. Twenty-per-cent of piglets were positive for MRSA on NS, 15% only on SS, and 15% for both NS and SS. A total of 30 MRSA were obtained from the 26-positive piglets. Of them, 28 isolates were typed as t011-SCCmeCV, one isolate t2346-SCCmeCV, and one isolate t1979-SCCmeC-non-typable. The *spa* types t011, t1197 and t2346 are within the ST398 group. Isolates t011 and t2346 showed a narrow resistance phenotype (one or two families of antibiotics, in addition to beta-lactams), however isolate t1197 presented a multi-resistant phenotype, harboring *tet(M), ermA* and ant(4′)-(4) genes. All MRSA studied were negative for PVL, genes. **Conclusion:** MRSA ST398 of different *spa* types is frequently detected among slaughter pigs in La Rioja (Spain), piglets showing a higher carriage rate and adult swine isolates a wider resistant profile. These microorganisms could be transmitted to humans, especially to persons in contact with these animals, and also could enter into the food chain representing a threat for human health.
found positive as well.

26B
DIAGNOSTIC VALIDITY OF POOLING ENVIRONMENTAL SAMPLES TO DETERMINE THE STATUS OF SOW-HERDS FOR PRESENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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Due to (inter)national approval of publication of these data, submission will be delayed. I already announced this by e-mail to Mireille Wulf (delegate of the scientific committee of the conference).

27A
UPDATE ON DRU-TYPING.ORG: AN INTERNET RESOURCE FOR THE SEQUENCE-BASED TYPING OF METHICILLIN-RESISTANT STAPHYLOCOCCI ANALYZING THE HYPERVARIABLE MEC-ASSOCIATED DIRECT REPEAT UNIT

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Background: Variable-number tandem repeat (VNTR) sequences have found important use in the epidemiological typing of problem bacterial pathogens. With Staphylococcus aureus, sequence analysis of the polymorphic X region of the protein A gene (spa), coupled with a uniform system of nomenclature, has resulted in a robust method for epidemiological analysis. In methicillin-resistant staphylococci the direct-repeat unit (dru) VNTR region adjacent to IS431 in SCCmec has also proved useful in the epidemiological analysis of highly uniform epidemic strains (e.g., EMRSA15 and -16) and in tracking the horizontal movement of SCCmec. Recently, more efficient use of dru typing has been facilitated by a proposed uniform system of nomenclature (Goering et al., 2008; Clin. Microbiol. Infect. 14:964-69). Optimum use of this typing approach is now facilitated by a convenient Internet-based means where newly generated data can be cataloged and compared in an internationally shared database. Methods: The establishment of a new Internet-accessible database freely available at http://www.dru-typing.org/search.php. Results: The dru-typing.org website allows investigators to enter user generated 40-bp repeat sequences which are then searched against the current database of dru repeats and identified, if known. Specific combinations of repeats may also be queried against the database and, if recognized, the resulting dru type will be identified. New dru repeat and/or dru type chromatograms can be submitted online for verification and inclusion into the database. At present, the growing database contains 52 different dru-repeat sequences and 151 dru types examples of which are found in staphylococcal strains of both human and animal origin and which can be downloaded for off-line reference. Conclusions: dru-typing.org represents the first freely-available Internet-accessible database for collecting and harmonizing dru-repeat and dru-type sequences. The website provides an interface which should assist in standardizing and facilitating the use of dru typing as a tool in the epidemiological and evolutionary analysis of methicillin-resistant staphylococcal strains.

28B
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS COLONIZATION OF VETERINARY PERSONNEL AT A SURGICAL CONFERENCE

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Objectives: Some studies of veterinary personnel and other individuals with animal contact have reported high rates of MRSA colonization, but further information about colonization of different groups and evaluation of factors associated with colonization is required to better understand zoonotic MRSA transmission and develop control programs. This study evaluated the prevalence of and risk factors for MRSA colonization among attendees of a veterinary surgeon specialty conference. Methods: Nasal swabs were collected from volunteers at the 2008 conference of the American College of Veterinary Surgeons in San Diego, USA. Enrichment culture was performed and isolates were characterized using standard techniques. Results: 341 individuals from 12 countries participated. MRSA was isolated from 59/341 (17.3%, exact 95% CI 13.4-21.7%) individuals; 53/308 (17%) veterinarians and 6/33 (18%) technicians (P=0.81). In the multivariable model, contact with small ruminants in the preceding 30 days (OR 2.2, 95% CI 1.1-5.6, P=0.032), having another person in their residence diagnosed with MRSA in the preceding year (OR 19.8, 95% CI 1.9-203, P=0.012) and working in a clinic where there is a specific person in charge of the infection control program (OR 1.9, 95% CI 1.1-3.5, P=0.035) were associated with colonization. The most common MRSA strain was spa type t002 or related types, which were PVL negative and accounted for 32 (54%) isolates. 16 (27%) were spa type t064 or related and PVL negative. 8 (14%) were t018 or related and PVL negative. 2 (3.7%) were t379 and PVL negative. 1 (1.9%) was t008 and contained PVL genes, consistent with the USA300 clone. Most individuals carrying spa type t002 or related were from small animal practices, while most people harboring t064 were from equine practice. ST398 strains were not isolated. Discussion: The colonization rate in this study was striking and provides further support suggesting MRSA exposure is an occupational risk for veterinary personnel. Unlike earlier studies, there were no differences between small animal and large animal personnel. The association of MRSA and small ruminant contact was unexpected and requires further investigation. The association with a person in charge of infection control presumably reflects the fact that facilities with infection control personnel at inherently at higher risk as opposed to a negative impact of infection control programs.
Background: To our knowledge the transmission of MRSA between humans and animals (dogs, horses, and pigs) has been reported, but rarely between cows and humans. This study attempts to investigate the transmission possibility among human and cows in Nigeria. Methods: To assess the spread, transmission rate and genetic characteristics of S. aureus and other Staphylococcus spp. in Southwest Nigeria, we investigated 351 isolates from clinical specimens (wound, postsurgical, conjunctival and otic swabs) including 70 MRSA isolates. The isolates were characterized by antibiogram analysis, spa typing, multilocus sequence typing (MLST), agr typing and toxin gene analysis; for MRSA isolates staphylococcal cassette chromosome mec (SCCmec) typing was performed. Results: The PVL gene was detected in methicillin-susceptible S. aureus (MSSA) and MRSA human isolates. PVGl-positive MSSA strains (n=93 of total 276) were found to belong to sequence types ST30 (CC30; n=33) and agr group 3. These types were different from those determined for MRSA ST88 which was PVG- and typed as agr group 3, ST250 (n=30) and ST241 (n=7): agr groups 1 and 2. PVGl-positive MSSA possessed a larger number of virulence factor genes (superantigens and hemolysins) than MRSA, although they were susceptible to more antimicrobials. Major antibiotic resistance profile was penicillin- cotrimoxazole-tetracycline for MSSA and penicillin-oxacillin- lysins) than MRSA, although they were susceptible to more antimicrobials. Major antibiotic resistance profile was penicillin- cotrimoxazole-tetracycline for MSSA and penicillin-oxacillin-
lysins) than MRSA, although they were susceptible to more antimicrobials. Major antibiotic resistance profile was penicillin-
doxycline for MSSA and penicillin-oxacillin-
lysins) than MRSA, although they were susceptible to more antimicrobials. Major antibiotic resistance profile was penicillin-

Conclusions: The findings suggest that the PVL gene is distributed to limited populations of S. aureus clones with specific genetic traits that are distinct from MRSA in Nigeria, but genetically close to CA-MRSA clones in the CC30 lineage reported in the United States, Oceania and European countries. ST88 was the most prevalent CA-MRSA in Ibadan community besides the HA-MRSA clones ST250 and ST241. The risk for spread of MRSA from bovine sources into the human population is low and could not be demonstrated in this study. Generally, persons are not at risk as long as raw milk is not consumed. However, persons in close contact with MRSA-infected cattle, including farmers, milkers, and persons working at slaughterhouses, may become colonized from the bovine source.
showed the occurrence of MRSA in 80% of the pig herds regularly visited by the practice. The five “trial” veterinarians wore gloves and respiratory masks (protecting against dust) for at least 30 farm visits or 30 days respectively. The two “control” veterinarians wore only gloves as regular routine of the veterinary practice. Nasal swabs were collected at a seven day interval and examined microbiologically including MLST and spa-typing (RK1). Ten masks per “trial” veterinarian were bacteriologically tested for MRSA after the herd visit. After 30 herd visits, the “trial” veterinarians worked without respiratory masks and were tested again after one week working without a mask. Results: The study showed a high MRSA-exposure for the veterinarians, since 68% of the masks (n=50) worn by the “trial” veterinarians were tested positive for MRSA. However, all 5 “trial” veterinarians stayed negative for two weeks, and only one of the “trial” veterinarians became positive after two weeks wearing the masks. Four vets didn’t return to MRSA-positive results while using the masks. After no masks were worn any more, two vets returned to colonisation soon. It took 5 respectively 6 farm-visits to become re-colonised. Further samples showed colonisation to be inconsistent. “Control” veterinarians turned positive after 26 and 54 days. Conclusions: ST 398 is wide spread in the pig farms visited regularly by the study vet practice and the high finding-rates of MRSA in the masks proof an enormous risk of nasal colonisation during routine work in pig herds. The results of the presented study show the potential of respiratory masks to prevent, at least to minimise the exposure of veterinarians to MRSA, provided the masks are used only once. Further details of the proper use of masks and the quantification of their protective potential need further studies on a larger scale.

32B
GENETIC BASIS OF MACROLIDE-LINCOASAMIDE RESISTANCE IN PORCINE MRSA ST398 ISOLATES: FIRST DETECTION OF THE GENE ERM(T)

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Background: Combined resistance to macrolides and lincosamides (ML antibiotics) in staphylococci is most frequently mediated by erm genes which code for rRNA methylases. Porcine methicillin resistant Staphylococcus aureus (MRSA) ST398 isolates have been reported to be resistant to ML antibiotics at varying frequencies. A recent study from Germany identified 24/54 (44.4%) porcine MRSA ST398 isolates as resistant to ML antibiotics. Analysis of these isolates identified the resistance genes erm(A), erm(B) and erm(C) alone or in different combinations in all but one isolate. In the present study, the molecular basis of the remaining ML-resistant ST398 isolate was investigated.

Methods: The MRSA ST398 isolate was investigated for its MIC values of the macrolides erythromycin, spiramycin, tilmicosin, tylosin, tulathromycin, and the lincosamides clindamycin and pirlimycin. PCR analysis for the ML resistance genes so far described to be present in staphylocoocci was performed. Plasmids were isolated and protoplast transformation into S. aureus RN4220 with subsequent selection on erythromycin was performed. Restriction fragments of the transformed plasmid were cloned and sequenced. Results: The MRSA ST398 isolate showed high MIC values of all ML antibiotics tested, but was negative in the PCR assays. Transformation experiments revealed that ML resistance was associated with a ca. 40-kb plasmid, designated pKKS25. Further investigation of the pKKS25 transformant showed that this plasmid also mediated resistance to tetracycline and trimethoprim via the genes tet(L) and dfrK, respectively. Sequence analysis of this plasmid confirmed that ML resistance was conferred by the rRNA methylase gene erm(T). The gene erm(T) was located in the opposite orientation 1.5 kb downstream of the tet(L)-dfrK cluster. Analysis of the erm(T) flanking regions showed identity to the erm(T) region of a Streptococcus pyogenes plasmid in a stretch of 123 bp upstream and 139 bp downstream of erm(T). The erm(T) gene of pKKS25 was expressed constitutively due to a 57-bp deletion in the erm(T) translational attenuator. The region harboring the genes erm(T), tet(L) and dfrK was flanked by two copies of the novel insertion sequence ISSau10. This insertion sequence consists of 793 bp, and shows 87% - 88% identity to IS431 or IS257. The transposase of 224 amino acids exhibits 92 - 93% amino acid identity to those from IS431 or IS257. The novel IS element carries inverted repeats 5’-GGTTCTGTT-GCAAGT-3’ at both termini.

Conclusions: This is the first report of the presence of erm(T) in staphylococci. Since erm(T) has previously been described in streptococci and lactobacilli, the finding of erm(T) in an MRSA strain underlines the presence of a gene flux between these bacteria and staphylococci. The observation that the erm(T)-dfrK-tet(L) gene region was flanked by IS elements suggests the mobility of this resistance gene region.

33A
MOLECULAR ANALYSIS OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATES FROM CASES OF MASTITIS IN DAIRY CATTLE

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) isolates have been identified in pet and companion animals, but also in food-producing animals. In contrast to the wealth of data on MRSA ST398 in swine, comparatively little is known about MRSA associated with bovine mastitis. Such strains occur very rarely in veterinary routine diagnostics. The aim of the present study was to investigate mastitis-associated MRSA isolates for their genomic relationships and their resistance properties.

Methods: During November 2008 to January 2009, six MRSA isolates from independent cases of bovine mastitis were identified in Lower Saxony. The isolates were subjected to PCR-directed SCCmec typing, macrorestriction analysis with SmaI and ApaI, and spa typing. Susceptibility testing was conducted by broth microdilution. Resistance genes were detected by specific PCR assays and checked for plasmid location by protoplast transformation into S. aureus RN4220.

Results: The six strains carried the gene mecA on SCCmec type V cassettes in four isolates and on a type IV,1 cassette in one isolate. The SCCmec type of the remaining isolate could not be determined by the PCR assays used. All six isolates had related ApaI-macrorestriction patterns differing in up to 9 bands, but were non-typeable by SmaI macrorestriction analysis. The results of spa typing showed t011 in five isolates and t034 in the remaining isolate. Susceptibility testing revealed that in
addition to \( \beta \)-lactam resistance, all six isolates were resistant to tetracycline via the gene \( \text{tet}(\text{M}) \), either alone or in combination with \( \text{tet}(\text{K}) \) and/or \( \text{tet}(\text{L}) \). Three isolates were resistant to macrolides/lincomamides and carried the genes \( \text{erm}(\text{C}), \text{erm}(\text{C}) + \text{erm}(\text{A}), \) or \( \text{erm}(\text{B}) \). Trimethoprim resistance was detected in four isolates with three of them harbouring the novel trimethoprim resistance gene \( \text{dfrK} \). Gentamicin resistance was detected in a single isolate which carried the gene \( \text{aacA}/\text{aphD} \). Small \( \text{erm}(\text{C}) \)-harboring plasmids of ca. 2.5 kb, a \( \text{tet}(\text{K}) \)-carrying plasmid of about 4.4 kb, and in two of the isolates harbouring \( \text{dfrK} \) larger plasmids which carried \( \text{tet}(\text{L}) \) and \( \text{dfrK} \) were identified. Based on their pheno- and genotypic characteristics, the six MRSA isolates from bovine mastitis closely resembled porcine ST398 isolates. Further inquiries at the respective farms revealed that pigs were kept in three farms and that the milker of a fourth farm worked part-time in a swine production unit. No obvious connection to swine production was seen in the remaining two farms. **Conclusions:** The finding of ST398-like MRSA isolates in cases of bovine mastitis was unexpected, but might be explained by either the presence of pigs on the same farms or the occupational exposure of the milker to pigs. These observations suggest a transfer of such strains between different animal species including humans.

### 34B

**NON-CFR-MEDIATED PLEUROMUTILIN RESISTANCE IN A PORCINE MRSA ST398 ISOLATE CONFERRED BY THE NOVEL ABC TRANSPORTER VGA(C)**

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates often exhibit resistance to several classes of antimicrobial agents in addition to \( \beta \)-lactam antibiotics. However to date, pleuromutilin resistance has been observed very rarely among MRSA isolates. So far, a single gene, \( \text{cfr} \), has been identified in MRSA isolates from animals and humans to confer pleuromutilin resistance. The gene \( \text{cfr} \) codes for a RNA methyltransferase which also confers resistance to penicillins, lincomamides, oxazolidinones and streptogramin A. In this study, we analyzed the multiresistance plasmid pKKS825 of a porcine MRSA ST398 isolate which does not carry \( \text{cfr} \) but mediates pleuromutilin resistance. **Methods:** Plasmid pKKS825 was transferred by protoplast transformation into *S. aureus*. The plasmid was sequenced completely by primer walking and analyzed for the resistance genes present. MIC testing was conducted by broth micro- or macrodilution according to the CLSI document M31-A3. **Results:** Plasmid pKKS825 was 14,364 bp in size and carried three already known resistance genes: \( \text{dfrK} \) for trimethoprim resistance, \( \text{tet}(\text{L}) \) for tetracycline resistance, and \( \text{aadD} \) for kanamycin/neomycin resistance. These three resistance genes were organized in a cluster related to the \( \text{tet}(\text{L}) \)-\( \text{dfrK} \) cluster recently described on plasmid pKKS2187 from a porcine MRSA ST398 isolate. Furthermore, a fourth resistance gene, designated \( \text{vga}(\text{C}) \) was detected. It encoded an ABC transporter of 523 amino acids (aa). Structural comparisons identified closest similarity to the similar sized Vga(A) variants of staphylococci (62.3% - 65.5% aa identity) and only 39.2% aa identity to Vga(B). The Vga(C) protein revealed the typical features of a class 2 ABC-transporter consisting of two ATP-binding cassettes each with the Walker A and B motifs as well as the signature motif involved in ATP binding and hydrolysis. The Vga(C) protein conferred resistance to streptogramin A antibiotics (virginiamycin M1), lincomamides (clindamycin, lincomycin, pirlimycin), and pleuromutilins (tiamiculin, valnemulin) as confirmed by comparative MIC testing. This finding was in agreement with the observation that some of the Vga(A) variants also mediate reduced susceptibility or resistance to lincomamides and/or pleuromutilins in addition to streptogramin A resistance. **Conclusions:** This study describes a novel resistance gene \( \text{vga}(\text{C}) \) coding for an ABC transporter which exports streptogramin A as well as lincomamides and pleuromutilins. Moreover, the \( \text{vga}(\text{C}) \) gene was co-located on a plasmid which also carried a multiresistance gene cluster consisting of \( \text{aadD}, \text{tet}(\text{L}), \) and \( \text{dfrK} \). This co-localization might facilitate the dissemination and persistence of \( \text{vga}(\text{C}) \) in the presence of a selective pressure by tetracyclines or trimethoprim which are used at distinctly higher quantities than streptogramin A antibiotics, lincomamides or pleuromutilins.

### 35A

**COMPARISON OF VIRULENCE FACTORS HARBOURED BY METHICILLIN RESISTANT S. AUREUS OF ANIMAL AND HUMAN ORIGIN BY MICROARRAY**

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**Background:** In Belgium, molecular epidemiological surveillance showed that 90% of methicillin resistant *S. aureus* (MRSA) strains belong to the international pandemic lineages of MLST Clonal Complex (CC) 45, CC8, CC5, CC22, CC80 and CC30. Recently, ST398 MRSA strains have been recognized as agent of infection or colonization in horses, swine, poultry, bovines and humans. Data on genomic content and virulence gene repertoire of these livestock associated strains are limited. **Objective:** The present study aimed to characterize, by the DNA-microarray technique, the repertoire of virulence genes possessed by ST398 MRSA strains compared with strains belonging to « human-associated » MRSA epidemic clones. **Methods:** Representative strains of the most frequent hospital acquired (HA) and community acquired (CA)-MRSA epidemic clones (n = 26) from Belgium were selected from the National Reference Laboratory for Staphylococci collection. ST398 strains (n = 18) were selected from a wide range of host species (horses, swine, poultry, cattle and human). These strains were characterized by a microarray designed from eight *S. aureus* sequenced genomes and composed of 390 oligonucleotide probes targeting resistance (37%), virulence (31%) and adhesion factors (32%). **Results:** 50% of genes tested displayed a variable distribution among strains. Each MLST lineage presented a specific gene profile that was highly conserved between strains. ST398 strains displayed very homogenous gene profiles (>95% homology) independently of their host origin. The “ST398-specific” genomic profile was characterized by the absence of several virulence-associated genes harboured by « human-associated » MRSA strains, such as genes encoding proteases, haemolysins or adhesion factors. No enterotoxin gene was found among ST398 strains. Additionally, genes essential to the newly described Ess (ESAT-6
secretion system) secretion pathway (found to be implicated in \textit{S. aureus} persistent infections) were lacking in all ST398 strains tested. \textbf{Conclusion:} ST398 MRSA strains of diverse origins shared a genomic profile lacking several virulence associated genes that were found in \textit{S. aureus} human-associated strains. Further study of ST398-associated virulence genes identified here should lead to a better understanding of their colonizing and infecting capacities.

\section*{36B
IDENTIFICATION AND PRESENCE OF DIFFERENT STAPHYLOCOCCAL SPECIES WITHIN THE STAPHYLOCOCCUS INTERMEDIUS GROUP AMONG VARIOUS ANIMAL HOSTS

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\textbf{Background:} Three species belong to the \textit{Staphylococcus} (\textit{S.}) \textit{intermedius} group: \textit{S. intermedius}, \textit{S. delphini} and \textit{S. pseudintermedius}. Studies have shown, that \textit{S. pseudintermedius} is very common in dogs and is often involved in infections. Due to the close relatedness of these species, a phenotypic differentiation is problematic. Very recently, a molecular diagnostic approach, namely the amplification of an internal part of the \textit{pta} gene followed by MboI restriction analysis, has been described to show specific fragments for \textit{S. pseudintermedius} as well as for \textit{S. aureus}. \textbf{Methods:} A total of 76 isolates was obtained from dogs (n=10), cats (n=14), horses (n=17), pigeons (n=19), and mink (n=16). The isolates showed different macrorestriction patterns with Smal. Five reference strains were included in the investigations: \textit{S. intermedius} ATCC 29663, \textit{S. delphini} ATCC 49171, \textit{S. pseudintermedius} LMG 22219, \textit{S. aureus} ATCC 25923 and \textit{S. schleiferi} spp. coagulans ATCC 49545. In addition to MboI, other restriction enzymes for a digestion of the PCR product were tested to differentiate between the species. Selected PCR products were sequenced. The species was confirmed by sequencing of a part of the gene \textit{hsp60}. \textbf{Results:} Restriction of the \textit{pta} amplicon with MboI resulted in the two expected fragments (213 + 107 bp) for \textit{S. pseudintermedius}. However, the PCR product obtained from the \textit{S. aureus} reference strain was not digested. Data base searches revealed that most of the sequenced \textit{pta} genes of \textit{S. aureus} did not have the recognition site for MboI. The enzymes HindIII, PstI, DraI, PvuI, SspI, PvuII and AluI were tested in the reference strains. A single digest with \textit{AluI} proved to distinguish between all five coagulase-positive species. Two bands were seen in \textit{S. pseudintermedius} (232 + 88 bp), three in \textit{S. intermedius} (186 + 88 + 46 bp), \textit{S. aureus} (141 + 109 + 70), and in \textit{S. schleiferi} (173 + 75 + 72 bp), while four bands were present in \textit{S. delphini} (123 + 109 + 70 + 18 bp). All ten isolates from dogs proved to be \textit{S. pseudintermedius}. Among the isolates from cats 11 were \textit{S. pseudintermedius} and three \textit{S. schleiferi} isolates. The isolates from horses were \textit{S. delphini} (n=12), \textit{S. pseudintermedius} (n=4) and a single \textit{S. aureus} isolate. Isolates from pigeons proved to be \textit{S. delphini} (n=15), \textit{S. pseudintermedius} (n=2) and \textit{S. intermedius} (n=2). All 16 isolates from mink showed the same restriction pattern (123 + 109 + 88 bp). Sequencing of the two genes \textit{pta} and \textit{hsp60} revealed ca. 97% identity to the corresponding genes from \textit{S. pseudintermedius} and \textit{S. delphini}. \textbf{Conclusions:} This study revealed that a PCR with a subsequent restriction digest with one enzyme can identify \textit{S. pseudintermedius}, but can also distinguish between other coagulase-positive staphylococci. Unexpectedly, pigeons did also harbour \textit{S. pseudintermedius} isolates. All isolates from mink showed the same characteristics which differed from those of the other so far described species.

\section*{37A
MOLECULAR TYPING OF PORCINE METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ST398 ISOLATES

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\textbf{Background:} Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) isolates of sequence type ST398 have been identified to colonize and cause infections in animals and humans. These isolates have been identified frequently in pigs and in humans with exposure to animal husbandry, especially to swine farming. Isolates of this type were first detected in The Netherlands, but later on also in other European countries. The aim of this study was to determine the genomic relationships of porcine MRSA ST398 isolates in Germany. \textbf{Methods:} In total 54 independent MRSA ST398 isolates obtained from pigs from Germany in 2004/2005 (n=5) and 2008 (n=49) on the basis of one isolate per herd were included in this study. The isolates were subjected to \textit{spa} typing, macrorestriction analysis with Apal and Smal, and PCR-directed SCC\textit{mec} typing. Moreover, all strains were investigated for the most relevant virulence properties by a diagnostic microarray. \textbf{Results:} Fifty-three isolates harboured SCC\textit{mec} type V elements while the remaining one carried \textit{mec}-A and \textit{staph\textit{c}}\textit{mec}\textit{J}, but no recombinase gene. None of the 54 isolates harbored the Panton-Valentine leukocidin genes \textit{ lukF-PV} and \textit{ lukS-PV} or the genes \textit{ sak}, \textit{ etp}, and \textit{ sit} indicative for \(\beta\) haemolysin converting phages. The carriage of \(\alpha\) and \(\delta\) haemolysin genes, the protease genes \textit{ spa}-\textit{A}, \textit{ spB}, and \textit{ spP}, the \textit{ stl} and \textit{ set} genes, as well as MSCRAMM\textit{G} genes was uniform among all isolates. One isolate was positive for the enterotoxin \textit{B (sub)} gene, another three isolates for the enterotoxin \textit{K} and \textit{Q (sek, seq)} genes. Eight different \textit{spa} types were identified with \textit{t011} (n=39) being most predominant. The \textit{spa} types \textit{t034}, \textit{t571}, \textit{t1197}, \textit{t1250}, \textit{t1451}, \textit{t1456}, and \textit{t2510} were seen in one to five isolates. Detailed analysis of these \textit{spa} types suggested that they might have developed from \textit{t011} by recombinational events or even single basepair exchanges within one repeat. Isolates of \textit{spa} types \textit{t011} and \textit{t034} were disseminated all over Germany. All 54 isolates were non-typeable by Smal macrorestriction analysis, but produced a number of different fragment patterns upon Apal macrorestriction patterns. A total of six major patterns A - F with up to eight sub-patterns were identified upon cluster analysis using a cut-off at 80% similarity. Among them, isolates assigned to the predominant cluster A were found to be distributed all over Germany whereas the isolates of cluster C were only seen in the Eastern part of Germany. \textbf{Conclusions:} The results of \textit{spa} typing and macrorestriction analysis obtained in this study revealed a high degree of
diversity between the porcine MRSA ST398 isolates from Germany. ApaI proved to be a suitable and highly discriminatory restriction endonuclease for macrorestriction analysis of ST398 strains which are non-typeable by Smal. Moreover, the results obtained with the diagnostic microarray suitably supplemented the typing data.

38B
PHENOTYPIC AND GENOTYPIC ANALYSIS OF ANTIMICROBIAL RESISTANCE IN PORCINE METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ST398 ISOLATES FROM GERMANY

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) isolates of the sequence type ST398 have been identified to colonize and cause infections in animals and humans, especially in people with occupational exposure to swine farming. In addition to the resistance to β-lactam antibiotics, MRSA isolates have been described to show resistance to other antimicrobial agents. The aim of this study was to characterize and to compare MRSA ST398 from pigs for their resistance phenotypes and genotypes. Methods: A total of 54 independent MRSA ST398 isolates obtained from pigs from Germany on the basis of one isolate per herd were tested by broth microdilution for their susceptibility to 31 antimicrobial agents. A diagnostic microarray and additional PCR assays were used to detect resistance genes. Selected resistance genes were tested for their plasmid localisation by Southern blot and/or transformation experiments. Results: Nineteen different resistance phenotypes were seen with 29.6% of the isolates being resistant to β-lactam antibiotics and tetracyclines only. In total, 85.1% of the isolates exhibited resistance to two to four classes of antimicrobial agents while the remaining 14.9% showed more expanded resistance phenotypes. All 54 isolates were tetracycline-resistant with 40 isolates harbouring the resistance genes tet(M) + tet(K), 11 isolates tet(M) + tet(K) + tet(L), and single isolates tet(M), tet(L) or tet(M) + tet(L). Trimethoprim resistance was identified in 28 isolates; the genes dfrK and dfrG were detected in 14 and nine isolates, respectively. Macrolide/lincosamide resistance was seen in 24 isolates. The genes ermA(A), erm(B) and erm(C) alone were identified in two, six and 12 isolates, respectively, while a single isolate harbored the gene combination ermA(A) + erm(B) and two isolates ermA(A) + erm(C). The two chloramphenicol/florfenicol resistant isolates harbored the gene fceC-A. The genes fceC-A and tet(K) were located on the chromosome, while tet(L) was located on plasmids of 14 - 40 kb in 13 isolates. The gene dfrK was located on the same plasmid in all 13 cases or on a smaller plasmid (ca. 6 kb) in the remaining case. Seven of the tet(L)-dfrK-harboring plasmids carried also ermA(B). The gene ermA(A) was not shown to be plasmid-located, but 13 ermA(C)-positive isolates had a small plasmid of approximately 2.5 kb which carried the ermA(C) gene. Eight isolates were found to be gentamicin resistant and all of them carried the resistance gene aacA/aphD. Conclusions: This study revealed that porcine MRSA ST398 isolates from Germany differed distinctly in their resistance properties. In contrast to β-lactam and tetracycline resistance present in all isolates, resistances to other antimicrobial agents were detected in 3.7% to 51.9% of the isolates. Occasionally, different resistance genes or combinations of resistance genes were responsible for the same resistance phenotype.

39A
GENETIC ENVIRONMENT OF THE NOVEL TRIMETHOPRIM RESISTANCE GENE DFRK

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Background: The trimethoprim resistance gene dfrK has been identified recently in a porcine MRSA isolate from Germany. This study aimed at the identification of this gene among other staphylococcal isolates from Germany as well as the determination of the plasmid localization of dfrK. This study included in total 310 staphylococcal isolates: coagulase-positive and coagulase-variable staphylococci collected between 2004 and 2006 from pigs (n=90) or dogs/cats (n=158), MRSA isolates collected in 2008 from pigs (n=52) or in 2009 from cows (n=6), and coagulase-negative staphylococci from 2004 to 2006 from cows (n=9). Methods: A total of 58 isolates (44 isolates from pigs, 8 isolates from dogs/cats and 6 isolates from cows) was considered trimethoprim-resistant. The gene dfrK was detected by PCR. Plasmid location was confirmed by protoplast transformation into S. aureus RN4220. Transformants were further analyzed by susceptibility testing, PCR for the respective resistance genes, and restriction analysis of the plasmids with subsequent Southern blotting. The dfrK-flanking regions of selected isolates were cloned and sequenced. Results: The gene dfrK was identified only in isolates from pigs (n=22) or from cows (n=4), but not from dog/cats. The dfrK-positive isolates were 6 Staphylococcus lysicus, 15 S. aureus (14 MRSA and 1 MSSA), 1 S. pseudintemedius from pigs as well as 3 MRSA and a single S. chromogenes from cows. The plasmid location of dfrK was confirmed by protoplast transformation in 24 isolates. From these transformants 22 harboured also a tet(L) tetracycline resistance gene. The results of a combined PCR assay with primers from tet(L) and dfrK confirmed that the dfrK gene was in all 22 cases located immediately downstream of the tet(L) gene. Thirteen tet(L)-dfrK-harboring plasmids conferred additional resistances to neomycin/kanamycin (n=2), neomycin/kanamycin, gentamicin and macrolides/lincosamides (n=2), neomycin/kanamycin and macrolides/lincosamides (n=2), or macrolides/lincosamides (n=7). Sequence analysis revealed that most of the tet(L)-dfrK regions were flanked by insertion sequences which might have played a role in the mobility of the tet(L)-dfrK region. Conclusion: The novel trimethoprim resistance gene dfrK was detected in 22 (50.0 %) of the 44 porcine in 4 (66.7 %) of the 6 bovine trimethoprim resistant Staphylococcus spp. isolates. This study showed that plasmids carrying more than one resistance gene were commonly present in staphylococcal isolates from pigs or cows. The co-localisation of resistance genes coding for resistance to different classes of antimicrobial agents allows the acquisition of such multi-resistance plasmids under the selective pressure of the respective antimicrobial agents.
**40B**

### METHICillin RESISTANCE AMong COagulase-NEGATIVE STAPHYLOCOCCI ASSOCIATED wITH MASTITIS IN BELgIAN COWs

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Mastitis is one of the most important diseases affecting dairy cows worldwide. *Staphylococcus aureus* is among the major pathogens causing mastitis and in a previous study, we found a high prevalence of methicillin resistance in Belgian *S. aureus* isolates from clinical and subclinical cases of mastitis. Recently, the importance of coagulase-negative staphylococci (CNS) as causative agents of (mainly subclinical) mastitis has been stressed. Studies suggest that bovine CNS strains acquire antimicrobial resistance relatively easy, particularly penicillin resistance. The prevalence of methicillin resistance is less clear. Moreover, little is yet known on the types of SCCmec-elements present in methicillin-resistant CNS from mastitis. In Belgium, there is a general lack of data on antimicrobial resistance of CNS associated with mastitis. To investigate all this, we are analysing 89 isolates phenotypically identified as CNS, originating from successive isolations of different cases of mastitis in different farms. Fifteen isolates (17%) have been shown to carry mecA, the gene responsible for methicillin resistance. Isolates are being further identified with tDNA intergenic spacer analysis and 16S rDNA and rpoB gene sequencing. Characterisation of the methicillin-resistant strains will be done by antimicrobial susceptibility testing and SCCmec-typing.

**41A**

### ABSENCE OF MRSA IN CLINICAL MASTITIS SAMPLES RECOVERED FROM DAIRY CATTLE ACROSS ENGLAND AND WALES

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**Objective:** Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare associated infection and is under investigation as a zoonotic pathogen. The current study was conducted to determine the prevalence of MRSA in dairy cattle across England and Wales. In addition, the presence of toxin genes and susceptibility to other clinically important antimicrobials were investigated.

**Method:** *S. aureus* isolates (n=940) were recovered from mastitic milk samples, and screened by a duplex PCR for the species-specific *nuc* gene and the methicillin resistance gene, *mecA*. Plate dilution and disc diffusion susceptibility testing was performed against 10 clinically important antimicrobials. 205 strains were selected for PFGE-SmaI characterisation on the basis of antibiogram and geographical data. Of these, 100 isolates with distinct PFGE and/or antibiograms were examined by multiplex PCR for the presence of 14 genes encoding staphylococcal toxins (namely, enterotoxin A-E and G-J, exfoliative toxins A, B and D, toxic shock syndrome toxin-1 and Panton-Valentine Leukocidin toxin).

**Results:** All 940 isolates were *nuc* positive and *mecA* negative. Antimicrobial sensitivity data showed 412 isolates (46%) were resistant to >=1 antibiotics. The most common resistance was against penicillin (43%). Resistance to remaining antimicrobials was as follows; ciprofloxacin (6%), oxacillin (2.6%), erythromycin (2%), amoxicillin/clavulanate (1%), cefoxitin (1.1%), tetracycline (2%) and gentamicin (0.3%). PFGE analysis demonstrated 63.3% identity amongst the 205 strains. Toxin characterisation revealed 51.5% of isolates encode at least one toxin. Staphylococcal enterotoxins and TSST-1 were detected as follows; sea (1%), seb (1%), sec (13%), sed (1%), seg (18%), seh (1%), sei (18%), sej (1%) and tst (12%). All isolates were negative for PVL and exfoliative genes (eta and etb).

**Conclusion:** The findings from this study suggest dairy cattle are not reservoirs of MRSA and associated PVL toxin across England and Wales. Dairy cattle could be a potential reservoir of TSST-1 and enterotoxins but not exfoliative toxins. Resistance to penicillin was found to be high, this has been shown in many similar animal studies and relates to the widespread usage of penicillin. There is a need for continued surveillance of other farmed livestock to detect any changes in the epidemiology of MRSA in the UK.

**42B**

### MOLECULAR CHARACTERIZATION OF THE FIRST CLINICAL INFECTIONS OF “LIVE-STOCK ASSOCIATED” ST398 METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN CANADA

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**Introduction:** The high prevalence of “live-stock associated” ST398 methicillin-resistant *Staphylococcus aureus* (MRSA) with pigs and pig farmers was first reported in the Netherlands in 1995 and has since been identified in Canada and the United States of America. Despite the high prevalence of MRSA colonization on select tested farms/production facilities, no human or animal infections resulting from this ST398 strain have previously been reported in North America.

**Methods:** A total of 2343 clinical MRSA isolates, submitted to the Saskatchewan Disease Control Laboratory (SDCL) (Jan 2007-Oct 2008 (n=2008)) and the Cadham Provincial Laboratory in Manitoba (Oct 2007- Aug 2008 (n=350)), were characterized by *spa* typing at the National Microbiology Laboratory. Isolates with *spa* types associated with ST398 were further characterized by multi-locus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) of *SmaI* or *Cfr*91 digested genomic DNA, antimicrobial susceptibility testing, and real time PCR for the detection of the Panton-Valentine Leukocidin toxin.

**Results:** A total of 5 MRSA isolates submitted to the SDCL were found to contain *spa* types associated with the ST398 strain (534 (n=4) and 1250 (n=1)). One of these isolates was obtained from a screening nasal swab whereas the remaining 4 were obtained from skin and soft tissue infections. Further molecular characterisation of these 5 isolates using MLST, RT-PCR, and PFGE determined that they were all ST398, negative for the PVL encoding genes, and non-typeable by PFGE using *SmaI*, respectively. Using a neoschizomer of *SmaI*, *Cfr*91, 4/5 isolates
were highly related by PFGE. Available patient information did not reveal any specific links geographically, as the five patients all resided in different health regions of the province of Saskatchewan. **Conclusions:** This is the first report of human infections associated with the “live-stock associated” ST398 MRSA strain in Canada. National surveillance of MRSA since 1995, through the Canadian Nosocomial Infection Surveillance Program, has identified only two additional patients who were colonized with ST398 in 2003. With the five additional cases identified in 2007–2008 presented in this study, the prevalence of this strain in Canada appears to be rare. Additional surveillance involving rural communities, farms, farmers, and production facilities across Canada is warranted.

**43A**
**RETROSPECTIVE STUDY OF METHICILLIN-RESISTANT AND METHICILLIN-SUSCEPTIBLE STAPHYLOCOCCUS PSEUDINTERMEDIUS INFECTIONS IN DOGS**

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**Background:** Methicillin-resistant S. pseudintermedius (MRSP) has emerged as an increasingly common and problematic canine pathogen. Despite this, detailed description of MRSP infections is lacking, as is information comparing MRSP with methicillin-susceptible (MSSP) infections and risk factors for MRSP versus MSSP infections. The objective of this study was to describe MRSP and MSSP infections in dogs, and identify factors associated with MRSP versus MSSP infection.

**Methods:** A retrospective case control study of Staphylococcus pseudintermedius diagnoses in dogs at the Ontario Veterinary College (OVC) and the University of Tennessee Veterinary Teaching Hospital was performed. MRSP cases were matched with 2 MSSP cases; infections that were diagnosed immediately preceding and following the MRSP case.

**Results:** 26 MRSP cases and 76 have been evaluated to date. The skin and/or ears were the most common MRSP infection site; 13 (36%) of cases. Soft tissue infections (predominantly surgical site infections) accounted for 10 (28%) while the stifle joint accounted for 5 (14%), all post-TPLO surgery infections. For MSSP infections, skin and/or ear infections were most common (27/76, 36%), followed by the bladder (11, 14%) and stifle joint (9, 12%). Using univariable analysis, animals with MRSP infections were more likely to have been treated with antimicrobials in the 30 days prior to onset of infection both overall antimicrobial administration (P<0.0001), and administration of cephalosporins (P<0.0001) and beta-lactams (P<0.0001). MRSP cases were more likely to have had outpatient visits (P=0.0094), been hospitalized (P<0.0001) or had surgery (P<0.0001) during that time period. There was no association identified between the presence of atopy (P=1.0), flea allergy (P=1.0), food allergy (P=0.66), Cushing’s disease (P=0.55), neoplasia (P=0.59), hypothyroidism (P=1.00), recurrent disease (P=0.77), corticosteroid therapy (P=0.33) or immunosuppressive therapy (P=1.0). There was no difference in outcome (survival/non-survival) (P=0.28). Dogs with MRSP infections were more likely to require additional hospitalization than those with MSSP infections (P=0.04), with 76% of dogs with invasive MRSP infection requiring further hospitalization. With preliminary multivariable analysis using stepwise forward logistic regression, antimicrobial administration within 30 days was the only significant variable (OR 12, P<0.0001).

**Discussion:** MRSP infections are increasingly common and can be problematic because of limited antimicrobial options. Dogs with MRSP infections were more likely to require further hospitalization. This study has demonstrated a clear association between recent antimicrobial administration and MRSP versus MSSP infection. While intuitive, the evidence provided here supports efforts to reduce non-essential and inappropriate antimicrobial use in dogs.

**44B**
**METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM HEALTHY HORSES IN CENTRAL MAINE, USA**

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Methicillin Resistant Staphylococcus aureus (MRSA) is an emerging equine and human pathogen that has become more common in both species in recent years. Colonization of pet animals with MRSA is considered a potential reservoir for human infection. We obtained nasal and pastern samples from healthy pet horses living on mid-sized (20-40 horse) commercial farms and on small (2-4 horse) private farms in central Maine, USA. Our objectives were to determine what percentage of the sampled horses carried MRSA, and to begin to define risk factors that might correlate with MRSA colonization in equines. MRSA was isolated from 20 of the 39 horses sampled. All of the MRSA-positive horses lived on commercial farms. Of the horses colonized with MRSA, 50% had recently travelled to other farms or travelled regularly, 10% had been hospitalized during the previous 12 months, and 20% had been on antibiotics during the previous 12 months. All of the MRSA isolates tested so far in this study (10 of the 20 isolates) carried the MecA gene as determined by PCR and agarose gel electrophoresis. Our preliminary results suggest that horses at commercial farms are often colonized with MRSA, and that exposure to higher numbers of horses and humans is a greater risk factor for MRSA colonization in horses than hospitalization or treatment with antibiotics.

**45A**
**PREVALENCE OF STAPHYLOCOCCUS AUREUS AND METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ON RETAIL MEAT IN IOWA**

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Recent research from the Netherlands indicated high levels of methicillin-resistant Staphylococcus aureus (MRSA) in retail-available meat. However, to date few studies have investigated MRSA in meat in the United States. With our recent isolation of MRSA from swine, the logical question arose as to the presence of these bacteria within retail-available meat in Iowa. Contamination of the meat could potentially occur during the slaughtering and processing of MRSA-positive animals, or post-slaughter via colonized workers. The aim of this study was to determine the presence of both methicillin-sensitive S.
**Poster Abstracts**

**46B**

**SURVEILLANCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN HORSES AT A VETERINARY TEACHING HOSPITAL**

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**Introduction:** Methicillin-resistant Staphylococcus aureus (MRSA) is endemic in horses in many regions and has caused outbreaks in numerous equine hospitals internationally. In human medicine, active surveillance of patients is often used as part of an infection control program. In response to the identification of community-associated (CA) and hospital-associated (HA) infection and colonization in horses, as well as zoonotic infections of staff, routine screening of equine patients is performed at the Ontario Veterinary College. This involves the collection of nasal swabs from horses at the time of admission, weekly during hospitalization, and at the time of discharge.

The objective of this study was to analyze MRSA surveillance data of equine patients from OVC. **Methods:** Surveillance data collected from January 2003 to May 2009 were analysed. Cases were classified as CA if the admission sample was positive; HA if the first sample was negative and a subsequent sample was positive; and indeterminate if the first sample was positive but was collected more than 48 hours following admission. Monthly MRSA rates were calculated at positive cases per 1000 admissions. **Results:** A total of 9886 horses were admitted during the surveillance period. MRSA was isolated on 1 or more occasions from 204 (2.1%) horses. Ninety-four (46%) and 96 (47%) of MRSA cases were classified as CA and HA, respectively, while 14 (6.9%) MRSA cases were indeterminate. One farm accounted for 15 (15%) of CA cases. The monthly overall MRSA rate ranged from 0.225/1000 admissions (median 10.3, mean 19.8, SD 31). Monthly CA-MRSA rates ranged from 0-127/1000 admissions (median 4.6, mean 9.5, SD 18) while HA-MRSA ranged from 0-98/1000 (median 5.0, mean 9.1, SD 14.9). Surgical services accounted for 62% of HA-MRSA but only 33% of CA-MRSA. There were no significant changes in monthly incidence rates over time for total MRSA (P=0.23), CA-MRSA (P=0.18) or HA-MRSA (P=0.77). Several temporal clusters were apparent, suggestive of both community and hospital outbreaks. There was an association between CA- and HA-rates, with HA-MRSA rates increasing with increasing CA-MRSA rates (P<0.0001). There was no association between caseload and monthly CA- or HA-MRSA rates (P=0.964 and 0.237, respectively). **Conclusion:** MRSA is an endemic pathogen in the horse population in Ontario, Canada, and can be found in a veterinary hospital as both a CA and HA pathogen. Active surveillance allows for prompt identification of colonized animals and implementation of infection control practices. It can also provide useful information for identification of endemic CA and HA rates, as well as for identification of clusters of infection and high-risk farms, information that can be used for infection control purposes. Identification of CA clusters might be a means of predicting increased risk for HA transmission.

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**47A**

**LONGITUDINAL EVALUATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PIGS**

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**Objectives:** The potential public health concerns of MRSA in food animals are receiving increasing attention, yet little is known about the dynamics of colonization in pigs. Cross-sectional studies have provided information about the prevalence of colonization however an understanding of MRSA colonization over time is required to better evaluate the ecology and epidemiology of this organism. The objective of this study was to characterize the prevalence of MRSA in piglets over time on a commercial swine farm. **Methods:** A 500 sow farrow-to-feeder swine farm that was known to have had MRSA colonized pigs in the past was enrolled. Pigs are produced for an antimicrobial-free pork market so there is no routine use of antimicrobials, including medication in the starter ration. Nasal swabs were collected from 10 sows 2 weeks prior to farrowing, then from piglets from the 3 MRSA positive and 7 MRSA negative sows at 1, 3, 7, 14, 21, 28, 42, 56 and 70 days of life. Enrichment culture for MRSA was performed. **Results:** MRSA colonization rates were low initially, but increased over time, with 79% of piglets positive on at least one occasion. The prevalence of MRSA colonization on days 1, 3, 7, 14 and 21 was 1%, 6.2%, 8.5%, 4.4%, 20%, respectively, with 35% of piglets positive prior to weaning. 34%, 64%, 50% and 41% of pigs were colonized on days 28, 42, 56 and 70, respectively. Of piglets surviving to weaning 84% of piglets from negative sows and 100% of piglets from positive sows were positive on at least 1 sample. A piglet from an MRSA positive sow was 1.4 times more likely to be colonized than a piglet from a negative sow (P=0.037). There was a significant association between sow and piglet colonization. The age of the piglet was significantly associated with the probability of colonization. No piglets or sows received antimicrobials during the study period. The first 21 isolates were typed and were all spa type 539/r034. Further typing was not performed because of the apparent clonal nature of MRSA on this farm. **Discussion:** MRSA was common in piglets from positive and negative sows, despite no antimicrobial exposure. While there was an influence of the sow’s MRSA status on colonization of its piglets, colonization rates were still very high in piglets from negative sows, so screening of sows and cohorting of piglets from positive and negative sows does not appear to be a useful control measure, at least as a sole measure. The changes in prevalence over time were impressive and indicate that age of animals must be considered when designing and comparing studies. The decrease in...
prevalence that was noted at the end of this study needs to be followed further to see if it continues to the time of slaughter, because that age is likely most relevant in terms of foodborne risks.

48B METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) SURVEILLANCE IN SLAUGHTER-AGE PIGS AND FEEDLOT CATTLE

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Background: MRSA has been isolated from pigs in various countries internationally, but many previous studies have involved pigs from limited geographic ranges and therefore potential biases such as clustered sampling may exist. Contact with cattle has been identified as a risk factor for MRSA infection or colonization in some regions and contamination of retail beef has been reported, but there has been only limited investigation of MRSA colonization in cattle. The objectives of this study were to determine the prevalence of MRSA colonization in pigs approaching slaughter age across Canada and to determine the prevalence of MRSA colonization of feedlot cattle. Methods: Pigs; 23 farms from 4 provinces were enrolled. Nasal swabs were collected from 10 non-co-mingled grower-finisher hogs from each farm, shortly before the time of slaughter. Cattle; Nasal swabs and fecal samples were collected from cattle from multiple farms at the time of arrival to feedlot. Selection for MRSA was performed using broth enrichment. Isolates were typed by spa typing. Results: MRSA was isolated from 9/230 (3.9%) pigs on 23 farms. MRSA was present in pigs from only 2/23 (8.7%) of herds, with the on-farm prevalence ranging from 0-70%. Spa type 539/t014, a sequence type 398 strain, was the most common, accounting for 7/9 (78%) of isolates; 7/7 isolates from one farm. Two other strains were found on the other farm; spa 2/t002 and spa 7/t064. MRSA was not isolated from any of 365 cattle enrolled. Discussion: The prevalence of MRSA in pigs was quite low compared with previous studies. Reasons for this are unclear. Sampling only pigs of slaughter age could be one factor because of the prevalence of MRSA may decrease with age, but further study is required. While spa 539/t014 was common, non-ST398 strains were also present. Spa 2/t002 is a common human epidemic clone that has previously been found in pigs in Canada. Spa 7/t064 is an uncommon human epidemic clone that is commonly isolated from horses and which has been previously found in retail pork in Canada. It was surprising that MRSA was not isolated from cattle, considering the association between MRSA and cattle contact that has been reported in Europe and the presence of MRSA in retail beef in Canada. Further study of cattle from different regions and management types is required to determine whether MRSA is present in the Canadian cattle population and explain this apparent discrepancy. This study demonstrates that MRSA is uncommon in feedlot cattle and late grower-finisher pigs. Investigation of these age groups is important because the prevalence in animals at the time of slaughter is probably of greatest relevance for MRSA contamination of food. Concurrent study of slaughter-age animals and retail meat is required. This study, combined with studies of retail meat in Canada, has identified discrepancies in both prevalence and strain distribution between animals and meat.

49A METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) CONTAMINATION OF RETAIL MEAT; CANADA

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Introduction: Reports of MRSA in food animals have created concerns about the potential for food to act as a source of MRSA infection in humans. Recent studies in The Netherlands and the United States have identified MRSA retail meat, but sources and relevance are unclear. The objectives of this study were to determine the prevalence of MRSA in retail meat in Canada and characterize recovered strains. Methods: Retail meat samples were purchased from 4 Canadian provinces as part of the active retail sampling component of the Canadian Integrated Program for Antimicrobial Resistance Surveillance. Pork, beef and chicken were tested. Enrichment culture was performed. Quantitative culture was also performed for later studies. Isolates were typed by PFGE and spa typing. Results: MRSA was isolated from 31/402 (7.7%), 95% CI 5.5-10.7% samples in the first study of retail pork; 23/296 (7.7%) pork chops, 7/94 (7.4%) ground pork and 1/12 (8.3%) pork shoulders (P=0.99). 3 related spa types (t064, t008 and a new related type) that were classified as Canadian epidemic MRSA-5 (CMRSA-5, an ST8 clone) by PFGE and consisted of 3 related spa types (t002, t045 and a new type). MRSA was isolated from 10/179 (6.0%) ground beef samples in the first study using quantitative methods. Four (40%) samples were only positive on enrichment culture. The remaining samples had 20-340 CFU/g. All isolates were spa 24/t242. Three different but related PFGE patterns, consistent with the CMRSA-2 (USA100) were present amongst those isolates. A second study of retail pork, using qualitative and quantitative methods, identified MRSA in 8/127 (6.3%) ground pork samples and 12/102 (12%) pork chops (P=0.16). Quantitation was possible in 6 (75%) of the positive ground pork samples and 18 (90%) of positive pork chops, with levels ranging from 20-340 CFU/g in ground pork and 10-3590 CFU/g I pork chops. Eighteen (90%) of isolates were spa 24/t242, with two different PFGE patterns. Two isolates were spa 2/t002. MRSA was not identified in any of 130 chicken samples. Discussion: MRSA is relatively common in retail beef and pork in Canada, yet the concentration of MRSA in meat is typically low. The strain distributions varied between studies and were not always representative of the strain distribution in food animals in Canada. This discrepancy requires further study, particularly the commonness of CMRSA-2, a human clone in both pork and beef yet recent failure to identify MRSA in any feedlot cattle in Canada. The predominance of CMRSA-5 in the first pork study was also surprising since this
human/equine clone has only been previously found in a single pig. Clearly, further investigation of sources of contamination, along with clinical relevance, is required.

50B
LOW PREVALENCE AND WIDE GEOGRAPHICAL DISTRIBUTION OF LIVESTOCK-ASSOCIATED STAPHYLOCOCCUS AUREUS ST398 IN HOSPITALISED PATIENTS IN BELGIUM, 2008

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Background: MRSA ST398 is frequently carried by swine farm workers in Belgium. The aims of this study were to determine the frequency and geographical distribution of MRSA and MSSA of clonal complex CC398 among S. aureus strains from hospitalised patients during 2008.

Methods: Belgian hospitals were invited to collect consecutive, non-duplicate S. aureus strains (MRSA = 3; MSSA = 2) from hospitalised patients during 2008. Identification was confirmed by PCR for nuc, mecA and 16S rRNA genes. All S. aureus strains were genotyped by spa-typing, a representative subset of strains was typed by PFGE and MLST. SCCmec-type was determined by PCR for MRSA isolates. The susceptibility to 20 antimicrobials was tested by agar dilution. Resistance genes and genes encoding TSST-1 and PVL toxins were tested by PCR.

Results: A total of 531 strains from 107 hospitals including 316 MRSA and 215 MSSA were recovered from blood (n = 35), screening swabs (n = 155), skin wound samples (n = 151), sputum (n = 87), puncture fluids (n = 42) and other samples (n = 61). Of these, two (0.6%) MRSA isolates and five (2.3%) MSSA isolates from 7 hospitals were non-typeable by PFGE and belonged to ST398. MRSA isolates showed spa-t011 and carried SCCmec type IV and V whereas MSSA isolates belonged to spa-t571. These isolates were collected from wounds (n = 3), sputum (n = 2), blood (n = 1) and MRSA screening swabs (n = 1). All CC398 isolates were found in patients admitted to hospitals in the different regions of Belgium. MRSA isolates were resistant to tetracyclines (100%) whereas MSSA isolates were resistant to erythromycin only (100%). No ST398 isolate carried either the PVL or TSST-1 gene. Conclusion: Although MRSA CC398 strains are highly prevalent in Belgian pig farmers, it only accounts for a small proportion in patients admitted to Belgian hospitals. Surprisingly, MSSA ST398 isolates were more frequently found than MRSA ST398 isolates. Isolates were recovered from different hospitals scattered in Belgium. The MRSA isolates were resistant to tetracycline, like the strains recovered from animal husbandry in Belgium. Both MRSA and MSSA were recovered from cases of invasive infection.
52B PERSISTENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN VEAL GROWERS IN THE NETHERLANDS: A LONGITUDINAL STUDY

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Objectives: A recent study shows that veal calf farming is a risk factor for Livestock associated (LA) MRSA colonization in humans. The duration of animal contact is identified as a risk factor. However, no information is available on the dynamics and persistence of LA-MRSA colonization in veal growers. To explore this, we conducted a longitudinal study among 155 farmers and family members in which repeatedly nasal- and throat swabs were taken for MRSA detection. Periods with and without or reduced animal contact were included. Methods: Randomly, 50 veal calf farms were selected and visited from June - December 2008. Farmers and family members were asked to fill in questionnaires (n=155) to identify potential risk factors. Nasal and throat swabs were repeatedly taken from each participant, in the morning and the evening of sampling days. Swabs were analysed for MRSA by selective enrichment, culturing and confirmed by MecA pcr. Spa types of the isolates were identified. Data were analyzed using multilevel logistic regression analysis taking clustering into account. Results: MRSA prevalence was 38% in calf farmers and 16% in family members. Number of working hours in the calf stables is associated with human MRSA colonization. MRSA prevalence was strongly reduced in periods with reduced or absence of animal contact. The prevalence decreased from 40% to 33.5% in farmers during periods with reduced animal contact (-16%). In family members the prevalence dropped from 17% to 11.5% (-32%). Only 7% (n=11) of the study population revealed to be persistent carrier. In addition a large heterogeneity in spa-types was found. Conclusions: Only a small group of persistent MRSA carriers exists, mainly among farmers. The association between human carriageerness and working hours in the calf stable and the drop in prevalence during the low exposure period both indicate that prolonged MRSA colonization only occurs in people with intensive animal contact. Secondary exposed persons (family members, who only lived on the farm and not or less working) seemed to be less vulnerable for persistent carriage. Detailed analyses of the spa-type heterogeneity are ongoing.

Keywords: NT-MRSA, colonization, exposure

53A METHICILLIN RESISTANCE IN STAPHYLOCOCCUS EPIDERMIDIS ISOLATES FROM DIFFERENT SOURCES IN THE CZECH REPUBLIC

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Staphylococcus epidermidis has been recognized as one of the most important opportunistic pathogens of the genus Staphylococcus, in which an increasing antimicrobial resistance rate was observed as well. A total of 121 S. epidermidis isolates originated from human patients (community related patients, hospitalized patients, n = 30), dairy farms (dairy cattle, farmers, n = 36), and a dairy plant (final milk products, surface scrapings, n = 55) were obtained during the period of 2006-2008 within one district in the Czech Republic. All the isolates were tested for their susceptibility to selected antimicrobial agents. Methicillin resistant S. epidermidis (MRSE) isolates were also identified and their staphylococcal chromosomal cassettes (SCCmec) were characterized. The highest level of resistance to the tested antimicrobial agents was noticed in the human clinical isolates. This finding correlated with a high number of MRSE isolates (23/30; 76.7%) found in human patients. Besides the human clinical isolates, a higher prevalence of MRSE was also recorded in the isolates from healthy farmers (6/14; 42.9%), among which a relatively increased resistance to various antimicrobials was observed, too. Another important finding in this study is the fact that majority of the isolates from raw milk of dairy cattle were identified as MRSE (16/22; 72.7%). No MRSE isolates were found in the dairy plant. By the SCCmec typing, the majority (40/45; 88.9%) of the MRSE isolates could not be assigned to any of the known SCCmec types. The occurrence of two or more sequences, each specific for a different SCCmec type, in a single isolate was observed quite often. Also four Reference Strains of S. aureus (N315, 85/2087, JCSC4744, WIS) tested in this study were non-typeable. A high variability of the SCCmec profiles among the MRSE isolates, together with the fact that no significant correlation between particular sequences was observed, led us to propose a new typing system of the SCCmec using numeric codes.

54B METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ST59 ISOLATES FROM DOGS, CATS AND THEIR OWNERS

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Methicillin-resistant Staphylococcus aureus (MRSA) is a well-known nosocomial pathogen and also emerges as a community pathogen recently. MRSA develops not only in human but also in companion and livestock animals. Thus, the aim of this study is to characterize ST59 MRSA isolates from companion animals and their owners by molecular typing analysis. One thousand nasal swab samples were collected from dogs, cats and their owners from northern Taiwan. The molecular genetic similarity of MRSAs were compared with pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), staphylococcal chromosomal cassette mec (SCCmec) typing, Panton-Valentine leukocidin (pvl) gene detection and 18 enterotoxin genes detection by PCR. The results showed that 13 different MRSA isolates were identified and 12 of these strains were ST59. Three major clusters of PFGE patterns were suggested with 80% of similarity. Two major PFGE clusters were ST59/SCCmecV (5) and ST59/SCCmecIV (7). The pvl gene was only found in ST59/SCCmecV MRSA (4). Enterotoxin genes carried by ST59 MRSA were sel, sek, seq (8), or sel, sek, seq, sep(2), or sep only(1); one ST59 MRSA did not show any enterotoxin gene. SCCmec type V / pvl (+) had higher antibiotic resistance to erythromycin, clindamycin and tetracy-
cline; SCCmec type IV / pel (-) had higher antibiotic resistance to clindamycin and ciprofloxacin. The results of current study are consistent with previously reported studies showing that MRSA can be detected on pig farms in the Netherlands. Ongoing studies are important to further elucidate the role of MRSA and other rodent-dwelling staphylococci in the transmission of zoonotic diseases.

**55A OCCURRENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN RATS LIVING ON PIG FARMS**


**Introduction:** In the Netherlands, ST398 MRSA has emerged in hospitals and human carriers have been associated with exposure to pigs and cattle. High prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in pigs and pig farmers have been determined and the transmission routes of MRSA on pig farms need to be elucidated. Recently, the black rat (*Rattus rattus*) has emerged as a prominent rodent on livestock farms in the south of the Netherlands. Rats are recognized for their role in the transmission of zoonotic agents, such as *Salmonella* spp., *Campylobacter* spp. and *Toxoplasma gondii*, on livestock farms. In this study, the occurrence of MRSA as well as methicillin sensitive *Staphylococcus aureus* (MSSA) in rats living on livestock farms was determined.

**Methods:** From March till May 2008, rats living on livestock farms in the south of the Netherlands and the north of Belgium were randomly collected in the context of a survey on the occurrence of zoonotic agents in rodents. A total of 40 black rats and 3 brown rats were collected at 12 farms. Colonies suspected to be MRSA or MSSA, were tested by multiplex PCR for the *S. aureus* specific DNA-fragment, the *mecA* gene and the Panton-Valentine leucocidin (*pvl*) genes. Staphylococcal protein A (*spa*)-typing and Multi-locus sequence typing (MLST) were performed on all MSSA and MRSA isolates.

**Results:** MRSA was isolated from 5 of the 40 black rats and no MRSA was isolated from the 3 brown rats. Four of the 5 MRSA-positive rats showed *spa*-type t011 and ST398, whereas the MRSA-isolate from one rat showed a rare *spa*-type t1236 and ST97. Furthermore, a total of 13 MSSA were isolated from 12 black rats and one brown rat. The MSSA isolates yielded various *spa*- and MLST-types which differed from the MRSA isolates. None of the isolates possessed the *PVL*-genes.

**Conclusions:** MRSA was isolated from black rats living on pig farms and 4 of the 5 isolates yielded ST398 and *spa*-type t011 showing that livestock related MRSA has also emerged among rodents on pig farms. Since rodents are well known for their role in the transmission and persistence of zoonotic bacteria on livestock farms, these findings suggest that rats might play a role in the spread and persistence of MRSA on pig farms. Strategies to control MRSA on pig farms should take the control of rats and other rodents into account.

**56B DIVERSITY OF MECA AND CCRB IN SCCmec TYPE III OF METHICILLIN-RESISTANT STAPHYLOCOCCI**

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**Background:** Our group recently showed that SCCmec type III is by far the most common type amongst methicillin-resistant coagulase-negative staphylococci (CoNS) of animal origin, in particular isolates within the *Staphylococcus sciuri* group (*S. sciuri*, *S. vitulinus*, *S. lentus* and *S. fleurettii*). Type III cassettes are the largest of all known SCCmec elements and could to be the progenitor of other SCCmec elements since they harbour mec complex type A with intact regulatory genes. The objective of this study was to investigate trends in the evolution of SCCmec III elements. Variations in *mecA* and *ccrB* were used as markers to study evolution and possible transfer of SCCmec III across distinct staphylococcal species. **Materials and Methods:** A collection of genetically and epidemiologically diverse animal and human *S. aureus* (*n=5*), *S. fleurettii* (*n=5*), *S. capitis* (*n=4*), *S. pseudintermedius* (*n=2*), *S. vitulinus* (*n=2*), *S. sciuri* (*n=2*), *S. haemolyticus* (*n=1*) and *S. equorum* (*n=1*) harbouring SCCmec III elements was subjected to partial sequence analysis of *mecA* and *ccrB* according to previously published protocols (Oliveira et al., JAC, 2006; Stephens et al., AAC, 2007). The sequences were analyzed by neighbour-joining analysis to assess phylogenetic relationships between and within species. **Results:** Most (78%) *ccrB* sequences were identical and corresponded to the prototype allele 300 found in all *S. aureus* SCCmec III described in the current database (www.ccrbtyping.net). Four CoNS isolates (*S. sciuri*, *S. equorum*, *S. haemolyticus* and *S. vitulinus*) had *ccrB* sequences displaying only 75-80% similarity to allele 300, and three *S. fleurettii* isolates yielded no *ccrB* bands. *mecA* was highly conserved overall (>99% similarity), yet 10 alleles were defined based on variation in 11 nucleotide positions. As for *ccrB*, species-specific clustering of *mecA* sequences was not observed as exemplified by *mecA* allele 5 being present in four species (*S. pseudintermedius*, *S. sciuri*, *S. haemolyticus* and *S. vitulinus*). Similarly, *mecA* allele 1 was found in three species (*S. aureus*, *S. capitis* and *S. pseudintermedius*). Although various combinations of *mecA* and *ccrB* alleles were detected, seven isolates representing *S. aureus* (*n=2*), *S. pseudintermedius* (*n=2*) and *S. capitis* (*n=3*) had the same combination of *ccrB* and *mecA* alleles, suggesting interspecies horizontal transfer of SCCmec III. **Conclusion:** The occurrence in distinct staphylococcal species of SCCmec type III that are undistinguishable on the basis of *ccrB* and *mecA* sequencing suggests that this large genetic element has been transferred horizontally between distantly related species within the genus. Ongoing studies on sequence diversity of the SCCmec junkyard regions in these isolates will be used to further test this hypothesis.
INVESTIGATION OF THE PREVALENCE OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS FROM VET VISITING DOGS IN GREAT BRITAIN

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Background: Carriage of meticillin-resistant Staphylococcus aureus (MRSA), and other meticillin-resistant staphylococci (MRS), have been reported for both diseased and healthy dogs, however most studies have been conducted within the veterinary hospital environment. The prevalence of MRSA carriage however, has not been determined for the dog population in Great Britain. **Aims:** To investigate the nasal carriage prevalence of MRSA in vet visiting dogs in Great Britain. **Methods:** A cross sectional study was conducted and nasal swabs were taken from 672 dogs, visiting 87 randomly selected veterinary practices. Staphylococci were isolated by enrichment in nutrient broth with 6% NaCl, followed by culture on mannitol-salt agar and oxacillin resistance screening agar. Isolates were selected based on morphology and were subjected to Gram staining, catalase and staphylyase (Pro-Lab, UK) tests. Meticillin resistance was confirmed by disc diffusion susceptibility testing and isolates were screened for further antimicrobial resistance using the guidelines set out by the British Society for Antimicrobial Chemotherapy. The **femA** and **mec** PCR assays were carried out on staphylyase positive isolates to confirm them as S. aureus and meticillin resistant isolates were tested for the presence of the **mecA** gene by PCR. All MRSA isolates were subjected to SCC**mec** typing using a multiplex PCR assay. **Results:** A total of 481 staphylococcal isolates were obtained from the nasal swabs of 394 dogs. The prevalence of MRS was 6.3% (n=42), of which 0.9% samples (n=6) were positive for MRSA. A total of eight MRSA isolates were recovered. Five (10.9%) MRS isolates displayed multidrug resistance (resistance to three or more drug classes). High prevalences of resistance were observed to ciprofloxacin (28.3%), tetracycline (21.7%) and co-trimoxazole (17.4%). All MRSA isolates were resistant only to ciprofloxacin. **SCCmec type could only be determined for four MRSA isolates and these were found to carry type IV cassettes.** 

Conclusions: This study demonstrated a very low prevalence (0.9%) of MRSA carriage in the vet visiting dog population in Great Britain. The carriage rate of MRSA was also relatively low, but higher than that for MRS. Multidrug resistance was not common in the MRS isolates and all MRSA isolates were resistant only to beta-lactams and ciprofloxacin, characteristic of EMRSA-15 strains, which have previously been found in dogs in Great Britain. **SCCmec type IV was identified in four of the MRSA isolates, but could not be determined in all isolates.** **Acknowledgements:** DEFRA and veterinary practices who took part in this study.

THE PILGRIM PROJECT: PREVENTING COMMUNITY AND NOSOCOMIAL SPREAD AND INFECTION WITH MRSA ST 398 - INSTRUMENTS FOR ACCELERATED CONTROL AND INTEGRATED RISK MANAGEMENT OF ANTIMICROBIAL RESISTANCE

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Methicillin-resistant Staphylococcus aureus (MRSA) is of increasing concern both as a community- and hospital-acquired pathogen. Since 2005, a specific type, MRSA ST398, has occurred in livestock populations and occupationally exposed persons resulting in human infection and disease in Europe and North America. A major concern is that MRSA ST398 is transferring into care facilities increasing the risk of nosocomial infection. This animal-adapted MRSA offers opportunities for investigation of factors affecting host-specificity in resistant bacteria and evaluation of control strategies. To address these issues, a specific research project (PILGRIM) was funded by the EU within the 7th Framework Programme. This project is coordinated by the Royal Veterinary College and brings together clinical, genetic, microbiological and public health partners from 6 EU countries. The project’s aim (http://www.fp7-pilgrim.eu) is to provide a range of novel control measures for the accelerated identification and control of these emerging bacteria. A series of epidemiological and physiological studies of MRSA ST398 will be undertaken as well as molecular approaches in closely comparable animal models and humans in animal and healthcare settings to: 1) investigate its biology and ecology, 2) identify and characterize factors determining the transmission pathways and risks from animal to human and between humans, 3) establish genetic differences, host-range and virulence of adhesive and non-adhesive strains as well as differences between MRSA ST398 and other MRSA, 4) identify specific genes for the development of new rapid tests to identify specific MRSA strains, 5) provide a Technology Testing platform for developing and assessing decolonisation and environmental sanitation approaches and 6) integrate results in policy and practice guidelines. This research will facilitate rapid and cost-effective measures to combat MRSA ST398 strains in order to prevent and eradicate community and hospital infections for better protection of citizens and patients in Europe and beyond.
59A
SUSCEPTIBILITY OF STAPHYLOCOCCUS SPP., ISOLATED FROM DOGS WITH PYODERMA TO ANTISTAPHYLOCOCCAL PROTEIN P128


Pyoderma is one of the most common pyogenic bacterial skin infections in dogs. *S.intermedius*, a commensal on canine skin is a coagulase positive Staphylococcus spp. (COPS) and known to be a common etiological agent in this disease. *S.aureus* and *S.schleiferi* sub sp *coagulans* are the other reported emerging causative species. In this study the objective was to determine the activity of P128, an antistaphylococcal chimeric protein under development at Gangagan, against Staphylococci isolated from dogs with pyoderma. To evaluate the spectrum of Staphylococci in pyoderma infections, we analysed 87 samples collected from 80 dogs with pyoderma and a history of previous antibiotic treatment. We isolated 56 Staphylococci of which 45 (80%) were COPS, and further speciated as 21 (47%) *S.schleiferi* sub sp *coagulans*, 21 (47%) *S.intermedius* and 3 (6%) *S.aureus*.

Coagulase negative Staphylococcus spp. (CONS) constituted 11 (20%) and were speciated as *S.epidermidis*, *S.hominis*, *S.xylosus*, *S.intermedius* and *S. haemolyticus*. We tested all these isolates for sensitivity to the protein P128 *in vitro*, by spot titration and determining MIC. We found 45/45 (100%) of COPS and 11/11 (100%) of CONS to be susceptible to the protein by spot titration. MIC of P128 was determined on a panel of COPS and CONS and ranged from <0.125µg/ml to 64µg/ml.

60B
PRIORITISATION OF MRSA RESEARCH IN DOGS: A RISK MODELLING APPROACH

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important nosocomial and community-acquired pathogen with zoonotic potential. This study utilised current knowledge to implement a quantitative stochastic simulation model with attendant uncertainty and sensitivity analyses in the area of MRSA acquisition in pet dogs. The model described the acquisition of MRSA in pet dogs over 24 hours and attempted to identify priority data gaps for future research. It was hypothesized that the use of quantitative risk assessment methods would enable a transparent and defensible approach in this emerging area of concern. A model structure was developed that defined potential pathways for exposure and transmission of MRSA to dogs, considered human, animal and environmental acquisition (encompassing carriage and infection) of MRSA and was stratified for veterinary clinic attendance. The quantitative model was specified as a second-order nested stochastic simulation model. Parameterisation was achieved using published data and expert opinion that was elicited using a non-convergent expert opinion questionnaire technique, with results obtained from 15 experts and modelled using modified beta distributions. A sensitivity analysis was undertaken, with the intention of identifying which inputs of the model were most influential on the output, using logistic regression modelling with consideration of interaction terms. The outcome of the simulation model was dominated by uncertainty (over variability) which should, in theory, be reducible by future research. The median probability of acquisition of MRSA on any given day (incidence) was estimated to be 1.5%, which lies within the binomial confidence intervals surrounding previously published estimates of prevalence. The effect of the environment dominated the sensitivity analyses, and was ranked as the most influential predictor of MRSA acquisition within the home and veterinary clinic. However, as expected, significant interactions existed between the environment and alternative sources (human and/or animal) as these are not independent. Exposure to and transmission from MRSA positive family members were also found to be influential, along with veterinary clinic attendance, although it was difficult to differentiate between the importance of independent sources of MRSA within the veterinary clinic. The findings of this study imply that community and veterinary routes are both important in acquisition of MRSA in dogs and, while it cannot be concluded that other sources of MRSA are unimportant, the role of the environment and of family members, were found to be deserving of further study. The implementation of logistic regression represents a novel application of a variance based sensitivity analysis technique in the area of veterinary medicine and is a new approach to prioritising research in an emerging area of animal health.

61A
ANTIMICROBIAL RESISTANT STAPHYLOCOCCUS AUREUS IN GROUND MEAT PRODUCTS OF THE WASHINGTON DC AREA

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From March to August 2008, ground retail meat samples (n=694, including 300 pork, 198 beef, and 196 turkey) from grocery stores in the Washington DC area were cultured for the presence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). A total of 200 isolates of *S. aureus* was identified and examined for susceptibility to 22 antimicrobials including methicillin, chloramphenicol, erythromycin, clindamycin, daptomycin, oxacillin, streptomycin, gentamicin, ampicillin, cefoxitin, linezolid, penicillin, rifampin, vancomycin, trimethoprim/sulfamethoxazole, levofloxacin, ciprofloxacin, quinupristin/dalfopristin, tigecycline, nitrofurantoin, tetracycline, and moxifloxacin. One MRSA isolate was identified in a pork sample. A large number of *S. aureus* isolates were resistant to tetracycline (68%), and to pencillin (26%). None of the *S. aureus* isolates was resistant to DAP, STR, LZD, RIF, VAN, SXT, LEVO, CIP, TGC, NIT, MXF, or GEN. More *S. aureus* isolates from turkey (19%) and pork (15%) were resistant to antimicrobials than those from beef.
62B
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM THERAPY DOGS AND HANDLERS WITHIN A HOSPITAL SETTING

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There are cited medical benefits regarding pet ownership and animal-assisted interventions (e.g. therapy dogs). These interactions are positive, contributing to the overall health and happiness of hospitalized patients. However, there is a potential for zoonotic disease transmission, especially with vulnerable patients. One agent of concern is methicillin resistant Staphylococcus aureus (MRSA). MRSA is endemic in United States’ hospitals causing skin abscesses and invasive, life threatening infections. Our objective was to document the occurrence of MRSA in therapy dogs and their handlers who visited a local hospital.

Methods: Weekly nasal and rectal swabs were collected from 10 dogs involved in a local hospital program. Samples were collected from handlers on weeks 1, 5, and 10. Samples were tested for the presence of the mecA gene and isolated by previously published protocols. Isolates were characterized by spa type and PFGE. Weekly surveys were conducted to document handler and dog activities.

Results: Primary locations that were visited by the therapy dogs were Emergency (77%) and Trauma (89%) waiting areas. Other areas included Intensive Care waiting room (18%), Oncology (34%), Rehabilitation (22%), and Physical Therapy (14%). 182 canine nasal and rectal swabs were collected. 26 handler nasal swabs were collected. 13 samples were positive for the mecA gene. Six of the 13 samples were identified as Staphylococcus aureus. Five isolates were confirmed as MRSA. MRSA was isolated from 2 handlers and 3 therapy dogs. Handlers who were colonized with MRSA also had therapy dogs colonized with MRSA (2 dog-handler pairs).

Conclusions: Therapy dogs may acquire MRSA. It is unclear as to their role in on-going transmission in the hospital environment. However, colonization appears to be transient in handlers and dogs. Measures to reduce interspecies transmission in hospital setting should be encouraged.

63A
INFECTION CONTROL AND MRSA INFECTIONS AT A VETERINARY TEACHING HOSPITAL

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Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is recognized as an important human pathogen and is the leading cause of suppurative infections in humans, including superficial skin infections such as boils and furuncles as well as more serious infections such as bloodstream infections, pneumonia, osteomyelitis and endocarditis. Until recently, most human infections were acquired after hospitalization. Within the past decade the emergence of community-associated strains have been recognized. Methicillin-resistant Staphylococcus aureus (MRSA) infections from dogs, horses, pigs, cattle, and cats are increasingly being reported. Our veterinary teaching hospital has seen an increasing number of infections in our companion animal referrals. These recent MRSA infections in animals have created diagnostic, treatment, and infection control challenges for staff veterinarians. Our objective is to summarize the clinical cases and the associated infection control issues.

Methods: Routine laboratory surveillance identified MRSA cases. Case follow-up was done with clients regarding potential MRSA risk factors. Infection control interventions were established for each hospitalized case. Isolates were sent to the Minnesota Department of Health for confirmation, antimicrobial susceptibility testing, and subtyping by pulsed-field gel electrophoresis.

Results: From 2003 through 2008, 29 MRSA infections were identified at the University of Minnesota, Veterinary Medical Center. Isolates were obtained from 20 dogs, 7 cats, and 2 horses. Fourteen PFGE subtype patterns have been identified from 24 isolates. The majority of isolates are characterized as clonal group USA100, representing health-care associated strains. Five temporal/clonal clusters were observed suggesting nosocomial transmission. MRSA was also recovered from environmental surfaces and a veterinary surgeon. Most clients of infected pet were recently hospitalized or had on-going severe illnesses (i.e. chemotherapy), or were health care providers.

Discussion: With the identification of MRSA in animals, appropriate precautions to prevent further infection are important. This includes both employee and owner education about potential risks, precautions, and the need for good hand hygiene. With the identification of temporal/clonal clusters of MRSA infections, it is important to incorporate “standard precautions” to protect staff, clients, and pets to prevent continued transmission of MRSA. This includes appropriate isolation and disinfection protocols.

64B
TYPOING RESULTS OF MRSA AT DIFFERENT PIG PRODUCTION LEVELS


The objective of the study was to analyse the spa-type patterns of MRSA isolated from pigs and dust samples from pig holdings at different levels of the production pyramid. Isolates were collected from nasal swabs of pigs at slaughter (n=630) in five big abattoirs and from dust samples in fattening (n=107) and breeding pig herds (n=84). MRSA were isolated from nasal swabs using selective enrichment as described by de Neeling et al. (2007). Dust samples were analysed according to the method laid out in Dec. (EC) No. 2008/55. All isolates were confirmed as MRSA using the triplex PCR by Poulsen et al. (2003). Spa-typing was carried out according to Shopsin et al. (1999). Overall, 22 different spa-types were identified. Spa-types t011 and t034 together accounted for 90% of the isolates. The proportion of different spa-types differed among the production levels. While in dust samples collected in holdings of pigs t011 was the predominant spa-type, t034 was more prevalent in slaughter pigs. However, in slaughter pigs there were also differences between regions concerning the proportion of spa-types. t011 was the most prevalent spa-type in one
abattoir and t034 was more frequent in the other abattoirs that were located in a different region. The differences between these regions were not observed in fattening and breeding pig holdings. Comparison of spa types within the same region revealed that t034 tended to be more prevalent in slaughter pigs in both regions than in breeding pigs (Region A) or in breeding and fattening pigs (Region B). The reason for this difference is not clear. Most spa-types belong to the livestock associated Multi-Locus-Sequence-Type (MLST) ST398. Only four isolates revealed spa-types not related to this sequence type. They were from MLST ST9 (1 isolate), ST39 (2 isolates) and ST97 (1 isolate). All four isolates were detected in dust samples. Our results confirm the predominance of t011 and t034 among MRSA isolates of pigs from different production levels.

65A MALIGNANT RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN FRENCH SLAUGHTERED PIGS

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MRSA is considered as a major human pathogen, involved in both hospital acquired and community-associated infections. Recently, studies from different countries demonstrated the presence of MRSA in pig and suggested a link between MRSA strains from pig and farmers. In order to assess the prevalence of MRSA carriage by French fattening pigs, AFSSA, in collaboration with the French Ministry of Agriculture and local veterinary services, sampled 165 batches of 10 pigs by nasal swabbing between January and September 2007 in 21 slaughterhouses. Sampling was representative of national production. The prevalence study demonstrated that 29.0% (IC 95% [22.8 - 35.2]) of batches and 13.3% (IC 95% [8.2 - 18.4]) of pigs were MRSA carriers at slaughterhouse. The majority of MRSA strains were resistant to tetracycline. Half of them were resistant to macrolides. Differing from human isolates, most of them were susceptible to fluoroquinolones. Spa-typing on all strains (n=204) showed 12 following spa-types: t011 (65.5%), t002 (8.9%), t1184 (6.9%), t899 (5.9%), t2370 (3.9%), t034 (2%), t108 (2%), t4146 (1.5%), t1456 (1%), t509 (1%), t588 (1%), t430 (0.5%). MLST on a panel of strains (n=32) selected randomly as one spa-type per slaughterhouse revealed only 4 MLST-types. ST398 was predominant with 81%, and ST5, ST8 and ST1348 reached 6.3% each. ST398 was retrieved for 8 spa-types (t011, t034, t108, t899, t899, 1184, 1456, 2370) while ST5 was for one spa-type (t002), ST8 for t430 and t4146, and ST1348 for t002 and t509. Overall, MRSA was isolated from French fattening pigs at slaughterhouse but was less prevalent and more diverse than in others countries. ST398 was, as described in others countries, the more predominant ST in fattening pigs. MRSA carriage by French pigs at farm level is probably lower than in slaughterhouses (data not shown) and requires specific investigations.

66B MRSA COLONIZATION IN HORSES: IT DOESN'T STOP AT THE NOSE

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Introduction: Up till now, equine methicillin-resistant Staphylococcus aureus (MRSA) screening protocols have been limited to the detection of nasal colonization. However, given the nature of Staphylococcus aureus, skin colonization is to be expected as well. Hence, the aim of this study was to enhance equine MRSA screening by determining preferential sampling locations. Method: Thirty healthy horses permanently residing in the Large Animal Hospital of Ghent University, were included in the study. Using two sampling techniques at each location, all horses were tested at the nose and 8 skin sites (neck, withers, croup, perineal, medial carpus, pastern, flank and medial thigh). Enrichment and phenotypic identification were performed using an established equine screening protocol. MRSA presence was confirmed using oxacillin disk diffusion and triplex PCR targeting the 16S rRNA, mecA and nuc genes. As soon as a sample was positive, the location and horse from which it was taken were regarded as positives. Results: Since one horse objected to nasal sampling, 538 samples were obtained. Twelve animals (40%) carried MRSA on at least one location with 10 (33.3%) testing positive in the nose and 9 (30%) at skin level. Respectively 3 and 2 of them were colonized exclusively in the nose and skin. MRSA was isolated from every sampled location with the highest isolation rate found in the nose (33.3%), followed by carpus (16.7%), neck, withers and croup (13.3%), periungual and perianal skin samples (10.0%) and finally flank and thigh (6.7%). Nasal sampling only resulted in the detection of 10 out of 12 colonized horses (sensitivity = 83.3%). Sampling both nose and neck increased the sensitivity to 91.7%. Only the combination of nose, neck and perineum resulted in 100% sensitivity. Conclusion: In concordance with human literature, a high percentage of individuals permanently residing in a long-term care facility was found to be MRSA positive. In addition, we demonstrated both nasal and multi-locus skin colonization in horses, which should be accounted for in screening protocols.

67A ANTIMICROBIAL RESISTANCE AMONG S. AUREUS ISOLATED FROM PEOPLE AND DOGS IN SASKATOON, CANADA

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Background: Methicillin resistant Staphylococcus aureus (MRSA) is a very important pathogen in people. Since 2006, nine dogs infected with MRSA have been seen at the Western College of Veterinary Medicine (WCVM) in Saskatoon, Cana-
Methicillin-Resistant Staphylococci in Animals

68B DIAGNOSIS OF MRSA COLONIZATION IN HORSES: CAN A SWAB MAKE THE DIFFERENCE?

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Introduction: Little data comparing equine methicillin-resistant Staphylococcus aureus (MRSA) isolation protocols is available. Since sample collection constitutes the basis of each diagnostic protocol, the aim of this study was to compare a classic cotton-tipped swab with a novel human collection device (ESwab) for MRSA detection in horses. Method: Thirty healthy horses permanently residing in the Large Animal Hospital of Ghent University were screened for MRSA carriage (nose and 8 skin sites). Two swab types were used at every location: a conventional cotton-tipped swab embedded in solid Stuart’s medium and the Copan nylon-flocked ESwab in modified Liquid Amies medium. Samples were processed using an established equine screening protocol. MRSA confirmation was achieved through oxacillin disk diffusion and triplex PCR (16S rRNA, mecA and nuc genes). As soon as a sample was positive, the location and horse from which it was taken were considered to be colonized. Results: With one horse refusing nasal sampling, a total of 269 locations were tested. Both sampling techniques were able to deliver positive results at several body sites. Nevertheless, the conventional swab demonstrated a higher sensitivity than the ESwab in all but two locations (see Table 1). In addition, only 37.9% of the locations testing positive with the conventional swab were confirmed using the ESwab, as opposed to 57.9% of positives resulting from ESwabs being verified with a positive conventional swab. Table 1: sensitivity of the conventional and E swab sampling technique per body site P> Discussion: The results clearly demonstrate a higher sensitivity of the conventional swab compared to the ESwab for MRSA detection in horses. On the other hand, only a limited level of agreement was found between positive results from both methods. Awaiting further research, usage of the conventional swab seems preferable when using the isolation protocol applied here.

69A ANTIBIOTIC RESISTANCE PATTERNS OF STAPHYLOCOCCUS SPECIES ISOLATED FROM HEALTHY DOGS AND DOGS WITH OTITIS, PYODERMA OR BOTH

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Introduction: Staphylococcus is a major pathogen and commensal in dogs. Skin disease, including otitis externa, is a primary feature of staphylococcal infection. Treatment in diseased animals will commonly include the use of antibiotics. The predominant Staphylococcus species isolated from healthy or diseased dogs is S. intermedius (most isolates of canine S. intermedius are now believed to be S. pseudointermedius). However other species can be isolated, such as S. aureus and S. schleiferi. Antibiotic resistance varies between and within these staphylococcus species and is related to a variety of molecular resistance determinants. Methicillin resistance is increasingly associated with staphylococci isolated from dogs and presents public health and treatment concerns. Objective: The purpose of this study was to investigate patterns of antibiotic resistance in Staphylococcus isolated from skin, aural canal, nasal cavity from healthy dogs and dogs with otitis, pyoderma or both. Methods: One hundred and forty eight staphylococcal isolates were obtained from 72 dogs. Staphylococcal species were identified based upon colony appearance, hemolytic pattern, gram stain and BBL Crystal Gram Positive ID kit. Antibiograms were determined by broth dilution or disc diffusion and interpreted according to CLSI standards. Results: Staphylococcus species were identified as S. intermedius (n = 126), S. schleiferi (n = 18) and S. aureus (n = 4). Isolation sites included: skin (n = 51), ear (n = 42), nose (n = 55). Antibiotic resistance among all isolates was highest for ampicillin (74%) and lowest for amikacin (2.7%). Twelve isolates were resistant to oxacillin of...
which 9 were *S. intermedius*, 2 were *S. schleiferi* and 1 *S. aureus*. Oxacillin resistant isolates were mostly likely also resistant to clindamycin, enrofloxacin, erythromycin, gentamicin, and trimethoprim/sulphamethoxazole (p < 0.05). Oxacillin co-resistance was highest with clindamycin and erythromycin (67% each). Most oxacillin resistant isolates were obtained from the skin (9/12). Oxacillin resistance was not significantly related to Staphylococcal species. **Conclusions:** Oxacillin resistance in dogs is more likely to be found in Staphylococcus isolated from skin versus ear or nose. *Staphylococcus intermedius* is equally likely to be oxacillin resistant as other staph found in dogs. If the Staph isolate is oxacillin resistant, it is likely to be resistant to a macrolide as well. Further characterization of these isolates for presence of mecA and Staphylococcal cassette chromosome (SCC) elements will aid in understanding the development of methicillin resistance in dogs.

**70B NASAL CARRIAGE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS BY AUSTRALIAN VETERINARIANS**

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A recent Danish study screened veterinarians at several national conferences for methicillin-resistant *Staphylococcus aureus* (MRSA) and found a prevalence rate of 3.4%, with the major subtypes identified having previously been associated with horses (ST8), small animals (ST22), and livestock (ST398). In Australia, geographic isolation, quarantine restrictions and a conservative approach to registration of antimicrobials for use in food animals are presumed to have protected veterinarians from occupational exposure to resistant pathogens, including MRSA. However, the majority of the veterinary workforce deals predominantly with companion or performance animals in clinical settings, thus giving rise to concerns that the risk of MRSA infection is greater for these groups of practitioners. Therefore, in this work we undertook the first large-scale MRSA screening study involving Australian veterinarians. Delegates attending the Australian Veterinary Association Annual Scientific Conference in May 2009 were invited to provide a nasal swab for MRSA culture and to take part in a web-based questionnaire profiling their professional activity, recent hospitalisation and antimicrobial history as well as contact with specific animal groups. Respondents who identified themselves as veterinarians working in clinical practice were asked to provide more detailed information, so that we could ascertain if specific sub-groups were at higher risk of MRSA infection compared to veterinarians engaged in non-clinical roles. Swabs and completed questionnaires were obtained for 459 delegates, a response rate exceeding 50%. The overall prevalence of MRSA nasal carriage was 2.8% (n=13), with delegates working in clinical practice (11/333; 3.3%) having a higher prevalence compared to those in non-clinical professions (2/126; 1.6%) giving a relative risk of 2.08 (95% CI = 0.47 to 9.26). More than half of the positive carriers (55%, n=6) were primary accession clinical veterinarians who predominantly worked with dogs and cats; four of the carriers (36%) worked in mixed practice and one in equine practice (7.7%). None of the positive carriers had been admitted to hospital within the last 12 months, or were currently on antibiotics, or had recently travelled abroad. Three strains of MRSA were multi-resistant whilst the remainder conformed to typical community-associated isolates that are not multi-resistant. In conclusion, this study confirmed the presence of MRSA strains in Australian veterinarians, with a higher prevalence recorded in individuals working in clinical veterinary practice compared to other non-clinical veterinary professions. The molecular characteristics of the MRSA strains in comparison to other common MRSA subtypes are currently being determined.

**71A METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN HORSES ADMITTED TO A REFERRAL HOSPITAL IN ATLANTIC CANADA**

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**Background:** Carriage of methicillin resistance Staphylococcus aureus (MRSA) has been reported to be of increasing prevalence in the horse population. At the Ontario Veterinary College 2.7% of horses harbored MRSA in their nasal cavity on admission to the hospital. A recent study using a convenience sample failed to identify any MRSA in horses in the Atlantic region of Canada (New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland), suggesting the prevalence of MRSA in this region may be very low. The Atlantic Veterinary College is the referral veterinary hospital for Atlantic Canada. Presumably a population of horses at higher risk of carrying MRSA would present to this institution compared to the general horse population of the region. The purpose of this study was to determine the prevalence of MRSA among horses that presented to the Atlantic Veterinary College. **Methods:** Horses admitted to the Veterinary Teaching Hospital from June 2007 to May 2009 had nasal swabs collected upon admission. Swabs were incubated in a mannitol salt enrichment broth for 24 hrs and then an aliquot of broth was plated onto a mannitol salt agar. Colonies of appropriate morphology that were coagulase-positive were tested with the Staph aureus latex agglutination test to identify if the isolate was S. aureus. All S. aureus isolates were tested for methicillin resistance using the PBP2' latex agglutination test and an oxacillin agar disk diffusion test. **Results:** Samples were collected from 549 horses admitted to the hospital during the 2 year time period. S. aureus was isolated from 95 (17.3%) of the horses sampled. Only two (0.36%) horses were positive for MRSA. Additionally 34 (6.2%) of the horses carried a coagulase positive Staphylococcus sp. that was not S. aureus. Further confirmation of the MRSA isolates with a qPCR for the mec gene as well as typing of the two isolates are pending. **Conclusions:** Carriage of S. aureus in the nasal passages was common in horses admitted to the Atlantic Veterinary College but carriage of MRSA was rare.
72B
MULTISTRAIN S. AUREUS MICROARRAYS TO INVESTIGATE HOST SPECIFICITY
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Comparative genome hybridisation (CGH) studies using microarrays have acted as a powerful application in increasing our understanding of bacterial genome evolution. Studies have shed light on the genomic content of bacteria and identified key differences that exist between bacterial genomes, such as the presence or absence important genes. We have previously used a well-validated 7-strain Staphylococcus aureus microarray to compare the genomes of 161 human S.aureus isolates with 56 S.aureus isolates that caused infection in cows, horses, goats, sheep and a camel. Animal-associated S.aureus isolates are clustered into 10 dominant lineages each that possess unique combinations of surface proteins. A set of immune evasion genes carried on a bacteriophage were highly associated with human but not animal strains (Sung et al. 2008). We are developing, in order to investigate S.aureus specific genes, a novel 24-strain S.aureus microarray that harbours three 60-mer probes for every ORF in each of the 16 complete S.aureus genome projects, and for 8 complete but unannotated genome sequences deposited in the GenBank database. The sequenced strains include strain RF122 (also referred to as ET-3), a bovine mastitis isolate that belong to the animal dominant ST151 lineage, and an ST398 isolate from a pig. For highly variable ORFs a single probe is designed to hybridise to the conserved gene region whilst a probe is designed within the variable region of each allele of the ORF. Special focus is placed on ORFs that encode surface or secreted proteins. We aim to perform an extensive CGH study to investigate gene distribution in S.aureus populations and lineages, and we aim to identify S.aureus allelic variants that are associated with host specificity. We further aim to characterise key S.aureus proteins involved in determining the host specificity of S.aureus by generating knock-out mutants of candidate genes for animal colonisation and infection studies as part of the EU PILGRIM consortium.

73A
RETROSPECTIVE STUDY ON THE OCCURRENCE OF STAPHYLOCOCCUS AUREUS CLONAL COMPLEX 398 AMONG PORCINE ISOLATES FROM THE 1970S
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Introduction and objective: The emergence of methicillin-resistant Staphylococcus aureus (MRSA) clonal complex (CC) 398 in pig farming has raised a strong interest in this lineage. Although CC398 has been isolated from a wide range of animals such as cattle, poultry and horses, pigs are generally assumed to be the natural reservoir. In this study, the occurrence of CC398 was retrospectively investigated by genotyping a collection of porcine isolates from the 1970s as well as by biotyping of recent CC398 isolates from distinct animal species. The objective was to determine the frequency of CC398 in S. aureus isolated from pigs in the past and to investigate the hypothesis that pigs are the natural reservoir of this genetic lineage. Materials and methods: Nineteen S. aureus strains isolated from pigs in the 1970s (Devriese 1984), representing human and poultry ecovars, and non-host specific (NHS) biotype, were characterized genotypically by spa typing. Thirteen CC398 strains isolated from pigs, poultry and horses in 2006 (Hermans et al., 2008; Nemati et al., 2008) were biotyped as described by Devriese (1984). Results: Nine spa types (t318, t012, t526, t337, t2112, t1236, t156, t213, t127, t008) associated with six clonal complexes (CC30, CC9, CC97, CC12, CC1, CC8) were detected among the old isolates. Surprisingly, the vast majority (11/13) of the CC398 isolates from 2008, including the porcine isolates, displayed the ovine biotype. Such a biotype was not reported in pigs by Devriese (1984), nor by an older study by Hájek and Maršálek (1971), who extensively studied the distribution of S. aureus biotypes in different animal species and found this ecovar to be associated with sheep and goats. One CC398 isolate from poultry belonged to the NHS biotype, which was originally described as one of the predominant biotypes in pigs. The remaining equine isolate produced staphylokinase and could not be assigned to any of the ecovars described by Devriese (1984). Conclusions: 1) The lack of CC398 among the available isolates from the old Devriese’s collection indicates that this S. aureus lineage was not present or occurred at low frequency in pigs at that time. This notion was further supported by the fact that CC398 isolates from pigs did not display any of the biotypes described by older studies on porcine S. aureus. 2) Irrespective of host origin, recent CC398 isolates generally belonged to the ovine ecovar, which has previously been associated with sheep, goat and occasionally cattle. A study investigating the population structure of S. aureus in small ruminants is needed to determine if they are a natural reservoir of CC398.

74B
ANTI-MRSA ACTIVITY OF ETHANOLIC EXTRACTS OF LEAVES OF PARKIA BIGLOBOSA AND VANDA ROXBURGII
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Background to the Study: Despite concerted global efforts, methicillin-resistant Staphylococcus aureus (MRSA) continues to be a serious public health problem earth-wide. Although Vancomycin and Daptomycin have proved effective in the treatment of MRSA infections in humans, these drugs are quite expensive and not readily available in most hospitals in the developing countries, such as Nigeria. This necessitated an inward look for locally available cheap and quality alternatives. In an earlier report, ethanolic extract of leaves of Parkia biglobosa was found to exhibit significant anti-Staphylococcal activity, hence we decided to investigate the anti-MRSA potentials of this plant. Vanda roxburghii, a plant used in the treatment of wound sepsis in folk medicine in Nigeria, is also being studied for anti-
MRSA activity. Materials and Methods: Ethanolic extracts of leaves of *Parkia biglobosa* and *Vanda roxburghii* were tested for activity against 9 isolates of MRSA (5) and Methicillin susceptible *Staph. aureus*, MSSA (3). The tests were carried out by agar dilution methods, using Mueller - Hinton's agar and suitable control organism (*Staph. aureus* ATCC 29213). Results: Results showed that all the isolates of MRSA and MSSA were inhibited by the medicinal plant extracts tested. Minimal inhibitory concentration of *P. biglobosa* ranged from 0.098-0.78mg/ml for both MRSA and MSSA. On the other hand, *Vanda roxburghii* recorded MIC range of 0.78-3.13mg/ml for MRSA and 0.098 - 3.13mg/ml for MSSA. Conclusion: Based on the results, it could be concluded that tropical medicinal plants hold a great promise as potential source of anti-MRSA drugs. The plants or their extracts could be used to free animals of their burden of MRSA, particularly in low resource countries; this, in turn, will reduce the chances of spread of MRSA from animals to the human population.

75A EVALUATION OF TOPICAL BIOCIDE AND ANTIMICROBIAL SUSCEPTIBILITY OF *STAPHYLOCOCCUS PSEUDINTERMEDIUS* FROM DOGS

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Background: *Staphylococcus pseudintermedius* is an important canine pathogen, and the emergence and widespread dissemination of methicillin-resistant strains (MRSP) is of significant concern. Multidrug resistant infections may require alternative approaches, such as the use of topical therapy, but there is minimal information about in vitro susceptibility of methicillin-susceptible *S. pseudintermedius* (MSSP) and MRSP to biocides and topical antimicrobials. The objective of this study was to determine susceptibility of canine MRSP and MSSP to various biocides and topical antimicrobials. Methods: The minimum inhibitory concentration (MIC) of chlorhexidine digluconate, benzalkonium chloride, trielosan, accelerated hydrogen peroxide, geranium oil, tea tree oil, and grapefruit seed extract against 25 MRSP and 25 MSSP isolates from dogs was assessed using the agar dilution method. MICs of fusidic acid, bacitracin and mupirocin were determined using Etests. Results: Trielosan demonstrated excellent activity against all bacterial isolates with no growth at the lowest concentration evaluated (MIC ≤0.5 ug/mL). Conversely, grapefruit seed extract did not uniformly inhibit growth at the highest concentration tested (MIC >3.84 ug/mL). The remainder of the biocides MICs reported include: chlorhexidine digluconate (MRSP; range: 4-16 ug/mL, MSSP: 0.06 ug/mL, MRSP: range: 8 ug/mL, MSSP: range: 0.06 ug/mL), benzalkonium chloride (MRSP: range: 2-16 ug/mL, MSSP: range: 2 ug/mL, MRSP: range: 8 ug/mL, MSSP: range: 4-8 ug/mL), accelerated hydrogen peroxide (MRSP: range: 8-32 ug/mL, MSSP: range: 16-32 ug/mL, MRSP: range: 16-32 ug/mL, MSSP: range: 32 ug/mL, MRSP: range: 2 ug/mL, MSSP: range: 32 ug/mL), germicidal oil (MRSP: range: 0.06-0.12 %, MSSP: range: 0.06-0.12 %, MRSP: range: 0.06-0.12 %, MSSP: range: 0.06-0.12 %, MRSP: range: 0.06-0.12 %, MSSP: range: 0.06-0.12 %, MRSP: range: 0.06-0.12 %, MSSP: range: 0.06-0.12 %), and tea tree oil (MRSP: range: 0.12-0.96 %, MSSP: range: 0.48 %, MRSP: range: 0.06-0.12 %, MSSP: range: 0.96 %). Antimicrobial data were as follows (all values ug/mL): mupirocin, MRSP: range 0.125-0.19, MIC50 0.19, MIC90 0.25; MSSP: 0.094-0.38, MIC50 0.19, MIC90 0.25; fusidic acid, MRSP: 0.094-0.19, MIC50 0.19, MIC90 0.25; MSSP 0.023-0.25, MIC50 0.19, MIC90 0.25; bacitracin, MRSP: 24-96, MIC50 and MIC90 96; MSSP: 16-96, MIC50 and MIC90 90. There was no difference between MRSP and MSSP for any biocide or antimicrobial (all P>0.17). Discussion: While determination of the clinical relevance MIC data is difficult for topical therapies, an understanding of the in vitro susceptibility to different compounds may be useful for empirical therapy in the absence of controlled studies of topical therapy. This study demonstrated good in vitro susceptibility to most tested compounds, but some differences need to be considered. Variations in susceptibility to individual biocides indicate that further study of biocide resistance determinants is indicated.

76B OUTBREAK OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* IN A LITTER OF PUPPIES: EVIDENCE OF VERTICAL PERINATAL AND HORIZONTAL TRANSMISSION

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Objectives: Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) has recently emerged as an important animal health problem. In this study we describe the epidemiology of a MRSP outbreak in a litter of Boxer puppies. All puppies (n=12) were born healthy but showed signs of severe septicaemia during the first week and eventually died 10 days after birth. Pathological examination revealed multifocal haemorrhagic lesions in various organs. Methods: Standard PCR tests were performed with DNA extracted from organs to identify *Brucella canis* and common neonatal viral pathogens such as Canine Herpesvirus and Canine Parvovirus type 1. Pure cultures of β-haemolytic staphylococci were isolated from all organs (liver, spleen, kidneys and blood taken directly from the heart). In the following weeks, staphylococci were also isolated from the bitch, the puppies’ father, and the two owners, giving a total of 16 isolates. All isolates were identified as *S. pseudintermedius* by restriction fragment length polymorphism (RFLP) of the pta gene and were shown to be methicillin-resistant by PCR detection of mecA. Routine antimicrobial susceptibility testing was performed by the microbroth dilution using Sensititre. Smal-pulsed field gel electrophoresis (PFGE) and spa typing was done on five isolates originating from blood taken directly from the heart of one puppy, the vaginal canal of the bitch, and the nasal cavities of the father and the two owners. Staphylococcal Cassette Chromosome *mec* (SCCmeC) was typed using M-PCR 1 and 2 as described by Kondo et al. (2007). Results: None of the samples were positive for Canine Herpesvirus, Canine Parvovirus type 1 and 2 as described by Kondo et al. (2007). Pure cultures of *Staphylococcus pseudintermedius* were cultured from the bitch, the puppies’ father, and the two owners, giving a total of 16 isolates. All isolates were identified as *S. pseudintermedius* by restriction fragment length polymorphism (RFLP) of the pta gene and were shown to be methicillin-resistant by PCR detection of mecA. Routine antimicrobial susceptibility testing was performed by the microbroth dilution using Sensititre. Smal-pulsed field gel electrophoresis (PFGE) and spa typing was done on five isolates originating from blood taken directly from the heart of one puppy, the vaginal canal of the bitch, and the nasal cavities of the father and the two owners. Staphylococcal Cassette Chromosome *mec* (SCCmeC) was typed using M-PCR 1 and 2 as described by Kondo et al. (2007).
indistinguishable band patterns, indicating that the bitch, the
father and the two owners were carriers of the same MRSP strain cause death of the puppies. All isolates harboured SC-
Cmec type III and belonged to spa type t02, which is the most common European MRSP clone. Conclusions: 1. This study shows that MRSP can be a cause of fatal neonatal outbreaks in dogs. 2. The fact that the same clone was isolated from the vaginal mucosa of the bitch strongly indicates vertical perinatal transmission of MRSP. 3. Isolation of the same MRSP clone from two owners suggests that these bacteria can be horizontally transferred to humans living in close contact with dogs and that dog owners may act as vehicles for MRSP dissemination.

77A PRESENCE OF METHICILLINE-RESISTANT STAPHYLOCOCCUS AUREUS ST398 IN RATS ON BELGIAN PIG FARMS

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In the past, rodents have been associated with transmission of different diseases: Salmonella spp., Campylobacter spp., Leptospira spp., Trichinella spp. and Toxoplasma spp. (1). However, rats have not yet been associated with Methicillin-Resistant Staphylococcus aureus (MRSA). The objective of this current study is to investigate the prevalence of MRSA sequence type (ST) 398 in rats on pig farms. In this preliminary study, 15 rats have been sampled. From each rat one swab was taken from the anterior nares, another swab was taken from the skin behind the ear, the fur on the abdomen and back, and the tail. The rats came from 3 different pig farms. The first 10 rats originated from farm A and were pooled in 2 groups (5 rats in each). One rat came from farm B and 4 rats came from farm C. Rats from farm B and C were treated separately. Swabs were enriched in nutrient broth (Oxoid) supplemented with 6.5% NaCl. After 24 hours of incubation, the broth was sub-cultured on a selective chromogenic media for MRSA. Characteristic colonies were confirmed by using a multiplex-PCR for 16S rRNA, mecA and nuc (2). The MRSA isolates were further characterized by staphylococcal protein A (spa) typing, staphylococcal chromosome cassette (SCC) mec typing and Multilocus sequence typing (MLST). After multiplex-PCR, farm A and C were found positive for MRSA. In farm A, two different spa types were identified, type t011, a common type in pigs and t4872. In farm C, all isolates belonged to spa type t011. After SCCmec typing, type IVa and V were found in farm A, and only type V in farm C. MLST was performed on one of each spa type and revealed ST398. Our results indicate that MRSA in rats is highly prevalent and can be isolated both from pooled and single samples. This indicates that rats may play an important role in the dissemination of the animal associated MRSA ST398. The finding that also rats, next to several other animal species, including humans, can carry strains from this clonal complex indicates that this strain has little to no host specificity. Striking is the first detection of a new spa type t4872 that has not been detected before in Belgium. This may be indicative for a constant evolution of the strain, perhaps under the influence of colonizing different animal species. Although this study is only preliminary, it is indicative that rodents play an important role as reservoir of MRSA ST398. Further study results will be available in the coming months.

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78B ANTIMICROBIAL RESISTANCE TO METHICILLIN IN PATHOGENS RECOVERED FROM SHEEP DAIRY FARM

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Introduction: As a consequence of the extensive use of antimicrobials, the occurrence of resistance has been observed in a wide variety of bacteria and animals. Antimicrobial resistance (AR) survey is still scarce in sheep dairy farm, where antimicrobials, including â-lactams, are frequently used for therapy.

Materials and Methods: In the period 2006-07, 227 S. aureus and 351 not-aureus Staphylococci (NAS) were tested in-vitro for oxacillin (OXA) (methicillin) resistance. Tests were performed by disc diffusion using a 1 µg OXA disc (Oxoid) and categorized in resistant (R), intermediate (I) or susceptible (S) according to CLSI M31-A2 Document. AR patterns were then evaluated by using the following discs: penicillin (PEN) 10 U, clindamycin (CLI) 2 µg, enrofloxacin (ENR) 5 µg, erythromycin (ERY) 15 µg, gentamicin (GEN) 10 µg, kanamycin (KAN) 30 µg, streptomycin (STR) 10 µg, tetracycline (TET) 30 µg, sulfamethoxazole-trimethoprim (SXT) 1.25/23.75 µg, vancomycin (VAN) 30 µg and â-lactamase (Nitrocefin, BBL). Minimal inhibitory concentrations (MIC) of strains for OXA were tested by the E-test (AB Biodisk). The presence of mecA gene was searched by PCR. Amplicons were purified by a QiAquick Gel Extraction kit (Qiagen) and sequenced. Sequences were then compared to the GenBank database. Results: Eighteen strains of NAS were phenotypically resistant to OXA (5%); 9 strains resistant also to PEN and 3 strains resistant to 4 antibiotics at the same time (PEN, TET, STR, CLI and VAN in various combinations with OXA). Four NAS strains (S. epidermidis) were characterized by MIC and gene detection and resulted mecA carriers with MICs between 0.5-2 µg/ml. All of them were also â-lactamase producers. Two strains of S. aureus resulted phenotypically resistant to OXA. Both resulted also R to PEN, CLI, KAN, STR, and ENR (1 I), ERY (both I), TET (1 R), GEN (1 I and 1 R). It was not possible to genetically characterized such strains. No strain was resistant to VAN or SXT.

Discussion: Our survey showed the presence of phenotypical OXA resistance in Staphylococci. Strains of NAS harboured the mecA gene. The contemporary presence of multiple AR
79A  
**VANCOMYCIN MEDIATED MODIFICATIONS IN CYTOPLASMIC MEMBRANE LIPID OF RESISTANT STRAIN OF STAPH. AUREUS (VRSA/MRSA)**  
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Vancomycin has pronounced effect on cell wall and morphology of *Staph. aureus*. In this pretext a project has been designed to analyze the effect on membrane structure and chemistry i.e. fatty acids metabolism on resistant strain of *S. aureus*. Therefore 3 local strains isolated from patients and a highly resistant strain of *Enterococci* (VRE) was used in this study. CP1 & CP2 strains are resistant to 5µg & 16µg of vancomycin respectively. Where as PSA a 3rd strain is sensitive to it. *Enterococci* has exhibited resistance to ≥1000µg of vancomycin. Comparative analysis of cytoplasmic membrane’s (CM) fatty acids (F.A) profile was done by GC mass spectrometry of these strains grown in presence and absence of vancomycin. CP1 & CP2 have shown diversified range of changes i.e. Desaturation, Cyclization and long chain alkane synthesis in resistant strain of *S. aureus*. Octadecanoic acid and its unsaturated isomers C19:1 were found to be major components of CM in resistant strains of *S. aureus*. In CP2 cis and trans isomers of C19:1 were found to be common. GC mass spectrum of CP2 has indicated diverse species of polyunsaturated octadecanoic acid in addition to multiple double bonds at different positions were generated e.g. 19-12, 12-14-17 and 9-12-15 in unsaturated and 6,7,8,9 and 19 in monounsaturated species. However position 9 in carbon chain of this acid is persistent choice for desaturation in this strain of *S. aureus*. Such modifications were not noticed in sensitive strain PSA or in VRE. Carbon chain length was another significant feature i.e. in CP1 & CP2 > 72% of total F.As are long chain (C18-C39). *Enterococci* has shown C8-C35 long F.A. in addition to significant amount of short chain (C7-C8)F.A. A common feature shared by VRSA and VRE is the formation of branched chain fatty acids (BCFA). These F.As account for about 50% of total F.A in CP2, 39% in CP1 and in VRE > 60%. *Anteiso* C19:0 & C19:1 is the major BCFA in CP2 and CP1. Other outstanding feature of CP1 & CP2 is the formation of long chain alkanes i.e. hexadecane and pentadecane.

Other implication of vancomycin intervention in the lipid metabolism is the cyclization of F.A. by VRSA. Presumably polyene polymers of Octadecanoic acid are the precursors for the following steps of cyclization. Vancomycin is a glycopeptide which may have potential to activate the modification pathway of F.A. which lead to cyclization. Interestingly in VRSA strains Octadecanoic acid and its poly unsaturated derivatives seem to be precursor for these enzymes. CP2 strain is harbored with a plasmid carrying VanA cassette. Our study has supported the hypothesis that glycopeptide intervene in lipid metabolism as well and implicates its effects on cytoplasmic membrane. Concurrent expression of vancomycin resistance genes influence the cell wall and bacterial morphology.

80B  
**TREATMENT AND OUTCOME OF MRSA INFECTION IN 11 DOGS**  
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Methicillin-resistant *Staphylococcus aureus* (MRSA) infections of dogs have been reported to be successfully treated with a variety of systemic antimicrobials (trimethoprim/sulfadiazine, clindamycin, fluoroquinolones, clindamycin, cephalaxin and amoxicillin/clavulanic acid) along with wound management and removal of surgical implants where indicated. Medical records of all dogs with MRSA infection at a tertiary referral veterinary hospital over a 17-month period (2007-2008) were retrospectively reviewed to determine treatments and outcome. Bacterial isolates were identified as *Staphylococcus aureus* based on colony morphology and biochemical testing. Methicillin resistance was determined by oxacillin disk diffusion testing and mecA PCR. Eleven dogs with MRSA were identified; 6/11 following orthopedic surgery, 2/11 following soft tissue surgery, 2/11 associated with traumatic wounds, 1/11 associated with chronic otitis and atopic dermatitis. Treatments included; removal of surgical implant (n=5) or bone sequestrum (n=1) with debridement of affected tissue, doxycycline (n=5: 5-10 mg/kg PO q 12 hrs for 4-8 weeks), amikacin (n=2: 15-21 mg/kg IV or SQ q 24 hrs for 5 days), chloramphenicol (n=2: 38-43 mg/kg PO q 8 hrs for 2-3 weeks), cephalaxin (n=1: 25 mg/kg PO q 12 hrs for 2 weeks), amoxicillin-clavulanic acid (n=2: 12.5-18 mg/kg PO q 12 hrs for 4 weeks), enrofloxacin (n=2: 8.5-10 mg/kg PO q 24 hrs for 4 weeks), trimethoprim/sulfadiazine (n=1: 24 mg/kg PO q 12 hrs for 2 weeks) and topical medication (chloramphenicol 1% ointment [n=1] and EpiOtic® [n=2]). A single systemic antibiotic was administered in 5 cases (doxycycline 3/5, chloramphenicol 1/5 and cephalaxin 1/5), multiple systemic antibiotics in 4 cases (doxycycline/amikacin 1/4, doxycycline/amoxicillin-clavulanic acid/enrofloxacin 1/4, amoxicillin-clavulanic acid/enrofloxacin 1/4, and amikacin/chloramphenicol 1/4), and topical therapy alone in 1 case (1% chloramphenicol ointment and EpiOtic®). One dog was euthanized prior to commencement of antibiotic therapy while the other 10 dogs had complete resolution of infection. To the authors’ knowledge, this is the first report of successful treatment of MRSA in dogs with doxycycline, chloramphenicol or amikacin, and further emphasizes the importance of removal of surgical implants and aggressive tissue debridement.

81A  
**CLINICAL EVALUATION OF THE BIO-RAD MRSASELECT™ FOR DETECTION OF MRSA IN PIG FARMERS**  
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Objectives: To assess the sensitivity and specificity of Bio-Rad MRSASelect™ for the detection of MRSA in samples of pig farmers. Methods: Nasal samples were taken from pig farmers, co-workers and their household members. Samples were inoculated directly onto MRSASElect™ (Bio-Rad). In addition, subcultures of an enrichment broth were inoculated onto...
both MRSASelect™ and MRSA ID (bioMérieux). Both sets of plates were read after 18-24 h incubation at 35°C. Growth of colonies showing pink coloration on MRSASelect™ or growths of colonies with green coloration on MRSA ID were considered to be indicative for MRSA. When isolates were identified as S. aureus a duplex PCR for the mecA gene and coagulase gene was performed and this was considered as the gold standard.

Results: A total of 257 freshly collected nasal samples were analysed in this study. When combined results for all media were analysed, 61 of the 257 (23.7%) samples were identified as positive for MRSA. The sensitivity of MRSASelect™ and MRSA ID are shown in Table 1. In combination with an enrichment broth the sensitivity of MRSASelect™ was comparable to MRSA ID. The directly inoculated MRSASelect™ was statistically significant less sensitive than the two media after enrichment broth. After enrichment broth, MRSASelect™ was grown 9 and MRSA ID grew 10 false positive strains. This results in specificities of 95.4% and 94.9% respectively (P = 1.0). The specificity of the directly inoculated MRSASelect™ medium was also 95.4%. Conclusions: MRSASelect™ and MRSA ID can detect MRSA strains and are sensitive and specific tools for differentiation between micro-organisms and MRSA in samples from pig farmers. However, an enrichment broth is required to obtain an acceptable sensitivity. The quality of MRSASelect™ medium directly inoculated and after enrichment broth compared with MRSA ID after enrichment broth.

Method MRSA detected MRSA not detected Sensitivity. MRSASelect™ directly inoculated 39 22 63.9%. Enrichment broth MRSASelect™ 60 1 98.4%. Enrichment broth MRSA ID 61 0 100%

82B
GENETIC COMPARISON OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN U.S. MID-ATLANTIC HORSES AND THEIR LOCAL ENVIRONMENTS

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Introduction: In the past 10 years, MRSA isolation from companion and farm animals has been reported with increasing frequency, comparable to the rise seen in community MRSA colonization and infection rates in human populations. Despite this increase in veterinary importance of MRSA, little is known about the regional and community-level epidemiology of MRSA in horses. Work in the Netherlands has demonstrated MRSA in nasal swabs from pigs and in environmental samples from pig barns in confined settings and has shown that environmental sampling can be used in place of more invasive methods. This study examined a novel sampling method for characterizing MRSA in the equine environment by determining molecular characteristics and relatedness of MSSA and MRSA strains cultured from horses and their local environments. Materials and Methods: Nasal swabs (NS) from 13 racehorses and 26 pleasure horses were obtained from two farms where MRSA had not been previously diagnosed. Environmental samples (ES) were taken from fences and stalls around sampled horses using sterilized Swiffer™ swabs. Both NS and ES samples were incubated using a double-enrichment protocol of Mueller Hinton Broth + 6.5% NaCl, followed by Tryptic Soy Broth + Aztreonam + Cefoxitin. Positive colonies were identified using a selective agar (MRSA Select™) and confirmed by a specific MRSA quadruplex PCR (Nuc, mec A, 16S, PVL). MRSA isolates confirmed by the presence of mec A gene were further typed by PFGE and tested for the presence of TSST. Results: On the pleasure horse farm 0/26 samples were positive. On the racehorse farm, 8/13 (61%) nasal swabs were MRSA (+), 1/13 (7.7%) was MSSA (+) and 5/7 (71%) of environmental samples were MRSA (+). In a related study, animal-environment pairs were examined based on sampling proximity and environment-animal samples were correlated (X² of 4.38, p=0.04). All positive samples were USA 500, PVL (-), TSST (-). PFGE SmaI restriction fragment length polymorphism patterns were grouped based on Tenover criteria; strains all had greater than 95% pattern similarity and were considered clonal. Discussion: In this study MRSA was found in both equine and environmental samples. Based on molecular characteristics, proximal environmental samples were found to be representative of horses. This was true for both indoor and outdoor areas. Given that horses have been found to be potential sources for human MRSA colonization in those with close proximity and that colonization can lead to greater morbidity, a better understanding of equine MRSA epidemiology is needed. In order to more fully characterize equine MRSA, more farms need to be sampled and longitudinal studies conducted to determine persistence of MRSA in both the environment and animals. The methods presented in this study may aid in future work to understand equine MRSA at the regional and community level.
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